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ORIGINAL PAPER

Survival outcome and prognostic factors in patients with chemotherapy-naive metastatic castration-resistant prostate cancer treated with abiraterone acetate – real-world experience in Vietnam

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ABSTRACT

Introduction and aim. In real life, metastatic castration-resistant prostate cancer patients (mCRPC) had more complex clinical presentation than patients in the COU-AA-302 trial. This study primarily aimed to describe the overall survival of chemotherapy-naive mCRPC treated with abiraterone acetate plus prednisone (AAP). Other relevant outcomes and baseline characteristics of these patients were also evaluated.

Material and methods. This retrospective, observational study collected data from chemotherapy-naive mCRPC patients treated with AAP in Vietnam. Kaplan-Meier curves were used to estimate time to treatment failure (TTF), and overall survival (OS). The impact of baseline characteristics on OS was explored using univariate and multivariate Cox proportional hazard models.

Results. Data from 65 eligible patients were analyzed. The rate of PSA response was 73.8%, median PSA PFS was 10.5 months (95% CI: 7.4–13.6), median TTF was 15 months (95% CI: 11.1–18.9), and median OS was 24.9 months (95% CI: 18.9–30.9). Shorter OS was significantly associated with a higher Gleason score (≥8), shorter time from ADT start to mCRPC (<12 months), visceral metastases, and <50% PSA decline ($p<0.05$).

Conclusion. Abiraterone acetate plus prednisone is well tolerated and effective for chemotherapy-naive mCRPC patients in clinical practice. Moreover, Gleason score, visceral metastasis, time from ADT start to mCRPC, and PSA response are the independent indicators for predicting the OS of mCRPC patients in both univariate and multivariate analyses.

Keywords. abiraterone acetate, metastatic castration-resistant prostate cancer, overall survival, real-world evidence

Introduction

Prostate cancer (PCa) is one of the most common cancers in males, especially in developed countries. According to the estimates from GLOBOCAN 2020, PCa ranks second in terms of the number of new cases with 1,414,259, and fifth in terms of mortality with 375,304 cases.[1] In Vietnam, PCa ranks fifth in the incidence rate and seventh in mortality with 6,248 new cases and 2,628

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deaths reported in 2020.[1] In the United States, where PCa screening with PSA (prostate-specific antigen) and prostate biopsy is well-implemented, the rate of stage IV PCa is only 8%.[2,3] Therefore, for all stages combined, the 5-year relative survival rate for prostate cancer is 98%.[2,3] However, the rate of patients with stage IV prostate cancer in Vietnam is stated to be over 75%. This significantly compromises the overall prognosis of prostate cancer patients and amplifies the financial burden associated with treatment.

For nearly eight decades, androgen deprivation therapy (ADT) has served as the cornerstone of systemic treatment for men with metastatic prostate cancer. The antitumor effects of ADT improve quality of life by reducing bone pain and complication rates. However, following a median of 18–24 months of ADT, almost all patients progressed to mCRPC. A phase III pivotal study of chemotherapy-naïve mCRPC patients (COU-AA-302) demonstrated improvements in median radiographic progression-free survival (rPFS) from 8 to 16 months and the median overall survival (OS) from 30.3 to 34.7 months with AAP as compared with prednisone plus placebo.[4] However, in real life, mCRPC patients had more complex clinical presentation than those patients in the COU- AA-302 trial. The majority of mCRPC patients treated in clinical practice are elderly and have poor ECOG PS, and comorbidities such as cardiovascular disease, hypertension, and diabetes mellitus are thus common. Such patients along with those with visceral metastases may be under-represented in RCTs of mCRPC. Especially in Vietnam, mCRPC patients often have severe clinical symptoms and poor medical care conditions. Therefore, the outcome of mCRPC patients is often poor.

Aim

The main objective of this retrospective observational study was to add to the body of knowledge related to AAP by primarily describing the OS of chemotherapy-naïve mCRPC patients treated with AAP in routine clinical practice in Vietnam.

Material and methods

Study design and eligibility

This was a retrospective, observational cohort study conducted at Vietnam National Cancer Hospital from January 2014 to May 2023. The data collection period for each patient ranged from the initial date of prostate cancer diagnosis up to the date of data collection. The start of AAP treatment was considered as the baseline. Data from eligible patients treated with AAP were extracted from their medical records and entered into electronic case report forms. Patients were eligible if they had documented mCRPC and had received AAP for the treatment of mCRPC, were naive to chemotherapy, had an Eastern Cooperative Oncology Group (ECOG) performance status grade of 0 to 3, and hematological and chemical laboratory values that met predefined. Patients were excluded if they had received any chemotherapy or cytotoxic agent for the treatment of mCRPC or novel hormonal therapies before initiation of AAP, had short survival time (older people with many co-morbidities), and had second cancer.

All objects of the protocol in this study was approved by the Science and Ethical Committee of Hanoi Medical University, Vietnam as number: 6811/QD-DHYHN. Written informed consent was applied to all patients before enrolling them in the study. Patients could withdraw from the study at any time without any threats or disadvantages and for no stated reasons.

Data collection and outcomes of interest

Patient characteristics at the time of AAP treatment initiation were collected. These factors included age, comorbidities, Gleason score, the time from ADT start to abiraterone, initial diagnosis, Eastern Cooperative Oncology Group Performance Status (ECOG-PS), the Brief Pain Inventory – Short Form (BPI – SF), location of metastases, prostate-specific antigen [PSA], hemoglobin.

The main endpoint of the study was overall survival (OS) with AAP. OS was defined as the time from the start of AAP treatment to treatment death for any reason. The secondary endpoint of the study was the time to treatment failure with AAP. Time to treatment failure (TTF) was defined as the time to treatment until 2 out of 3 progression factors were biochemical, imaging, and clinical progression and was considered equivalent to the duration of treatment.

Statistical analysis

All patients who met the eligibility criteria were included in the data set for analysis by SPSS 20.0 statistical software. The objective of the study was primarily descriptive, and most of the outcomes were analyzed using descriptive statistics (for categorical variables number and percentage of patients per response option, based on non-missing data; for continuous variables, the median and the inter-quartile range [IQR] are reported).

Time-to-event endpoints were analyzed using Kaplan-Meier survival plots. For all time-to-event endpoints, patients who had not experienced the event of interest at the time of data collection were censored. The impact of covariates on OS was explored using univariate and multivariate Cox proportional hazard models.

Results

From January 2014 to May 2023 in Vietnam National Cancer Hospital, a total of 65 patients were recruited in the study. At the time of data collection after three years

of treatment, 63 patients (96.9%) discontinued AAP, and 51 patients (78.5%) died. After treatment failure with AAP, 40 patients (61.5%) received second-line therapy with docetaxel, and 10 patients (15.4%) received third-line therapy with enzalutamide. The majority of patients (86.4%) were treated with an anti-osteoporotic drug (zoledronic acid or denosumab).

Table 1 shows the patients' baseline clinical and paraclinical features. The median age of patients at mCRPC diagnosis was 70 (IQR: 64–76), the rate of patients who had comorbidities was 43.1% which cardiovascular disease accounted for 35.4%, and Gleason scores ≥8 was 78.5%. The rate of patients de novo was 75.4%, and the median time from ADT to mCRPC was 16.0 months (IQR: 11–23). Patients had ECOG PS status ≥2 was 24.6%, and pain symptoms of BPS-SF >3 was 36.9%. The rates of bone, lymph node, and visceral metastasis were 87.7%, 38.5%, and 16.9%. The median PSA was 34.7 ng/ml (IQR: 13–106.8), and the median hemoglobin was 126 g/l (IQR: 116–132).

Table 1. Patient characteristics with mCRPC

	n (%)
Median age (IQR)	70 (64–76)
Comorbidities n (%)	28 (43.1)
Cardiovascular disorders	23 (35.4)
Metabolic disorders	6 (9.2)
Other disorders	4 (6.2)
Gleason score n (%)	
<8	14 (21.5)
≥8	51 (78.5)
Diagnosis n (%)	
Recurrent	16 (24.6)
De novo	49 (75.4)
Time from ADT start to abiraterone (months), median (IQR)	16 (11–23)
ECOG PS n (%)	
0	18 (27.7)
1	31 (47.7)
≥2	16 (24.6)
BPI – SF n (%)	
BPI-SF 0–3	41 (63.1)
BPI-SF >3	24 (36.9)
Location of metastases n (%)	
Bone	57 (87.7)
Non-regional lymph nodes	25 (38.5)
Viscera	11 (16.9)
Median PSA (IQR)	34.7 (13–106.8)
Median hemoglobin (IQR)	126 (116–132)

The median duration of treatment was 15 months (IQR: 8–19.8). The rate of PSA response was 73.8%, median PSA OS was 10.5 months (95% CI: 7.4–13.6), median TTF was 15 months (95% CI: 11.1–18.9) (Fig. 1A), and median OS was 24.9 months (95% CI: 18.9–30.9) (Fig. 1B). However, the median overall survival between subgroups was heterogeneous.

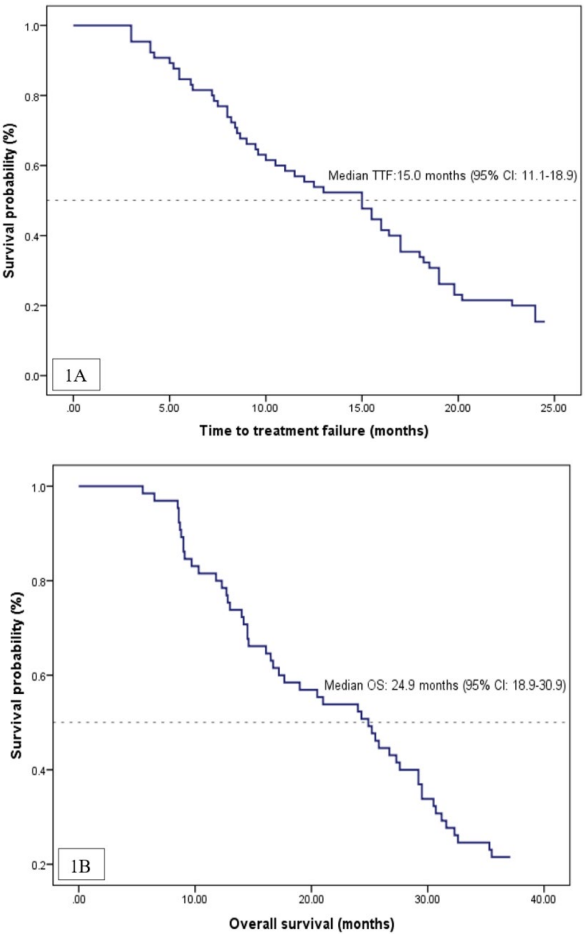

Fig 1. A: Time to treatment failure, B: overall survival during abiraterone treatment

The Kaplan-Meier analysis revealed significantly longer median OS for various factors: age <70 versus age ≥70 (30.7 vs 16.1 months) (Fig. 2A), ECOG PS 0-1 versus ECOG PS 2-3 (27.6 vs 11.8 months) (Fig. 2B), BPI-SF ≤3 versus BPI-SF >3 (29.5 vs 14.2 months) (Fig. 2C), Gleason score <8 versus Gleason score ≥8 (32.6 vs 19 months) (Fig. 2D), PSA ≤80 ng/ml (26.7 vs 12.8 months) (Fig. 2E), absence of visceral metastases versus presence of visceral metastases (27.6 vs 10.3 months) (Fig. 2F), "recurrent" status versus de novo status (29.2 vs 17.7 months) (Fig. 2G), time from ADT start to mCRPC ≥12 months versus time from ADT start to mCRPC <12 months (29.2 vs 12.7 months) (Fig. 2H), and PSA response with venous PSA response versus without PSA response (29.2 vs 9.1 months) (Fig. 2I).

In univariate analysis of the relationship between clinical characteristics at initiation and OS, higher age (≥70), higher ECOG PS (≥2), higher Gleason score (≥8), or higher BPI-SF (>3), or higher PSA (>80), or visceral metastases, de novo, or shorter time from ADT start to mCRPC (<12 months), and <50% PSA decline were all associated with shorter time to OS with AAP ($p<0.05$). However, in the multivariate analysis, only a high-

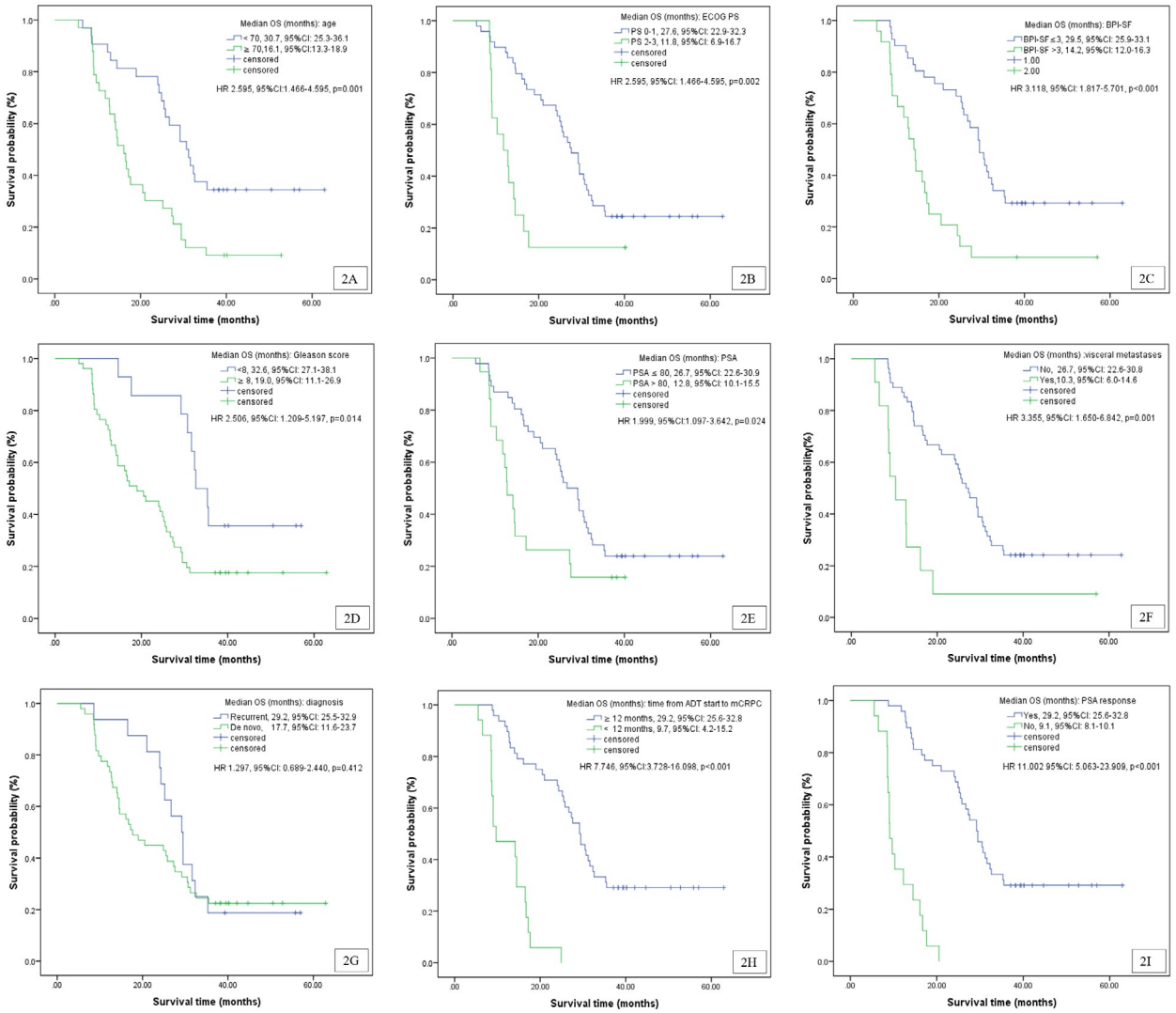

Fig. 2. Kaplan-Meier assessing the relationship between baseline characteristics and OS

er Gleason score (≥8), shorter time from ADT start to mCRPC (<12 months), visceral metastases, and <50% PSA decline were associated with shorter time to OS (Table 2).

During treatment with AAP, the most common symptoms were edema (23.1%), hypokalemia (15.4%), hypertension (10.7%), elevation of AST/ALT enzymes (7.7%), and hyperglycemia (7.7%). The majority of patients had mild to moderate adverse events, without patients had to discontinue the treatment due to adverse events of AAP.

Discussion

In the COU-AA-302 trial by Ryan et al. 1088 patients had asymptomatic or mildly symptomatic, ECOG PS 0-1, no visceral metastasis, no cardiovascular disease, and chemotherapy-naive prostate cancer.[4] The results showed that median overall survival was significantly longer in the abiraterone acetate group than in the placebo group (34.7 months vs. 30.3 months).[4] However, clinical practice shows that at the time of initial mCRPC diagnosis, patients have heterogeneous characteristics. Our sample has more complex characteristics than the COU-AA-302 trial. The median age was 70 (IQR: 64–76), cardiovascular disease was 35.4%, de novo was 75.4%, Gleason score ≥8 was 78.5%, and ECOG PS ≥2 was 24.6%. The rate of patients who had symptoms of pain BPS-SF >3 was 36.9% and visceral metastasis was 16.9%. The median PSA was 34.7 ng/ml (IQR: 13–106.8), and the median hemoglobin was 126.0 g/l (IQR: 116–132). In our study, PSA response was 73.8% higher than the COU-AA-302 trial (62%) but the median biochemical PFS was 10.5 months (95% CI: 7.4–13.6) lower than COU-AA-302 trial (11.1 months).

Prostate Cancer Clinical Trials Working Group 3 (PCWG3) evaluates the progression of bone metastases on bone scintigraphy according to the 2+2 rule.[5] However, in Vietnam, clinical practice conditions are often inadequate to assess bone metastases. In addition, the results of the COU-AA-302 trial by Rao et al. showed

that a substantial proportion (38%) of patients discontinued treatment for non-radiographic progression.[6] Therefore, in our study, we evaluated the time to treatment failure. The median time to treatment failure with AAP in our sample was 15 months (95% CI: 11.1–18.9), which was longer than the treatment duration of 13.8 months seen in the phase III COU-AA-302 trial by Ryan et al. and in other real-world studies by Pilon et al. and Boegemann et al. (6.8 and 10 months, respectively).[4,7,8]

Table 2. Univariate and multivariate analysis (Cox regression models) assessing the relationship between baseline characteristics and OS

		n (%)	Univariate HR (95% CI), p	Multivariate HR (95% CI), p
Age	<70	33 (50.2)		
	≥70	32 (49.8)	2.595 (1.466–4.595)	1.253 (0.629–2.494)
			p=0.001	p=0.512
PS	0–1	49 (75.4)		
	2–3	16 (24.6)	2.764 (1.465–5.216)	1.707 (0.775–3.736)
			p=0.002	p=0.715
Gleason	<8	14 (21.5)		
	≥8	51 (78.5)	2.506 (1.209–5.197)	3.136 (1.373–7.136)
			p=0.014	p=0.007
BPI–SF	≤3	41 (63.1)		
	>3	24 (36.9)	3.118 (1.817–5.701)	1.067 (0.522–2.184)
			p<0.001	p=0.858
PSA	≤80	46 (70.1)		
	>80	19 (29.9)	1.999 (1.097–3.642)	1.957 (0.906–4.227)
			p=0.024	p=0.88
Visceral metastases	No	54 (83.1)		
	Yes	11 (16.9)	3.355 (1.650–6.842)	2.735 (1.09–6.593)
			p=0.001	p=0.032
Diagnosis	Recurrent	16 (24.6)		
	De novo	49 (75.4)	1.297 (0.689–2.440)	0.439 (0.217–1.123)
			p=0.412	p=0.092
Time from ADT start to mCRPC	≥12 months	48 (73.8)		
	<12 months	17 (26.2)	7.746 (3.728–16.098)	3.883 (1.556–9.441)
			p<0.001	p=0.003
PSA response	Yes	48 (73.8)		
	No	17 (26.2)	11.002 (5.063–23.909)	6.425 (2.115–19.520)
			p<0.001	p=0.001

In the COU-AA-302 trial by Bjartell et al. 67% of patients after failure of AAP continued on second-line therapy, which was mainly docetaxel (48%) and third-line therapy was 36%.[9] The results showed that patients who received docetaxel second-line therapy improved prognosis compared with patients who received symptomatic treatment. In our study, after the failure of AAP, 40 patients (61.5%) received second-line therapy with docetaxel and 10 patients (15.4%) received third-line therapy with enzalutamide. The results showed that the median OS of 24.9 months (95% CI: 18.9–30.9) was similar to other real-world studies by Bjartell et al. and George et al. (27.1 months and 23.7 months, respectively) but shorter than the median OS of 34.7 months seen in the COU-AA-302 trial by Ryan et al.[4,9,10]

The results of the COU-AA 302 trial by Ryan et al. and real-world studies by Chen et al. and Valero et al. have indicated that the characteristics of patients at the time of initial mCRPC diagnosis, including age, Gleason score, ECOG performance status score, presence of visceral metastases, baseline PSA, hemoglobin, alkaline phosphatase, and PSA response, serve as significant prognostic factors for overall survival.[4,11,12] In our study, univariate analysis of the relationship between clinical characteristics at initiation and OS, higher age (≥70), or higher ECOG PS (≥2), or higher Gleason score (≥8), or higher BPI-SF (>3), or higher PSA (>80), or visceral metastases, de novo, or shorter time from ADT start to mCRPC (<12 months), and <50% PSA decline were all associated with shorter time to OS with AAP (p<0.05). However, in the multivariate analysis, only a higher Gleason score (≥8), shorter time from ADT start to mCRPC (<12 months), visceral metastases, and <50% PSA decline were associated with shorter time to OS.

The public health burden of prostate cancer treatment in elderly patients is anticipated to increase in the coming decades. Elderly patients often present with poor ECOG PS and numerous co-morbidities. Consequently, the effectiveness of treatment tends to be reduced compared to younger patients. However, many studies also showed that young patients often have more aggressive histological and molecular features than elderly patients. The study of Humphreys et al. involving 333 CRPC patients treated over 10 years, of age (>75) and (<55) at the time of initial prostate cancer diagnosis is associated with a statistically significant shorter OS.[13] In our study, the median OS of the subgroup of patients with age <70 was statistically significantly longer than the subgroup of patients with age ≥70 (30.7 months, 95%CI: 25.3–36.1 vs 16.1 months, 95%CI: 13.3–18.9; p=0.001).

Besides age, ECOG PS is an important factor in choosing therapy and has a great influence on treatment outcomes. Results from the COU-AA 302 trial by Ryan et al. showed that the subgroup of patients with PS 0 had statistically significantly higher OS than the subgroup of patients with PS 1.[4] In addition, the study of Chen et al. shows that patients with poor ECOG PS (PS 2-3) had statistically significantly lower OS than patients with good ECOG PS.[11] In our study, 24.6% of patients had poor ECOG PS (PS 2-3). The results indicated that the median overall survival of the subgroup with ECOG PS 0-1 was significantly longer than that of the subgroup with ECOG PS 2-3 (27.6 months, 95% CI: 22.9–32.3 vs. 11.8 months, 95% CI: 6.9–16.7; p=0.002).

The Gleason score is assessed based on the histopathology of prostate tumor biopsies, relying on the structural features of cancer cells and closely correlating with the patient's clinical characteristics. The Gleason sub-

type bears a strong relationship with the degree of clinical presentation, malignancy, time to progression, and survival of prostate cancer patients. In a study by Valero et al. involving 314 CRPC patients, the subgroup with a GS <8 exhibited significantly longer overall survival than the subgroup with a GS ≥8 (45 months vs. 34 months, p=0.009).[12] The results from our study demonstrated that the median overall survival of the subgroup with a Gleason score < 8 was notably longer than that of the subgroup with a Gleason score ≥ 8 (32.6 months, 95%CI: 27.1-38.1 vs. 19.0 months, 95%CI: 11.1–26.9; p=0.014).

Bone pain is one of the most common symptoms in mCRPC patients, greatly impacting their quality of life and treatment outcomes. Numerous studies consistently demonstrate that the extent of bone pain at the time of initial mCRPC diagnosis serves as a predictor of overall survival. In the study conducted by Fizazi et al. the subgroup of patients reporting no or mild bone pain exhibited significantly longer overall survival compared to the subgroup experiencing moderate to severe pain (p<0.001).[14] The results in our study showed that the median OS of the subgroup of patients with BPI-SF ≤3 was statistically significantly higher than the subgroup of patients with BPI-SF >3 (29.5 months, 95%CI: 25.9–33.1 vs 14.2 months, 95%CI: 12–16.3; p<0.001).

PSA is a valuable marker for screening, diagnosis, monitoring, and prognosis of prostate cancer. Patients with high PSA often have a worse prognosis than patients with low PSA. The study of Valero et al. showed that the subgroup of patients with PSA at the time of initial diagnosis mCRPC <50 ng/ml had statistically significantly longer OS than the subgroup of patients with PSA ≥50 (36 months vs. 24 months, p=0.008).[12] The results in our study showed that the median OS of the subgroup of patients with PSA ≤80 was statistically significantly higher than the subgroup of patients with PSA >80 (26.7 months, 95%CI: 22.6-30.9 vs 12.8 months, 95%CI: 10.1–15.5; p=0.024).

In our study, the rates of bone, lymph node, and visceral metastasis were 87.7%, 38.5%, and 16.9%. The results of many studies show that the site of metastasis prostate cancer is a prognosis factor for overall survival. The study of Mazzone et al. showed that patients with metastases involving only lymph nodes had superior survival compared to those with bone metastases only, or visceral metastases only and that harboring a combination of these sites at diagnosis was associated with poorer survival.[15] The results in our study showed that the median OS of the subgroup of patients without visceral metastases was statistically significantly longer than the subgroup of patients with visceral metastases (27.6 months, 95%CI: 22.6–30.8 vs 10.3 months, 95%CI: 6–14.6; p=0.001).

For nearly eight decades, androgen deprivation therapy (ADT) has served as the cornerstone of systemic treatment for men with metastatic prostate cancer. The antitumor effects of ADT improve quality of life by reducing bone pain and complication rates. Nevertheless, around 20% of patients respond poorly to ADT and this subgroup often also shows poor responses to second-line anti-androgens. The study of Wenzel et al. evaluated the impact of time to castration resistance (TTCR) in metastatic hormone-sensitive prostate cancer (mHSPC) patients on overall survival (OS).[16] The results of this study showed that the subgroup of patients with TTCR < 12 months had statistically significantly lower OS than the subgroup of patients with TTCR ≥ 12. The results of our study showed that the median OS of the subgroup of patients with time from ADT start to mCRPC <12 months was statistically significantly higher than the subgroup of patients with time from ADT start to mCRPC ≥12 months (29.2 months, 95%CI: 25.6–32.8 vs 9.7 months, 95%CI: 4.2–15.2; p<0.001).

In the United States, the majority of prostate cancer patients are diagnosed at an early stage; only 8% of patients are diagnosed at the metastatic stage.[2] The 5-year survival rate for patients is 98%.[3] However, at the metastatic stage, the 5-year survival rate is significantly reduced, to approximately 34%.[3] In the metastatic stage of the disease, patients who undergo radical treatment at the time of initial diagnosis have a better prognosis than de novo patients. The CHAARTED trial by Sweeney et al. and the GETUG-AFU 15 trials by Gravis et al. demonstrated that patients who underwent radical treatment at the time of initial diagnosis had significantly longer overall survival (OS) compared to de novo patients who relapsed.[17,18] In the study by Koura et al., 28.4% of patients who received radical treatment at the time of initial diagnosis showed significantly longer OS than de novo patients (HR 0.56, 95% CI: 0.33–0.93).[19] However, the results of our study showed that the median OS of the subgroup of patients with "recurrent" was not statistically significant compared to the subgroup of patients with de novo (29.2 months, 95%CI: 25.5–32.9 vs 17.7 months, 95%CI: 11.6–23.7; p=0.412).

PSA is widely used to monitor prostate cancer and its decline after chemotherapy and new-generation hormonal agents has been acknowledged as a valid surrogate for OS and PFS at 3 months. Retrospective studies have confirmed that patients with mCRPC who experience a PSA response exhibit a survival benefit compared to patients who do not achieve a PSA response. The study by Alvim et al. showed that median OS was significantly longer for patients with PSA response compared with patients without PSA response (29.3 vs. 9.7 months, p<0.001).[20] The results of our study showed that the median OS of the subgroup of patients with PSA response was statistically significantly higher than the subgroup of patients without PSA response (29.2

months, 95%CI: 25.6-32.8 vs 9.1 months, 95%CI: 8.1–10.1; p<0.001).

Abiraterone acetate inhibits CYP-17OH, leading to an increase in mineralocorticoid synthesis. As a result, it causes salt and water retention, hypokalemia, hypertension, edema, and an elevated risk of cardiovascular events. Additionally, AAP is metabolized by the liver and can lead to increased liver enzyme levels, a common occurrence within the first 3 months. In our study, the rate of patients experiencing adverse events due to AAP was lower than in the COU-AA-302 trial by Ryan et al.[4] The most frequent symptoms included edema (23.1%), hypokalemia (15.4%), hypertension (10.7%), elevation of AST/ALT enzymes (7.7%), and hyperglycemia (7.7%). The majority of patients experienced mild to moderate adverse events, and none had to discontinue the treatment due to AAP-related adverse events.

Study limitations

At present, some limitations still remained in the current study. In Vietnam, many mCRPC patients cannot be treated with AAP due to financial problems. Therefore, our study has a smaller sample size than other studies. In addition, this study was not designed to evaluate radiographic progression-free survival. Continued follow-up and analysis of more patients are planned to confirm the more therapy value of this regime in mCRPC patients.

Conclusion

The treatment with AAP is well tolerated and effective in mCRPC patients naïve to chemotherapy, even though in real life they are more vulnerable and have a high burden of disease such as visceral metastases and pain. Moreover, Gleason score, visceral metastasis, time from ADT start to mCRPC, and PSA response are the independent indicators for predicting the OS of mCRPC patients in both univariate and multivariate analyses.

Declarations

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Author contributions

Conceptualization, T.A.D. and L.D.N.; Methodology, T.A.D. and L.D.N.; Software, L.D.N.; Validation, T.A.D., H.T.T.N., H.X.N. and C.V.N.; Formal Analysis, C.V.N. and L.D.N.; Investigation, T.A.D. and L.D.N.; Resources, T.A.D., H.T.T.N., H.X.N. and L.D.N.; Data Curation, L.D.N.; Writing – Original Draft Preparation, C.V.N. and L.D.N.; Writing – Review & Editing, C.V.N. and L.D.N.; Visualization, C.V.N. and L.D.N.; Supervision, T.A.D., H.T.T.N., H.X.N. and C.V.N.; Project Administration, T.A.D. and L.D.N..

Conflicts of interest

The authors declare no potential conflicts of interest concerning this article's research, authorship, and/or publication.

Data availability

All data analysed during this paper are included in this article. Further enquiries can be directed to the corresponding author.

Ethics approval

All objects of the protocol's this study was approved by the Science and Ethical Committee of Hanoi Medical University, Vietnam as number: 6811/QD-DHYHN.

References

1. Gandaglia G, Leni R, Bray F, et al. Epidemiology and Prevention of Prostate Cancer. *Eur Urol Oncol*. 2021;4(6):877-892. doi: 10.1016/j.euo.2021.09.006
2. Cancer of the Prostate - Cancer Stat Facts. SEER. https://seer.cancer.gov/statfacts/html/prost.html. Accessed February 5, 2024.
3. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA Cancer J Clin*. 2021;71(1):7-33. doi: 10.3322/caac.21654
4. Ryan CJ, Smith MR, de Bono JS, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. *N Engl J Med*. 2013;368(2):138-148. doi: 10.1056/NEJMoa1209096
5. Scher HI, Morris MJ, Stadler WM, et al. Trial Design and Objectives for Castration-Resistant Prostate Cancer: Updated Recommendations From the Prostate Cancer Clinical Trials Working Group 3. *J Clin Oncol*. 2016;34(12):1402-1418. doi: 10.1200/JCO.2015.64.2702
6. Rao A, Scher HI, De Porre P, et al. Impact of clinical versus radiographic progression on clinical outcomes in metastatic castration-resistant prostate cancer. *ESMO Open*. 2020;5(6):000943. doi: 10.1136/esmoopen-2020-000943
7. Pilon D, Behl AS, Ellis LA, Emond B, Lefebvre P, Dawson NA. Duration of Treatment in Prostate Cancer Patients Treated with Abiraterone Acetate or Enzalutamide. *J Manag Care Spec Pharm*. 2017;23(2):225-235. doi: 10.18553/jmcp.2016.16233
8. Boegemann M, Khaksar S, Bera G, et al. Abiraterone acetate plus prednisone for the Management of Metastatic Castration-Resistant Prostate Cancer (mCRPC) without prior use of chemotherapy: report from a large, international, real-world retrospective cohort study. *BMC Cancer*. 2019;19(1):60. doi: 10.1186/s12885-019-5280-6
9. Bjartell A, Lumen N, Maroto P, et al. Real-World Safety and Efficacy Outcomes with Abiraterone Acetate Plus Prednisone or Prednisolone as the First- or Second-Line Treatment for Metastatic Castration-Resistant Prostate Cancer: Data from the Prostate Cancer Registry. *Target Oncol*. 2021;16(3):357-367. doi: 10.1007/s11523-021-00807-4

10. George DJ, Sartor O, Miller K, et al. Treatment Patterns and Outcomes in Patients With Metastatic Castration--resistant Prostate Cancer in a Real-world Clinical Practice Setting in the United States. *Clin Genitourin Cancer*. 2020;18(4):284-294. doi: 10.1016/j.clgc.2019.12.019
11. Chen WJ, Kong DM, Li L. Prognostic value of ECOG performance status and Gleason score in the survival of castration-resistant prostate cancer: a systematic review. *Asian J Androl*. 2021;23(2):163-169. doi: 10.4103/aja.aja_53_20
12. Valero J, Peleteiro P, Henríquez I, et al. Age, Gleason Score, and PSA are important prognostic factors for survival in metastatic castration-resistant prostate cancer. Results of The Uroncor Group (Uro-Oncological Tumors) of the Spanish Society of Radiation Oncology (SEOR). *Clin Transl Oncol Off Publ Fed Span Oncol Soc Natl Cancer Inst Mex*. 2020;22(8):1378-1389. doi: 10.1007/s12094-019-02274-w
13. Humphreys MR, Fernandes KA, Sridhar SS. Impact of Age at Diagnosis on Outcomes in Men with Castrate-Resistant Prostate Cancer (CRPC). *J Cancer*. 2013;4(4):304-314. doi: 10.7150/jca.4192
14. Fizazi K, Massard C, Smith M, et al. Bone-related Parameters are the Main Prognostic Factors for Overall Survival in Men with Bone Metastases from Castration-resistant Prostate Cancer. *Eur Urol*. 2015;68(1):42-50. doi: 10.1016/j.eururo.2014.10.001
15. Mazzone E, Preisser F, Nazzani S, et al. Location of Metastases in Contemporary Prostate Cancer Patients Affects Cancer-Specific Mortality. *Clin Genitourin Cancer*. 2018;16(5):376-384. doi: 10.1016/j.clgc.2018.05.016
16. Wenzel M, Preisser F, Hoeh B, et al. Impact of Time to Castration Resistance on Survival in Metastatic Hormone Sensitive Prostate Cancer Patients in the Era of Combination Therapies. *Front Oncol*. 2021;11:659135. doi: 10.3389/fonc.2021.659135
17. Sweeney CJ, Chen YH, Carducci M, et al. Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer. *N Engl J Med*. 2015;373(8):737-746. doi: 10.1056/NEJMoa1503747
18. Gravis G, Fizazi K, Joly F, et al. Androgen-deprivation therapy alone or with docetaxel in non-castrate metastatic prostate cancer (GETUG-AFU 15): a randomised, open--label, phase 3 trial. *Lancet Oncol*. 2013;14(2):149-158. doi: 10.1016/S1470-2045(12)70560-0
19. Koura M, Shiota M, Ueda S, et al. Prognostic impact of prior local therapy in castration-resistant prostate cancer. *Jpn J Clin Oncol*. 2021;51(7):1142-1148. doi: 10.1093/jjco/hyab019
20. Alvim CM, Mansinho A, Paiva RS, et al. Prognostic factors for patients treated with abiraterone. *Future Sci OA*. 6(2):FSO436. doi: 10.2144/fsoa-2019-0079

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ORIGINAL PAPER

Inference of oxidative stress in patients with hypothyroidism

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ABSTRACT

Introduction and aim. Oxidative stress is one of the complications that accompany defects in thyroid hormone levels. This study was designed to evaluate oxidative stress markers in patients with hypothyroidism.

Material and methods. This case control study was comprised of forty-two hypothyroid patients aged 36–46 years and forty age matched (35–43 years) healthy control participants randomly selected from the Endocrine Clinic of Al-Yarmook Hospital in Iraq. Blood levels of thyroid hormones malondialdehyde, glutathione, and paraoxonase-1 were assessed. Body mass index was calculated for each patient and control participant.

Results. Regarding the data of oxidative stress markers in hypothyroid patients compared to controls, a significant elevation was reported in blood levels of malondialdehyde and a significant reduction was found in blood levels of glutathione (p=0.031). On the other hand, the blood levels of paraoxonase-1 were significantly different in hypothyroid patients compared with the control.

Conclusion. Elevated blood levels of malondialdehyde and declined blood levels of glutathione in hypothyroid patients are a signal of oxidative stress and consequently increase the risk of cardiovascular complications.

Keywords. glutathione, hypothyroidism, malondialdehyde, oxidative stress, paraoxonase-1

Introduction

Thyroid hormone is an endocrine hormone released from the thyroid gland under the influence of thyroxine stimulating hormone. It regulates many processes inside the body that have an effect on metabolism, growth, and basal metabolic rate. Imbalance between the oxidative state and the anti-oxidative state leads to oxidative stress, which is mostly associated with thyroid diseases.[1]

Many studies reported elevated levels of lipid peroxidation and malondialdehyde in patients with hypothyroidism disorders.[2,3] Inconsistency between production and removal of reactive oxygen species that occur during the hypometabolic state may lead to oxidative stress, causing damage to proteins, DNA, and lipid damage.[4]

Oxidative stress may cause injury to endothelial cells leading to inflammation of the vascular cells, which could cause many cardiovascular complications by enhancing the release of cytokines such as osteoprotegerin and receptor activator of nuclear factor kappa-b ligand (RANKL).[5] Furthermore, the release of inflammatory cytokines may cause stimulation of angiotensin II type-1 receptors. Treatment with angiotensin II type-1 receptor blocker drugs may diminish these events in addition to reducing blood pressure.[6] Dyslipidemia and lipid peroxidation, another risk of hypothyroidism, affect endothelial cells by causing inflammation of the vascular cells which may progress to coronary artery disease and arteriosclerosis.[7,8]

Aim

The aim of this study was to quantify the levels of thyroid hormones and oxidative stress markers such as malond-

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ialdehyde, glutathione, and paraoxonase-1 in hypothyroid patients and apparently healthy individuals.

Material and methods

Study design, locus, and patient selection

In this case-control study, a total of 42 patients (19 males and 23 females) with hypothyroidism aged 36±10 years and 40 apparently healthy individuals (18 males and 22 females) aged 35±8 years, were recruited from the Endocrine Clinic of Al-Yarmook Hospital in Iraq. Questionnaires for inclusion in this study included many details such as age, gender, previous diseases, medications, and existence of chronic diseases in the family. The exclusion criteria included patients with other chronic diseases or disorders. The rules of this study obeyed instructions of the Helsinki Declaration for research and approved by the College Ethical Committee of the Pharmacy College (2/4/1236 on 22-11-2021).

All participants received printed informed consent with enclosed study details and agreements.

Sample collection and laboratory measurements

After 8–12 hours of fasting, blood samples were collected from each subject and dispensed into anticoagulant bottles containing lithium heparin and centrifuged at 3000 rpm for 10 minutes. After which the plasma samples were isolated and kept at -20°C until the assay. Body mass index (BMI) was calculated for all participants by dividing the weight of subjects in kilograms by height in squared meters.

The hormones tri-iodothyronine (T3) and tetra-iodothyronine (T4) were quantified using a radioimmunoassay kit (Institute of Isotopes, Budapest), while thyroid stimulating hormone (TSH) was quantified using an immunoradiometric assay kit (Institute of Isotopes, Budapest) according to the manufacturer's instructions. Malondialdehyde (MDA) was estimated using the thiobarbituric acid reactive substance method, while glutathione (GSH) was estimated using Ellman's reagent (5,5´-dithiobis- (2-nitro benzoic acid), DTNB), measured spectrophotometrically at 412 nm.[9,10] The activity of paraoxonase-1 (PON-1) was assessed using the substrate 4-nitrophenyl phosphate measured spectrophotometrically at 410 nm.[11]

Statistical analysis

Data were presented as mean ± standard deviation. Continuous variables evaluated by unpaired student t-test, two-tails with $p<0.05$. Pearson correlation analyses was used for assessing the correlation between variables with $p<0.05$. All statistical analyses were done using Microsoft Excel and the software Statistical Package for Social Sciences (SPSS) version 25 (IBM, Armonk, NY, USA).

Results

Table 1 shows the demographical properties of the studied groups and the clinical data of biomarkers and hormones. Concerning the blood levels of hormones, the hypothyroid patients had significantly decreased plasma levels of T3 ($p<0.0002$), and T4 ($p<0.0002$) and had significantly increased plasma levels of TSH ($p<0.001$) compared with the control individuals. Body mass index values was significantly elevated ($p=0.035$) in patients with hypothyroidism compared with control individuals.

Results of oxidative stress markers in hypothyroid patients compared with control showed a significant elevation ($p<0.0002$) in plasma levels of MDA and a significant reduction ($p=0.031$) in plasma levels of GSH, while plasma PON-1 levels showed no significant differences.

Table 1. Demographic outline and clinical data for all participants.[a]

Variables	Control	Hypothyroid patients	p
Number (n)	40	42	
Gender (male/female)	18/22	19/23	
Age (years)	35±8	36±10	
T3 (nmol/L)	2.2±0.22	0.28±0.9	<0.0002
T4 (nmol/L)	117.8±18.5	35.4±15.2	<0.0002
TSH (μ IU/mL)	1.9±0.41	26.6±12.1	<0.001
BMI (Kg/m^2)	28.34±2.62	33.8±2.75	0.035
MDA (μmol/L)	0.76±0.07	2.03±0.24	<0.0002
GSH (mg/dL)	40.5±3.5	31.5±4.3	0.031
PON-1 (IU/L)	6.25±0.42	6.18±0.5	

[a] Values are set as mean (±SD) for continuous variable, T3 – tri-iodothyronine, T4 – tetra-iodothyronine, TSH – thyroid stimulating hormone, BMI – body mass index, MDA – malondialdehyde, GSH – glutathione, PON-1 – paraoxonase

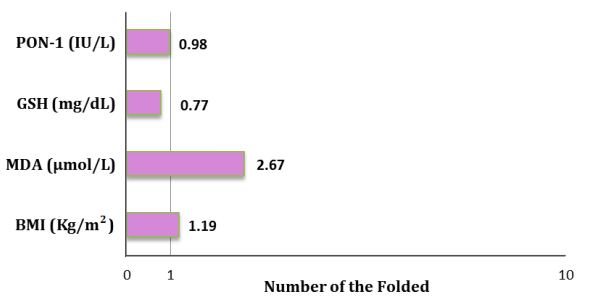

Fig. 1. The changes in the studied variables in hypothyroid patients versus controls.

Figure 1 shows the number of variable changes in hypothyroid patients versus normal control; the mean blood level of MDA was 2.67-fold higher in hypothyroid patients compared to control. On the contrary, the mean blood level of GSH was decreased 0.77-fold in hypothyroid patients compared to normal control.

Our data in Figure 2 demonstrates that there is a highly significant positive correlation (r=0.924, p=0.001) between MDA levels and BMI for hypothyroid patients and a significant positive correlation (r=0.364, p<0.05) between MDA levels and BMI for control individuals.

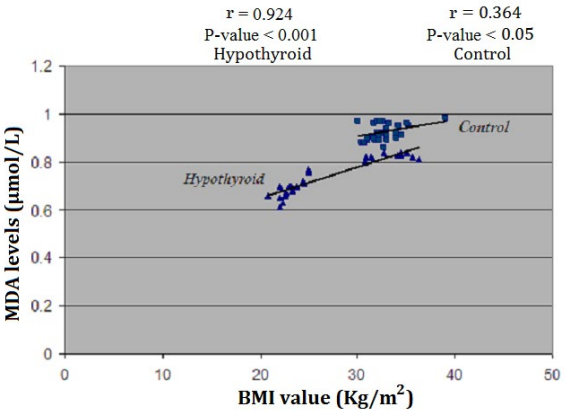

Fig. 2. The positive correlation coefficient between BMI value and MDA levels for control and hypothyroid groups.

Discussion

Oxidative stress is a state of disproportion of oxidative to antioxidative status that occurs in many disorders such as thyroid gland dysfunction and can lead to increases in the levels of reactive oxygen species such as hydrogen peroxide that cause protein damage and subsequent illness.[12] In hypothyroid patients, the risk of dyslipidemia, metabolic syndrome, and atherosclerosis are elevated because of an increased incidence of oxidative stress. In this state, lipid peroxidation, as consequence of hypercholesterolemia and elevated levels of low-density lipoprotein (LDL) cholesterol, are increasingly vulnerable to lipid peroxidation by free radicals.[13,14]

Decreased levels of thyroid hormone may diminish the rate of conversion of β-carotene- LDL to vitamin A - LDL that serve as antioxidants and subsequently enhance oxidation of LDL.[15] Dyslipidemia in hypothyroidism leads to an increase in the number and size of adipocytes (hypertrophy), and consequently, cytokine production such as IL-1, IL-6 and TNF-α by adipocytes will be escalated and enhance the incidence of pro-inflammatory states and oxidative stress.[16]

As noted in this study, BMI values for hypothyroid patients were positively correlated with MDA levels. This finding is in concordance with previous studies.[17] Reactive oxygen species cause lipid peroxidation and consequently the production of MDA indicates oxidative damage and oxidative stress inside the body.[18] Therefore, MDA can be used for diagnosis of oxidative stress accompanied hypothyroidism and for monitoring of hypothyroidism after treatment with L-thyroxine, as levels decline with treatment as documented by many studies.[19,20]

As verified in this current study, the level of GSH in hypothyroid patients was significantly less than those in normal controls; this outcome agreed with a study by Pasupathi, et al.[21] As thyroid hormone is important for the synthesis of antioxidant agents such as GSH, the decreased level of thyroid hormone causes a decline in biosynthesis of GSH.[22] Moreover, reduced levels of GSH can occur by other factors such as decreased levels of superoxide dismutase leading to accumulation of superoxide and oxidation of GSH.[23] Likewise, Ustundag and colleagues inferred that with an escalation of oxidative stress, the levels of antioxidant activity of superoxide dismutase and glutathione peroxidase diminish in corpulent humans.[24]

PON-1 is an enzyme present in high-density lipoprotein (HDL) and has antioxidant properties. Levels of PON-1 decline in many diseases related to autoimmune and connective tissue.[25] Concerning the level of paraoxinase-1 in this study, there was no significant reduction in the levels of PON-1 for hypothyroid patients compared with control individuals. This event was consistent with the studies of Roshni, et al. and Kebapcilar, et al.[26,27] In contrast, Duman, et al., and Cebeci, et al. documented a significant decline in the level of PON-1 in hypothyroid patients.[28,29] This variation may be due to genetic differences in enzyme expression or due to differences in blood levels of HDL.

Study limitations were the number of the subjects (sample size), the retrospective design of the study and one-center analysis. Therefore, additional studies with larger sample sizes in multiple centers are suggested.

Conclusion

Thyroid hormone is important for maintaining a normal oxidative status in the body. Therefore, any defect in this hormone causes imbalance between the oxidative and anti-oxidative state and may progress to oxidative stress. Our data confirms that overproduction of MDA, and a reduction of glutathione in hypothyroid patients, provides a signal of oxidative stress escalation since these markers are related to increased free radical formation causing lipid peroxidation and decreased antioxidant enzyme levels. Therefore, these biomarkers can be used for monitoring this impairment in hypothyroidism and to overcome complications.

Declarations

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Author contributions

Conceptualization, F.R.; Methodology, F.R.; Software, F.R.; Validation, F.R.; Formal Analysis, F.R.; Investigation, F.R.; Resources, F.R.; Data Curation, F.R.; Writing

– Original Draft Preparation, F.R.; Writing – Review & Editing, F.R.; Visualization, F.R.; Supervision, F.R.; Project Administration, F.R.; Funding Acquisition, F.R.

Conflicts of interest

The author declares no conflict of interest.

Data availability

The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request.

Ethics approval

The rules of this study followed the Helsinki Declaration for research on humans and was approved by the College Ethical Committee of the Pharmacy College (2/4/1236 on 22-11-2021).

References

1. Wang L, He W, Xu X, et al. Pathological changes and oxidative stress of the HPG axis in hypothyroid rat. *J Mol Endocrinol*. 2021;67(3):107-119. doi: 10.1530/JME-21-0095
2. Panda S, Dash MK, Thatoi PK, Dandapat J. Rath B. Oxidative stress correlates well with markers of metabolic syndrome in clinically hypothyroid cases: a hospital-based case-control study in a remote tribal district. *RUDN J Med*. 2021;25(1):55-65. doi: 10.22363 /2313-0245-2021-25-1-55-65
3. Torun AN, Kulaksizoglu S, Kulaksizoglu M, et al. Serum total antioxidant status and lipid peroxidation marker malondialdehyde levels in overt and subclinical hypothyroidism. *Clin Endocrinol*. 2009;70(3): 469-474.
4. Santi A, Duarte MM, deMenezes CC, Loro VL. Association of Lipids with Oxidative Stress Biomarkers in Subclinical Hypothyroidism. *Int J Endocrinol*. 2012:856359. doi: 10.1155/2012/856359
5. Rada FH. Impact of Osteoprotegerin and RANKL on Non-ST- segment Elevation Myocardial Infarction. *Int J Nutr Pharmacol Neurol Dis*. 2021;11:206-210. doi: 10.4103/ijnpnd.ijnpnd30_21
6. Rada FH. Effect of Angiotensin II Receptor Blocker Treatment on Adipokine of Corpulence. *Biomed & Pharmacol J*. 2020;13(2):957-963. doi: 10.13005 /bpj/1964
7. Hashim F. Compendious Review on Adipokines of Corpulence. *Research J Pharm and Tech*. 2022;15(9):4315-4318. doi: 10.52711/0974-360X.2022.00724
8. Rada FH. Association of lipid fractions levels with cardiovascular disease. *Asian J Pharm Clin Res*. 2017;10(3):180-182. doi: 10.22159/ajpcr. 2017.v10i3.15984
9. Sangeetha P, Das UN, Koratkar R, Suryaprabha P. Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. *Free Radic Biol Med*. 1990;8:15-19.
10. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and non-protein sulfydryls groups in tissue with Elman's reagent. *Anal Biochem*. 1968;25:192-205.
11. Beltowski J, Wójcicka G, Marciniak A. Species and substrate –specific stimulation of human plasma paraoxonase 1 (PON1) activity by high chloride concentration. *Acta Biochim Pol*. 2002;49:927-936.
12. Sankha S, Kumar YM, Madhuri AA, Kumar MT. Antioxidant status and oxidative stress in hypothyroidism. *J Datta Meghe Inst Med Sci Univ*. 2021;16:508-514. doi: 10.4103/jdmimsu. jdmimsu _ 13_21
13. Eddib I, Barhoumi L, Mahmoudi A, Ben Nasr H. Oxidative stress in thyroid dysfunction. *Endocrinol Metab Int J*. 2022;10(2):66-69. doi: 10.15406/emij.2022.10.00321
14. Kirac CO, Sirikci V, Findikli HA. Comparison of triglyceride-glucose index and HOMA-IR as indicators of insulin resistance in obese women with subclinical hypothyroidism. *Eur J Clin Exp Med*. 2022;20(4):412-416. doi: 10.15584/ejcem.2022.4.5
15. Reaven PD, Ferguson E, Navab M, Powell FL. Susceptibility of human LDL to oxidative modification: effects of variations in β-carotene concentrations and oxygen tension. *Arterioscler Thromb*. 1994;14:1162-1169. doi: 10.1161/01.atv.14.7.1162
16. Cotillard A, Poitou C, Torcivia A, et al. Adipocyte size threshold matters: link with risk of type 2 diabetes and improved insulin resistance after gastric bypass. *J Clin Endocrinol Metab.* 2014;99(8): 1466-1470. doi: 10.1210/jc.2014-1074
17. Nanda N, Bobby Z, Hamide A. Association of thyroid stimulating hormone and coronary lipid risk factors with lipid peroxidation in hypothyroidism. *Clin Chem Lab Med*. 2008;46:674-679. doi: 10.1515/CCLM.2008.139
18. Marrocco I, Altieri F, Peluso I. Measurement, and Clinical Significance of Biomarkers of Oxidative Stress in Humans. *Oxid Med Cell Longev*. 2017;2017:6501046. doi: 10.1155/2017/6501046
19. Ruggeri RM, Giovinazzo S, Barbalace MC, et al. Influence of Dietary Habits on Oxidative Stress Markers in Hashimoto´s Thyroiditis. Thyroid Off. *J Am Thyroid Assoc*. 2021;31:96-105. doi: 10.1089/thy.2020.0299
20. Chakrabarti SK, Ghosh S, Banerjee S, Mukherjee S, Chowdhury S. Oxidative stress in hypothyroid patients and the role of antioxidant supplementation. *Indian J Endocrinol Metab*. 2016;20(5):674-678. doi: 10.4103/2230-8210.190555
21. Pasupathi P, Latha R. Free radical activity, and antioxidant defense mechanisms in patients with hypothyroidism. *Thyroid Sci*. 2008;3(12):CLS1-6.
22. Ghosh S, Rahaman SO, Sarkar PK. Regulation of neurofilament gene expression by thyroid hormone in the developing rat brain. *Neuroreport*. 1999;10:2361-2365.
23. Benov L. How superoxide radical damages the cell. *Protoplasma*. 2001;217:33-36. doi: 10.1007/BF01289410
24. Ustundag B, Gungor S, Aygun AD, Turgut M, Yilmaz E. Oxidative status and serum leptin levels in obese prepubertal children. 2006;25(5):479-483. doi: 10.1002/cbf.1334

25. Bodolay E, Seres I, Szodoray P, et al. Evaluation of paraoxonase activity in patients with mixed connective tissue disease. *J Rheumatol*. 2008;35(2):237-243.
26. Roshni K, Prabhu A, Durga Rao Y, Sowndarya K, Nandini M. Assessment of Oxidative Stress Index in Sub-Clinical Hypothyroidism. *Biomed Pharmacol J*. 2021;14(2):739-748. doi: 10. 13005/bpj/2177
27. Kebapcilar L, Comlekci A, Tuncel P, et al. Effect of levothyroxine replacement therapy on paraoxonase-1 and carotid intima-media thickness in subclinical hypothyroidism. *Med Sci Monit*. 2010;16:41-47.
28. Duman G, Doğan HO. Serum NOX-2 concentrations and paraoxanase-1 activity in subclinical hypothyroidism: a pilot study. *Turk J Biochem*. 2020;45(3):271-276. doi: 10.1515/tjb-2018-0159
29. Cebeci E, Alibaz-Oner F, Usta M, Yurdakul S, Erguney M. Evaluation of oxidative stress, the activities of paraoxonase and arylesterase in patients with subclinical hypothyroidism. *J Investig Med*. 2012;60:23-28.

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ORIGINAL PAPER

The prevalence of incidental findings in computed tomography of the head in Pediatric Emergency Department

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ABSTRACT

Introduction and aim. Computed tomography (CT) is the first imaging modality in the evaluation of children in case of patients with head injury in pediatric Emergency Departments (EDs). Radiological CT reports include not only lesions that are the main cause of the child's complaints but also incidental findings. The objective of this study is to assess incidental findings observed in children who were admitted to the ED and had the head CT performed.

Material and methods. This retrospective, cross-sectional study enrolled 644 children under the age of 18, from 1st January 2021 to 31st June 2021. Each child could have had one or more incidental findings in CT

Results. Among all CT studies, incidental findings were found in 279 out of 644 (43.32%) cases, of which 73 (11.34%) had both lesions detected incidentally and related to the trauma.

Conclusion. Head CT is an incredibly useful tool in the assessment of some head emergencies. However, evaluation of the prevalence of incidental findings is difficult. Most of them require no specific further investigation. Pediatricians, who order CTs in children, must be prepared to interpret and communicate findings to families and introduce treatment in necessary situations.

Keywords. head CT incidental findings, pediatric ED, pediatric head CT

Introduction

The pediatric emergency department (ED) is an important place where children of all ages are brought by self-referral, by emergency medical services or referred from either primary or secondary care. The assessment of pediatric children in the emergency setting is difficult due to limited history and physical examination, which often yields findings that overlap with multiple disease entities. Therefore, diagnostic imaging has a significant role in the evaluation of pediatric patients in the EDs. Computed tomography (CT) scans are most frequently obtained in the evaluation of children, in whom imaging of the head region in the EDs is necessary. The indications to perform a CT scan fall under two categories, i.e. post-traumatic and non-traumatic.[1] After obtaining a medical history and physical examination, preliminary diagnoses of patients with non-traumatic reasons that may be an indication for head CT include headache, seizures, fever, confusion, hematoma, infarct, optic neuritis and arrest.[2] The simplicity and accessibility of this imaging tool have led to its overuse, especially in EDs, where a correct diagnosis must be made quickly.[3] Although

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this imaging method can show lesions, which enable the diagnosis of many diseases, it is also sensitive to a wide variety of incidental findings that may be previously unknown to the patient or family. This incidental information can lead to increased patient stress and additional diagnostic testing, whether or not it is ultimately clinically important.[4]

Aim

The objective of this study is to assess incidental findings observed in children, who were admitted to the ED and had the head CT performer and point out how many of these changes can have life-threatening consequences.

Material and methods

This retrospective, cross-sectional study enrolled 644 pediatric patients who underwent a CT scan of the head in the Pediatric Emergency Department at the Prof. Antoni Gebala Children's Hospital of Lublin over six months from 1st January 2021 to 31st June 2021. All study subjects were children under the age of 18, referred for a head CT scan from the ED. The children were categorized into four age groups: 0–1, 2–5, 6–11 and 12–18 years old, who had brain CT with post-traumatic and non-traumatic indications. Patients were excluded from this study if they had cerebral shunt or their primary complaint lasted more than three days. The imaging was performed on Siemens Definition AS+ 128 slices without administering an intravenous contrast agent. The purpose was to examine radiological reports for any notation of incidental findings. Each child could have had one or more findings. Statistical analysis was performed using Statistica 13.3 (Statsoft, Tulsa, OK, USA).

Results

A total of 644 pediatric patients admitted to the ED, on whom a CT scan of the head was performed, were included in this study. Of these patients, 364 (56.52%) were male, and 280 (43.48%) were female.

The age of children varied between 0 and 18, and the mean age of patients in the study group was 10.02±4.68. The largest proportion of the study group were children aged 12–18 years old – 243 patients (37.73%), with an equal proportion of middle childhood (36.96%). More information about the study group is provided in Table 1 and Table 2.

Table 1. Characteristics of study group

	Sex	Mean±SD	Median (Min; Max)	n	%
Age (years)	Female	9.90±4.72	9.91 (0-17)	280	43.48
	Male	10.19±4.64	10.24 (0-17)	364	56.52
Total		10.02±4.68	9.96 (0-17)	644	100

Of all patients, in 318 (49.38%), any lesions in CT of the head were not found, whether incidental or associated with trauma findings. 206 (31.99%) pediatric patients had incidental findings, and 73 (11.34%) had both lesions found incidentally and related to the trauma (Table 3).

Table 2. Study group by the age groups

Age group	n	%
Toddlers and infants (0-2 years old)	18	2.80
Early childhood (2-5 years old)	145	22.51
Middle childhood (6-11 years old)	238	36.96
Early adolescence (12-18 years old)	243	37.73
Total	644	100

Table 3. Head computed tomography scan findings in children treated in the Emergency Department

	n	%
Incidental findings	206	31.99
Trauma caused findings	47	7.30
Incidental + trauma caused findings	73	11.33
Normal	318	49.38

From the total of 279 children with incidental findings in brain CT, 215 (77.06%) had only one lesion, and in 64 (22.94%) patients, two or more findings were found. In all radiologist reports included in this study, there were documented 359 lesions, the largest group being intracranial calcifications, with a total number of 126 (35.10%). The frequency of incidental findings in brain CT is shown in Table 4.

Table 4. Frequency of incidental findings

	n	%
Intracranial calcifications	126	35.10
Skull base pneumatization	40	11.14
Sinus opacification	96	26.74
Cyst	31	8.63
Adenoidal hypertrophy	3	0.84
Ventricular abnormality	13	3.62
Tumor/mass	2	0.56
Cerebellar tonsillar ectopia	3	0.84
Extraaxial fluid	5	1.39
Other	40	11.14

Discussion

Unenhanced head CT is the most common of all requested CTs in ED, accounting for 70-80%, according to Wang et al.[5] It is a very useful tool in the assessment of pediatric patients to establish a particular diagnosis. However, despite much useful information, this imaging method provides us with, incidental findings are often presented in radiological reports. These findings are usually unrelated to the principal complaint and may not be pertinent to the immediate care of the patient.[6]

Our study demonstrates the prevalence of incidental findings on cranial CT in patients in whom a scan of the head in the ED was done. In this study, 43.32%

of children had incidental findings identified. In 73 (11.33%) cases, incidental findings were accompanied by lesions related to the trauma. Cranial CT-based studies reported prevalences that range from as low as 1% to as high as 19%. However, these studies pay particular attention to doing a follow-up examination.[4,7,8] There were also studies where the prevalence of incidental findings reached 85.1%, but the majority of them were innocuous and trivial.[6] Most of the research pertaining to incidental findings on cranial CT concentrate on the adult population, while pediatric studies represent a significant minority.

The most commonly found incidental lesion in radiological reports in the pediatric ED in our study is intracranial calcification (35.1%; 126/359 lesions). Ogbole et al. also identified calcifications as the most common, with a frequency of 67.7%. However, the study's group age varied between a few days of life to 95 years.[6] CT is a very sensitive method for depicting intracranial calcifications. In general, in conventional nonenhanced CT, any lesion with a density larger than 100 Hounsfield units is classified as calcification.[9,10] They are physiological and mostly found in choroid plexus or pineal gland.[11,12]

In studies in which only patients under the age of 18 were included, Rogers et al. pointed out that sinonasal abnormalities (19%) are the most common, and Ortega et al. and also Ghimire et al. reported sinus opacification (83.9% and 43.9%) as the most common in the pediatric population.[4,13,14] In our study, sinus opacification was noted in the second place (26.74%; 96/359).

The further clinical examination of pediatric patients with incidental findings has not been widely studied in our research. However, it is clear that lesions, such as a suspicious potentially cancerous mass, can have potentially serious consequences for patients and are an indication to extend the diagnosis. In our study, there were only 2 (0.56%) lesions, which should increase oncological awareness. Rogers et al. observed 12 (2.17%) cases of mass in CT that required follow-up examination.[4] With the development of technology and increased availability of neuroimaging, the incidence of incidentally detected brain tumors is increasing among children.[15,16]

Communicating incidental findings to the families of pediatric patients has been a topic of many studies.[17-19] Incidental findings still remain a challenge in terms of ethics and management in case of prospective study participants. Vast number of patients with incidental findings will not require treatment, but it is crucial to provide an appropriate follow-up.[20,21] In our study we point out that incidental findings are common so proper communicating them to the patients and their families is necessary.

Conclusion

Evaluation of the true prevalence of incidental findings in an ED setting is difficult. However, most of them are benign and require no specific follow-up. Pediatricians in the EDs who order CTs in children must be prepared to interpret and communicate findings to families and introduce treatment in necessary situations. It is important to balance the ethical and medical implications of this unexpected information.

Declarations

Funding

No funding received.

Author contributions

Conceptualization, M.M.W. and J.L.; Methodology, J.L.; Software, Ł.L.; Validation, M.K., J.L. and I.K.; Formal Analysis, M.M.W.; Investigation, J.L.; Resources, Ł.L.; Data Curation, Ł.L.; Writing – Original Draft Preparation, J.L., M.K., I.K.; Writing – Review & Editing, M.M.W.; Visualization, J.L.; Supervision, M.M.W.; Project Administration, M.M.W.; Funding Acquisition, M.M.W.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data presented in this study are available on request from the corresponding author.

Ethics approval

Not applicable. The study was conducted in accordance with the Declaration of Helsinki. In accordance with the law in force in the Republic of Poland, retrospective studies do not require the opinion or consent of the Bioethics Committee, as they are not a medical experiment in which human organisms would be interfered with. For this reason, we did not seek the consent of the Commission. What's more, the results of the study did not affect the management of patients at any stage, so the above-mentioned procedure was followed.

References

1. Nishtar T, Ahmad T, Noor N, Muhammad F. Rational use of Computed Tomography Scan head in the Emergency Department of a high volume tertiary care public sector hospital. *Pak J Med Sci*. 2019;35(2):302-308. doi:10.12669/pjms.35.2.719
2. Dogan GM. Comparison of the Efficiency of the Paediatric Brain CTs with Trauma and Non-Trauma Related Indications in the Paediatric Emergency Department. *Disaster Emerg Med J.* 2021;6(2):80-84. doi: 10.5603/DEMJ.A2021.0014
3. Novoa Ferro M, Santos Armentia E, Silva Priegue N, Jurado Basildo C, Sepúlveda Villegas CA, Del Campo Estepar

S. Brain CT requests from emergency department: reality. *Radiologia (Engl Ed)*. 2020;S0033-8338(20):30123-30125. doi: 10.1016/j.rx.2020.08.005
4. Rogers AJ, Maher CO, Schunk JE, et al. Incidental findings in children with blunt head trauma evaluated with cranial CT scans. *Pediatrics*. 2013;132(2):e356-e363. doi: 10.1542/peds.2013-0299
5. Wang X, You JJ. Head CT for nontrauma patients in the emergency department: clinical predictors of abnormal findings. *Radiology*. 2013;266(3):783-790. doi: 10.1148/radiol.12120732
6. Ogbole GI, Adeleye AO, Owolabi MO, Olatunji RB, Yusuf BP. Incidental cranial CT findings in head injury patients in a Nigerian tertiary hospital. *J Emerg Trauma Shock*. 2015;8(2):77-82. doi: 10.4103/0974-2700.155499
7. Sanei TM, Hemandi H, Sajadi NM, Jalali AH, Eftekharpour D. Evaluation of incidental findings in brain CT scans of mild head trauma patients (GCS: Thirteen to Fifteen). *Iran J Cancer Prev*. 2010;3:32-35.
8. Thompson RJ, Wojcik SM, Grant WD, Ko PY. Incidental Findings on CT Scans in the Emergency Department. *Emerg Med Int*. 2011;2011:624847. doi: 10.1155/2011/624847
9. Gonçalves FG, Caschera L, Teixeira SR, et al. Intracranial calcifications in childhood: Part 1. *Pediatr Radiol*. 2020;50(10):1424-1447. doi: 10.1007/s00247-020-04721-1
10. Go JL, Zee CS. Unique CT imaging advantages. Hemorrhage and calcification. *Neuroimaging Clin N Am*. 1998;8(3):541-558.
11. Al Hajri F, Sirasanagandla SR, Boudaka A, Al Dhuhli H, Al Ajmi E. Physiological Intracranial Calcifications in Children: A computed tomography-based study. *Sultan Qaboos Univ Med J*. 2023;23(2):227-232. doi: 10.18295/squmj.9.2022.058
12. Kıroğlu Y, Callı C, Karabulut N, Oncel C. Intracranial calcifications on CT. *Diagn Interv Radiol*. 2010;16(4):263-269. doi:10.4261/1305-3825.DIR.2626-09.1
13. Ortega HW, Vander Velden H, Reid S. Incidental findings on computed tomography scans in children with mild head trauma. *Clin Pediatr (Phila)*. 2012;51(9):872-876. doi: 10.1177/0009922812450508
14. Ghimire P, Paudel N, Koirala D. Incidental Findings in CT scan in Pediatric Populations with head injury. *Journal of Nepalgunj Medical College*. 2018;16:45-47. doi: 10.3126/jngmc.v16i1.24228
15. Soleman J, Kozyrev DA, Ben-Sira L, Constantini S, Roth J. Management of incidental brain tumors in children: a systematic review. *Childs Nerv Syst*. 2020;36(8):1607-1619. doi: 10.1007/s00381-020-04658-8
16. Graf WD, Kayyali HR, Abdelmoity AT, Womelduff GL, Williams AR, Morriss MC. Incidental neuroimaging findings in nonacute headache. *J Child Neurol*. 2010;25(10):1182-1187. doi:10.1177/0883073809353149
17. Vander Wyst KB, Olson ML, Bailey SS, et al. Communicating incidental and reportable findings from research MRIs: considering factors beyond the findings in an underrepresented pediatric population. *BMC Med Res Methodol*. 2021;21(1):275. doi: 10.1186/s12874-021-01459-8
18. Wolf SM. Introduction: the challenge of incidental findings. *J Law Med Ethics*. 2008;36(2):216-218. doi: 10.1111/j.1748-720X.2008.00265.x
19. Roane JL, Mio M, Viner J, et al. Incidental Findings Among Youth Participating in Multimodal Imaging Research: Characteristics of Findings and Description of a Management Approach. *Front Pediatr*. 2022;10:875934. doi: 10.3389/fped.2022.875934
20. Kim BS, Illes J, Kaplan RT, Reiss A, Atlas SW. Incidental findings on pediatric MR images of the brain. *AJNR Am J Neuroradiol*. 2002;23(10):1674-1677.
21. Seki A, Uchiyama H, Fukushi T, Sakura O, Tatsuya K; Japan Children's Study Group. Incidental findings of brain magnetic resonance imaging study in a pediatric cohort in Japan and recommendation for a model management protocol. *J Epidemiol*. 2010;20, 2(2):498-504. doi: 10.2188/jea.je20090196

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ORIGINAL PAPER

The relationship between knowledge levels of HPV and health literacy in youth – an example from Türkiye

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ABSTRACT

Introduction and aim. Human papillomavirus (HPV) is a common infection responsible for many cancers. This study was conducted to determine the relationship between the knowledge level of HPV and the health literacy of youth living in Türkiye.

Material and methods. The study sample comprised 543 youth aged 18 and 30 living in Türkiye. The data were assessed with a questionnaire, the human papilloma virus knowledge scale (HPV-KS), and the health literacy scale (HLS) and the correlation between them.

Results. 50.6% of the youth informed that they are aware of HPV, and 54% of the youth know about the transmission mode of HPV. It was determined that knowing HPV increased 1.839 times by being a woman, 1.949 times by being married, and 14.339 times by knowing STIs. The HPV-KS total score average of the youth was low at 14.26±7.04, and the total score average of HLS was high at 107.91±16.81. A positive and significant correlation was found between HPV-KS total score, all sub-factor scores, HLS total score, and all sub-factors total score($p<0.001$).

Conclusion. Information studies on HPV should be increased by taking advantage of the generality of health literacy.

Keywords. health education, health literacy, HPV, sexually transmitted infections, youth

Introduction

Human papillomavirus (HPV) is the world's most common sexually transmitted disease. Transmission spreads through genital fluids or genital area contact, contaminated surfaces, perinatal transmission, and a chap on skin and mucosa. HPV is typically asymptomatic and diagnosed by an HPV DNA test. Its treatment includes surgical excision of papilloma, topicals, and immune modulators. HPV results in skin, pharyngeal, vulvar, vaginal, penile, and anal cancers, especially cervices cancer.[1-3]

It is known that every year, 530,000 women are infected with HPV worldwide, and 266 thousand of women die from this virus. It is estimated that the prevalence of HPV-infected individuals is around 11.7%. Cervical cancer caused by HPV takes fourth place in terms of prevalence among cancers in women. Data from 2018 revealed that 570,000 women were diagnosed with cervical cancer and 311,000 women died from this disease.[4,5] Türkiye has a population of 32.4 million women aged 15 and over at risk of cervical cancer. It is estimated that 2,532 women are diagnosed with cervical cancer annually, and 1,245 women die from this disease.[6] The World Health Organization (WHO) data shows that women who die from cervical cancer in Türkiye are 5.9 per 100 thousand.[7]

Vaccination, screening, early diagnosis, and treatment effectively reduce this virus's spread. WHO rec-

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ommends that girls between the ages of 9 and 14 be vaccinated against HPV, in addition to screening and treatment services to reduce the risk of cancer in women.[5] However, it is difficult to access the vaccine in our country because the HPV vaccine is not included in the free vaccine program, and the costs are high. Regular screening of women between the ages of 30 and 49, treatment and follow-up of infected women, and palliative care is recommended to eliminate HPV.[5] Other ways to prevent HPV infection are monogamy, the popularization of condom use, the development of safe sexual behaviors, and increasing health education.[2,3]

It is essential to prepare appropriate, cost-effective, easy-to-understand, and accessible materials and convey accurate information, especially to youth in the education and counseling of STIs. The concept of health literacy developed in recent years is known as a person's ability to read, understand and apply the information he/she has reached.[8] Demand for preventive health services and vaccines includes knowing how and when to use prescription drugs or certain behaviors, including asking healthcare professionals about medical problems. Health literacy is essential in understanding why health behaviors do not improve. As a result of studies conducted in Türkiye, it is known that health literacy is in good condition.[9-11] On the other hand, although information about HPV and its vaccine has been widely available in recent years, it is known that youth have insufficient knowledge of the subject.[12,13] HPV is risky for young people, one of the risk groups for sexually transmitted infections, due to reasons such as being unheard of, ignorance and inadequacies in vaccination and lack of understanding of the importance of safe sexual intercourse. Reducing the spread of HPV will reduce associated cancers and reduce sexually transmitted infections and other opportunistic infections.

Aim

For all these reasons, this research was conducted to determine the relationship between knowledge levels of HPV and the health literacy of youth among the risk groups for HPV.

Material and methods

This study was conducted in a descriptive and cross-sectional type to determine the relationship between knowledge levels of HPV and health literacy of youth aged 18 and 30 living in Türkiye. The research population consisted of youth between 18 and 30 living in Türkiye. The study was conducted between 10 May and 30 August 2022.

A total of 16,940,475 people between the ages of 18 and 30 from 'Results of Address Based Population Registration System 2021' of the Turkish Statistical Institute (TUIK) and the formula ($n= N t^2 p q / d^2 (N-1) + t^2 p q$), where the population is known, determined that the prevalence of HPV in the population was 15% in line with the literature and this determined the number of samples.[14,15] The number of samples was 543, with a 95% confidence interval and a 0.03 sensitivity. Data were collected online as this study was conducted during the COVID-19 pandemic, and maximum diversity was aimed. We targeted Turkish-speaking people between the ages of 18 and 30 living in Türkiye and recruited through Instagram, Facebook and Linkedin. Each researcher shared a web link on their social media page. We implemented a continuous recruitment strategy from October – December 2023 until the target number of participants were enrolled. Participants were not compensated. The survey was anonymous and the young people could leave the study at any time. Young people were able to move on to other questions based on the questions covering the inclusion and exclusion criteria at the beginning of the form. Those who did not complete these questions were excluded from the study because they could not see the other questions. Young people who answered all questions were considered to have completed the survey. Data was collected through a secure method of Google surveys and each survey took an average of 5–10 minutes to complete. Ethical approval of the study (Date: 08.04.2022 Issue: E-14679147-663.05-266758) was obtained.

Introductory information form examining demographic data, human papilloma virus knowledge scale, and health literacy scale were used as data collection tools.

Introductory information form

It consists of 15 questions developed by the researchers to determine the socio-demographic status of youth.[16]

Human papilloma virus knowledge scale (HPV-KS)

Developed by Waller et al. in 2013 and which validity and reliability of the Turkish version were studied by Demir and Özdemir in 2019, HPV-KS was developed to measure individuals' knowledge levels of HPV, HPV vaccine, and screening tests. In the scale, it is researched whether individuals have heard of HPV, HPV vaccine, and HPV screening tests before and to what extent they know these issues. The total score to be obtained from HPV-KS is between 0 to 35, and a high score indicates a high knowledge level of HPV, HPV screening tests, and the HPV vaccine. The Cronbach α value of the scale was calculated as 0.96.[17,18]

Health literacy scale (HLS)

HLS was developed by Toçi et al. in 2013, and a validity and reliability study of the Turkish version made by Aras and Bayık Temel in 2017. The scale has four sub-dimensions: accessing information, understanding infor-

mation, appraising, and applying. Low scores indicate insufficient, problematic, and poor health literacy, while high scores indicate adequate and very good health literacy. The higher the score is, the higher the individual's health literacy level is. The Cronbach α value of the scale was calculated as 0.91.[19,20]

The data were evaluated by entering SPSS 21.0 package program (IBM, Armonk, NY, USA). Data were analyzed by number, percentage, mean, Standard deviation, Anova, t test, Logistic regression, and Pearson correlation coefficient.

In our study, the normality of the distribution of the variables was examined with the Kolmogorov-Smirnov test, and the homogeneity of the variances was evaluated with the Levene test. The Cronbach Alpha value of HPV-KS was found to be 0.90, and the Cronbach Alpha value of the Health Literacy Scale was found to be 0.96.

Results

The average age of the youth participating in the study was 23.91±6.35, 69.1% of the participants (n=375) were women, 30.9% (n=168) were men, 45.3% (n=246) in the 21–24 age group, 82.5% (n=448) were single, 42.7% (n=232) were high school graduates and 47.5% (n=258) were university graduates or higher. It was determined that 66.5% (n=361) of the youth had a middle income, and 24.7% (n=134) were working in wage-earning employment.

67.4% (n=366) of the participants stated that they have knowledge of STIs and 57.8% (n=314) stated that they need health education for STIs. 50.6% (n=275) of the youth informed that they had heard of HPV, and 54% (n=293) informed that they knew the transmission mode of HPV.

The participants' knowledge of HPV and STIs is given in Figure 1.

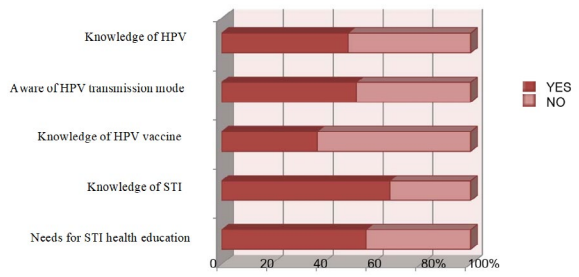

Fig. 1. Participants' knowledge status of HPV and STIs

When the socio-demographic characteristics of the youth and the score averages of HPV-KS and HLS were compared, the difference between HPV-KS total score average and employment status was found to be statistically significant (p<0.05). The difference between HLS total score average and gender, marital status, and age was found to be statistically significant (p<0.05) (Table 1).

Table 1. HPV-KS and HLS score averages of the youth participating in the study by socio- demographical characteristics (n=310)[a]

			HPV-KS		HLS	
	n	%	$\bar{X} \pm SD$		$\bar{X} \pm SD$	
Gender						
Woman	226	72.9	14.37±6.89	t= -0.47	109.91±14.39	t= 2.961
Men	84	27.1	13.95±7.46	p=0.642	102.50±21.21	p=0.004*
Age						
18–20 years	93	30	13.24±6.53	F=1.526	104.87±2.04[b]	F=3.229
21–24 years	144	46.5	14.53±6.84	p=0.219	108.87±19.69	p=0.041*
25 years and over	73	23.5	15.03±7.94		111.49±15.06[a]	
Marital status						
Single	261	84.2	14.10±6.90	t= -0.892	107.10±17.30	t= -2.348
Married	49	15.8	15.08±7.79	p=0.373	112.20±13.24	p=0.021*
Education						
Maximum secondary school graduates	19	6.1	12.32±5.68	F=0.785	104.53±12.79	F=0.434
High school graduates	142	45.8	14.31±7.68	p=0.457	108.36±17.03	p=0.648
University and higher graduates	149	48.1	14.46±6.54		107.91±17.09	
Family income status						
Income more than expenses	42	13.6	16.55±8.89	F= 2.672	109.57±16.60	F= 0.703
Income equals expense	205	66.1	13.99±6.67	p=0.071	108.20±17.22	p=0.496
Income less than expenses	63	20.3	13.60±6.64		105.86±15.63	
Working status						
Working	79	25.5	15.96±7.85	t= 2.319	110.32±15.57	t= 1.479
Not working	231	74.5	13.68±6.66	p=0.022*	107.08±17.17	p=0.14

[a] X – mean, SD – standard deviation, F – One Way Anova, t – Student T, * – p<0.05, * – a-b: difference between groups

According to the logistic regression analysis, it was determined that the state of knowing HPV increased 1.839 times by being a woman, 1.949 times by being married, and 14.339 times by knowing STIs (p<0.05) (Table 2).

Table 2. Results of logistic regression analysis on HPV knowledge of youth[a]

	B	S.E.	Wald	df	Sig.	95% C.I.for EXP(B)		
						Exp(B)	Lower	Upper
Gender	0.609	0.219	7.748	1	0.005	1.839	1.198	2.825
Age	-0.038	0.181	0.044	1	0.834	0.963	0.675	1.374
Marital status	0.667	0.313	4.554	1	0.033	1.949	1.056	3.597
Education	-0.019	0.166	0.013	1	0.909	0.981	0.709	1.358
Working status	0.106	0.274	0.149	1	0.699	1.112	0.650	1.902
Knowledge of STI	2.663	0.249	113.976	1	<0.001	14.339	8.794	23.379

[a] B – regression coefficient, SE – standard error, Wald – Chi-square value, df – degree of freedom, p – significance level (p<0.05), Exp (B) – odds ratio (OR)

HPV-KS total score average of the youth was found to be 14.26±7.04, and the HLS score average was 107.91±16.81. Youth's HPV-KS and Health Literacy

Scales Total Score and Sub-Factors Total Score Averages are given in Table 3.

Table 3. HPV-KS and HLS total scores and sub-factors score averages of youth

	Average±SD	Min–max	n
HPV-KS total score	14.26±7.04	0–33	310
HPV-KS sub-factor 1: General knowledge of HPV	9.05±3.50	0–16	310
HPV-KS sub-factor 2: Knowledge of the current HPV vaccination schedule	1.24±1.52	0–6	310
HPV-KS sub-factor 3: General knowledge of the HPV vaccine	2.20±1.76	0–5	310
HPV-KS sub-factor 4: General knowledge of HPV screening test	1.77±1.68	0–6	310
HLS total score	107.91±16.81	25–125	310
HLS sub-factor 1: Access to Information	21.51±3.86	5–25	310
HLS sub-factor 2: Understanding information	30.71±4.89	7–35	310
HLS sub-factor 3: Appraisal/evaluation	34.64±5.99	8–40	310
HLS sub-factor 4: Application/use	21.05±4.18	5–25	310

A positive and significant correlation was found between the HPV-KS total score and all sub-factor scores of the youth, and the HLS total score and all sub-factors total scores (p<0.001) (Table 4).

Table 4. The relationship between total scores of HPV-KS and HLS[a]

		HPV-KS	HPV-KS SUB 1	HPV-KS SUB 2	HPV-KS SUB 3	HPV-KS SUB 4	HLS	HLS SUB1	HLS SUB2	HLSSUB3
HPV-KS sub factor 1	r	0.903**								
	p	0.000								
HPV-KS sub factor 2	r	0.730**	0.511**							
	p	0.000	0.000							
HPV-KS sub factor 3	r	0.802**	0.602**	0.517**						
	p	0.000	0.000	0.000						
HPV-KS sub factor 4	r	0.804**	0.605**	0.544**	0.587**					
	p	0.000	0.000	0.000	0.000					
HLS	r	0.292**	0.281***	0.153*	0.262**	0.223**				
	p	0.000	0.000	0.007	0.000	0.000				
HLS sub factor 1	r	0.332**	0.310***	0.181*	0.271**	0.295**	0.876**			
	p	0.000	0.000	0.001	0.000	0.000	0.000			
HLS sub factor 2	r	0.242**	0.237**	0.115*	0.214**	0.192**	0.887**	0.759**		
	p	0.000	0.000	0.043	0.000	0.001	0.000	0.000		
HLS sub factor 3	r	0.258**	0.264**	0.118*	0.236**	0.178**	0.936**	0.762**	0.757**	
	p	0.000	0.000	0.038	0.000	0.002	0.000	0.000	0.000	
HLS sub factor 4	r	0.214**	0.189**	0.145*	0.217**	0.145*	0.835**	0.622**	0.613**	0.744**
	p	0.000	0.001	0.011	0.000	0.011	0.000	0.000	0.000	0.000

[a] r – Pearson correlation coefficient, * – p<0.05, ** – p<0.01

Discussion

It was determined that 50.6% of the participants were aware of HPV, but only 39.4% were aware of the HPV vaccine. With this result, it can be concluded that almost one out of every two young people has no knowledge about HPV. 93% of the students had heard of HPV in a study conducted with midwifery students. In comparison, 16.8% of the students stated that they had heard of HPV before in another study conducted with university students studying other than health.[13,21] A study conducted with university students in Indonesia reported that 90% of the youth have heard of HPV, and 68% have knowledge of the HPV vaccine.[22] It was determined that 89.7% of the students studying in the health department in Romania have heard of HPV, and 32.2% have poor knowledge of the HPV vaccine.[23] In a study conducted in Türkiye, it was reported that 58.6% of the youth had heard of the HPV vaccine before, and 57.1% were doubtful about whether the HPV vaccine would be protective or not.[12] The youth's knowledge level of HPV and its vaccine differs between studies. It can be said that the reason for this is related to the differences between the panel, countries, and the studied departments. HPV vaccines are not sufficiently promoted in Türkiye. The fact that the vaccine is provided for a fee and that not every individual has the financial means to afford this vaccine are barriers to vaccination. We may attribute the insufficient knowledge of HPV of the youth participating in our study to the fact that HPV is not common yet, and the HPV vaccine is not included in the vaccination schedule in Türkiye.

Vaccination against HPV is the primary protection, and it is recommended by WHO.[5] Nearly half of the participants have heard of the HPV vaccine, and 38.3% have knowledge of the vaccine. In a study conducted in Italy, although 42.1% of the participants have heard of HPV, only 15.3% know that the vaccine is available in the country.[24] It was determined that 67.4% of the youth in our study know STIs, and more than half of them need health education. This indicates that youth do not have enough knowledge of HPV and other infections. The study conducted with young Thai women revealed that the scoring average of knowledge of HPV is moderate, and there is a greater need for health education.[25] Studies indicate that even if there is knowledge of HPV, it is not enough. For this reason, it may be said that youth, a significant risk group, need a comprehensive education. Although the majority of the participants in the study were university graduates, it is noteworthy that their knowledge of HPV and other STIs was insufficient. University students need to be informed about sexual health.

Our study determined that being a woman, being married, and knowing STIs increased the knowledge level of HPV. Women generally do not tend to share their sexual experiences when they are not married. On the other hand, married women may be sensitive about gynecological examinations. It is likely that they have

been informed about HPV screening during gynecological examinations. Men, on the other hand, may feel more comfortable about HPV transmission. This may be due to men's lack of knowledge about HPV and its risks. A study conducted with youth between 18 to 30 in Sweden revealed that education level, gender, and economic income are associated with knowledge of HPV. In the same study, most participants knew that HPV is an STI and that both men and women can be infected. However, it was reported that they did not know that HPV can cause cancers other than cervical cancer.[26] A study of university students in Poland reported that almost three quarters of young people did not know that HPV can cause oral cancer. However, HPV is responsible for oral cancers as well as cervical cancer.[27] According to data from Türkiye, it is estimated that HPV-related cancers affect women more and are approximately five times higher in women than men.[28] Women are more exposed to the negative consequences of HPV and other sexually transmitted infections. In Türkiye, women who apply to gynecology outpatient clinics in Türkiye are also informed about and administered pap-smears. However, there is no platform to inform men about this issue. Therefore, men are still one of the effective factors in increasing the spread.

In our study, the HPV-KS total score average of the youth was found to be 14.26±7.04. In different studies conducted in Türkiye, it was reported that the HPV-KS total score average of midwifery students was 21.21±4.69, and university students was 14.3±9.7. It is thought that the education of midwifery students for genital infections and women's cancers is reflected in their scores.[16,21] Similar to this situation, in another study, it was found that young women studying in the fields of medicine and biology had better HPV knowledge than other women.[29] It is thought that the low score average of the youth participating in our study is due to the difference in the number of participants, and the participants of people from all educational levels.

In different studies conducted in our country, it is seen that health literacy is at a reasonable level. When HLS scores were examined in studies conducted in Türkiye, it was determined that surgical patients scored 100.82±15.62, old individuals scored 113.03±12.24, and nursing students scored 107.3±15.1.[9,10,11] It was determined that the HLS total score of youth participating in our study was 107.91±16.81, which was good. Health literacy may be defined as the individual's understanding and interpretation of the information and acting accordingly when a patient is intended to be provided with medical information. Therefore, it may be said that the health education given to youth in our country will improve their health behaviors. When developing health education programs, especially sexual health should be included in the curriculum. This will reduce the prevalence of cervical and other HPV-related cancers in Türkiye in the long term. Deaths, health and economic losses due to cancers will improve.

In our study, a positive and significant correlation was found between HPV-KS total score and all sub-factor scores of the youth, and HLS total score and all sub-factors total score ($p<0.01$). In two similar studies conducted at different times, it was determined that health literacy increased as young university students' knowledge level of HPV increased.[30,31] Although high health literacy is a social advantage, HPV and similar STIs should be brought to the agenda in broader fields. Information about HPV will play an active role in screening tests and the spread of the vaccine. Introducing HPV to society will inform youth about its possible risks and consequences and demand more protection, treatment, or care.

Study limitations

The limitations of the study include the lack of face-to-face interviews with young people and lack of motivation to fill out the study forms. This study cannot be generalized due to the lack of access to young people in different regions of the country.

Study strengths

The strengths of this study are that the number of samples representing the population was reached and the scarcity of similar studies in Türkiye.

Conclusion

It was determined that youth in Türkiye have insufficient knowledge of HPV and its vaccine. Our study revealed that the knowledge level of HPV is low in people with low health literacy, men, singles, and those who do not know about STIs, and the risk groups that should be focused on in solving the problem were determined.

It should benefit from a good health literacy level, and studies should be conducted to inform the youth. Information studies should be maintained by public service announcements and short notes, primarily through social media tools used by youth actively. The centers providing screening services should be increased and promoted. On the other hand, the HPV vaccine, sold for a fee, is recommended to be included in the national vaccination program.

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Declarations

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Author contributions

Conceptualization, M.A.S. and E.Y.A.; Methodology, E.Y.A.; Software, M.A.S. and E.Y.A; Validation, M.A.S. and E.Y.A.; Formal Analysis, M.A.S.; Investigation, E.Y.A; Resources, E.Y.A;; Data Curation, X.X.; Writing – Original Draft Preparation, X.X.; Writing – Review & Editing, M.A.S..; Supervision, M.A.S.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

Data availability

Data available on request from the authors.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University Dicle (Date:11.04.2022/ No: E-14679147-663.05-266750).

References

1. Çevik E, Coşkun AM. Current Approach to HPV Infection and the role of the midwife. *KSHED.* 2021;7(3):215-229.
2. Alp Avcı G, Bozdayı G. Human papilloma virus. *Kafkas J Medical Sci*. 2013;3(3):136-144. doi: 10.5505/kjms.2013.52724
3. Tanner E, Alexander CJ. Genital Kanal Enfeksiyonları. In: *Johns Hopkins Jinekoloji ve Obstetri El Kitabı*. Fortner KB, Szymanski LM, Fox HE, ed. Güneş Tıp Kitabevi; 2008:289.
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA. Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer J Clin*. 2018;68:394-424. doi: 10.3322/caac.21492
5. World Health Organization site. Cervical cancer 2022. https://www.who.int/news-room/fact-sheets/detail/cervical-cancer. Accessed October 11, 2023.
6. HPV Information Center 2021. Türkiye human papillomavirus and related cancers, fact sheet; 2021. https://hpvcentre.net/statistics/reports/TUR_FS.pdf. Accessed October 10, 2023.
7. World Health Organization site. Türkiye cervical cancer profile 2021. https://cdn.who.int/media/docs/default-source/ncds/ncd-surveillance/cxca/cxca-profiles/cxca-profiles-en.pdf?sfvrsn=d65f786_23&download=true. Accessed October 14, 2023.
8. Yeşildal M, Kaya ŞD. The relationship between digital literacy and health literacy in adult individuals: The case of Konya. *Sağlık Bilimleri Dergisi.* 2021;30:174-181.
9. Çetin F, Yılmaz E. The effects of health literacy levels of patients in surgical clinic on health perception and surgical fear. *İzmir Katip Çelebi University Faculty of Health Science Journal.* 2022;7(1):61-67.
10. Tuğut N, Yılmaz A, Yeşildağ Çelik B. The relationship between nursing students' health literacy levels and healthy lifestyle behaviors. *Journal of Cumhuriyet University Health Sciences Institute.* 2021;(6)2:103-111.
11. Bakır S, Yağız Altıntaş R, Şahin S, Ertem G. determining the health literacy level of students at the university of the third age. *Avrasya Sağlık Bilimleri Dergisi.* 2022;5(3):12-18.
12. Dönmez S, Gümüşsoy S. Adolescents' knowledge, attitude, behavior and barriers regarding human papillomavirus vaccine and cervical cancer. *International Anatolia Academic Online Journal Health Sciences.* 2020;6(3):323-336.
13. Çınar İ, Ozkan S, Aslan G, Alatas E. Knowledge and behavior of university students toward human papillomavirus and vaccination. *APJON.* 2019;6(3):300.
14. Turkish Statistical Institute. Address based population registration system results, 2021. https://data.tuik.gov.tr/Bulten/Index?p=45500. Accessed October 11, 2023.
15. Usubütün A, Alemany L, Küçükali T, et al. Human papillomavirus types in invasive cervical cancer specimens from Türkiye. *International Journal of Gynecological Pathology.* 2009;28:541-548.
16. Karahan S, Agadayı E, Koç MM, et al. Knowledge, attitudes, and behaviors of medical school students about human papilloma virus (HPV) and HPV vaccine. *KSU Medical Journal*. 2023;18(3):78-85.
17. Waller J, Ostini R, Marlow L, McCaffery K, Zimet G. Validation of a measure of knowledge abouthuman papillomavirus (HPV) using item response theory and classical test theory. *Prev Med.* 2013;56(1):35-40. doi: 10.1016/J.YPMED.2012.10.028
18. Demir Bozkurt F, Özdemir S. Validity and reliability of a Turkish version of the human papillomavirus knowledge scale: a methodological study. *J Turk Ger Gynecol Assoc.* 2023;24:177-186.
19. Toci E, Burazeri G, Jerliu N, et al. Health literacy, self-perceived health and self-reported chronic morbidity among older people in Kosovo. *Health Promot Int.* 2015;30(3):667-674. doi: 10.1093/heapro/dau009
20. Aras Z, Bayık Temel A. Evaluation of validity and reliability of the Turkish version of health literacy scale. *FNHD.* 2017;25(2):85-94.
21. Genç Koyuncu R. Analysis of midwifery students' knowledge of human papilloma virus: A descriptive survey study. *And Bül.* 2022;24:118-126.
22. Khatiwada M, Kartasasmita C, Mediani HS, Delprat C, Hal GV, Dochez C. Knowledge, attitude and acceptability of the human papilloma virus vaccine and vaccination among university students in Indonesia. *Front Pub Health.* 2021;9:616456. doi: 10.3389/fpubh.2021.616456
23. Voidăzan TS, Budianu MA, Rozsnyai FF, Kovacs Z, Uzun CC, Neagu N. Assessing the level of knowledge, beliefs and acceptance of hpv vaccine: a cross-sectional study in Romania. *Int J Environ Res Public Health.* 2022;19:6939. doi: 10.3390/ijerph19116939

24. Di Guiseppe G, Abbate R, Liguori G, Albano L, Angelillo IF. Human papillomavirus and vaccination: knowledge, attitudes, and behavioral intention in adolescents and young women in Italy. *Br J Cancer.* 2008;99(2):225-229. doi: 10.1038/sj.bjc.6604454
25. Juntasopeepun P, Davidson PM, Suwan N, Phianmongkhol Y, Srisomboon J. Human Papillomavirus Vaccination Intention among Young Women in Thailand. *Asian Pac J Cancer Prev.* 2011;12(12):3213-3219.
26. Dahlström AL, Sundström K, Young C, Lundholm C, Sparén P, Tran NT. Awareness and knowledge of human papillomavirus in the Swedish adult population. *J Adolesc Health*. 2012;50(2):204-206. doi: 10.1016/j.jadohealth.2011.05.009
27. Lewandowski B, Czenczek-Lewandowska E, Pakla P, et al. Awareness of Polish undergraduate and graduate students regarding the impact of viral infections and high-risk sexual behaviors on the occurrence of oral cancer. *Medicine.* 2018;97(41):12846. doi: 10.1097/MD.0000000000012846
28. T.R. Ministry of Health. Türkiye kanser istatistikleri 2017. https://hsgm.saglik.gov.tr/depo/birimler/kanser-db/istatistik/Turkiye_Kanser_Istatistikleri_2017.pdf. Accessed October 14, 2023.
29. Węgorowski P, Michalik J, Turkowska K, et al. Evaluation of the knowledge of the issues related to human papillomavirus infections within young women. *Eur J Clin Exp Med.* 2018;16(4):308-316. doi: 10.15584/ejcem.2018.4.7
30. Albright AE, Allen RS. HPV Misconceptions among college students: the role of health literacy. *J Community Health.* 2018;43:1192. doi: 10.1007/s10900-018-0539-4
31. Kitur H, Horowitz AM, Beck K, Wang MQ. HPV Knowledge, Vaccine Status, and Health Literacy Among University Students. *J Cancer Educ.* 2021;37:1606-1613. doi: 10.1007/s13187-021-01997-1

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ORIGINAL PAPER

Characteristics of patients with cerebral aneurysms and arteriovenous malformations

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ABSTRACT

Introduction and aim. Aneurysms and arteriovenous malformations (AVMs) are both vascular abnormalities that can occur simultaneously or separately and increase the risk of intracerebral hemorrhage. The aim of this research was to characterize patients with intracranial aneurysms and AVMs.

Material and methods. This retrospective research was based on analysis of Digital Subtraction Angiography. The research group consisted of 118 patients. StatSoft STATISTICA 13 was used for the statistical analysis of parameters, such as dimensions and vascularization pattern of vascular abnormalities.

Results. The total number of aneurysms described was 168. In addition, 33 patients with cerebral aneurysms were also diagnosed with cerebral AVMs. The average dimensions of the aneurysms were 6.33mm, 95% CI (5.47 7.18) × 4.76mm, 95% CI (4.09, 5.43). The abnormalities were found in the extent of circulation of ACA (anterior cerebral artery) 50 (29.76%), ICA (internal carotid artery) 48 (28.57%), MCA (middle cerebral artery) 26 (15.48%), PCA (posterior cerebral artery) 23 (13.69%). There were 76 (64%) patients with diagnosed single aneurysms and 42 (36%) with multiple.

Conclusion. Aneurysms are more likely to occur singly than multiply. The majority are located within the vascularization of the ACA. Aneurysms can coexist with AVMs, which increases the risk of rupture.

Keywords. aneurysm, arteriovenous malformation, digital subtraction angiography

Introduction

Intracranial aneurysms are defined as pathological dilatations of cerebral vessels characterized by a relatively frequent occurrence.[1] This vascular pathology affects approximately 2–5% of the adult population.[2] Although cerebral aneurysms occur in both men and women, the female gender, especially in the postmenopausal age, is thought to predispose to their formation.[3] Aneurysms within the cerebrum can be divided into congenital lesions, which are in the minority, and acquired lesions, which occur far more frequently and whose formation is particularly associated with risk factors such as older age, atherosclerosis, hypertension, smoking, alcoholism and connective tissue diseases.[4,5] In addition, the significant aspect associated with the formation of brain aneurysms are genetic factors, primarily family occurrence, hereditary diseases such as polycystic kidney disease and tuberous sclerosis, as well as some genetic syndromes such as Ehlers-Danlos syndrome and Marfan syndrome.[6-9] Brain aneurysms are also classified according to their vascular structure (angioarchitecture). A distinction is made between saccular and fusiform aneurysms.[10] The coexistence of arteriovenous malformations (AVMs) with cerebral aneurysms is another significant issue. AVMs are vascular malformations that involve the formation of fistulas between arterial and venous vessels

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without an intermediate capillary bed.[11] Cerebral aneurysms are more common in people diagnosed with AVMs than in those without such lesions.[12] Intracranial aneurysms are a significant medical problem because, due to rupture, they can lead to intracranial hemorrhages, potentially resulting in death or severe disability.[13] The previously mentioned coexistence of aneurysms and AVMs is a significant risk factor for such bleeding.[14] Unruptured Intracranial Aneurysms can cause nonspecific symptoms such as headache, nausea, or dizziness. Aneurysms of larger size can compress the cranial nerves, which may lead, for example, to visual impairments such as double vision, drooping of the eyelid or deterioration of visual acuity. Unfortunately, a significant number of aneurysms do not cause any symptoms for many years, which leads to many patients not being diagnosed before a life-threatening event occurs. It is only at the time of rupture that the patient experiences a sudden, very severe headache described as" the most intense headache of one's life", which may be accompanied by nausea, vomiting, nuchal rigidity, photophobia, convulsions and impaired consciousness. A great majority of cases of aneurysm rupture result in unconsciousness.[15]

Since unruptured brain aneurysms may not cause any symptoms, their diagnosis is significantly more difficult. For this reason, they are often diagnosed incidentally. Imaging examinations such as computed tomography (CT), digital subtractive angiography (DSA) or magnetic resonance imaging (MRI) are used in detecting both aneurysms and AVMs.[16-17] Considering the severe consequences of ruptured aneurysms and AVMs, there are attempts to look for other diagnostic methods that would help detect these vascular pathologies in the prodromal period. Among the potential options for early diagnosis of aneurysms are biomarkers from blood or cerebrospinal fluid (CSF) samples. In the process of aneurysm formation and rupture, inflammatory and cell adhesion molecules, enzymes or hormones are involved. Some of those that are associated with the inflammatory process, such as MPO, GM-CSF, MCP-1 and other cytokines could potentially be used in the future as biomarkers for the diagnosis of cerebral aneurysms.[18] A potential inflammatory parameter that could be useful in the diagnosis of brain aneurysms is interleukin-6, which is increased in the cerebrospinal fluid of patients with unruptured cerebral aneurysms.[19] Another example of such biomarkers could be proteins that build the walls of blood vessels. In the study titled: Intracranial Aneurysm Biomarker Candidates Identified by a Proteome-Wide Study, researchers observed increased levels of the ORM1 glycoprotein in a group of patients with unruptured aneurysms.[20] There is reason to suspect that ORM1 shows potential to be used as a biomarker in future screening of unruptured aneurysms.[20] The inflammatory process, as in the case of aneurysms, has an important role in the pathogenesis of the formation of AVMs. Significant activity of proinflammatory cytokines can be observed, resulting in overexpression of cell adhesion molecules. It leads to the recruitment of leukocytes secreting metalloproteinase-9, which damages the walls of AVMs, finally causing the rupture of vascular pathology. Because of this, it can be assumed that there is a possibility of using inflammatory parameters, such as proinflammatory cytokines or metalloproteinase-9, as biomarkers for the diagnosis of AVMs.[21] In addition, with the progressive development of technology, the opportunity to use artificial intelligence (AI) in the diagnostic process of intracranial aneurysms is being recognized.[22] Given the high risk of death or injury from hemorrhage caused by ruptured aneurysms, patients diagnosed with aneurysms must be qualified for intervention. Several treatment approaches can be identified. First of all, surgical techniques and percutaneous embolization procedures are distinguished. The choice of the type of therapy depends on many factors, such as the morphology of the vascular lesion, the patient's medical condition and preferences.[23] In addition to surgery, other forms of treatment for aneurysms are being investigated. Pharmacological treatment may be considered as one of the options. A number of research suggests that some of the commonly used drugs for cardiovascular disease (statins, calcium channel blockers and angiotensin receptor antagonists) may have a application in the preventive treatment of unruptured intracranial aneurysms.[24] A significant clinical problem involving the treatment of patients with cerebral aneurysms is the issue of SARS-CoV-2 virus infection. Due to the high infectivity that SARS-CoV-2 virus has, many patients with vascular disease are at risk of coronavirus infection, including its severe course. SARS-CoV-2 enters cells through angiotensin-converting enzyme 2 (ACE2), which serves as a receptor for the virus. SARS-CoV-2 attacks epithelial cells and induces inflammation, leading to damage of the vascular endothelium. For this reason, people with cerebral aneurysms have a risk of developing vascular complications. Because of this danger, people with such vascular pathologies, should be especially considered as a group at higher risk for a more severe course of SARS-CoV2 virus infection.[25]

Aim

To characterize and compare vascular abnormalities in patients diagnosed with intracranial aneurysms. To investigate whether aneurysm diameter depends on the presence of AVMs.

Material and methods

The research was based on analysis of anonymized archival data obtained by digital subtractive angiography (DSA) imaging performed by multi-row CT scanner (GE

Optima CT 660) with usage of Seldinger wire technique. All examinations were performed at the University Hospital in Krakow. The data was collected from descriptions provided by experienced radiologists. The data included age and gender of patients, the size of diagnosed intracranial aneurysms, considering the maximum and minimum dimensions of vascular lesions, the presence of single and multiple aneurysms, their vascularization and the presence of arteriovenous malformations.

The research group consisted of 118 patients who were diagnosed with intracranial aneurysms, 33 of whom also had coexisting cerebral arteriovenous malformations. All patients were hospitalized in the University Hospital of Krakow between 2015 and 2022. The patients were divided into two groups:

Group 1 – patients diagnosed only with intracranial aneurysms,

Group 2 – patients diagnosed with both aneurysms and AVMs.

In addition, the relationship of aneurysm vascularization to AVMs was analyzed in Group 2. Group 2 was divided into subgroups as follows:

Subgroup 2.1 intranidal aneurysms,

Subgroup 2.2 flow-related aneurysms,

Subgroup 2.3 aneurysms unrelated to the shunt flow to the AVM.

Intranidal aneurysms are those located inside the boundaries of the AVM nidus. Flow-related aneurysms are those occurring along the course of arteries implicated in the perfusion of the nidus and, as such, are hemodynamically related to the AVM. The last group involves aneurysms unrelated to the shunt flow to the AVM, meaning they are located beyond the arteries vascularizing the AVM.

Since coexistence of AVM and aneurysm is well documented, it is very important to evaluate whether there is correlation between size of aneurysm and the coexistence with AVM.[12] Therefore, a comparison of the dimensions of the aneurysms between Group 1 and Group 2 was analyzed.

StatSoft STATISTICA 13 software was used to statistically analyze the collected data regarding the investigated cerebral vascular abnormalities. Significance level was set at 5% ($p<0.05$). Nonparametric test was used due to uneven sample size.

Results

The structure of analyzed population is presented in Table 1. The dimensions of the aneurysms analyzed in the research are presented in Table 2.

The prevalence of single and multiple aneurysms was one of the factors investigated. The whole research group included 76 (64%) patients with single aneurysms detected and 42 (36%) patients diagnosed with multiple aneurysms. There were 56 patients (66%) in the group 1, and 20 (61%) patients with diagnosed single aneurysms in the group 2. In contrast, patients with multiple intracranial aneurysms were 29 (34%) in the group 1 and 13 (39%) in the group 2.

Table 1. Structure of analysed population

Characteristics	Group 1		Group 2		All patients	
Patients, n (%)	85 (72%)		33 (28%)		118	
Gender, n (%)	Women	Men	Women	Men	Women	Men
	53 (62%)	32 (38%)	20 (61%)	13 (39%)	73 (62%)	45 (38%)
Age in years, means	60		44		55	
	61	58	42	45	56	54

Table 2. The dimensions of the aneurysms analyzed in the research

Characteristics	Cases, n	Maximum dimension (millimeters)	95% CI	Minimum dimension (millimeters)	95% CI
All patients	168	6.33	5.47, 7.18	4.76	4.09, 5.43
Group 1	119	6.22	5.26, 7.19	4.65	3.89, 5.41
Group 2	49	6.51	4.68, 8.34	5.12	3.68, 6.56

In the overall studied population, multiple aneurysms were more common in women (22% of all women) than men (14% of all men).

In group 1, 119 aneurysms were diagnosed in patients. Of these, 24 patients had 2 aneurysms and 5 patients had 3 aneurysms.

In group 2, 49 aneurysms were diagnosed in patients. Of these, 10 patients had 2 aneurysms and 3 patients had 3 aneurysms coexisting with AVMs. The number of intranidal aneurysms (Subgroup 2.1) was 11 (22.5%), flow-related aneurysms (Subgroup 2.2) was 27 (55%) and aneurysms unrelated to the AVM's vasculature (Subgroup 2.3) was 11 (22.5%).

The next feature analyzed was the vascularization of the aneurysms. The vascular abnormalities investigated had their arterial supply among the following vessels:

- anterior cerebral artery (ACA),
- middle cerebral artery (MCA),
- posterior cerebral artery (PCA),
- cerebellar arteries,
- internal carotid artery (ICA),
- other arterial vessels.

Among the entire group of patients, the most common arteries that provided aneurysmal vascularization were the anterior cerebral artery (50 aneurysms vascularized) and the internal carotid artery (48 aneurysms vascularized). Other aneurysms were found in the extent of circulation of MCA (26 aneurysms vascularized), PCA (23 aneurysms vascularized), cerebellar arteries (19 aneurysms vascularized) and other arterial vessels (2 aneurysms vascularized). Also in the group 1, both the ACA and ICA were the most numerous arterial supplies (40 and 39 vascularized aneurysms, respectively).

In contrast, in the group 2, the cerebellar arteries (12 vascularized aneurysms) and the ACA (10 vascularized aneurysms) were the most common arterial supplies.

Aneurysms dimensions were compared between group 1 (49 observations) and 2 (119 observations). Neither of them were proven to be statistically significant different using Mann-Whitney U test, z(dimension 1) = -0.434, p(dimension 1) = 0.6657, z(dimension 2) = 0.687, p(dimension 2) = 0.4942.

Discussion

According to the existing knowledge on the epidemiology of aneurysms, females predominate also among the research group, especially in the postmenopausal age.[3,5] In both analyzed groups, the distribution of patients by gender is similar, with a female predominance. The patients with coexisting aneurysms and AVMs had a lower age of lesion detection. This may indicate a predisposition to aneurysm formation among patients with AVMs. In this study, there was a correlation between the presence of an aneurysm and arteriovenous malformation to a significant extent (77.5% of aneurysms associated with AVM vascularization), which is also confirmed by other studies.[26] It is worth noting that the size of aneurysms in patients with AVMs is not statistically larger than those without. However average age of people in this group is significantly lower than in the group without AVMs, which may lead to the conclusion that there is a particular cause such as a genetic component that lowers the age of developing an aneurysm. There is also a theory that increased blood flow to the AVM results in the creation of an aneurysm.[27] Greater dimensions of aneurysms may potentially affect higher risk of their rupture.[28] There is a significant proportion of individuals with multiple aneurysms in the research population, which could indicate the presence of specific syndromes that predispose to aneurysm formation. In addition, the incidence of multiple aneurysms in the group with malformations is only slightly higher than in the group without malformations. This raises the observation that the presence of malformations significantly increases the size of aneurysms, but not necessarily the number of aneurysms. Multiple aneurysms pose a significant problem because with each subsequent aneurysm the risk of rupture and thus hemorrhage increases.[29] Among the group 1, the most common arteries associated with aneurysms were the internal carotid artery and the anterior cerebral artery (which the research also included the anterior communicating artery), which may be related to the tendency for aneurysms to form at the bifurcations.[30] Also notable is the higher proportion of cerebellar artery aneurysms in people with arteriovenous malformations. This may be related to a greater susceptibility to changes in the structure of this artery in people with a concomitant tendency to form malformations. The study highlights the increased prevalence of multiple aneurysms compared to other studies, which may raise further questions about the reasons for this prevalence in the study population.[31] Higher number of multiple aneurysms were present both in women and men. It shows that it is important to search for more aneurysms if one is detected. Moreover, the paper points out the need to follow-up people with AVMs because of the risk of future aneurysms.

There are limitations to this study, which are mainly due to the lack of access to clinical data of the patients included in the analysis and the lack of follow-up. Part of the reason for this is the fact that patients who have imaging examinations performed at the Radiology Department of the University Hospital are not necessarily being hospitalized there while referrals for examinations rarely include detailed clinical data of the patient.

Conclusion

Intracranial artery aneurysms are one of the most common vascular abnormalities of the brain. Rupture of an aneurysm can cause serious complications, primarily being a life-threatening condition due to subarachnoid hemorrhage. Aneurysms occur in a variety of locations and sizes. These abnormalities more often occur singly than multiply, however the amount of multiple aneurysms is significant. The majority of aneurysms are located within the vascularization of the ACA and ICA. With advances in radiology, there are increasing numbers of opportunities for early detection of aneurysms and appropriate management before developing complications, especially in patients at greater risk of their development. However, more research is needed to specifically focus on early detection of patients at risk of vascular abnormalities and their proper treatment.

Declarations

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Author contributions

Conceptualization, J.O. and M.S.; Methodology, J.O., M.S. and M.W.; Software, M.W.; Validation, J.O. and M.S.; Formal Analysis, J.O.; Investigation, J.O., M.S. and M.W.; Resources, J.O.; Data Curation, M.S.; Writing – Original Draft Preparation, J.O., M.S., M.W. and S.S.; Writing – Review & Editing, J.O. and S.S.; Visualization, J.O.; Supervision, J.O.; Project Administration, J.O.

Conflict of interest

The authors declare no conflict of interest.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval
Not applicable.

References

1. Krings T, Mandell DM, Kiehl TR, et al. Intracranial aneurysms: from vessel wall pathology to therapeutic approach. *Nat Rev Neurol*. 2011;7(10):547-559. doi: 10.1038/nrneurol.2011.136
2. Xu Z, Rui YN, Hagan JP, Kim DH. Intracranial aneurysms: Pathology, genetics, and molecular mechanisms. *Neuromolecular Med*. 2019;21(4):325-343. doi: 10.1007/s12017-019-08537-7
3. Turan N, Heider RAJ, Zaharieva D, Ahmad FU, Barrow DL, Pradilla G. Sex differences in the formation of intracranial aneurysms and incidence and outcome of subarachnoid hemorrhage: Review of experimental and human studies. *Transl Stroke Res*. 2016;7(1):12-19. doi: 10.1007/s12975-015-0434-6
4. Jersey AM, Foster DM. *Cerebral Aneurysm*. StatPearls Publishing; 2023.
5. Ellamushi HE, Grieve JP, Jäger HR, Kitchen ND. Risk factors for the formation of multiple intracranial aneurysms. *J Neurosurg*. 2001;94(5):728-732. doi: 10.3171/jns.2001.94.5.0728
6. Wilkinson DA, Heung M, Deol A, et al. Cerebral aneurysms in Autosomal dominant polycystic kidney disease: A comparison of management approaches. *Neurosurgery*. 2019;84(6):352-361. doi: 10.1093/neuros/nyy336
7. Jung KH. New pathophysiological considerations on cerebral aneurysms. *Neurointervention*. 2018;13(2):73-83. doi: 10.5469/neuroint.2018.01011
8. Olubajo F, Kaliaperumal C, Choudhari KA. Vascular Ehlers-Danlos Syndrome: Literature review and surgical management of intracranial vascular complications. *Clin Neurol Neurosurg*. 2020;193(105775):105775. doi: 10.1016/j.clineuro.2020.105775
9. Schievink WI, Parisi JE, Piepgras DG, Michels VV. Intracranial aneurysms in marfan's syndrome: An autopsy study. *Neurosurgery*. 1997;41(4):866-871. doi: 10.1097/00006123-199710000-00019
10. Pritz MB. Cerebral aneurysm classification based on angioarchitecture. *J Stroke Cerebrovasc Dis*. 2011;20(2):162-167. doi: 10.1016/j.jstrokecerebrovasdis.2009.11.018
11. Ozpinar A, Mendez G, Abla AA. Epidemiology, genetics, pathophysiology, and prognostic classifications of cerebral arteriovenous malformations. In: *Handbook of Clinical Neurology*. Elsevier; 2017:5-13.
12. Wilkins RH. Natural history of intracranial vascular malformations: A review. *Neurosurgery*. 1985;16(3):421-430. doi: 10.1227/00006123-198503000-00026
13. Findlay JM, Deagle GM. Causes of morbidity and mortality following intracranial aneurysm rupture. *Can J Neurol Sci*. 1998;25(3):209-215. doi: 10.1017/s031716710003403x
14. Platz J, Berkefeld J, Singer OC, et al. Frequency, risk of hemorrhage and treatment considerations for cerebral arteriovenous malformations with associated aneurysms. *Acta Neurochir (Wien)*. 2014;156(11):2025-2034. doi: 10.1007/s00701-014-2225-3
15. Cianfoni A, Pravatà E, De Blasi R, Tschuor CS, Bonaldi G. Clinical presentation of cerebral aneurysms. *Eur J Radiol*. 2013;82(10):1618-1622. doi: 10.1016/j.ejrad.2012.11.019
16. Pereira VM, Bijlenga P, Marcos A, Schaller K, Lovblad KO. Diagnostic approach to cerebral aneurysms. *Eur J Radiol*. 2013;82(10):1623-1632. doi: 10.1016/j.ejrad.2012.10.014
17. Sebök M, Dufour JP, Cenzato M, et al. When Is Diagnostic Subtraction Angiography Indicated Before Clipping of Unruptured and Ruptured Intracranial Aneurysms? An International Survey of Current Practice. *Acta Neurochir Suppl*. 2021;132:9-17.
18. Hussain S, Barbarite E, Chaudhry NS, et al. Search for biomarkers of intracranial aneurysms: A systematic review. *World Neurosurg*. 2015;84(5):1473-1483. doi: 10.1016/j.wneu.2015.06.034
19. Kamińska J, Dymicka-Piekarska V, Chrzanowski R, et al. IL-6 quotient (the ratio of cerebrospinal fluid IL-6 to serum IL-6) as a biomarker of an unruptured intracranial aneurysm. *J Inflamm Res*. 2021;14:6103-6114. doi: 10.2147/jir.s335618
20. Sharma T, Datta KK, Kumar M, et al. Intracranial aneurysm biomarker candidates identified by a proteome-wide study. *OMICS*. 2020;24(8):483-492. doi: 10.1089/omi.2020.0057
21. Tanzadehpanah H, Modaghegh MHS, Mahaki H. Key biomarkers in cerebral arteriovenous malformations: Updated review. *J Gene Med*. 2023;25(12):3559. doi: 10.1002/jgm.3559
22. Din M, Agarwal S, Grzeda M, Wood DA, Modat M, Booth TC. Detection of cerebral aneurysms using artificial intelligence: a systematic review and meta-analysis. *J Neurointerv Surg*. 2023;15(3):262-271. doi: 10.1136/jnis-2022-019456
23. Zhao J, Lin H, Summers R, Yang M, Cousins BG, Tsui J. Current treatment strategies for intracranial aneurysms: An overview. *Angiology*. 2018;69(1):17-30. doi: 10.1177/0003319717700503
24. Shimizu K, Imamura H, Tani S, et al. Candidate drugs for preventive treatment of unruptured intracranial aneurysms: A cross-sectional study. *PLoS One*. 2021;16(2):0246865. doi: 10.1371/journal.pone.0246865
25. Tanzadehpanah H, Lotfian E, Avan A, et al. Role of SARS-COV-2 and ACE2 in the pathophysiology of peripheral vascular diseases. *Biomed Pharmacother*. 2023;166(115321):115321. doi: 10.1016/j.biopha.2023.115321
26. Rammos SK, Gardenghi B, Bortolotti C, Cloft HJ, Lanzino G. Aneurysms associated with brain arteriovenous malformations. *AJNR Am J Neuroradiol*. 2016;37(11):1966-1971. doi: 10.3174/ajnr.a4869
27. Brown RD, Wiebers DO, Forbes GS. Unruptured intracranial aneurysms and arteriovenous malformations:

frequency of intracranial hemorrhage and relationship of lesions. *J Neurosurg*. 1990;73(6):859-863. doi: 10.3171/jns.1990.73.6.0859

28. Wiebers DO. Unruptured intracranial aneurysms: natural history, clinical outcome, and risks of surgical and endovascular treatment. *Lancet*. 2003;362(9378):103-110. doi: 10.1016/s0140-6736(03)13860-3
29. Shen X, Xu T, Ding X, Wang W, Liu Z, Qin H. Multiple intracranial aneurysms: Endovascular treatment and complications. *Interv Neuroradiol*. 2014;20(4):442-447. doi: 10.15274/inr-2014-10037
30. Gao BL, Hao H, Hao W, Ren CF, Yang L, Han Y. Cerebral aneurysms at major arterial bifurcations are associated with the arterial branch forming a smaller angle with the parent artery. *Sci Rep*. 2022;12(1):5106. doi: 10.1038/s41598-022-09000-7
31. Kaminogo M, Yonekura M, Shibata S. Incidence and outcome of multiple intracranial aneurysms in a defined population. *Stroke*. 2003;34(1):16-21. doi: 10.1161/01.str.0000046763.48330.ad

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ORIGINAL PAPER

Encapsulation of genistein in glycidylated G3 polyamidoamine dendrimers enables diffusion of genistein through biological membranes and anti-nematode activity of the encapsulate

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ABSTRACT

Introduction and aim. Poorly soluble isoflavonoid genistein is known as an anti-nematode agent and also it decreases the risk of certain types of cancer. The biological activity of genistein is limited mostly by its low solubility. Therefore many attempts to increase genistein solubility in water were reported. We applied a polyamidoamine dendrimer, modified its surface by glycidylation, and used this macromolecule as a guest for genistein.

Material and methods. Polyamidoamine dendrimer 3rd generation was substituted with 64 glycidol residues to obtain a macromolecule host for genistein. The stoichiometry of this host-guest complex was determined. The complex was tested for skin model permeability, toxicity on fibroblast (BJ) and keratinocyte (HaCaT) cell lines *in vitro* and anthelmintic activity on the *Caenorhabditis elegans* nematode.

Results. The partition coefficient of genistein between octanol and water was determined ($K_{O/W}$). The 1:1 host-guest complex was isolated and used as drug delivery system for genistein delivery. PAMAM G3 glycidyled dendrimer containing genistein indicated an anthelmintic activity at 50 μM concentration.

Conclusion. The solubility of genistein in water increases 640 times in presence of an equimolar concentration of the dendrimer. One molecule of host dendrimer encapsulates 3 molecules of genistein. The encapsulate is an efficient anti-nematode formulation.

Keywords. *C. elegans*, fibroblast BJ toxicity, genistein, keratinocyte HaCaT toxicity, PAMAM dendrimer

Introduction

Genistein (5,7-dihydroxy-3-(4'-hydroxyphenyl) chromen-4-one) is a trihydroxyisoflavone (Fig. 1) which is considered as a phytoestrogen of rather low bioavailability due to its hydrophobic character.[1] Soy based diets deliver isoflavonoids (IFLs) into the digestion system. Among them, genistein (GEN) and its 7-O-glucosides are absorbed in the small intestine (10%) within the first 2 hours and then in the large intestine (90%) within 4-6 hours, where gut bacteria release GEN. Detailed studies (absorption, distribution, metabolism, and excretion) of IFLs after soy intake were performed based on urinary and plasma levels of IFLs.[1] Biological activity of IFLs in humans is rather low. Generally IFLs are suggested to

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protect against many chronic diseases (including coronary heart diseases, subclinical atherosclerosis, type 2 diabetes), and decrease the risk of certain types of cancer such as breast and prostate cancers.

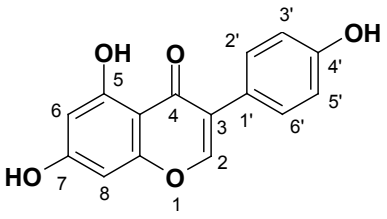

Fig 1. Formula of GEN with atom numbering

On the other hand, IFLs including GEN are anti-nematode agents. IFLs were demonstarted to impede soybean cyst nematode (SCN) by influencing sex ratios and the number of female eggs. GEN was shown to strongly influence the physiological index of SCN in a dosage and time dependent way.[2] When SCNs were exposed to 0.37 mM GEN (100 μg/mL) aqueous solution (with 1% DMSO), a 50% reduction of SCN mobility (measured by reversal frequency assay), ca 35% reduction of oxygen consumption, and severe body leaking after 24 hours treatment were observed.

Another nematode model organism, a transgenic Aβ-expression *Caenorhabditis elegans* strain CL4176 was used to determine the ability of IFLs to alleviate Aβ expression-induced paralysis in *C. elegans*, which correlated with a reduced level of hydrogen peroxide and β amyloid.[3] This relationship was observed only for glycitein, not for GEN. The authors concluded that the neuroprotective effect of phytoestrogens is due to their anti-oxidative activities.

Inspired by the potential health beneficial effects of GEN intake and reported high membrane permeability of GEN across Caco-2 cell monolayers and effective efflux of GEN,[4] Shen et al. designed a Drug Delivery System for GEN delivery.[5] GEN was entrapped in micelles prepared from Soluplus® and α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) in order to improve GEN solubility and thus oral bioavailability of GEN. Micelles 185 nm in size were loaded with GEN up to almost 4% DL and then tested *in vitro* for GEN delivery. Thus, GEN was dispersed in the mixed micelles (M) to an apparent concentration of 1.5 mg/mL, while GEN solubility in water is much lower (1.3 μg/L).[6] GEN was released from GEN-M dissolved in methanol in dialytic conditions into saline buffer with surfactants with 4-fold higher efficiency than GEN alone. The apical-basolateral permeability through Caco-2 cell monolayer was slightly higher for GEN-M than that for GEN, which resulted in decrease in efflux ratio from 0.84 for GEN into 0.73. Finally, the pharmacokinetic studies on rats revealed the 4-fold increase of GEN levels in plasma with a maximum at 0.7 hour for GEN-M in comparison with 1.0 hour T_{max} for GEN alone. Many other DDSs for GEN delivery were discovered, which slightly improved solubility and permeability of GEN, and the subject was recently reviewed.[7]

Aim

We have designed the DDS for GEN delivery based on a modified polyamidoamine (PAMAM) dendrimer generation 3. The native dendrimer was used before to encapsulate riboflavin and 8-methoxypsoralene for transdermal delivery.[8,9] However, recently, we modified the G3 dendrimer by glycidylation in order to eradicate primary amine groups of native G3.[10] Those $G3^{gl}$ dendrimers were very well soluble in water and efficiently internalized into normal human fibroblasts and two cancer lines within 1 hour.

Material and methods

Materials

Genistein (>98% purity) was purchased from Merck KGaA (Darmstad, Germany). Third generation polyamidoamine dendrimer (G3) was synthesized according to Tomalia's protocol and then modified by conversion of primary amine groups into bis-2,3-dihydroxypropylamine groups as was described before to yield $G3^{gl}$ (molecular weight 11645 Da).[10,11] HaCaT keratinocytes were purchased from CLS Cell Lines Service GmbH (Eppelheim, Germany) and BJ normal human fibroblasts from American Type Culture Collection (Manassas, VA 20108, MA, USA). Dulbecco's Modified Eagle Medium supplemented with sodium pyruvate, L-glutamine, high glucose (DMEM) was provided by Biological Industries (Cromwell, CO, USA), and fetal bovine serum by Gibco (Waltham, MA, USA). Penicillin and streptomycin solution, phosphate-buffered saline (PBS) with and without magnesium and calcium ions were provided by Thermo Fisher Scientific Inc. (Waltham, MA, USA). Trypsin–EDTA solution, 0.4% trypan blue solution, dimethylsulfoxide (DMSO) for molecular biology, 5-fluoro-2′-deoxy-uridine (FUdR) and other chemicals and buffers were purchased from Merck KGaA (Darmstadt, Germany). Alamar Blue test was purchased from Sigma, (Life Technologies, Bleiswijk, The Netherlands). Cell culture dishes and materials were from Nunc (Roskilde, Denmark), Corning Inc. (New York, NY, USA) or Googlab Scientific (Rokocin, Poland). Reagents used to culture of *C. elegans* nematode were supplied by Sigma-Aldrich (Saint Louis, MO, USA) or Carl Roth GmbH & Co., KG (Karlsruhe, Germany).

Methods

The 1-D ^{1}H and ^{13}C NMR spectra and 2-D ^{1}H-^{1}H correlations spectroscopy (COSY), ^{1}H-^{13}C heteronuclear

single quantum correlation (HSQC), and heteronuclear multiple bond correlation (HMBC) spectra were recorded in DMSO-d_6 using Bruker 300 MHz instrument (Rheinstetten, Germany) at College of Natural Sciences, University of Rzeszow.

UV-Vis spectra were recorded with Hitachi U-1900 spectrometer (Tokyo, Japan).

The permeation GEN and GEN@G3^{gl} was studied using Franz diffusion assembly (Thermo Scientific (UK) model DC 600) equipped with 6 cm^3 acceptor compartments. Polyvinydifluoride (PVDF, 0.125 mm thickness) or prepared rabbit skin (RS, 0.55 mm thickness) model membranes were used for permeation studies.

Partition of GEN between octanol and water containing host G3^{gl}

Analytical protocols used in partition experiments and transdermal diffusion experiments (vide infra) required determination of extinction coefficients in various solvents. The UV-vis spectra were taken in water, ethanol, octanol, and in 70% aqueous phosphate buffered pH 7.4 and 30% ethanol (receiving solution in diffusion tests) are shown at Figure 2.

Solubility of GEN in octanol was determined by suspending 5.1 mg GEN in 5 mL octanol, stirring the mixture at 25 °C for 12 hours, filtration off the undissolved octanol, and determination of GEN concentration using previously determined extinction coefficient at 336 nm, $e_{336}=3.52\times10^3$ mol$^{-1}\times$dm$^3\times$cm^{-1}. Finally, the 3.77 M concentration of GEN in octanol saturated solution was elucidated.

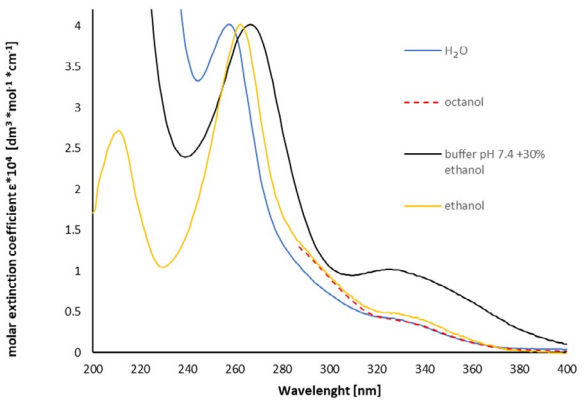

Fig. 2. The UV-Vis spectra of GEN in various solvents. Only part of GEN spectrum in octanol is shown (dashed line) due to high absorption of octanol below 290 nm

In o/w partition experiments, 60 mM solutions of GEN in octanol was prepared. Several partition experiments were performed by equilibration of 10 ml GEN in octanol against 10 ml of water, 0.21 mM G3^{gl} (aq), 0.63 mM G3^{gl} (aq), 0.63 mM G3^{gl} (aq), 1.91 mM G3^{gl} (aq), and 5.70 mM G3^{gl} (aq) with vigorous magnetic stirring at 25C for 48 hours. Then, the aqueous and octanol phases were separated and concentration of GEN in octanol (l=336 nm, $e_{336}=3.52\times10^3$ mol$^{-1}\times$dm$^3\times$cm^{-1}) and in water (l=255 nm, $e_{255}=3.82\times10^4$ mol$^{-1}\times$dm$^3\times$cm^{-1}) were determined spectrophotometrically. Thus the partition coefficients $\log K_{OW}=\log(c_o/c_w)$ were obtained in function of concentration of G3^{gl}. The results are presented graphically in the Figure 3.

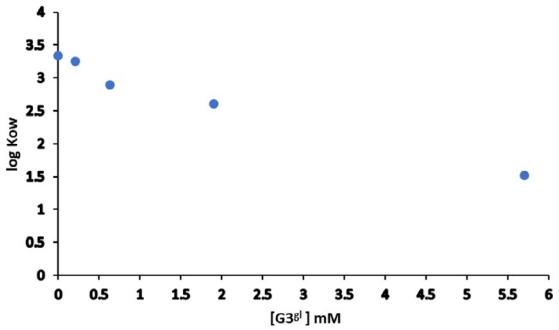

Fig. 3. The values of octanol/water partition coefficient in function of concentration of the host dendrimer G3^{gl} in the water phase

Transdermal permeation of GEN and GEN@G3^{gl} encapsulate

To analyze the ability of GEN to diffuse through skin models, synthetic polyvinyldifluoride (PVDF) and prepared rabbit skin (RS) were studied using hydroxyethylcellulose-based hydrogel as delivery form and a mixture of phosphate-buffered water pH 7.4 (70 %) and ethanol (30 %) as receiving solutions. The ability of GEN (guest) to release from the dendrimer (host) and from hydrogel followed by permeation of skin model was monitored spectrophotometrically at 266 nm (l_{max}, $e_{266}=4.0\times10^4$ mol$^{-1}\times$dm$^3\times$cm^{-1}). The delivery gels were prepared as follows: 1:1 G3^{gl} : GEN solution was prepared by dissolving 0.7386 g G3^{gl} in 4.0 ml water, 17.1 mg GEN in 1.5 mL ethanol and mixing the solutions. The mixture was stirred at 50°C for 2 hours and then cooled to room temperature. The final concentrations of GEN and G3^{gl} were both 11.5 mM. In the meantime, the hydrogel was prepared by addition of 0.75 g hydroxyethylcellulose to phosphate-buffered water (25 mL). After homogenization, 0.5 mL of 11.5 mM GEN or G3^{gl} : GEN solution was added to freshly prepared gel and homogenized rapidly to obtain the delivery system. Typically 0.32 g of delivery sample was mounted topically on membrane of a Franz chamber filled with 6 ml receiving solution below the membrane (PVDF or RS). The initial amount of GEN in the mounted sample was between 16 and 20 μg (ca 75 nmoles) and equimolar amounts of G3^{gl}. The receiving solution (6 mL) was removed in 2 hr time intervals and replaced with new portions. The concentration of GEN in receiving solution was quantified by measurements of absorption at 266 nm. The results are presented as cumulative percentage of released GEN in time for PVDF and RS vs time (vide infra).

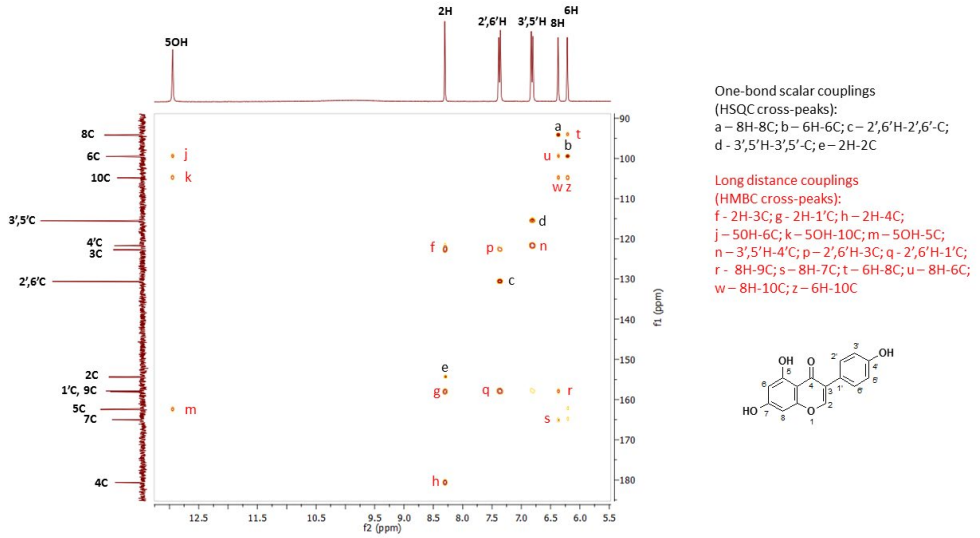

Fig. 4. The 2-D HSQC/HMBC combined map and 1-D spectra of GEN in DMSO-d_6 with spectral assignments. For atom numbering, the semi-structural formula of GEN is shown

Cell cultures

BJ human normal skin fibroblasts, doubling time 1.9 day, and HaCaT human immortalized keratinocytes, doubling time 24 hours, were propagated in a DMEM medium supplemented with 10% FBS, and 100 U/ml penicillin, 100 µg/ml streptomycin. Cells were cultured at 37°C in 5% CO_2 atmosphere and 95% humidity, with growth medium changed every 2–3 days and passaged at 60-80% confluence, using 0.25% trypsin-0.03% ethylenediaminetetraacetic acid in PBS (calcium- and magnesium-free). Cell morphology was checked under the Nikon Eclipse TE2000 inverted microscope (Tokyo, Japan) equipped with phase contrast. The number and viability of cells were estimated by the trypan blue exclusion test, with Automatic Cell Counter TC10™ (Bio-Rad Laboratories, Hercules, CA, USA).

Cytotoxicity of GEN, G3^{gl} and GEN@G3^{gl} encapsulate

Alamar Blue assay

BJ or HaCaT cells were seeded in flat-bottom 96-well culture plates at a density of 1×10^4 cells/well (200 µL cell suspension/well) and allowed to attach for 24 hours. After culture medium removal, GEN, G3^{gl} and GEN@G3^{gl} in the concentration range 1–100 µM were added (100 µL/well) and incubated for 24 hours. Then, tested drugs solutions (GEN, G3^{gl} and GEN@G3^{gl}) were aspirated and 60 µM resazurin solution was added (100 µL/well). The plates GEN@G3^{gl} were incubated at 37°C for 2 hours. After that, the fluorescence was measured at λ=570 nm with a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). The control sample were cells (BJ and HaCaT separately) grown in DMEM medium without addition of tested samples, for which viability was assumed to be 100%. An experiment was performed in three independent assays in triplicate.

Toxicity against Caenorhabditis elegans

The *C. elegans* nematode wild-type culture (strain N2, variety Bristol) was used to estimate the *in vivo* activity of the G3^{gl} and GEN@G3^{gl} as described earlier.[12] After the transfer of nematodes to a 96-well plate (about 20 individuals in 50 µL), the solutions of the studied compounds in a complete medium were added (50 µL/well). The maximal DMSO final concentration was equal to 0.2% and had no significant influence on nematode viability. Five replicates were made for each concentration. The plate was incubated at 21 °C for seven days. During this time, live and dead worms were counted under an inverted microscope (Delta Optical IB-100).

Statistical analysis

To estimate the differences between treated samples and non-treated controls for the cell culture assays, a nonparametric Kruskal–Wallis test was performed because the data did not show signs of a normal distribution. To analyze differences in nematode viability between the control and the nematodes incubated with dendrimer and encapsulate, the Kaplan-Meier estimator was used. Statistically significant differences against thee control were determined with Gehan's Wilcoxon test. The $p<0.05$ was considered statistically significant. Analyses were performed with Statistica 13.3 software (StatSoft, Tulsa, OK, USA).

Results and discussion

Stoichiometry of host-guest encapsulates

Genistein (GEN) was characterized by the 1-D ^1H, ^{13}C, and heteronuclear HSQC, and HMBC NMR spectroscopy. 2-D NMR measurements enabled us to unambiguously assign the ^1H and ^{13}C resonances (Fig. 4).

Then the G3^{gl} dendrimer in D_2O was titrated with GEN in order to determine the ability of the host to encapsulate the guest. The starting spectrum of G3^{gl} in D_2O

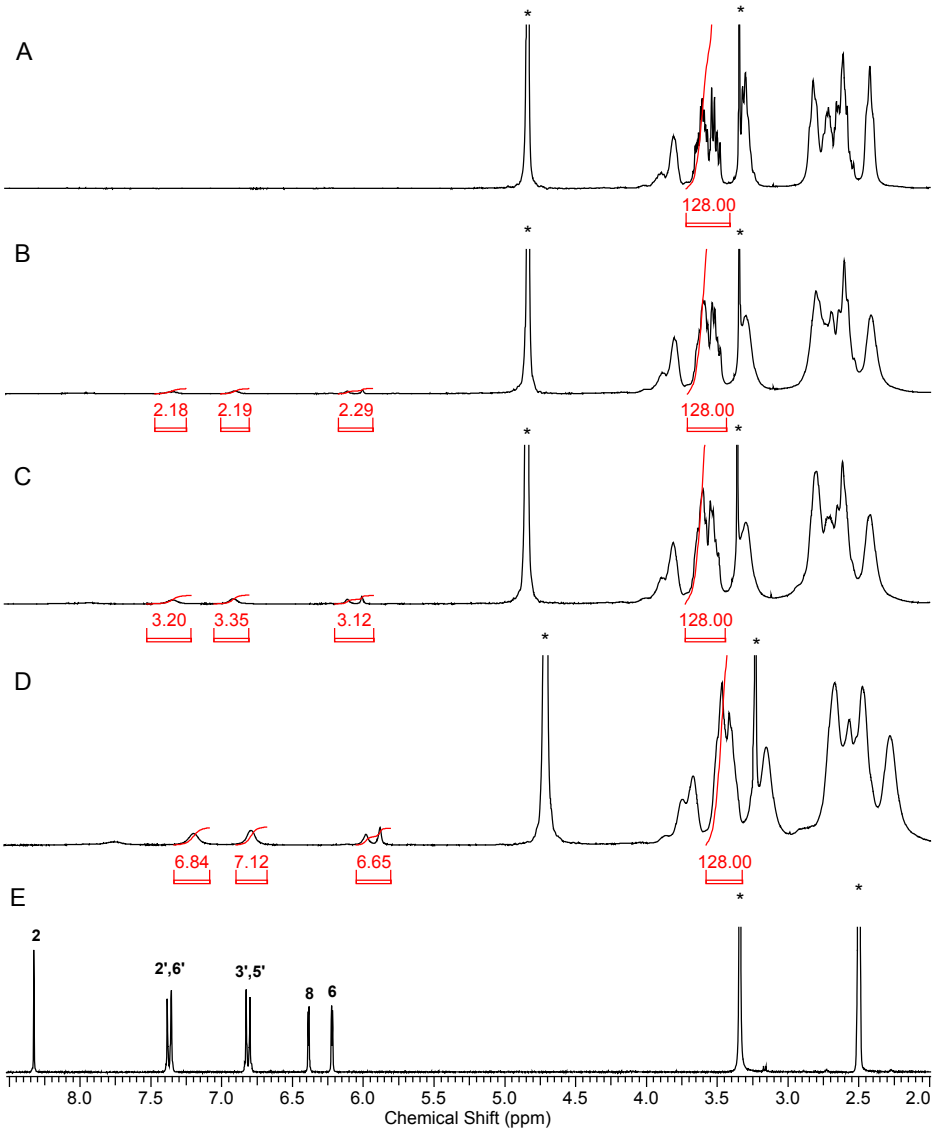

Fig. 5. ^{1}H NMR titration of G3^{gl} (in D_2O) with GEN (in DMSO-d_6). A – spectrum of G3^{gl} in D_2O (ca 9 mM concentration); B – D: spectra of solution containing G3^{gl} and 1.1, 1.6, and 3.4 equivalents of GEN; E – spectrum of GEN in DMSO-d_6. The residual resonances of HDO, DMSO-d_5, and methanol are labeled with asterisks. The [128H] integral intensity signal of 2,3-dihydroxypropyl substituents (64 residues) centered at 3.5 ppm were used as an internal reference

(9 mM) is presented at Figure 5A. To this, a solution GEN in DMSO-d_6 (125 mM) was added stepwise until no precipitate was formed. The ^{1}H NMR spectra of the solutions are presented at Figure 5, traces B-D. We found that the maximum concentration of GEN in the mixture was 31 mM (Fig. 5D); above this, the GEN (or GEN@G3^{gl} encapsulate) precipitated upon addition to the aqueous solution. Thus, we concluded that solubility of GEN in water increased from 4.8 μM to 31×10^3 μM concentration in presence of G3^{gl} host, i.e. four orders of magnitude. Furthermore, the molar stoichiometry of the GEN@G3^{gl} encapsulate was finally at least 3.5:1 GEN:G3^{gl} (Fig. 5D).

Skin permeation

GEN is known to be highly hydrophobic. We measured the $K_{O/W}$ parameter using standard procedure of partition between octanol and water.[13] We obtained $\log K_{O/W}$=3.34, which was close to the value of 3.04 determined before.[13] Then we determined the $\log K_{O/W}$ in presence of various concentrations of the G3^{gl} host in the water phase (Fig. 3). We found that $K_{O/W}$ decreased almost two orders of magnitude in presence of 5.7 mM G3^{gl} in water phase, namely from 2157 to 33.7 (corresponding to $\log K_{O/W}$ 3.34. and 1.53, respectively). We have assumed that the highly hydrophobic G3^{gl} host was absent in the octanol phase, which is reasonable considering that $\log K_{O/W}$=-2.24 in case of PAMAM G3 dendrimer,[14] indicating that solubility of that hydrophilic dendrimer in water is 5754 times higher than in octanol. From partition experiments, we conclude that GEN@G3^{gl} encapsulate loses the guest GEN molecule, which is promising in terms of biological conditions

which might be met when GEN@G3^{gl} in water is delivered into tissue.

Considering the determined stoichiometry achievable in aqueous environment (at least 3 GEN per 1 G3^{gl} host) we obtained the encapsulate 1:1 GEN:G3^{gl} and tested this as DDS in skin permeation experiments, as well as toxicity against human cell lines and *C. elegans*.

Skin permeation of GEN was tested using hydroxyethylcellulose hydrogel as a donor, polyvinyldifluoride (PVDF) and freshly prepared rabbit skin (RS) membranes as skin models, and phosphate buffered water/ethanol receiving solutions. The amount of transferred GEN from donor into receiver was determined spectrophotometrically within 48 hours (Fig. 6 and 7). The cumulative amount of GEN permeated through PVDF indicated 3-fold enhancement of GEN release from encapsulate GEN@G3^{gl} donor in comparison with GEN alone in donor. The time of 10% GEN transfer, $t_{0.1}$ was used as comparative parameter. Thus the estimated $t_{0.1}$ was 3.2 ± 0.2 hour in case of GEN@G3^{gl}, while 6.50.2 hour in case of GEN alone (Fig. 6). The values of $t_{0.1}$ for GEN@G3^{gl} were shorter than those obtained in similar delivery system for 8-methoxypsoralen in PAMAM G3 and G4 dendrimers, which were 6.5 and 9.0 hours,[9] respectively as well as riboflavin in PAMAM G3,5 dendrimer, which was determined as 6.5 hours.[8] The release of GEN from both hydrogel and from GEN@G3^{gl} in hydrogel was relatively fast; the control experiment after 24 hours indicated that permeation was completed within the first 12 hours.

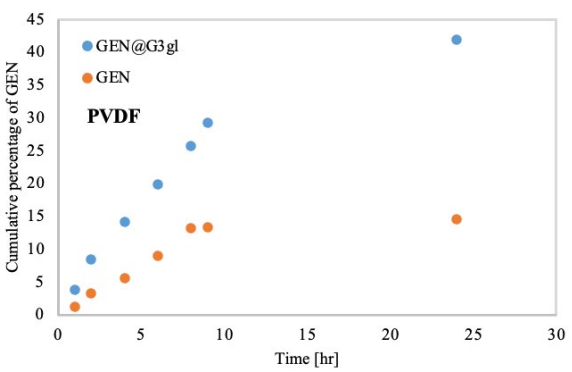

Fig. 6. The permeation of GEN from hydrogel containing GEN and GEN@G3^{gl} encapsulate through PVDF. Standard deviation for 5 times repeated experiments was not higher than 1.5%

In case of permeation through rabbit skin, the release of GEN from hydrogel and GEN@G3^{gl} was stopped after 32 hours of experiments (Fig. 7). No statistically relevant enhancement of GEN release from GEN@G3^{gl} versus GEN alone in the hydrogel donor was found, although the release of GEN from encapsulate was ca 1% faster than from GEN in hydrogel within first 30 hours. Because no 10% of transfer was achieved in either case, the time of 3% transfer was chosen for comparison with other results, $t_{3\%}$. Thus the $t_{3\%}$=7.5±0.2 hour was determined for GEN release from GEN@G3^{gl}, while it was 12.0±0.2 hour for GEN alone. For comparison $t_{3\%}$=1.5±0.1 hours was determined for riboflavin release from riboflavin – PAMAM G3 encapsulate in o/w emulsion through porcine skin membrane and 7.4±0.1 hour for 8-methoxypsoralen in PAMAM G3 in an analogous experiment.[8,9] Generally, only a slight increase of rabbit skin permeation was observed in case of GEN@G3^{gl} encapsulate in comparison with GEN alone. Presumably the reason is highly hydrophobic conditions within the skin, promoting GEN release already in the rabbit skin tissue. The kinetics of GEN permeation is thus limited by diffusion of GEN though RS.

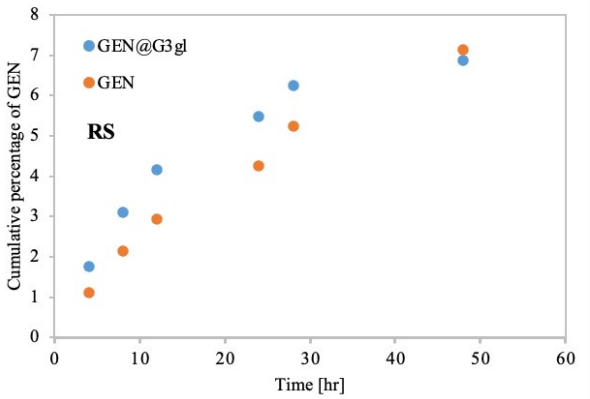

Fig. 7. The permeation of GEN from hydrogel containing GEN and GEN@G3^{gl} encapsulate through rabbit skin. Standard deviation for 5 times repeated experiments was not higher than 0.3%

Kitagawa et al. used w/o microemulsions prepared from isopropyl myristate (oil phase), 150 mM NaCl(aq), ethanol and Tween 80 surfactant to enhance the solubility of GEN and other isoflavones.[15] Using various microemulsions, they were able to enhance the GEN dispersion 1300–2000 times. The microemulsions were then used as GEN delivery system to accumulate GEN in guinea pig dorsal skin and Yukatan micropig skin. The amount of accumulated GEN increased 25-59 times in comparison with GEN in a NaCl(aq) delivery experiment. Accumulated GEN in skin significantly inhibited lipid peroxidation *in vitro* dose-dependently. Furthermore, pretreatment of guinea pig dorsal skin with GEN containing microemulsions prevented UV irradiation-induced erythema formation. As described here, solubilization effects for GEN using G3^{gl} is more efficient than the aforementioned microemulsion dispersion.

***In vitro* toxicity of GEN, G3^{gl} and GEN@G3^{gl}**

In order to estimate the biological properties of GEN, G3^{gl} and GEN@G3^{gl} encapsulates, we performed the toxicity Alamar Blue assay on BJ normal human fibro-

blasts and immortalized human keratinocytes (HaCaT cell line). We determined that neither GEN, nor G3gl or GEN@G3^{gl} exerted any toxic effect in range of 1–100 μM concentration (Fig. 8).

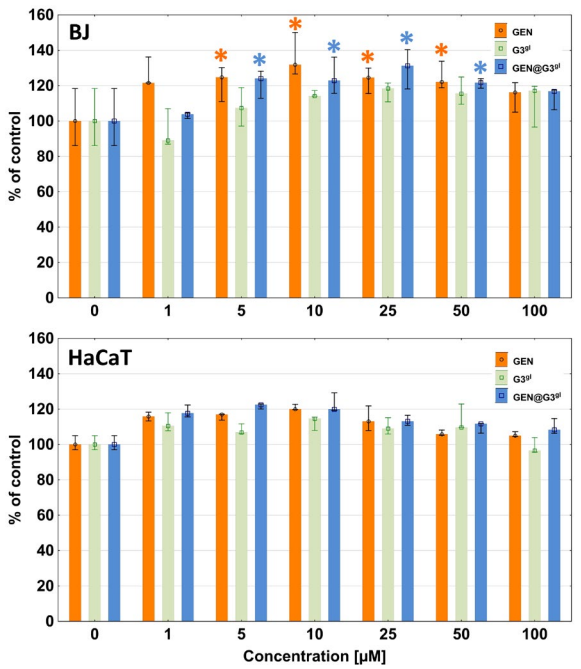

Fig. 8. Biological activity of GEN, G3^{gl}, and GEN@G3^{gl} against normal human BJ fibroblasts and immortalized HaCaT keratinocytes after 24 hours incubation. Results are presented as medians (percentage of non-treated control). Whiskers indicate the lower (25%) and upper (75%) quartile ranges. * p≤0.05, Kruskal–Wallis test (against non-treated control)

Moreover, a slight increase of cell viability over the entire range of used concentrations (1–100 μM) was noted, which could be related to the trophic effect of the substances used. Obtained results were consistent with others, where GEN did not influence on HaCaT cell viability up to 100 μM concentration after 24 h incubation (MTT assay or Sulforhodamine B (SRB) assay).[16,17] Also Pawlicka et al. indicated that genistein was not toxic up to 150 μM concentration against BJ fibroblasts, and observed that genistein at lower concentrations (10–100 μM) stimulated fibroblast growth (MTT assay, 24 h incubation).[18]

It is worth mentioning that increases of cell viability under the influence of GEN@G3^{gl} was probably an effect of presence of GEN in encapsulate, since viability after incubation with GEN and GEN@G3^{gl} was always higher than G3^{gl} alone at appropriate concentrations (Fig. 8).

In vivo toxicity against C. elegans

Due to previously reported anti-nematode activity of GEN, we have tested the GEN@G3^{gl} biological effect on *C. elegans* in comparison with G3^{gl}.[2,3] We found that after 7 days incubation, glycidylated G3 PAMAM dendrimer did not influence on *C. elegans* lifetime (Fig. 9).

A similar pattern was observed for GEN@G3^{gl} up to 25 μM concentration. At 50 μM concentration, viability of nematodes decreased significantly, achieving a value of 63% after 7 days incubation. It was interesting that 100 μM concentration of both G3^{gl} and GEN@G3^{gl} had a slighter effect than 50 μM concentration. In summary, at a 50 μM concentration, the genistein delivered as GEN@G3^{gl} encapsulate induced an anthelmintic effect.

Conclusion

Third generation polyamidoamine dendrimers modified by exhausting glycidylation, act as a solubilizer for the highly hydrophobic isoflavonoid genistein. The host dendrimer macromolecule is able to encapsulate more than 3 molecules of guest genistein. Water solubility of genistein increased about 27 times in presence of the host. The 1:1 genistein : dendrimer encapsulate promotes skin permeation of genistein. It was non-toxic against normal BJ fibroblast and immortalized HaCaT keratinocyte human cells. The 1:1 genistein : dendrimer

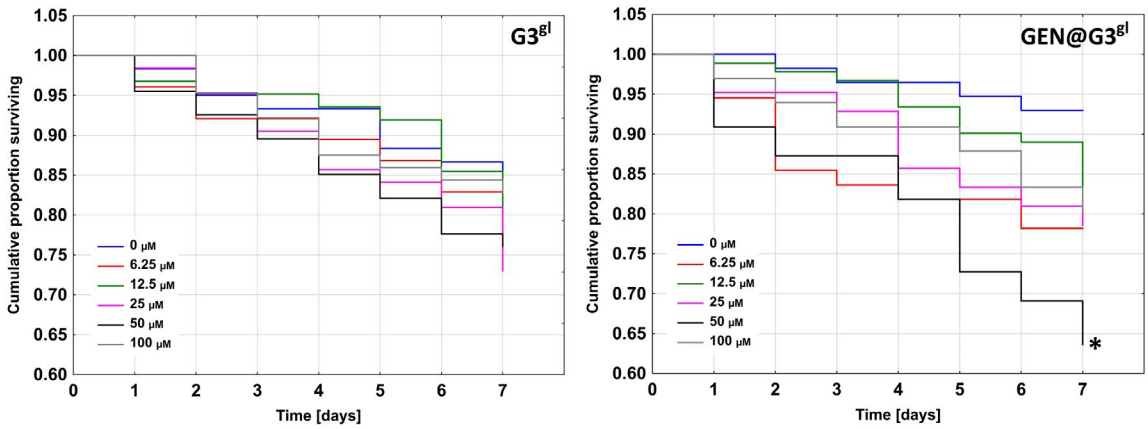

Fig. 9. The Kaplan–Meier survival curves of *C. elegans* after 7 days of incubation with G3^{gl} and GEN@G3^{gl}. Results are presented as cumulative proportion surviving. Statistically significant differences against DMSO-treated control (0.2%) obtained by Gehan's Wilcoxon test are marked with asterisks * (p≤0.05) in the colors corresponding to the tested concentrations

encapsulate indicated anthelmintic action at 50 μM concentration against *C. elegans*.

Declarations

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Author contributions
Conceptualization, A.F-R. and S.W.; Methodology, S.W. and Ł.U.; Software, Ł.U.; Validation, S.W., Ł.U. and A.F-P.; Formal Analysis, S.W.; Investigation, J.D., A.F-R, M.Z-D, and S.W.; Resources, Ł.U.; Data Curation, Ł.U.; Writing – Original Draft Preparation, Ł.U., S.W., and A.F-R; Writing – Review & Editing, S.W.; Visualization, S.W. and Ł.U.; Supervision, S.W.; Project Administration, S.W.; Funding Acquisition, S.W.

Conflicts of interest
Authors declare no conflict of interest.

Data availability
Raw data are available from corresponding author on reader demand.

Ethics approval
There are no ethic approvals needed for experimental work presented.

References

1. Franke AA, Lai JF, Halm BM. Absorption, distribution, metabolism, and excretion of isoflavonoids after soy intake *Arch Biochem Biophys*. 2014;559:24-28. doi: 10.1016/j.abb.2014.06.007
2. Ma Y, Yuan R, Sikandar A, Zhu X, Duan Y, Wang Y. Genistein and Daidzein effects on the physiological indices of Soybean Cyst Nematodes. *Sci Agric*. 2022;79:20200298. DOI: doi: 10.1590/1678-992X-2020-0298
3. Gutierrez-Zepeda A, Santell R, Wu Z, et al. Soy isoflavone glycitein protects against beta amyloid-induced toxicity and oxidative stress in transgenic *Caenorhabditis elegans. BMC Neuroscience.* 2005;6:54. doi: 10.1186/1471-2202-6-54
4. Papaj K, Rusin A, Szeja W, Grynkiewicz G. Absorption and metabolism of biologically active genistein derivatives in colon carcinoma cell line (Caco-2). *Acta Pol Pharm*. 2014;71:1037-1044.
5. Shen H, He D, Wang S, Ding P, Wang J, Ju J. Preparation, characterization, and pharmacokinetics study of a novel genistein-loaded mixed micelles system. *Drug Dev Ind Pharm*. 2018;44:1536-1542. doi: 10.1080/03639045.2018.1483384
6. Nan G, Shi J, Huang Y, et al. Dissociation Constants and Solubilities of Daidzein and Genistein in Different Solvents. *J Chem Eng Data.* 2014;59:1304-1311. doi: 10.1021/je4010905
7. Jaiswal N, Akhtar J, Singh SP, Badruddeen Ahsan F. An Overview on Genistein and its Various Formulations. *Drug Res*. 2018;69:305-313. doi: 10.1055/a-0797-3657
8. Filipowicz A, Wołowiec S. Solubility and in vitro transdermal diffusion of riboflavin assisted by PAMAM dendrimers. *Int J Pharm.* 2011;408:152-156. doi: 10.1016/j.ijpharm.2011.01.033
9. Borowska K, Laskowska B, Magoń A, Myśliwiec B, Pyda M, Wołowiec S. PAMAM dendrimers as solubilizers and hosts for 8-methoxypsoralene enabling transdermal diffusion of the guest. *Int J Pharm.* 2010;398:185-189. doi: 10.1016/j.ijpharm.2010.07.019
10. Malinga-Drozd M, Uram Ł, Wróbel K, Wołowiec S. Chiral recognition of homochiral poly(amidoamine) dendrimers substituted with R- and S-glycidol by keratinocyte (HaCaT) and squamous carcinoma (SCC-15) cells in vitro. *Polymers*. 2021;13:1049. doi: 10.3390/polym13071049
11. Tomalia D, Baker H, Dewald J, et al. A new Class of Polymers: Starburst-Dendritic Macromolecules. *Polym J*. 1985;17:117-132. doi: 10.1295/polymj.17.117
12. Uram Ł, Wróbel K, Walczak M, et al. Exploring the Potential of Lapatinib, Fulvestrant, and Paclitaxel Conjugated with Glycidylated PAMAM G4 Dendrimers for Cancer and Parasite Treatment. *Molecules*. 2023;28:6334. doi: 10.3390/molecules28176334
13. Rothwell JA, Day AJ, Morgan MRA. Experimental Determination of Octanol-Water Partition Coefficients of Quercetin and Related Flavonoids. *J Agric Food Chem*. 2005;53:4355-4360. doi: 10.1021/jf0483669
14. Dugiri J, Diallo MS, Goddard III WA, Dalleska N, Fang X, Tang Y. Partitioning of Poly(amidoamine)Dendrimers between *n*-Octanol and Water. *Environ Sci Technol.* 2009;43:5123-5129. doi: 10.1021/es9003747
15. Kitagawa S, Inoue K, Teraoka R, Morita S. Enhanced Skin Delivery of Genistein and Other Two Isoflavones by Microemulsion and Prevention against UV Irradiation-Induced Erythema Formation. *Chem Pharm Bull.* 2010;58:398-401. doi: 10.1248/cpb.58.398
16. Smolińska E, Moskot M, Jakóbkiewicz-Banecka J, et al. Molecular action of isoflavone genistein in the human epithelial cell line HaCaT. *PLoS ONE*. 2018;13(2):0192297. doi: 10.1371/journal.pone.0192297
17. Rendón JP, Cañas AI, Correa E, et al. Evaluation of the effects of genistein in vitro as a chemopreventive agent for colorectal cancer-strategy to improve its efficiency when administered orally. *Molecules*. 2022;27(20):7042. doi: 10.3390/molecules27207042
18. Pawlicka MA, Zmorzyński S, Popek-Marciniec S, et al. The effects of genistein at different concentrations on MCF-7 breast cancer cells and BJ dermal fibroblasts. *Int J Mol Sci*. 2022;23(20):12360. doi: 10.3390/ijms232012360

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ORIGINAL PAPER

Effectiveness of health education on knowledge, attitude and practice regarding junk food consumption among interns in a tertiary health care center in Chennai, India – a quasi-experimental study

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ABSTRACT

Introduction and aim. Junk foods are defined as any of various pre-packaged foods that are high in calories but low in nutritional value. This study was done to evaluate the effect of health education intervention on the knowledge, attitude, and practices regarding junk food consumption among MBBS interns at the tertiary health care center in Chennai, India.

Material and methods. This was a quasi-experimental study conducted from January to March 2023 among 105 CRMIs of a Government medical college in Chennai, India. After getting Institutional Ethics Committee approval, data was collected using a pretested self-administered semi structured questionnaire before and after health education intervention.

Results. The majority (55.2%) of the respondent interns were hostellers. There was a significant increase in the knowledge ($p<0.001$), healthy attitude ($p<0.001$), and healthy practice ($p<0.001$) concerning junk food intake following health education intervention.

Conclusion. Routine repeated sessions promoting health should be implemented in the medical curriculum in order for our future physicians to live a long healthy life as well as to set a good example

Keywords. attitude, junk foods, knowledge, MBBS interns, practice

Introduction

Public health interventions aimed at improving health often involve promoting healthy lifestyle by addressing the behavioral risk factors in the general population. Giving advice and educating the patient is viewed as a professional responsibility by all general practitioners and are expected by the patients.[1] It has been observed that if physicians do not engage in these healthy behaviors, they are less likely to encourage such behaviors in their patients.[2] Junk foods are defined as any of various pre-packaged snack foods that are high in calories but low in nutritive value. The term 'Junk food' was coined by Michael Jacobson, director of the Center for Science in 1972 in the public interest who wanted to raise pub-

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Mary B, Murugan A, Ramasubramanian R, Kamali R, Ragavan MUV, Sathishkumar K. Effectiveness of health education on knowledge, attitude and practice regarding junk food consumption among interns in a tertiary health care center in Chennai, India – a quasi-experimental study. *Eur J Clin Exp Med.* 2024;22(2):300–305. doi: 10.15584/ejcem.2024.2.9.

lic attention about the issue of foods with a high caloric value and a low nutritional value. Junk food contains high level of refined sugar, white flour, trans fat, polyunsaturated fat, salt, and numerous food additives such as monosodium glutamate (MSG) and tartrazine, and lacking in protein, vitamin, and fiber. Junk food is popular because of their simplicity of manufacture, consumption, their taste and they have a long shelf life which may not require refrigeration.[3,4] The prevalence of overweight has risen in India from 2 to 17.1%.[5] The fundamental cause of obesity is an energy imbalance between calories consumed and calories expended. Excessive intake of junk food, lesser intake of fruits and vegetables and lack of regular physical exercise are the major causes for obesity and overweight. Junk food is a quick unhealthy food, which is easy to be made and to be consumed. It lacks nutritional value and is a caloric dense. Junk food is rich in refined sugar, white flour, polyunsaturated fats, salts, and numerous food additives, but low in protein, vitamins and fibers.[6] Junk food cause cerebrovascular accidents, systemic hypertension, type II diabetes mellitus, angina, myocardial infraction, raised LDL cholesterol levels, and colon cancer. It also leads to weight gain and obesity.[7] University life creates a different environment for students with little time for meal preparation, planning, and eating. Students may have the feeling of independence, with preference for eating whenever they want.[8] Youth consume junk food meals more than older people.[9,10] Taste, time constrains, and costs are considered the main predisposing factors that make medical students consume junk food.[11]

Aim

Given the significant potential for negative outcomes to a physician's own health as well as the health and safety of their patients, examination of the acculturation process about the development of related health-promoting/risking lifestyle patterns over the continuum of medical training is critical to the improvement of the health care delivery system. Hence, this study was done to evaluate the effect of health education intervention on the knowledge attitude and practices regarding junk food consumption among MBBS interns at tertiary care center in Chennai, India.

Material and methods

This was a quasi-experimental (before and after intervention) study conducted among all the 105 interns of both genders at a tertiary care hospital in Chennai, India from January to March 2023 using a self-administered semi-structured questionnaire. After getting approval from the Institute Ethical Committee, (IEC approval No: 68/IEC/GOMC/2022) data collection was started. The nature and purpose of the study was explained to the interns and a written informed consent was obtained, confidentiality was ensured and data was collected using a self-administered semi-structured questionnaire.

Study procedure

A questionnaire was prepared after extensive review of the literature by database searches from Google Scholar, Medline (PubMed), Academic search Complete (EBSCO host) and Medline (EBSCOhost), and Cochrane online library. These were meticulously searched for quality research literature published in English from 2001–2022 using the terms junk food, medical students, knowledge, attitude and practices, and health education. The 4-section semi structured questionnaire with a set of sociodemographic questions, 10 knowledge measuring questions, 8 attitude measuring questions and 3 practices assessing questions were used. A knowledge component dealt with an in-depth understanding of a medical intern about junk food, its composition, and its ill effects. Whereas the attitude component investigated how junk foods were perceived in an intern's point of view. The practices section assessed the frequency, energy consumption in kilocalories by dietary recall method and amount of money spend on junk food in one week. The questionnaire was pretested on 10 interns who were also part of the study sample.

Data collection and analysis

The study was conducted in two phases. Baseline data on knowledge attitude and practices regarding junk food consumption among interns were collected using a pretested semi-structured questionnaire. All the interns were assembled into three groups over a period of one week. On completion of the questionnaire, health education regarding junk food consumptions its ill effects, healthy alternatives were given in a lecture session which lasted for two hours. Health education IEC material was also distributed via online platforms. Post intervention data was collected using the same semi structured questionnaire two months after the intervention and was compared with the baseline data.

About 105 interns were given semi structured questionnaires. The data was analyzed according to the scoring pattern. Based on scores allotted to each question in the pre and post intervention questionnaire, total scoring for each component and total score of pre and post intervention responses were calculated. This EXCEL was fed into IBM SPSS software Version22 for data analysis (Armonk, NY, USA). Total scores after both pre and post intervention was compared using Wilcoxon Signed Rank Test and percentage of increase in score was calculated for everyone.

Scoring pattern of questionnaire

– the knowledge component comprises of: 10 multiple choice questions with single correct answers. Each question carries 1 mark with no negative marks,

- the attitude component comprises of 8 questions. They were multiple choice questions with options in the form of Likert scale as Strongly disagree, disagree, neutral, agree and strongly agree. They were scored as 5,4,3,2,1 marks according to healthy practice to unhealthy practice respectively,
- the practice component had 3 MCQS with single option with options in the form of Likert scale graded 4,3,2,1 according to healthy practice to unhealthy practice respectively.

Results

A total of 105 interns participated in this study. Table 1 shows the sociodemographic characteristics of the respondents. Majority (84.8%) of them are in the age range of 22-25 years, followed by (14.3%) in age range of more than 25 years. Majority of the interns were female (51.5%) with 64.8% of participants residing in urban areas. About 44.8% were hostellers.

Table 1. Socio-demographic characteristics of the participants

Variable	Categories	Frequency	Percent
Age in years	18 to <22	1	0.9
	22 to <25	89	84.8
	≥25 years	15	14.3
Gender	Male	52	49.5
	Female	54	51.5
Residence	Rural	37	35.2
	Urban	68	64.8
Current place of stay	Day scholar	47	44.8
	Hostel	58	55.2

Table 2. Comparison of scores before and after intervention*

	Change in scores		
Component	Pre-Intervention median score	Post-Intervention median score	p*
Knowledge	7 (5–8)	9 (9–10)	<0.001
Attitude	16 (14–17)	23 (22–25)	<0.001
Practice	7 (6–9)	11 (10–11)	<0.001

* An analysis was done by Wilcoxon Signed rank test

Table 2 shows the comparison of knowledge, attitude and practice scores before and after intervention. There was a significant (p<0.001) increase in the median knowledge score after intervention. There was a drastic change in median score of attitudes towards junk food intake from 16 to 23 which was also found to be statistically significant (p<0.001). The healthy practice of avoiding junk food also improved as there was a significant increase (p<0.001) in the practice score. Overall score also improved significantly (p<0.001) following health education intervention.

Figures 1 to 6 give a schematic representation of the change in knowledge, attitude and practice scores of junk food intake by the interns before and after health education.

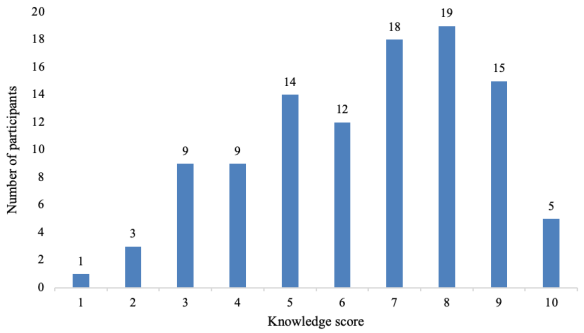

Fig. 1. Pre-test knowledge scores

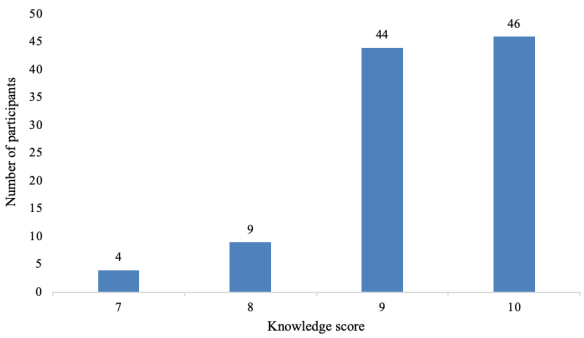

Fig. 2. Post-test knowledge scores

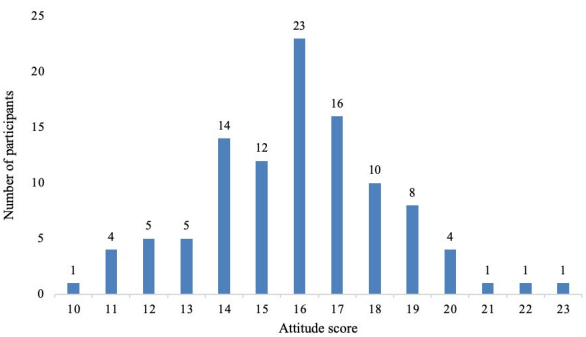

Fig. 3. Pre-test attitude scores

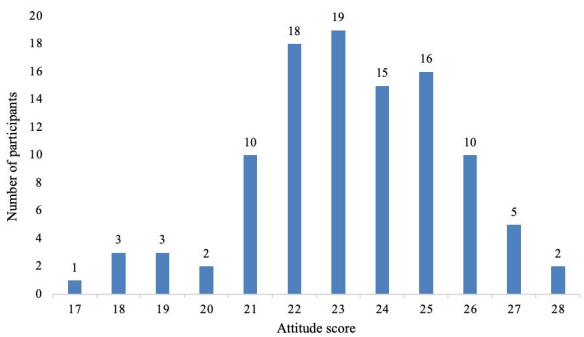

Fig. 4. Post-test attitude scores

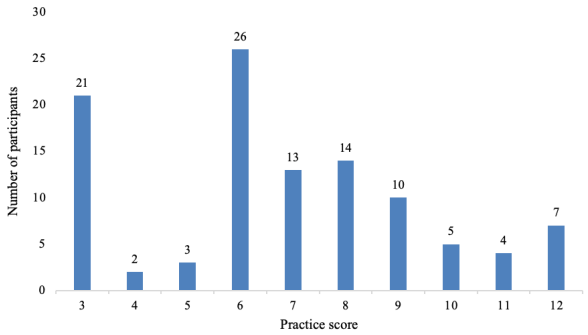

Fig. 5. Pre-test practice scores

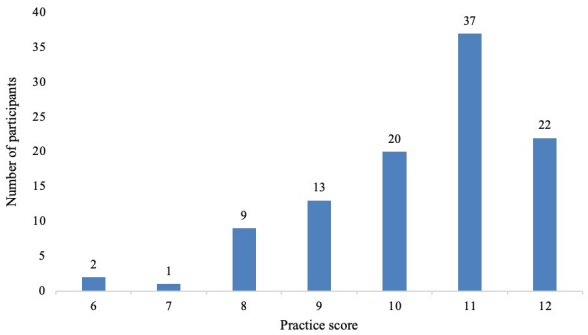

Fig. 6. Post-test practice scores

Discussion

This study showed that there was a significant increase in knowledge, healthy attitude and healthy practice towards junk food intake. This finding was also replicated in previous studies. In a study done among higher secondary school students in Himachal Pradesh, mean post-test attitude score 75.40 was significantly higher than the mean pre-test attitude score 64.18. The knowledge and attitude among students were significantly improved in the study.[12] The results of our investigation were consistent with those of related research carried out by Yadav B et al., Amoldeep et al., and Mishra R et al. among college going students.[13-16] Several studies conducted among school students by Poor Abdollahi et al., Vakili et al., Choobineh et al., Mazloomi et al., and Hosseini et al., Singh UK et al., Hajivandi L et al. also give similar findings.[17-23] These findings demonstrated a significant increase in knowledge, attitude, and performance regarding the consumption of junk food following the intervention; this finding points to the beneficial effects of education on enhancing knowledge, attitude, and performance in reducing junk food intake. The results of Pour Abdollahi and Vakili et al. support similar findings; however, the latter study's finding that the participant's performance score increased to a non-significant degree differs from the current investigation.[17,18] Additionally, research by Chobineh et al. supported the impact of education on students' performance and understanding in the area of eating.[19] These outcomes are also consistent with the findings of a 2003 study conducted by Hoffman et al. on 70 male and female guidance school students over the course of five weeks in an effort to increase fruit and vegetable consumption.[24] In order to improve children's knowledge and attitudes about fruits and vegetables, Heidari et al. used education on diet in their study.[25] They did this by employing educational resources such as newsletters designed specifically for children, parents, and teachers. The participant's knowledge and attitude toward fruit intake significantly improved nine months after the intervention, according to the researchers. These variations can be attributed to different study populations our being medical community already well educated regarding the junk food consumption, settings, designs, differences in the tools used to measure the KAP and methods of data collection.[26,27] Moreover, differences in the performance of health systems in different countries could also explain the differences. There is a dramatic transformation in an intern's dietary patterns, most notably an increase in consumption of processed foods such as hamburgers, cheeseburgers, deep-fried chicken, french fries, pizza, donuts, carbonated drinks etc. Several studies have shown that adolescents staying away from home are associated with increased consumption of fast food with high calorie intake, poorer diet quality, finally ending up in weight gain. With day- by-day increase in the number of fast-food outlets, and online food delivery apps and above all peer pressure our future medical practitioners are themselves on the path of metabolic syndrome. High salt content foods can be acting as addictive substances that stimulate dopamine receptors in brain, leading to increase in craving and hunger.[28,29] It leads to increase in appetite, calorie consumption, overeating, obesity, and related illnesses. Many a times despite being medicos, they are not aware about the high calorie content of such items since it is not easily accessible in streetside fast-food outlets. In addition to increased genetic and environmental susceptibility to diabetes mellitus, hypertension, coronary heart diseases, poor eating habits seals one's fate.

Study limitations

There are some limitations in our study. The duration of the intervention was short. The study was limited to a single set-up and hence may not be representative of all the MBBS interns in the state or the country. Moreover, the study was done without a control group and hence the placebo effect could not be ascertained.

Conclusion

This study revealed that there was a significant improvement in the attitude knowledge and practices among regarding health hazards of junk food after implementation structured teaching program. Hence routine repeated health promoting sessions should be implemented in medical curriculum in order for our future physicians to live a long healthy life as well as set a good example.

Declarations

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Author contributions

Conceptualization, B.M., A.M., R.R., R.K., M.U.V.R. and K.S.; Methodology, B.M. and A.M.; Software, B.M. and K.S.; Validation, B.M., A.M. and R.K.; Formal Analysis, B.M. and M.U.V.R.; Investigation, B.M. and R.R.; Resources, B.M. and A.M.; Data Curation, B.M., A.M. and K.S.; Writing – Original Draft Preparation, B.M., A.M. and K.S.; Writing – Review & Editing, B.M. and K.S.; Visualization, R.R.; Supervision, R.K., M.U.V.R. and K.S.; Project Administration, A.M. and R.R.;

Conflicts of interest

The author declares no conflicts of interest.

Data availability

Data will be made available on request.

Ethics approval

Ethical approval obtained from the Institutional Ethics Committee, Government Medical College, Omandurar Government Estate, Chennai (IEC approval No: 68/IEC/GOMC/2022).

References

1. Ampt AJ, Amoroso C, Harris MF, McKenzie SH, Rose VK, Taggart JR. Attitudes, norms and controls influencing lifestyle risk factor management in general practice. *BMC Fam Pract.* 2009;10:59. doi: 10.1186/1471-2296-10-59
2. Frank E, Rothenberg R, Lewis C, Belodoff BF. Correlates of physicians' prevention-related practices. Findings from the Women Physicians' Health Study. *Arch Fam Med*. 2000;9(4):359-367. doi: 10.1001/archfami.9.4.359
3. Frank E, Breyan J, Elon L. Physician disclosure of healthy personal behaviors improves credibility and ability to motivate. *Arch Fam Med*. 2000;9(3):287-290. doi: 10.1001/archfami.9.3.287
4. Junk food. English meaning - Cambridge Dictionary. Cambridge Dictionary. http://www.dictionary.cambridge.org/dictionary/english/junk-food. Accessed September 29, 2023.
5. Unnikrishnan AG, Kalra S, Garg MK. Preventing obesity in India: Weighing the options. *Indian J Endocrinol Metab*. 2012;16(1):4-6. doi: 10.4103/2230-8210.91174
6. Cizza G, Rother KI. Beyond fast food and slow motion: weighty contributors to the obesity epidemic. *J Endocrinol Invest*. 2012;35(2):236-242. doi: 10.3275/8182.
7. Zhu SP, Ding YJ, Lu XF, et al. Study on factors related to top 10 junk food consumption at 8 to 16 years of age, in Haidian District of Beijing. *Zhonghua Liu Xing Bing Xue Za Zhi*. 2008;29(8):757-762.
8. Stockton S, Baker D. College Students' Perceptions of Fast Food Restaurant Menu Items on Health. *American Journal of Health Education*. 2013;44(2):74-80. doi: 10.1080/19325037.2013.764242
9. Nielsen SJ, Siega-Riz AM, Popkin BM. Trends in food locations and sources among adolescents and young adults. *Prev Med*. 2002;35(2):107-113. doi: 10.1006/pmed.2002.1037
10. Paeratakul S, Ferdinand DP, Champagne CM, Ryan DH, Bray GA. Fast-food consumption among US adults and children: dietary and nutrient intake profile. *J Am Diet Assoc*. 2003;103(10):1332-1338. doi: 10.1016/s0002-8223(03)01086-1
11. Driskell JA, Kim YN, Goebel KJ. Few differences found in the typical eating and physical activity habits of lower-level and upper-level university students. *J Am Diet Assoc*. 2005;105(5):798-801. doi: 10.1016/j.jada.2005.02.004
12. Sharma D, Pathania P & Kumari M. A quasi-experimental study to assess the effectiveness of the structured teaching programme on knowledge and attitude regarding health hazards of the junk food among students in selected school of Himachal Pradesh. *World Journal of Advanced Research and Reviews*. 2023;18:673-683. doi: 10.30574/wjarr.2023.18.1.0342
13. Yadav B, Mandeep K. Knowledge Regarding Health Hazards of Junk Food and Its Prevention Among Adolescents. *Journal of Nursing Research, Education and Management*. 2019;1(2):57-64. doi: 10.5281/zenodo.3346467
14. Amoldeep, Kumari P. Effectiveness of planned teaching program on knowledge regarding harmful effects of junk food among adolescents. *Int J Health Sci Res*. 2017;7(5):176-182.
15. Mishra R, Ghosh SS. Effect Of Computer Based Teaching Module On Knowledge Level Regarding Healthy Lifestyle Practices Among Adolescent School Going Girls At Kolkata-A Pre-Experimental Study. *International Journal of creative research thoughts*. 2020;8(2):273-237. doi: 10.13140/RG.2.2.35237.45287
16. Mishra R, Kurian B, Nair D, Singh K, Bawari M, Joseph S. Effect of educational intervention on awareness regarding health hazards of junk food consumption among engineering students. *IP International Journal of Medical Paediatrics and Oncology*. 2021;7:73-78. doi: 10.18231/j.ijmpo.2021.015
17. Pour-Abdollahi P, Zarati M, Razavieh SV, Dastgiri S, Ghaem Maghami SJ, Fathi Azar E. The effect of nutrition education on the knowledge and practice of elementary school children regarding junk food intake. *Zanjan Univ Med Sci J*. 2004;13:13-20.
18. Vakili M, Baghiani-Moghadam MH, Pirzadeh A, Dehghani M. Assessing the effect of education on knowledge, attitude and practice of guidance school students about milk and dairy products. *Knowl Health J*. 2007;2:41-47.
19. Choobineh MA, Hesari SN, Hossain D, Haghighizadeh MH. Study of nutritional knowledge of Ahwaz high school girls and the education effect. *Birjand Univ Med Sci J*. 2009;16:23-30.

20. Mazloomi -Mahmoodabad S, Moein-Taghavi A, Barkhordari A, Alidoosti F. Effect of role modeling through theater show in oral health education. *J Islamic Dent Assoc Iran.* 2009;21:138-142.
21. Hosseini M, Shojaeizadeh D, Chaleshgar M, Pishva H. A study of educational intervention on knowledge, attitude, practice about iron deficiency anemia in female adolescent students. *Gorgan Univ Med Sci J.* 2006;8:37-43.
22. Singh UK, Gautam N, Bhandari TR, Sapkota N. Educational Intervention of Intention Change for Consumption of Junk Food among School Adolescents in Birgunj Metropolitan City, Nepal, Based on Theory of Planned Behaviors. *J Nutr Metab*. 2020;2020:7932324. doi: 10.1155/2020/7932324
23. Hajivandi L, Noroozi M, Mostafavi F, Ekramzadeh M. Assessing the impact of an educational intervention program based on the theory of planned behavior on the nutritional behaviors of adolescents and young adults with PCOS in Iran: a field trial study. *BMC Pediatr*. 2021;21(1):316. doi: 10.1186/s12887-021-02784-z
24. Blom-Hoffman J, Kelleher C, Power TJ, Leff SS. Promoting healthy food consumption among young children: Evaluation of a multi-component nutrition education program. *J Sch Psychol.* 2004;42:45-60.
25. Heydari GH, Moslemi S, Montazeri Far F, Heydari M. Efficacy of education on knowledge, attitude and practice of type II diabetic patients in relation to correct nutrition. *Tabib-e-Shargh, J Zahedan Univ Med Sci Health Serv.* 2003;4:207-213.
26. Smith AF. *Encyclopedia of Junk Food and Fast Food*. Greenwood; 2006:212-231.
27. Rang HP, Dale MM, Ritter JM, Moore PK. *Pharmacology.* 5th Ed.Delhi: Churchill Livingstone; 2006:394-400.
28. Wiles NJ, Northstone K, Emmett P, Lewis G. 'Junk food' diet and childhood behavioural problems: results from the ALSPAC cohort. *Eur J Clin Nutr*. 2009;63(4):491-498. doi: 10.1038/sj.ejcn.1602967
29. Joseph R, Keller C, Adams MA, et al. Print versus a culturally-relevant Facebook and text message delivered intervention to promote physical activity in African American women: a randomized pilot trial. *BMC Womens Health.* 2015;15:30. doi: 10.1186/s12905-015-0186-1

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ORIGINAL PAPER

Relationship between perceived fear of COVID-19 and self-care management in heart failure patients

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ABSTRACT

Introduction and aim. The fear of contracting COVID-19, which affects human health biopsychosocially, is expected to affect the self-care management of patients with heart failure (HF). This study was conducted to investigate the relationship between perceived fear of COVID-19 and self-care management in HF patients.

Material and methods. The study was conducted with 115 HF patients. "Sociodemographic characteristics form", "Fear of COVID-19 tool" and "Nine-item European Heart Failure Self-care Behavior Scale (EHFScB Scale-9)" were used to collect data.

Results. 51.3% of the participants in the study are in the 40–60 age range, 53.7% are male, 92.2% live with their family, 64.3% have a diagnosis of HF for less than 5 years. Levels of COVID-19 fear, and self-care management were found to be high in the participants who are 40 years old or younger, has higher education level, diagnosed within 1 to 3 years, received psychological support during the pandemic process, received training from doctor or nurse about COVID-19. Fear level and self-care levels were moderately positively correlated.

Conclusion. During the COVID-19 pandemic, it is necessary to develop effective care strategies to identify individuals with diseases such as HF who should have high self-care management skills.

Keywords. COVID-19 fear, heart failure, self-care management

Introduction

Heart failure (HF) is a prevalent chronic disease affecting millions worldwide, characterized by a gradual decline in cardiac pumping ability and ventricular function. HF is associated with high mortality and morbidity rates.[1] Decreased ventricular function results in failure of the circulation to meet systemic needs. With the increased workload of the heart, the emergence of symptoms such as dyspnea, chest pain, pulmonary oedema, peripheral oedema, cough, fatigue, and palpitations result in decreased physical mobility, activity intolerance, and limitations in cognitive functions.[1,2] This shows the

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importance of individual disease management in patients with HF.

In December 2019, a novel coronavirus, SARS-CoV-2, was identified as the causal agent of a pneumonia outbreak in Wuhan, China. This marked the inception of the COVID-19 epidemic. COVID-19 poses heightened risks, particularly for older adults and individuals with chronic diseases like heart failure.[3] The virus's impact has underscored the critical need for vigilance and specialized care in managing vulnerable populations with pre-existing health conditions, including those with heart-related issues.[4] The ongoing pandemic, marked by the emergence of COVID-19, has indeed instilled significant fear, especially among individuals with chronic conditions such as heart failure has contributed to increased concerns about mortality. In addition to the strong evidence of the effects of COVID-19 on the cardiac system, the psychosocial effects of COVID-19 such as phobia, fear, anxiety, and depression have made it challenging for HF patients to maintain self-care.[5-7] This unprecedented global health crisis has emphasized the importance of addressing not only the physical health aspects of individuals with heart failure but also the psychological and emotional well-being.[8] In this process, it is essential for individuals with HR to possess sustainable self-care skills to maintain their daily living activities, which is crucial for both physiological and psychological health, and it is essential to maintain it.[9] Self-care that involves lifestyle changes, adherence of medication and regular monitoring HR patients is crucial for managing the condition and enhancing well-being. Key aspects include maintaining a heart-healthy diet, managing fluid intake, engaging in moderate physical activity, monitoring symptoms, attending regular medical check-ups, practicing stress management techniques, and ensuring good sleep hygiene.[10] Quarantine processes implemented worldwide caused a decrease in physical activity, which is important in managing chronic diseases.

In a study involving 1050 patients in England in 2021, the level of patients' perceived anxiety about COVID-19 was found to be significantly higher compared to HF. Additionally, patients reported that hospital appointments were cancelled or postponed, medication prescriptions were disrupted, and they had difficulties reaching HF teams. In the same study, HF patients were reluctant to go to the hospital because of the concerns they experienced.[11] We examined the relationship between perceived fear of COVID-19 and self-care management in HF patients.

Aim

The research aims to provide a comprehensive understanding of the factors shaping self-care behaviors in HF patients during the COVID-19 era, ultimately contributing valuable insights for the development of targeted interventions and support mechanisms.

Material and methods

Design and sample

This descriptive and cross-sectional study was conducted in a cardiology hospital in Istanbul between December 2021 and April 2022. The selection of a cardiology hospital was crucial for reaching patients who are regularly monitored. The study's sample comprised 115 patients with HF. These individuals applied to the hospital during the study period, met the sample selection criteria, and both verbally and in writing agreed to participate in the research. Inclusion criteria comprised individuals aged 18 years or older, diagnosed with HF for a minimum of one year, able to communicate verbally, not diagnosed with any psychiatric disorder, and who agreed to participate in the study. Researchers collected data within an average of 15 minutes through face-to-face interviews on weekdays when the patients and the researchers were available.

The Scientific Research Ethics Committee approved this research under the decision numbered 2021-40034-30, dated 12 July 2021. Participation was voluntary, anonymous, and did not involve any compensation. Informed consent was obtained from all the patients who were willing to participate in the study.

Research questions

1. What is the relationship between the level of fear related to COVID-19 and the level of self-care management observed in patients with heart failure?
2. Are there specific demographic factors that moderate the association between the fear of COVID-19 and self-care management in individuals with heart failure?

Data Collection Tools

Information form

The researchers designed this questionnaire to ascertain the sociodemographic and medical characteristics of patients with HF. The form facilitated the collection of information, including age, gender, marital status, education level, cohabitants, duration since the HF diagnosis, history of COVID-19 disease, and the current COVID-19 vaccination status of the participants.

Fear of COVID-19 scale

This valid scale was developed by Ahorsu et al. to measure the fear levels of individuals due to COVID-19.[12,13] The scale has a single-factor structure and consists of seven items rated on a five-point Likert scale (1=I strongly disagree; 5=I strongly agree). The total score on the scale ranges from 7–35, with higher scores indicating higher levels of fear of COVID-19. The internal consistency of the scale was 0.82. In this study, the Cronbach's alpha value was found to be 0.90.

Nine-item European Heart Failure Self-care Behavior Scale
This valid scale was created by Jaarsma et al. by revising the 12-item Heart Failure Self-Care Behavior Scale to a 9-item scale.[14,15] The scale was developed to determine the self-care behaviors of individuals with HF. The items of the scale are measured using a five-point Likert scale (1=I totally disagree, 5=I totally agree). Although the sub-dimensions of the scale are adherence to treatment and counselling, the score that can be obtained varies between 9 and 45. A high score on the scale indicates that self-care management is high. The reliability was determined as 0.82. For this study, the Cronbach's alpha value was 0.92.

Statistical analysis
Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows (SPSS Inc, Chicago, Illinois, USA). Continuous variables were expressed as mean ±standard deviation (SD), and categorical variables as percentages. The Kolmogorov-Smirnov test was used to assess whether the data was normally distributed. When the data was non-normal, the Mann-Whitney U test was used to compare two independent groups, and the Kruskal-Wallis test was used to compare three or more independent groups. The Pearson correlation coefficient was used to determine the relationship between two variables. The reliability analysis of the scales was evaluated with the Cronbach's alpha reliability coefficient. Statistical significance was considered at $p < 0.05$.

Results

Of the participants, 51.3% were 41–49 years old, 47% were women, 73% were married, 39.1% were primary school graduates, 92.2% lived with their families, and 24.5% were retired. Most participants did not smoke (91.3%) or drink alcohol (98.3%). Of the participants, 50.4% were diagnosed with HF between 1–3 years, and 90.4% had a chronic disease in addition to HF, such as chronic obstructive pulmonary disease, renal failure, and hypertension. Moreover, 95.7% of the participants were vaccinated against COVID-19, 13.9% had COVID-19, and 3.5% were hospitalized when suffering COVID-19. Additionally, 91.3% of the participants did not receive psychological support, only 27.8% received training from their physician and nurse on the relationship between COVID-19 and HF, and 83.5% followed the news about COVID-19 (Table 1).

The COVID-19 fear scale mean score of the study participants was 20.78±5.68, and the self-care scale mean score was 29.71±7.79 (Table 2).

The mean scores of the participants on the fear of COVID-19 scale and the self-care scale were higher in those under the age of 40 (p=0.002, p<0.00a) and those with a bachelor's degree (p=0.001, p=0.000). In addi-

Table 1. Demographic and clinical characteristics of patients

Categories		n	%
Gender	Female	54	47
	Male	61	53
Age, years	< 40	23	20
	41–59	59	51.3
	≥ 60	33	28.7
Marital Status	Married	64	73
	Single	51	27
Educational Status	Illiterate	12	10.4
	Primary school	45	39.1
	High school	32	27.8
	University	26	22.6
Employment	Employed	87	75.5
	Retired	28	24.5
Income status	High income	4	3.5
	Middle income	21	18.3
	Low income	90	78.3
Smoking status	Yes	10	8.7
	No	105	91.3
Alcohol use	Yes	2	1.7
	No	113	98.3
Cohabitant	Alone	9	7.8
	Family or partner	106	92.2
Heart failure duration, years	1–3	58	50.4
	4–6	40	34.8
	≥ 7	17	14.8
Any other chronic diseases?	Yes	104	90.4
	No	11	9.6
Have you had COVID-19?	Yes	16	13.9
	No	99	86.1
Have you been vaccinated against COVID-19?	Yes	110	95.7
	No	5	4.3
Have you received training from your physician and nurse about the relationship between COVID-19 and heart failure?	Yes	32	27.8
	No	83	72.2
Time to follow news about COVID-19, hours	< 1	96	83.5
	1–2	15	13
	≥ 3	4	3.5

Table 2. Fear of COVID-19, self-care scale sub-dimensions and total score*

		Mean±SD	The range value that can be obtained from the scales	Cronbach's alpha
Fear of COVID-19 tool		20.78±5.68	7–35	0.90
Nine-item European Heart Failure Self-care Behaviour Scale	Adherence regimen	13.86±3.67	4–20	0.56
	Consulting behaviour	15.85±4.49	5–25	0.87
	Total	29.717.79	9–45	0.92

* SD – standard deviation

Table 3. Comparison of COVID-19 fear, self-care sub-dimensions, and total score averages by the sociodemographic and clinical characteristics of the participants[a]

(n=115)		Fear of COVID-19	Nine-item European Heart Failure Self-care Behaviour Scale		
			Adherence to treatment	Consultation Behavior	Total
Gender	Female	21.98±5.69	14.403.48	16.42±4.64	30.83±7.67
	Male	19.72±5.51	13.37±3.80	15.34±4.33	28.72±7.82
		p=0.061**	p=0.303**	p=0.288**	p=0.282**
Age (year)	< 40	24.04±4.51	16.17±2.60	19.08±4.10	35.26±6.53
	41–59	20.67±5.56	13.643.42	15.33±4.29	28.98±7.54
	≥ 60	18.69±5.74	12.63±4.09	14.51±4.13	27.15±7.52
		p=0.002*	p=0.003*	p=0.001*	p<0.001*
Marital Status	Married	20.42±5.68	13.553.66	15.41±4.33	28.97±7.59
	Single	21.74±5.68	14.67±3.63	17.03±4.79	31.70±8.11
		p=0.509**	p=0.195**	p=0.130**	p=0.120**
Educational Status	Illitarate	16.83±6.24	11.504.01	11.83±3.37	23.33±5.97
	Primary school	19.44±5.56	12.753.56	14.44±3.63	27.20±6.72
	High school	21.56±5.16	14.00±3.13	15.87±4.14	29.87±7.06
	University	23.96±4.36	16.69±2.60	20.11±3.52	36.80±6.06
		p=0.001*	p<0.001*	p=0.000*	p<0.001*
Income status	High income	22.75±6.18	15.25±2.98	13.75±4.92	29.00±5.29
	Middle income	19.85±5.16	13.66±4.83	15.76±5.74	29.42±9.99
	Low income	20.91±5.81	13.84±3.41	15.96±4.17	29.81±7.37
		p=0.343*	p=0.679*	p=0.662*	p=0.954*
Smoking status	Yes	21.40±4.00	14.50±3.17	15.80±4.93	30.30±7.67
	No	20.72±5.83	13.80±3.72	15.85±4.47	29.65±7.84
		p=0.731**	p=0.514**	p=0.780**	p=0.508**
Alcohol use	Yes	20.50±3.53	17.50±0.70	22.00±1.41	39.50±2.12
	No	20.78±5.72	13.79±3.67	15.74±4.45	29.53±7.57
		p=0.881**	p=0.066**	p=0.043**	p=0.046**
Cohabitant	With family or partner	20.62±5.52	13.81±3.53	15.59±4.33	29.40±7.55
	Alone	22.66±7.46	14.44±4.82	18.88±5.51	33.33±10.09
		p=0.381**	p=0.800**	P=0.085**	p=0.278**
Heart failure duration, years	1–3	22.65±5.03	14.82±3.42	17.27±4.50	32.10±7.54
	4–6	19.70±4.64	12.90±3.49	14.75±3.95	27.65±7.11
	≥ 7	16.94±7.50	12.82±4.23	13.58±4.21	26.41±8.00
		p=0.001*	p=0.015*	p=0.002*	p=0.002*
Any other chronic diseases	Yes	20.67±5.83	13.88±3.80	15.83±4.62	29.72±8.04
	No	21.81±4.19	13.63±2.29	16.00±3.13	29.63±5.14
		p=0.603**	p=0.519**	p=0.871**	p=0.879**
Have you had COVID-19?	Yes	20.75±6.00	14.43±3.91	16,37±4,39	30.81±8.03
	No	20.78±5.66	13.76±3.65	15.76±4.52	29.53±7.78
		p=0.903**	p=0.482**	p=0.673**	p=0.630**
Where did you get over COVID-19?	Resting at home	21.50±6.38	14.66±3.74	17.58±4.50	32.25±8.15
	In hospital	19.50±3.69	14.75±4.57	14.25±2.98	29.00±7.34
		p=0.428**	p=0.579**	p=0.160**	p=0.300**
Have you been vaccinated against COVID-19?	Yes	20.81±5.78	13.84±3.69	15.85±4.51	29.70±7.82
	No	20.00±3.16	14.20±3.76	15.80±4.65	30.00±8.03
		p=0.616**	p=0.972**	p=0.783**	p=0.837**
Have you received training from your physician and nurse about the relationship between COVID-19 and heart failure?	Yes	23.00±4.02	16.31±2.74	19.37±3.70	35.68±6.36
	No	19.92±6.01	12.95±3.56	14.49±4.02	27.40±7.06
		p=0.001**	p<0.001**	p<0.001**	p<0.001**
Time to follow news about COVID-19, hours	< 1	20.17±5.66	13.41±3.63	15.41±4.33	28.83±7.59
	1–2	24.66±5.15	16.80±2.30	19.53±3.77	36.33±5.70
	≥ 3	20.75±2.06	13.50±4.50	12.50±4.20	26.00±8.28
		p=0.024*	p=0.002*	p=0.001*	p=0.001*

[a] * – Kruskal Wallis test, ** – Mann Whitney-U test

tion, the mean score of the self-care scale (p=0.046) was higher in alcohol users than in non-users. The mean scores of the COVID-19 fear scale and the self-care scale were high in the following groups: those with a disease duration of 1–3 years (p=0.001, p=0.002), those who received psychological support during the pandemic process (p=0.017, p=0.001), those who received training by their physicians and nurses about COVID-19 (p=0.001, p=0.000), and those who had 1–2 hours to follow the news about COVID-19 (p=0.024, p=0.001) was high (Table 3).

Table 4. The relationship between COVID-19 fear and self-care sub-dimensions and total scores[a]

	Fear of COVID-19	Adherence regimen	Consulting behaviour	Total scores of EHFScB scale-9
	r	r	r	R
Fear of COVID-19 tool	1	0.667**	0.681**	0.707*
Adherence regimen		1	0.818**	0.943**
Consulting behaviour			1	0.962**
Total scores of EHFScB scale-9				1

[a] * – p<0.05, ** – p<0.001, r – Pearson correlation coefficient, EHFScB – the European Heart Failure Self-care Behavior Scale

Discussion

Many studies investigated the relationship between chronic diseases and COVID-19 during the pandemic. In a study published in the USA, including 8438 patients diagnosed with COVID-19, cardiovascular diseases were the leading comorbidities associated with COVID-19, 28.2% of the patients were diagnosed with hypertension, 8.6% had coronary artery disease, and 6.9% had HF.[16] In another study, 23% of patients hospitalized for COVID-19 were diagnosed with HF.[17] Individuals with cardiovascular disease diagnosed with COVID-19 have elevated overall and in-hospital mortality rates, patients with a history of HF have prolonged hospital stay the length of hospital stay in patients with a previous diagnosis of HF, and the risk of 30-day death is higher.[18,19]

Compliance with pharmacological treatment is essential in the self-care of cardiovascular diseases. A systematic review published by Ruksakulpiwat et al. in 2022 discussed the impact of COVID-19 on drug compliance. Concerns about COVID-19, medication shortage, travel restriction, financial restriction, and substance use have been reported as barriers to drug compliance, whereas compliance with health guidelines and health information has been reported to facilitate drug compliance.[20] In this study, the finding that patients who received training related to COVID-19 from their physician or nurse had higher drug compliance scores and self-care scores supports this situation.

In a study of diabetes and hypertension patients at risk of HF, the rate of poor drug compliance was reported as 72% during the pandemic period. In the same study, treatment adherence, going to a health centre, presence of comorbidity, and history of current substance use were strongly associated with drug non-adherence.[21] In this study, being 60 years of age or older, having a low level of education, having a diagnosis of HF for seven years or more, not receiving psychological support, and not receiving training from a doctor or nurse were determinants of poor drug compliance.

Psychosocial risk factors are known triggers of acute cardiovascular events.[22] Therefore, we discussed the relationship between perceived fear of COVID-19 and self-care management in HF patients is discussed. In the study, there was a moderate positive correlation between perceived COVID-19 fear levels and self-care levels in HF patients. Lifelong self-management is important in reducing the effects of the disease in patients with HF.[23] The presence of depressive symptoms may prevent patients with HF from participating in self-care.[24] In 2023, Kim et al. included 162 patients with coronary artery disease and investigated the relationship between fear of COVID-19 and healthcare behaviors. That study found that gender and the presence of comorbidities were found to be significant influential factors of fear of COVID-19.[25]

Studies examining the impact of COVID-19 on chronic diseases indicated that COVID-19 accounts for the observed psychosocial results. Bansal et al. found that during the COVID-19, the 30-day all-cause readmissions were higher in patients with psychosocial risk factors, and myocardial infarction-related readmissions were significantly higher in patients with psychosocial risk factors and acute myocardial infarction. The same study emphasized that early identification and reduction of psychosocial risk factors in patients with MI could reduce readmissions, especially during the pandemic.[26]

Alkouri et al. found that about half of HF patients were afraid of COVID-19, and increasing age, presence of angina, and having chronic lung disease in patients with HF were associated with fear of COVID-19 and coronavirus anxiety.[27] A study examining the fear of COVID-19 in patients with acute myocardial infarction found a positive correlation between age and the level of total fear of COVID-19.[28] In contrast, the present study determined that the fear of COVID-19 was higher in those under 40 years.

Moreover, it has been emphasized that undergoing self-care training via telemedicine and telenursing applications during the COVID-19 period may affect disease management positively.[29] The support of the monitoring step, an integral aspect of self-care, through telemedicine, telenursing, telerehabilitation, virtual home visits, and structured phone calls is crucial.[30] In addition, in a study, cardiology nurses stated that heart failure patients preferred telehealth applications by reducing clinic visits during the pandemic period, but patients' self-care abilities decreased. The study also highlighted challenges faced by nurses in collecting objective data.[31] Our study revealed that 72.2% of participants did not receive training from physicians or nurses and the self-care scores of the individuals who received training were higher. This suggests the potential benefits of considering telehealth applications for future periods.

A certain level of fear is beneficial in coping with the disease, but it becomes problematic when the fear is more than the actual threat.[28] Despite this, HF can increase the risk of contracting the virus and experiencing severe symptoms and complications.[27] The level of fear of the patients in this study is similar to the literature. Particularly in the younger population (40 years and under), those with a bachelor's degree, and patients who received training from their physician and nurse about COVID-19, the high level of fear may be associated with an increase in awareness. In addition, adaptation problems experienced during symptom management, the disease and treatment process in the early stages of the disease, and the overlap between HF and COVID-19 symptoms may have caused increased fear in patients.

Study limitations

This study was limited by the fact that it was a survey and was, therefore, prone to selection bias. Secondly, the study was carried out in a single center, and as a result, the findings may not be applicable to a broader context. Moreover, the research data were gathered using subjective data collection tools, which means objective assessment was not undertaken. Lastly, it should be noted that certain unexamined factors in this study could potentially influence the state of self-care.

Conclusion

This study determined a positive correlation between mean scores of the COVID-19 fear scale and the self-care scale. It found high levels of fear of COVID-19 and self-care management in the participants who were below 40 years, had a bachelor's degree, had a disease duration of 1–3 years, received psychological support during the pandemic, received training from a physician and nurse about COVID-19, and followed the news about COVID 19 for 1–2 hours. The findings of this study can help healthcare providers develop interventions for psychological support and self-management and may guide future pandemic processes.

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Declarations

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Author contributions
Conceptualization, T.Y. and M.Y.A.; Methodology, T.Y., E.B., S.E., S.T., M.Y.A., and A.H.; Software, T.Y., S.E., and S.T., ; Validation, S.T., E.B., and S.E., ; Formal Analysis, A.H., T.Y.; Investigation, A.H., and T.Y.; Resources, T.Y., M.Y.A., and E.B.; Data Curation, A.H., T.Y., and S.T.; Writing – Original Draft Preparation, T.Y., S.E., S.T.; Writing – Review & Editing, S.E., and S.T.; Visualization, T.Y.; Supervision, S.T.; Project Administration, T.Y.

Conflicts of interest
None declared.

Data availability
The data that support the findings of this study are available from the corresponding author, TY, upon reasonable request.

Ethics approval
The Scientific Research Ethics Committee approved this research under the decision numbered 2021-40034-30, dated 12 July 2021. Participation was voluntary, anonymous, and did not involve any compensation. Informed consent was obtained from all the patients who were willing to participate in the study.

References

1. Malik A, Brito D, Vaqar S, Chhabra L. Congestive Heart Failure. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; November 5, 2023.
2. McDonagh TA, Metra M, Adamo M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J.* 2021;42(36):3599-3726. doi: 10.1093/eurheartj/ehab368
3. Mohan BS, NambiarV. COVID-19: an insight into SARS-CoV-2 pandemic originated at Wuhan City in Hubei Province of China. *J Infect Dis Epidemiol.* 2021;6(4):146.
4. Shaik A. CAD Complications in Existing With COVID-19 Infections. In: *Clinical Practice and Post-Infection Care for COVID-19 Patients*. 1st ed. Rao GS and Dhamdhere-Rao S eds. Hershey, PA USA: IGI Global, 2023:17-41. doi: 10.4018/978-1-6684-6855-5.ch002
5. Mertens G, Gerritsen L, Duijndam S, Salemink E, Engelhard IM. Fear of the coronavirus (COVID-19): predictors in an online study conducted in March 2020. *J Anxiety Disord.* 2020;74:102258. doi: 10.1016/j.janxdis.2020.102258
6. Passavanti M, Argentieri A, Barbieri DM, et al. The psychological impact of COVID-19 and restrictive measures in the world. *J Affect Disord.* 2021;283:36-51.
7. Zhang Y, Ma ZF. Impact of the COVID-19 pandemic on mental health and quality of life among local residents in Liaoning Province, China: A cross-sectional study. *Int J Environ Res Public Health*. 2020;17(7):2381. doi: 10.3390/ijerph17072381
8. Mukhtar, S. Psychological health during the coronavirus disease 2019 pandemic outbreak. *Int J Soc Psychiatry.* 2020;66(5):512-516. doi: 10.1177/0020764020925835
9. van Het Bolscher-Niehuis MJ, den Ouden ME, de Vocht HM, Francke AL. Effects of self-management support programmes on activities of daily living of older adults: A systematic review. *Int J Nurs Stud.* 2016;61:230-247. doi: 10.1016/j.ijnurstu.2016.06.014
10. Riegel B, Moser DK, Anker SD, et al. State of the science: promoting self-care in persons with heart failure: a scientific statement from the American Heart Association. *Circulation*. 2009;120(12):1141-1163. doi: 10.1161/CIRCULATIONAHA.109.192628
11. Sankaranarayanan R, Hartshorne-Evans N, Redmond-Lyon S, et al. The impact of COVID-19 on the management of heart failure: a United Kingdom patient questionnaire study. *ESC Heart Fail*. 2021;8(2):1324-1332. doi: 10.1002/ehf2.13209
12. Ahorsu DK, Lin CY, Imani V, Saffari M, Griffiths MD, Pakpour AH. The Fear of COVID-19 Scale: Development and Initial Validation. *Int J Ment Health Addict*. 2022;20(3):1537-1545. doi: 10.1007/s11469-020-00270-8
13. Ladikli N, Bahadır E, Yumuşak FN, Akkuzu H, Karaman G, Türkkan Z. The Reliability and Validity of Turkish Version of The Fear of COVID-19 Scale. *Int J of Soc Sci.* 2020;3(2):71-80.
14. Jaarsma T, Arestedt KF, Mårtensson J, Dracup K, Strömberg A. The European Heart Failure Self-care Behaviour scale revised into a nine-item scale (EHFScB-9): a reliable and valid international instrument. *Eur J Heart Fail.* 2009;11(1):99-105. doi: 10.1093/eurjhf/hfn007
15. Yıldız E, Erci B. The Turkish version of Reliability and Validity of Nine Item-European Heart Failure Self-Care Behaviour Scale. *Kafkas J Med Sci.* 2018;8(1):55-60.
16. Kuno T, Takahashi M, Obata R, Maeda T. Cardiovascular comorbidities, cardiac injury, and prognosis of COVID-19 in New York City. *Am Heart J.* 2020;226:24-25. doi: 10.1016/j.ahj.2020.05.005
17. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* 2020;395(10229):1054-1062. doi: 10.1016/S0140-6736(20)30566-3
18. Rumery K, Seo A, Jiang L, et al. Outcomes of coronavirus disease-2019 among veterans with pre-existing diagnosis of heart failure. *ESC Heart Fail.* 2021;8(3):2338-2344. doi: 10.1002/ehf2.13291
19. Sokolski M, Trenson S, Sokolska JM, et al. Heart failure in COVID-19: the multicentre, multinational PCHF-COVI-CAV registry. *ESC Heart Fail*. 2021;8(6):4955-4967.
20. Ruksakulpiwat S, Zhou W, Phianhasin L, et al. The experience of caregivers of chronically ill patients during

the COVID-19: A Systematic Review. *Chronic Illn*. 2022;18(3):488-502.
21. Shimels T, Asrat Kassu R, Bogale G, et al. Magnitude and associated factors of poor medication adherence among diabetic and hypertensive patients visiting public health facilities in Ethiopia during the COVID-19 pandemic. *PLOS ONE*. 2021;16(4):0249222. doi: 10.1371/journal.pone.0249222
22. Pimple P, Lima BB, Hammadah M, et al. Psychological distress and subsequent cardiovascular events in individuals with coronary artery disease. *J Am Heart Assoc*. 2019;8(9):011866. doi: 10.1161/JAHA.118.011866
23. Aghajanloo A, Negarandeh R, Janani L, Tanha K, Hoseini-Esfidarjani SS. Self-care status in patients with heart failure: systematic review and meta-analysis. *Nurs Open*. 2021;8(5):2235-2248. doi: 10.1002/nop2.805
24. Lee KS, Lennie TA, Yoon JY, Wu JR, Moser DK. Living arrangements modify the relationship between depressive symptoms and self-care in patients with heart failure. *J Cardiovasc Nurs*. 2017;32(2):171-179. doi: 10.1097/JCN.0000000000000327
25. Kim K, Yu J. Fear of COVID-19 and social distancing on the health behavior of coronary heart disease patients. *Appl Nurs Res*. 2023;69:151664. doi: 10.1016/j.apnr.2022.151664
26. Bansal K, Majmundar V, Muthyala A, et al. Association between psychosocial risk factors and readmissions after acute myocardial infarction: role of COVID-19 pandemic. *Curr Probl Cardiol*. 2023;48(10):101881. doi: 10.1016/j.cpcardiol.2023.101881
27. Marotta M, Gorini F, Parlanti A, et al. Fear of COVID-19 in patients with acute myocardial infarction. *Int J Environ Res Public Health*. 2021;18(18):9847. doi: 10.3390/ijerph18189847
28. Mertens G, Gerritsen L, Duijndam S, Salemink E, Engelhard IM. Fear of the coronavirus (COVID-19): Predictors in an online study conducted in March 2020. *J Anxiety Disord*. 2020;74:102258. doi: 10.1016/j.janxdis.2020.102258
29. Satici S, Iyngkaran P, Andrew S, et al. Rethinking heart failure care and health technologies from early COVID-19 experiences - A narrative review. *RCM*. 2021;22(1):105-114. doi: 10.31083/j.rcm.2021.01.272
30. Tersalvi G, Winterton D, Cioffi GM, et al. Telemedicine in Heart Failure During COVID-19: A Step Into the Future. *Front Cardiovasc Med*. 2020;7:612818. doi: 10.3389/fcvm.2020.612818
31. Prasun MA, Blakeman JR, Vuckovic K, et al. Perceptions of changes in practice patterns and patient care among heart failure nurses during the COVID-19 pandemic. *Heart Lung*. 2022;52:152-158. doi: 101016/j.hrtlng.2022.01.004

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ORIGINAL PAPER

Evaluation of the knowledge, attitudes and behavior of healthcare workers concerning influenza vaccination in a training and research hospital in Türkiye

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ABSTRACT

Introduction and aim. Although increasing vaccination rates among healthcare workers (HCWs) is crucial for protecting their own health and preventing the spread of infections to patients, vaccination rates remain low. The purpose of this study is to evaluate the knowledge, attitudes, and behavior of healthcare workers concerning seasonal influenza vaccination in a training and research hospital in Türkiye.

Material and methods. This cross-sectional descriptive study was conducted among 364 healthcare workers working in a training and research hospital in Ankara, Türkiye. A face-to-face questionnaire was completed by selected participants that included questions about their sociodemographic characteristics, receiving seasonal influenza vaccination, reasons for not receiving vaccination, attitudes and behavior towards seasonal influenza vaccination recommendations for their surroundings, knowledge of who should get the vaccine, and their immunization history.

Results. Among the participants, 58.5% stated that they had never received an influenza vaccination, 35.7% mentioned not receiving the regular influenza vaccination, and only 5.8% reported receiving the influenza vaccination regularly every year. Reasons for not getting vaccinated included not trusting the influenza vaccine's protection (60.1%), not believing they are in the risk group (38.9%), and not finding a suitable time to get vaccinated (36.1%). A total of 57.1% of the healthcare workers recommended the influenza vaccine for their surroundings.

Conclusion. Influenza vaccination rates among healthcare workers are quite low. To maximize influenza vaccine uptake, awareness programs are needed to correct the misconceptions health care workers have about the vaccine, and diverse strategies should be implemented to encourage them to get vaccinated, thereby promoting influenza vaccination.

Keywords. attitude, behavior, healthcare workers, influenza vaccine, knowledge

Introduction

Influenza is a major health concern that can lead to serious complications in individuals with risk factors.[1] Yearly, influenza epidemics have the potential to impact 5% to 15% of the global population, resulting in approximately 4-5 million severe cases and causing 250,000 to 500,000 fatalities.[2] Influenza is a markedly contagious acute respiratory infection disease distinguished by symptoms such as fever, cough, headache, muscle and joint discomfort, pronounced malaise, sore throat, and nasal congestion.[3] The course of influenza can be mild or severe, depending on various factors and conditions (i.e., age, immune status, comorbidity, and seasonal flu strain).[1] Children under 5 years of age, chronically ill

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and immunocompromised patients, the elderly (>65 years), and pregnant women are at high risk of complicated influenza courses.[1-4]

Annual vaccination is the primary measure to prevent influenza and its complications.[5] For this reason, the World Health Organization (WHO) recommends annual vaccination for pregnant women at any stage of pregnancy, children aged between 6 months to 5 years old, elderly individuals (aged more than 65 years), individuals with chronic medical conditions, and healthcare workers (HCWs).[6]

Healthcare workers who are typically healthy adults, are not at high risk of experiencing severe complications after contracting an influenza infection. However, they constitute a recommended target group for vaccination against seasonal influenza, as per the guidelines outlined by the WHO.[7] Healthcare workers are at high risk of both contracting influenza and spreading the virus to vulnerable patients. This situation increases the global burden of the disease and particularly affects healthcare services.[8,9] Nevertheless, despite efforts to improve influenza vaccination coverage among HCWs for many years, vaccination is still low in this group in many European countries.[4,10]

Similar to other vaccines, it is widely recognized that the attitudes of individuals toward receiving the influenza vaccination play a significant role in determining the effectiveness of vaccination. The specific advantages of vaccination for HCWs have not been thoroughly documented; however, existing studies indicate a minor reduction in sickness absenteeism (around 0.5 days) and a diminished likelihood of contracting the influenza virus.[10-12]

Aim

The aim of this study is to evaluate the vaccination rates, the knowledge and behaviors of HCWs related to seasonal influenza vaccination.

Material and methods

This cross-sectional and descriptive study was conducted among HCWs employed at a training and research hospital in Ankara, Türkiye. Currently, our tertiary hospital has a total of 1437 HCWs in service. The sample size for the study was determined to be 369 individuals out of 1437 HCWs, with a 95% confidence interval, an error margin of 0.05, and 50% unknown frequency, according to the simple random sample calculations with 6 epi-info sample package programs. Three hundred and sixty-four out of the 369 individuals comprised the sample.

The pre-prepared questionnaire was administered to the participants through face-to-face interviews to collect data on sociodemographic features, seasonal influenza vaccination status, seasonal influenza vaccination recommendations, and knowledge of vaccination. Those serving as HCWs who willingly agreed to participate were included in the research. Healthcare workers who could not allocate time due to a busy work schedule or those who declined to respond to our inquiries were not included in the research. Surveys were administered after providing participants with detailed information about the study's content and obtaining voluntary consent through signed consent forms. In the initial section of the survey, participants were asked questions related to the study, including socio-demographic characteristics, years of professional experience, work units, seasonal influenza vaccination status, recommendation practices, to whom the vaccine should be administered, and the timing of vaccination. The second part of the survey consisted of 15 questions aimed at assessing the participants' knowledge regarding seasonal influenza vaccination. Participants were asked to mark the correct option if they believed the given statements were accurate, select the incorrect option if they thought the statements were false, or choose the "I don't know" option if they had no opinion on the matter. In this stage, one point was awarded for each correct answer, while zero points were assigned for incorrect answers or selecting the "I don't know" option. Following the assessment, the total scores for each participant were calculated within the range of zero to fifteen, where each participant received a score based on the number of correct answers. The total knowledge score was obtained by summing the correct answers provided by the HCWs included in the study. Accordingly, the average knowledge level of the participants in the research was determined to be 8.1±2.8, with a median of 8 (0–15). No open-ended questions were included in the evaluation questions.

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the local Ethics Committee of the hospital (date: 16.01.2017; number 34/14).

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp. released in 2013, Armonk, NY). Descriptive statistics are presented as the mean and standard deviation (minimum-maximum), median, frequency distribution and percentage. The normal distribution suitability of the variables was assessed using both visual methods (histogram and probability plots) and analytical methods (Kolmogorov-Smirnov). For the variables that were determined not to be suitable for normal distribution, the Mann-Whitney U Test was employed for the comparisons between two independent groups, and the Kruskal Wallis Test was used for statistical analysis among three independent groups. Categorical variables were analyzed using Pearson Chi-square Fisher's exact test. The statistical significance level was set at $p<0.05$.

Results

In our study, a total of 364 HCWs were interviewed. Among the participants, 261 (71.7%) were female, and 103 (28.3%) were male. The mean age was 30.3 9.8 years (min= 17, max: 60 years). A total of 207 (56.9%) were physicians, and 157 (43.1%) were other HCWs.

The distribution of the influenza vaccine status of the participants is presented in Table 1.

Table 1. The distribution of seasonal influenza vaccination status of the healthcare workers (n=364)[a]

	n (%)
Seasonal influenza vaccination status	
Never received	213 (58.5)
Irregularly received	130 (35.7)
Regularly received (every year)	21 (5.8)
Would like to receive seasonal influenza vaccination	
Yes, would like to	115 (31.6)
No, would not like to	249 (68.4)
The reasons for never received seasonal influenza vaccination (n=213) *	
Would not trust the influenza vaccine protection	128 (60.1)
Not be thinking to be in the risk group	83 (38.9)
Could not find the time	77 (36.1)
The side-effects of the vaccine	71 (33.3)
To think that influenza is dangerous	44 (20.6)
To think that there are harmful components in the vaccine	35 (16.4)
Needle phobia	7 (3.3)
To think he/she has natural immunity	4 (1.8)
Afraid of being infected after the influenza vaccination	3 (1.4)
The change of vaccine strains every year	3 (1.4)
The vaccine does not contain all strains	2 (0.9)
There is no need to be immunized	2 (0.9)
Allergic reactions after vaccination	1 (0.4)
Would like to develop natural immunity	1 (0.4)

[a] * – given more than one answer, percentage

Among the participants, 213 (58.5%) had never received the seasonal influenza vaccine. The reasons for their preference not to get vaccinated are listed in the same table. When all 364 participants were asked whether they wanted to receive seasonal influenza vaccination, 249 (68.4%) HCWs stated that they did not want to get vaccinated. When examining subgroups of HCWs, the annual rates of those who receive regular vaccination were as follows: among doctors, it was 8.7% (n=18), among allied HCWs, it was 2% (n=3). The rates of irregular vaccination were found to be 37.7% (n=78) among doctors and 33.1% (n=52) among allied HCWs. In both groups, the rates of not having received the seasonal influenza vaccine were 53.6% (n=111) among doctors and 64.9% (n=102) among allied HCWs respectively. For all three conditions, statistically significant values were found among doctors and other allied health personnel ($p<0.001$).

When examining the distribution of recommendations for the seasonal influenza vaccine among the study participants, 156 (42.9%) of the participants did not recommend the seasonal influenza vaccine. Among the reasons for not recommending the seasonal influenza vaccine, 85 (54.4%) stated that they did not think the vaccine was protective, 49 (31.4%) stated that they did not find the influenza vaccine safe, and 43 (27.5%) stated that they did not think the patients they encountered were in the risk group. One hundred eighty (49.5%) of the participants did not recommend the influenza vaccine to their relatives. The most prevalent reasons for not recommending were as follows: 102 (56.6%) stated that they did not think the vaccine was protective, 58 (32.2%) stated that they did not think their relatives were in the risk group for influenza, and 57 (31.6%) of them thought the side effects of the vaccine were too much, respectively.

The distribution of the answers in the study regarding the questions evaluating their knowledge level is shown in Table 2.

Table 2. The distribution of the answers towards the questions for evaluating the knowledge level of healthcare workers (n=364)

The questions for evaluating the knowledge level	Correct answer n (%)	Incorrect answer n (%)
Inactivated and live-attenuated types of influenza vaccines exist	214 (58.8)	150 (41.2)
Influenza is a disease caused by bacteria	289 (79.4)	75 (20.6)
The vaccination of the individuals who have a chronic disease is very important	317 (87.1)	47 (12.9)
The most common side-affect seen after vaccination is localized erythema and induration on the vaccination area	226 (62.1)	138 (37.9)
The live influenza vaccine is applied as intramuscular	155 (42.6)	209 (57.4)
Preservation is maximum in healthy individuals who are younger than 65 years	118 (32.5)	246 (67.5)
Influenza disease can be transmitted through small droplets that spread by coughing or sneezing	333 (91.5)	31 (8.5)
H1N1 which is one of the subtypes of Influenza A virus is called bird flu	102 (28.1)	262 (71.9)
In the influenza disease, the symptoms usually start 2 days after the contact with the virus and disappear within 1 week	222 (61.09)	142 (39.0)
Inactive vaccine is not given to children who are taking aspirin treatment	89 (24.5)	275 (75.5)
After the influenza vaccine is given, the protective antibody level occurs in usually 10-15 days and reaches the highest level in the 3rd week	218 (59.9)	146 (40.1)
Antiviral drugs and influenza vaccine are not taken together	115 (31.6)	249 (68.4)
Since influenza vaccine disrupts the blood sugar regulation, it should not be applied to patients with diabetes mellitus	194 (53.3)	170 (46.7)
Live-attenuated vaccine is not given to people who have egg allergy	195 (53.6)	169 (46.4)
Live-attenuated vaccine can be given to pregnant women	174 (47.8)	190 (52.2)

The statement with the highest percentage of correct answers was 'Influenza disease can be transmitted through small droplets that spread by coughing or sneezing,' with a rate of 333 (91.5%) participants answering correctly. The most common incorrect answer, at a rate of 89 (24.5%), was given to the statement 'Inactive vaccine is not given to children who are taking aspirin treatment.' The overall knowledge score

was calculated by summing up the correct answers given by HCWs. The participants' overall knowledge scores had an average of 8.1± 2.8 and a median of 8 (range=0–15).

A statistically significant difference in knowledge level was observed among occupational groups based on the total scores of the participants ($p<0.01$). The average total knowledge score of doctors was found to be 9.1±2.5, while that of other HCWs was 6.7±2.6. The total knowledge score of doctors was significantly higher than that of other HCWs.

When the distribution of total scores of physicians was analyzed regarding seasonal influenza vaccination status, it was found that the total score of those who had never received was 9±2.7, the total score of those who received regularly every year was 9.6±2.7, and the total score of those who received irregularly was 9.3±2.2. There was no statistically significant difference between the distribution of total scores regarding seasonal influenza vaccination status among physicians ($p=0.547$).

The analysis of the distribution of total scores regarding the seasonal influenza vaccination recommendation status of physicians, revealed that the average overall knowledge level score of those who recommended seasonal influenza vaccination was 9.5±2.3, while those who did not recommend had a score of 8.2±2.8. It was determined that the overall knowledge score of those who recommended seasonal influenza vaccination was higher ($p=0.003$).

Discussion

Vaccination is the most effective way to prevent influenza outbreaks. Although vaccination is an effective and cost-effective method, anti-vaccine opinions are becoming increasingly common not only in the general population, but also among HCWs.[13]

In a study conducted in Türkiye, it was determined that 6.7% of HCWs are regularly vaccinated every year, and fifty-five percent have never been vaccinated against influenza before.[14] Our findings were consistent with these results. In a study conducted by Lang et al. among HCWs in Switzerland, the rate of unvaccinated HCWs was found to be 59.8%.[15] A similar study conducted in Denmark showed that the non-vaccination rate among HCWs was 49%.[16] The situation remains unsatisfactory in low- and middle-income countries like Africa (6.5%) and Asia (8.84%), where a lower rate of influenza immunization among healthcare professionals (HCPs) has been documented.[17,18] In another study conducted in Finland, the vaccination rate among HCWs was found to be significantly higher at 83.7%, in contrast to the results observed in two other European countries, low- and middle-income countries, and our own study.[19]

When the vaccination rates of doctors and other HCWs were compared, similar results were in favor of doctors were obtained, consistent with the findings of studies conducted in other countries and our study.[15-19]

When the reasons for not getting vaccinated were examined, not trusting the effectiveness of the vaccine, not considering oneself in the risk group, and not finding a suitable time were the most common reasons, respectively. Although the rankings of the reasons for not being vaccinated vary in similar studies, their contents show significant similarities. In similar studies, it has been stated that the most common reasons for not getting vaccinated include not believing in the necessity of the vaccine and fearing potential side effects.[14-17] Hofmann et al.'s study revealed that HCPs concerns about adverse reactions may serve as a primary barrier to vaccination.[20] Although many studies in the literature have shown that such fears have no basis, the fear of side effects is still considered one of the main causes of low vaccination rates for all vaccines.[13,18] Moreover, it is known that the most crucial reasons necessitating HCWs to receive the vaccine include protecting themselves, and their patients and ensuring the protection of family members.[15,22,23,24]

According to the answer given to the question "whom should influenza vaccine be recommended to?" by HCWs who participated in our study, the rate of the answer "healthcare workers" 195 (53.6%) was less than expected. Unlike our study, in a study conducted in southern India, 93.28% of respondents were aware that the seasonal influenza vaccine was recommended for health workers, but only 52.1% expressed their intention to be vaccinated next year.[24] This study reveals a disconnection between knowledge of HCWs and their decision to get vaccinated. Interventions or strategies aimed at enhancing vaccination rates may need to address not only awareness but also other factors influencing HCWs' decisions, such as attitudes, perceptions, or systemic barriers. When examining the factors influencing the decision not to recommend vaccination, responses indicated a lack of belief in the protective efficacy of the vaccine, concerns about its safety, and the perception that the encountered patients were not in the high-risk group. It was observed that the participants had scientifically unproven worries and negative thoughts about influenza vaccine. On a global scale, the influenza vaccination rates for HCWs are estimated to vary between 2% and 44%. If HCWs are vaccinated at the recommended rates, the protection rate of their patients from this infection reaches 90%.[10] Adequate data supports the conclusion that influenza vaccines are both effective and safe.[10] In Eastern Europe, although confidence in vaccines among HCWs is high, there is still some hesitancy towards recommending seasonal influenza vaccines, particularly.[25] Negative thoughts and attitudes causing vaccine hesitancy have been observed in other similar studies in the literature.[23,26] HCWs with negative attitudes toward vaccinations tend to recommend them less often.[27,28] Therefore, increasing the knowledge

levels and awareness of HCWs may be useful in boosting both vaccination rates and vaccination recommendation.

When the knowledge level of the HCWs was evaluated, the most correct answer was given to the statement, "Influenza disease can also be transmitted through small droplets scattered around by coughing and sneezing." In a similar study conducted by Luo et al., HCWs were asked the same question, and it was observed that 98.65% answered correctly.[29] The total average score of all HCWs participating in our study is a maximum of 8.1 out of 15. The median value of the total score used to determine the level of knowledge is 8. The result that 52.4% of the HCWs who participated in our study remained below the median makes us think the participants had an insufficient knowledge level about influenza vaccination. In a cross-sectional study conducted in China, findings revealed that merely 50% of HCWs in Chongqing possessed a satisfactory understanding of influenza and its corresponding vaccine.[29] However, significant deficiencies in knowledge were also discerned; only 50.57% of participants believed that wearing masks can limit the spread of influenza, and just 58% agreed that the immunity afforded by the influenza vaccine is better than natural immunity.[29] Moreover, in some studies, a majority of HCWs believed that influenza can only be transmitted by symptomatic patients.[17,18,30] Therefore, it is necessary to ensure that opportunities are provided for HCWs to regularly update their information about influenza and vaccination.

The overall knowledge score of physicians was significantly higher than that of other occupational groups. There was no significant difference in the distribution of total scores due to the seasonal influenza vaccination status of physicians ($p>0.05$). Additionally, the study showed that although physicians with a moderate to high knowledge level constituted 86% of the participants, their regular vaccination rates were found to be less than half of the participants. The knowledge level of physicians who recommended seasonal influenza vaccination to their patients was higher than that of those who did not recommend. In the study conducted by Chen et al. in China, it was shown that the knowledge and attitudes of HCWs about the influenza vaccine had a meaningful relationship with their behavior.[31] Therefore, it was concluded that as HCWs' knowledge of the influenza vaccine increases, there is a likelihood that the rates of recommending the influenza vaccine to patients will also increase.

Our study has several limitations. Firstly, the study is based on a cross-sectional design; therefore, the findings cannot result in causality, as HCWs are evaluated in just one time frame. Secondly, the number of HCWs evaluated in the study is far below the total number of employees in our tertiary hospital. Generalizations of the results presented here should be done with caution. Third, vaccination data were collected using a self-reported survey, and the accuracy of the data from these HCWs was not verified based on their medical records.

Conclusion

This study revealed low vaccination rates for the influenza vaccine among HCWs. It is of great importance to increase the vaccination rates of HCWs, both in order to protect their own health and to protect other patients and employees from infections that may be transmitted. It is necessary to prioritize education on influenza vaccination to increase low vaccination rates and to determine the reasons for reluctance to get vaccinated. It is important to explain the necessity, effectiveness, and low side effects of the vaccine. It is essential to employ innovative strategies related to vaccines and use methods for encouraging vaccination in the community, as well as in HCWs, through communication channels that will enable us to reach many people. Through all these efforts, the knowledge and awareness levels of HCWs regarding the influenza vaccine can be increased, and the vaccination rates in the community can be raised.

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Declarations

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Author contributions

Conceptualization, M.A., S.D. and C.A.; Methodology, M.A., S.D. and C.A.; Software, M.A., S.D. and C.A.; Validation, M.A., S.D. and C.A.; Formal Analysis, M.A., S.D. and C.A.; Investigation, M.A., S.D. and C.A.; Resources, M.A., S.D. and C.A.; Data Curation, M.A., S.D. and C.A.; Writing – Original Draft Preparation, M.A., S.D. and C.A.; Writing – Review & Editing, M.A., S.D. and C.A.; Visualization, M.A., S.D. and C.A.; Supervision, M.A., S.D. and C.A.; Project Administration, M.A., S.D. and C.A.

Conflicts of interest

The authors declare no conflicts of interest in the research reported in this paper.

Data availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the local Ethics Committee of the hospital (date: 16.01.2017; number 34/14).

References

1. Uyeki TM, Hui DS, Zambon M, Wentworth DE, Monto AS. Influenza. *Lancet*. 2022;400 (10353):693-706. doi: 10.1016/S0140-6736(22)00982-5
2. Guillari A, Polito F, Pucciarelli G, et al. Influenza vaccination and healthcare workers: barriers and predisposing factors. *Acta Biomed.* 2021;92(2):2021004. doi: 10.23750/abm.v92iS2.11106
3. Gualano MR, Corradi A, Voglino G, et al. Healthcare workers' (HCWs) attitudes towards mandatory influenza vaccination - A systematic review and meta-analysis. *Vaccine*. 2021;39(6):901-914. doi: 10.1016/j.vaccine.2020.12.061
4. Schumacher S, Salmanton-Garcia J, Cornely OA, Mellinghoff SC. Increasing influenza vaccination coverage in healthcare workers: a review on campaign strategies and their effect. *Infection*. 2021;49(3):387-399. doi: 10.1007/s15010-020-01555-9
5. Grohskopf LA, Alyanak E, Ferdinands JM, et al. Prevention and control of seasonal influenza with vaccines: Recommendations of the advisory committee on immunization practices, United States, 2021-22 influenza season. *MMWR Recomm Rep.* 2021;70:1-28. doi: 10.15585/mmwr.rr7005a1
6. World Health Organization (WHO) Influenza (Seasonal). Available at: https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal). Accessed January 20, 2024.
7. Van Hooste WLC, Bekaert M. To Be or Not to Be Vaccinated? The ethical aspects of influenza vaccination among healthcare workers. *Int J Environ Res Public Health.* 2019;16(20):3981. doi: 10.3390/ijerph16203981
8. Vanhems P, Voirin N, Bénet T, et al. Detection of hospital outbreaks of influenza-like illness based on excess of incidence rates compared to the community. *Am J Infect Control.* 2014;42(12):1325-1327. doi: 10.1016/j.ajic.2014.08.011
9. Mytton OT, Rutter PD, Mak M, Stanton EA, Sachedina N, Donaldson LJ. Mortality due to pandemic (H1N1) 2009 influenza in England: a comparison of the first and second waves. *Epidemiol Infect.* 2012;140(9):1533-1541. doi: 10.1017/S0950268811001968
10. Jędrzejek MJ, Mastalerz-Migas A. Seasonal influenza vaccination of healthcare workers: a narrative review. *Int J Occup Med Environ Health*. 2022;35(2):127-139. doi: 10.13075/ijomeh.1896.01775
11. Bianchi FP, Stefanizzi P, Di Lorenza A, et al. Attitudes toward influenza vaccination in heathcare workers in Italy: A systematic review and meta-analysis. *Hum Vaccin Immunother*. 2023;19(3):2265587. doi: 10.1080/21645515.2023.2265587
12. La Torre G, Scalingi S, Garruto V, Siclari M, Chiarini M, Mannocci A. Knowledge, attitude and behaviours towards recommended vaccinations among healthcare workers. *Healthcare (Basel)*. 2017;5(1):13. doi: 10.3390/healthcare5010013
13. Yaqub O, Castle-Clarke S, Sevdalis N, Chataway J. Attitudes to vaccination - a critical review. *Soc Sci Med*. 2014;112:1-11. doi: 10.1016/j.socscimed.2014.04.018
14. Korkmaz N, Nazik S, Gümüştakım RŞ, et al. Influenza vaccination rates, knowledge, attitudes and behaviours of healthcare workers in Türkiye: A multicentre study. *Int J Clin Pract.* 2021;75(1):13659. doi: 10.1111/ijcp.13659
15. Lang P, Wu CT, Le-Nguyen AF, Czock A. Influenza vaccination behaviour of healthcare workers in Switzerland: A cross-sectional study. *Int J Public Health*. 2023;68:1605175. doi: 10.3389/ijph.2023.1605175
16. Lindvig SO, Larsen L. Uptake of and attitudes towards influenza vaccination among Danish hospital healthcare workers. *Dan Med J.* 2021;68(3):A10200729.
17. James PB, Rehman IU, Bah AJ, Lahai M, Cole CP, Khan TM. An assessment of healthcare professionals' knowledge about and attitude towards influenza vaccination in Freetown Sierra Leone: a cross-sectional study. *BMC Public Health.* 2017;17(1):692. doi: 10.1186/s12889-017-4700-2
18. Ali I, Ijaz M, Rehman IU, Rahim A, Ata H. Knowledge, attitude, awareness, and barriers toward influenza vaccination among medical doctors at tertiary care health settings in Peshawar, Pakistan-A cross-sectional study. *Front Public Health*. 2018;6:173. doi: 10.3389/fpubh.2018.00173
19. Hammer CC, Lyytikäinen O, Arifulla D, Toura S, Nohynek H. Hight influenza vaccination coverage among healthcare workers in acute care hospitals in Finland, seasons 2017/18, 2018/19 and 2019/20. *Euro Surveill*. 2022;27(17):210011. doi: 10.2807/1560-7917.ES:2022.27.17.2100411
20. Hofmann F, Ferracin C, Marsh G, Dumas R. Influenza vaccination of healthcare workers: a literature review of attitudes and beliefs. *Infection*. 2006;34(3):142-147. doi: 10.1007/s15010-006-5109-5
21. Gasparini R, Panatto D, Lai PL, Amicizia D. The "urban myth" of the association between neurological disorders and vaccinations. *J Prev Med Hyg.* 2015;56(1):1-8.
22. Boey L, Bral C, Roelants M and et al. Attitudes, believes, determinants and organisational barriers behind the low seasonal influenza vaccination uptake in healthcare workers - A cross-sectional survey. *Vaccine.* 2018;36:3351-3358. doi: 10.1016/j.vaccine.2018.04.044
23. Karafillakis E, Dinca I, Apfel F, et al. Vaccine hesitancy among healthcare workers in Europe: A qualitative study. *Vaccine*. 2016;34(41):5013-5020. doi: 10.1016/j.vaccine.2016.08.029
24. Padmanabhan A, Abraham SV, Koul PA. Knowledge, attitude and practices towards seasonal influenza vaccination among healthcare workers. *Lung India*. 2022;39(5):437-442. doi: 10.4103/lungindia.lungindia_741_21
25. A Report for the European Commission, State of Vaccine Confidence in the EU+UK. Available online: https://ec.europa.eu/health/sites/default/files/vaccination/docs/2020_confidence_rep_en.pdf. Accessed January 20, 2024.
26. Little KE, Goodridge S, Lewis H, et al. Occupational vaccination of health care workers: uptake, attitudes and potential solutions. *Public Health*. 2015;129(6):755-762. doi: 10.1016/j.puhe.2015.02.031

27. Alshammari TM, Yusuff KB, Aziz MM, Subaie GM. Healthcare professionals' knowledge, attitude and acceptance of influenza vaccination in Saudi Arabia: a multicenter cross-sectional study. *BMC Health Serv Res*. 2019;19(1):229. doi: 10.1186/s12913-019-4054-9
28. Dubé E, Laberge C, Guay M, Bramadat P, Roy R, Bettinger J. Vaccine hesitancy: an overview. *Hum Vaccin Immunother.* 2013;9(8):1763-1773. doi: 10.4161/hv.24657
29. Luo Q, Gan L, Xiong Y, Li Q, Chen T, Tang X. Knowledge, attitudes and practices related to influenza and influenza vaccine among healthcare workers in Chongqing, China-a cross-sectional study. *Hum Vaccin Immunother.* 2021;2;17(12):5500-5508. doi: 10.1080/21645515.2021.2007013
30. Khan TM, Khan AU, Ali I, Wu DB. Knowledge, attitude and awareness among healthcare professionals about influenza vaccination in Peshawar, Pakistan. *Vaccine*. 2016;34(11):1393-1398. doi: 10.1016/j.vaccine.2016.01.045
31. Chen S, Jiang Y, Tang X and et al.Research on Knowledge, Attitudes, and practices of influenza vaccination among healthcare workers in Chongqing, China - Based on structural equation model. *Front Public Health*. 2022;10:853041. doi: 10.3389/fpubh.2022.853041

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ORIGINAL PAPER

Turkish cross-cultural adaptation of Pediatric Transition Experience Measure

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ABSTRACT

Introduction and aim. The period immediately after leaving the hospital is known as the transition home, which presents a critical time for parents to take on all the responsibilities and care of a child without the support of the hospital environment. The purpose of this study is to culturally adapt the Pediatric Transition Experience Measure (P-TEM) instrument into Turkish and test its validity and reliability.

Material and methods. We conducted this methodological study with parents of children who were discharged from the hospital between May 2021 and May 2022. We evaluated linguistic, content, construct, convergent validity, and internal consistency.

Results. The P-TEM exhibited a two-factor structure and accounted for 65% of the overall variability. The internal consistency reliability for the transition preparation and transition support subdimensions was 0.779 and 0.793, respectively, while the total measure had a reliability of 0.831. The item-total correlations for the P-TEM ranged from 0.493 to 0.671. Parents who received the highest P-TEM scores experienced a 5.1-point improvement (95% confidence interval: 1.7 to 8.6) in their satisfaction with healthcare services, which was greater than that of parents who reported lower P-TEM scores.

Conclusion. It is worth noting that the P-TEM has been validated and has high reliability in Türkiye.

Keywords. measure, parents, pediatric transition, reliability, validity

Introduction

Hospital transition occurs within 30 days in 38% of cases.[1] Most children who die post-hospital discharge, pass away in their homes, indicating that interventions before discharge are optimal for dealing with this overlooked fatality cause.[2] Families report issues during their child's transition from the hospital to home, including insufficient preparation for home care, inadequate discharge instruction, and a lack of support systems following discharge or challenges in accessing healthcare providers.[3]

The period immediately after leaving the hospital is known as the transition home, which presents a critical time for parents to take on all the responsibilities and care of the child without the support of the hospital environment. The experiences or psychosocial problems experienced by parents who are followed up for chronic or acute health problems may differ. Parents spend a lot of time caring for their children and meeting their needs, which can be challenging. Dealing with chronic illness for a long period of time makes it easier to adjust to care. However, children and parents who are hospitalized and discharged for an acute reason may need support in transition to home care.[4] Adequate information about the transition home process is crucial in preventing adverse events related to the child, such as

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home accidents, feeding difficulties, re-hospitalization, or even death.[2] Parents' experience of caring for their children after hospital discharge can be marked by anxiety in the initial days following the discharge. This anxiety often materializes as the feeling that they are not yet ready to move back home with their children. This signifies a lack of preparedness for discharge, indicating the importance of providing parents with training and preparation.[5]

In pediatrics, surveys completed by parents are frequently employed to evaluate patient and family experiences and gauge quality of care. These assessments are considered to be representative of the patient experience.[6] Parent-reported outcomes regarding the quality of the pediatric transition from hospital to home focus primarily on assessing particular aspects of the transition experience, such as the discharge process, preparedness for discharge, and coping with difficulties post-discharge, rather than the overall experience after discharge.[7] Patient or parent reported outcomes offer valuable insight into their perception of transitioning from hospital to home. It is important to maintain a logical flow throughout the paper by including causal relationships between statements. Use of objective language that is grammatically correct and avoids jargon, contractions, colloquial words or phrases, and ornamental language. The text should be free of errors in punctuation, spelling, and grammar. Lastly, avoid bias while maintaining a formal tone. These results can serve as useful metric.[8,9]

Aim

We examined the Turkish validity and reliability of the Pediatric Transition Experience Measure (P-TEM). The P-TEM was designed to evaluate parent-reported experiences of transitions from pediatric hospital care to home.

Material and methods

Design and participants

The research was carried out among parents whose child had been discharged from the Gynecology and Child Hospital in the Western Black Sea Region of Türkiye between May 2021 and May 2022. When it comes to improvement studies, the recommended sample size ranges from 5 to 10 times the total number of items.[10] As the P-TEM comprises eight items, the total number of parents was expected to be over 80. Due to the low response rate of screening questions in P-TEM, more parents were recruited. The study sample included a total of 127 parents, apart from those utilized in the pre-application, who met the study's inclusion criteria. These criteria were as follows: (1) Parents who speak Turkish (2) Patients who were discharged from a medical or surgical unit (3) Patients between the ages of 0 and 18 (4) Administered 2 to 8 weeks after hospital discharge.

Data collection

The purpose and scope of the study were explained to the parents during a telephone interview held after their child's discharge, and they were invited to participate. Data were collected by telephone because the follow-up was long and the parents did not come to the hospital at each follow-up. The data was collected using the Descriptive Information Form, P-TEM, and the PedsQL Health Care Parent Satisfaction Scale from a total of 127 children who met the sampling conditions, excluding those who underwent pre-application.

Descriptive Information Form

This form contains inquiries related to the age and gender of the patient, their length of stay in the hospital, the unit to which they were admitted, the frequency of their hospitalizations, as well as the gender, age, and education level of the parent.

Pediatric Transition Experience Measure

Desai et al. developed the Pediatric Transition Experience Measure (P-TEM) to assess parent-reported experiences of transitioning from hospital-to-home for their child.[9] P-TEM comprises eight items and includes two domains – transition preparation and transition support. Response options range from 0 to 10 on a Likert scale. Scores were computed as the mean of non-missing responses and converted on a linear scale of 0 to 100, where higher scores indicate higher quality.[8]

PedsQL Health Care Parent Satisfaction Scale (Version 3.0) (PedsQL)

It is a 25-question survey created by James W. Varni to assess contentment with healthcare provisions. Ulus and Kubilay subsequently localized it into Turkish.[11] The internal consistency coefficient of Cronbach's alpha reported by Ulus and Kubilay was α=0.93, whereas in the present study, it was found to be 0.89.[11] The internal consistency coefficient of Cronbach's alpha reported by Ulus and Kubilay was α=0.93, whereas in the present study, it was found to be 0.89.[11] Parents and patients were surveyed regarding their child's experience during the admission assessment and the seven days following discharge in the post-follow-up assessment.

Health Care Reuse

These measures encompassed healthcare reuse outcomes, defined as any unscheduled hospital revisit within 7 or 30 days. Data were collected from the hospital database.

Cross-cultural adaptation

We followed the guideline for cross-cultural adaptation of self-report measures which includes initial translation, synthesis of translations, back translation, pre-final version testing, and adaptation process.[12]

Translation

The initial adaptation phase entailed forward translation, with subsequent translation and back-translation to ensure content validity for P-TEM. During the first stage, two native Turkish translators independently translated the English version of P-TEM to generate a standardized version.[12] Each translator provided a written report of their completed translation. Subsequently, the two translators collaborated to synthesize the results of their translations. In the following stage, two translators, who were unaware of the original version, translated the scale items back into its original language. Back translations were executed by two English-speaking individuals without prior knowledge of the concepts discovered and preferably with no medical background. After approval was obtained, a draft scale was created.

Expert committee review (Content validity)

Nine experts in child health, nursing, diseases, and medical sciences were consulted for assessing the content validity of the P-TEM Turkish version.[12] The scale items were evaluated by experts using a four-point rating system. The content validity index (CVI) for P-TEM at the item level and the Lawshe content validity index for the scale level were utilized.

Pilot implementation

The pilot implementation marked the final stage of the adaptation process. The approach for a novel questionnaire aims to incorporate the pre-final version among children in the designated location.[12] To assess the comprehensibility of the Turkish form, we conducted a pilot study involving 30 parents whose children had been discharged from the hospital and agreed to participate. The pilot study was conducted with 30 people via telephone interviews to further test comprehensibility. Each parent completed a questionnaire and was subsequently interviewed to gain insight into their interpretation of the items and selected responses. No changes were made to the items after the pilot study. Data from the pilot study were not included in the sample.

Statistical analysis

Descriptive statistics, including frequencies, percentages, arithmetic means, and medians, were presented. Data analysis was conducted using the IBM SPSS Version 22.0 package program. Content validity was determined through analysis of CVI values, while Bartlett's Test of Sphericity and Keiser-Meyer-Olkin (KMO) tests were used to assess data adequacy and sample size for factor analysis. Principal component analysis was performed during the exploratory factor analysis (EFA) phase. The construct validity resulting from the EFA was verified via a CFA. Internal consistency was evaluated using McDonald's omega coefficient. To assess convergent validity, multivariable regression models were employed. Results were deemed statistically significant with a confidence interval of 95%, accepting $p<0.05$ as significant.

Ethical considerations

The study received ethical approval from the university ethics committee (Protocol no: 2021-SBB-0212, Decision no: 6, Dated 04.30.2021) and necessary permissions from the institution where the study took place, as well as the relevant provincial health directorate. In order to use the scale in the study, Arti Desai, the scale's developer, granted permission via e-mail. Written consent was obtained from all parents included in the study.

Results

Most of the children in the study were female (53.6%) and fell within the age range of 0 to <2 years (37%). Additionally, the majority of children were hospitalized for less than 3 days (76.3%) in medical units (80.3%). A significant majority of patients (69.3%) have been hospitalized more than once. The majority of parents (66.1%) were female and had a primary school education (51.1%) (Table 1). Table 2 shows the average scores and response rates of the scales used in the study.

Table 1. Demographic characteristics of children

Demographic variables	n	%
Patient age		
0–2 years	47	37
2–4 years	37	29.2
5–12 years	14	11
13–18 years	29	22.8
Patient gender		
Girl	68	53.6
Boy	59	46.4
Length of stay in hospital		
<3 days	97	76.3
≥3 days	30	23.7
Hospitalized unit		
Medical	102	80.3
Surgical	25	19.7
Number of hospitalizations of child		
Once	39	30.7
More than once	88	69.3
Parent age		
18–34 years	37	29.1
35–44 years	51	40.1
≥45 years	39	30.8
Parent gender		
Female	84	66.1
Male	43	33.9
Parent education		
Primary school graduate	65	51.1
High school graduate	40	31.4
University graduate	22	17.5

Nine experts were consulted to assess the content validity of the P-TEM. CVRs and CVI were then computed to enable statistical interpretation of expert opinion data. The minimum CVR was set at 75% given that there were nine experts involved in the study.[13] The CVI was calculated as 78 based on the total average of all item CVRs. Considering the content validity index (CVI) formula of CVI=sum of content validity ratio (CVR) divided by the number of items, as well as the provided CVI being equal to CVR, it can be concluded that the scale has a statistically significant content validity (Table 2).

Table 2. Scale items and scores*

	Mean±SD	Minimum	Maximum	Responses in top box (%)[a]
P-TEM total score	89.40±14.1	20	100	28.7
Transition preparation	90.42±13.39	0	100	46.8
Transition support	85.02±13.25	0	100	59.4
PedsQL				
PedsQL admission assessment	68.44±22.58	10	100	-
PedsQL follow-up assessment	72.76±20.59	10	100	-
Health care reuse				
Any 7-d revisit hospital	4.7%	-	-	-
Any 30-d revisit hospital	7.5%	-	-	-

* - – not applicable, P-TEM – Pediatric Transition Experience Measure, PedsQL – PedsQL Health Care Parent Satisfaction Scale, [a] – for the P-TEM total score, transition preparation subdimension score, and transition support subdimension score, top-box responses referred to the proportion of respondents with a score of 100; for the individual measure items, top-box responses referred to the proportion of respondents who selected 10 on the Likert scale (which corresponded to "strongly agree")

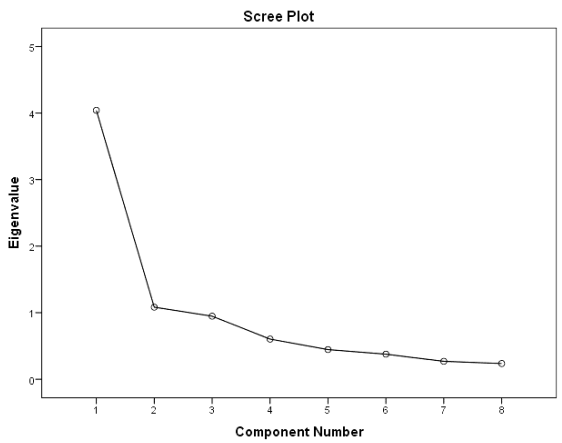

Fig. 1. Slope of scree plot

The KMO coefficient was determined to be 0.839, and the Barlett's Test of Sphericity yielded a value of 112.493 ($p<0.001$). According to the results of the EFA analysis, the P-TEM consists of two factors with an eigenvalue greater than 1 (Table 3, Figure 1). The eigenvalue of the first factor was 3.249, accounting for 40.611 % of the variance, while the second factor had an eigenvalue of 1.874 and accounted for 23.429% of the variance. The total variance accounted for by the two factors was 65.040% (Table 3).

Tablo 3. Exploratory factor analysis: pattern matrix

Items	Factors	
	Transition Preparation	Transition Support
Item 1	0.775	
Item 2	0.745	
Item 3	0.684	
Item 4	0.771	
Item 5		0.751
Item 6		0.857
Item 7		0.775
Item 8		0.612
Eigenvalue	3.249	1.874
Explained variance	40.611	23.429
Total variance explained	40.611	65.040

Figure 2 depicts the path diagram showcasing the standardized results garnered from CFA. All factor loadings recorded a p-value less than 0.001, indicating statistical significance. The goodness-of-fit indices for this investigation were as follows: Chi-square/sd=1.46, Root mean square error of approximation (RMSEA)=0.005, Standardized root mean square residual (SRMR)=0.025, Normed fit index (NFI)=0.91, Comparative fit index (CFI)=0.96, Adjusted goodness of fit index (AGFI)=0.96 and Goodness of fit index (GFI)=0.94.

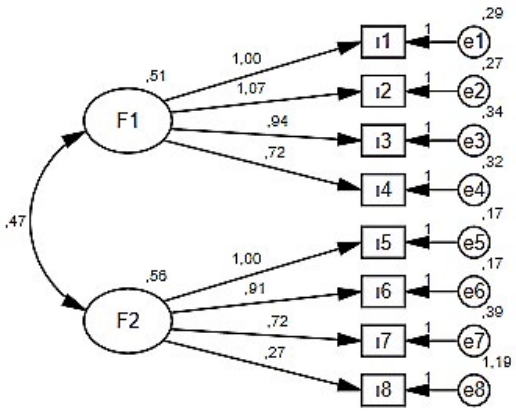

Fig. 2. Path diagram according to confirmatory factor analysis results

Participants who received the highest P-TEM scores (i.e., top-box responses) experienced an improvement of 5.1 points in healthcare parent satisfaction (95% confidence interval: 1.7 to 8.6). However, P-TEM scores did not show a significant association with 7 or 30 days of reuse, as indicated in Table 4.

Tablo 4. Difference in PedsQL and health care reuse outcomes for respondents with and without top-box responses on the P-TEM#

Predictor Variable	Change in PedsQL		Any 7-d revisit hospital		Any 30-d revisit hospital	
	n[a]	b (95% CI)[b]	n[a]	aOR (95% CI)[c]	n[a]	aOR (95% CI)[c]
P-TEM total score	127	5.1 (1.7 to 8.6)**	127	0.8 (0.3 to 1.9)	127	0.8 (0.4 to 2.1)
Transition preparation	127	4.6 (1.2 to 8.3)*	127	0.5 (0.2 to 1.8)	127	0.5 (0.3 to 2.0)
Transition support	96	4.9 (1.7 to 8.3)*	96	0.7 (0.3 to 2.6)	96	0.7 (0.4 to 1.9)

[#] aOR – adjusted odds ratio, CI – confidence interval, P-TEM – Pediatric Transition Experience Measure, PedsQL – PedsQL Health Care Parent Satisfaction Scale, [a] – the denominators for these analysis are dependent on the number eligible for the P-TEM transition support subdimension or are due to missing data for the PedsQL measure or covariates, [b] – b coefficient represents the difference in PedsQL score, comparing respondents with to those without top-box responses on the P-TEM; models were adjusted for parent age, parent education, length of stay in hospital, and PedsQL admission score, [c] – adjusted odds ratio represents the odds of either a 7- or 30-d revisit hospital, comparing respondents with top-box responses on the P-TEM to those without top-box responses on the P-TEM; models were adjusted for parent age, parent education, length of stay, * – p<0.01, ** – p<0.001

The "transition preparation" subdimension had an internal consistency reliability of 0.779, while the "transition support" subdimension had a reliability of 0.793. The overall measure had a reliability of 0.831. P-TEM item-total score correlations ranged from 0.493 to 0.671, as shown in Table 5.

Table 5. Internal consistency results

Items	Factors	
	Transition Preparation	Transition Support
Item 1	0.591	
Item 2	0.623	
Item 3	0.671	
Item 4	0.560	
Item 5		0.566
Item 6		0.665
Item 7		0.556
Item 8		0.493
McDonald's coefficient omega (factors)	0.779	0.793
McDonald's coefficient omega (total)	0.831	

Discussion

We tested the validity and reliability of the P-TEM for Turkish parents reporting on their experience of hospital-to-home transitions for pediatric patients. The Turkish version of the P-TEM indicated a two-factor structure with high internal consistency. These findings support the high validity of the P-TEM instrument for use in Türkiye.

The P-TEM possesses a two-factor structure, which was assessed by CFA. The data's goodness-of-fit indices were satisfactory in this research.[14–17] Examining both EFA and CFA is crucial for testing construct validity in scale adaptation and development studies.[18] Model fit statistics for each iteration of the two-factor CFA are reported by Desai et al.[8]

Parents with higher P-TEM scores experienced a 5.1-point improvement (95% confidence interval: 1.7 to 8.6) in their satisfaction with health care as compared to parents with lower P-TEM scores. Desai et al.[8] used four parameters, including PedsQL and health care reuse. Unfortunately, there is no standardized scale available in Türkiye to measure parent-reported experiences during a pediatric hospital-to-home transition. Similarly to Desai et al., we employed the PedsQL and health care reuse measures to assess convergent validity.[8]

In this study, the item total score correlations for the P-TEM ranged from 0.530 to 0.825. Item-total score correlation provides information about whether the item accurately measures the quality measured by the remaining items in the scale. An item with a lower total score correlation value has a smaller share in the scale.[19] The item-total score correlation coefficient should be positive and greater than +0.20. Items that do not meet this criterion should be eliminated from the scale, and the reliability of the remaining items should be reassessed.[20]

McDonald's coefficient omega was utilized to assess the P-TEM's internal consistency. Internal consistency reliability was 0.779 for the "transition preparation" subdimension, 0.793 for the "transition support" subdimension, and 0.831 for the overall measure. These results indicate that the P-TEM is highly reliable.[21] Higher internal consistency indicates greater compatibility among scale items and a stronger collaboration between them in measuring a specific feature.[22] Desai et al. reported internal consistency reliability scores of 0.87 for the "transition preparation" subdimension, 0.67 for the "transition support" subdimension, and 0.84 for the overall measure, determined by McDonald's coefficient omega. These omega values were similar to those found in the study of P-TEM.[8]

Given the high frequency of pediatric hospital-to-home transitions, there is a substantial need for measurement tools such as P-TEM.[23,24] P-TEM has been validated for reliability in Turkish in this study and is expected to be widely used and useful in assessing the pediatric hospital-to-home transition experience. In this study, we adapted P-TEM to the Turkish language and evaluated its validity and reliability in children discharged from hospitals. Future research can test its fea-

sibility in routine clinical practice. Thus, healthcare providers can use P-TEM to assess the parental experience during hospital-to-home transitions. A study on the validity and reliability of P-TEM should be conducted to assess the experience of hospital-to-home transitions for pediatric patients with various conditions, such as chronic diseases. This will ensure the widespread use of P-TEM by testing its efficacy and reliability in diverse contexts.

Study limitations

A limitation of this study is that the data was collected during the COVID-19 pandemic. Collecting data in a single center during this period led to a prolonged data collection process. Another limitation is that the hospitalizations of the children included in the study for acute and chronic reasons were not separated. Whether the child has an acute or chronic illness may affect the transition to home. Therefore, it is recommended to conduct psychometric analyses on this factor in future studies.

Conclusion

The P-TEM developed by Desai et al. demonstrates high levels of validity and reliability in Türkiye. Thus, it is advisable to implement this scale to evaluate parent-reported experiences of pediatric hospital-to-home transitions in Turkish society. Furthermore, it is suggested to evaluate its validity and reliability in children with chronic diseases by using a larger sample.

Declarations

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Author contributions

Conceptualization, A.K., F.D. and E.G.Ş. and A.K., F.D. and E.G.Ş.; Methodology, A.K., F.D. and E.G.Ş.; Software, A.K., F.D. and E.G.Ş.; Validation, A.K., F.D. and E.G.Ş.; Formal Analysis, A.K., F.D. and E.G.Ş.; Investigation, A.K., F.D. and E.G.Ş.; Resources, A.K., F.D. and E.G.Ş.; Data Curation, A.K., F.D. and E.G.Ş.; Writing – Original Draft Preparation, A.K., F.D. and E.G.Ş.; Writing – Review & Editing, A.K., F.D. and E.G.Ş.; Visualization, A.K., F.D. and E.G.Ş.; Supervision, A.K., F.D. and E.G.Ş.; Project Administration, A.K., F.D. and E.G.Ş.; Funding Acquisition, A.K., F.D. and E.G.Ş.

Conflicts of interest

The authors have no *conflicts of interest* to declare.

Data availability

Data available on request from the authors.

Ethics approval

The study received ethical approval from the Bartın University Social Sciences and Humanities Ethics Committee (Protocol no: 2021-SBB-0212, Decision no: 6, Dated 04.30.2021)

References

1. De Jesus-Rojas W, Mosquera RA, Samuels C, et al. The effect of comprehensive medical care on the long-term outcomes of children discharged from the NICU with tracheostomy. *Open Respir Med J*. 2018;12(1):39-49. doi: 10.2174/1874306401812010039
2. Nemetchek B, English L, Kissoon N, et al. Paediatric post-discharge mortality in developing countries: A systematic review. *BMJ Open*. 2018;8(12):023445. doi: 10.1136/bmjopen-2018-023445
3. Desai AD, Popalisky J, Simon TD, Mangione-Smith RM. The effectiveness of family-centered transition processes from hospital settings to home: a review of the literature. *Hosp Pediatr*. 2015;5(4):219-231. doi: 10.1542/hpeds.2014-0097
4. Eren SG, Şahin ÖÖ. Evaluate of anxiety status of children hospitalized for acute or chronic diseases and their mothers. *J Infant, Child Adolesc Heal*. 2023;1(1):1-11. doi: 10.5281/zenodo.7871132
5. Antolick MM, Looman WS, Cady RG, Kubiatowicz K. Identifying and communicating postdischarge goals for hospitalized children with medical complexity: A process improvement pilot in a specialty pediatric setting. *J Pediatr Heal Care*. 2020;34(2):90-98. doi: 10.1016/j.pedhc.2019.07.006
6. Porter KA, O'Neill C, Drake E, et al. Caregivers' assessment of meaningful and relevant clinical outcome assessments for Sanfilippo syndrome. *J Patient-Reported Outcomes*. 2022;6(1):40. doi: 10.1186/s41687-022-00447-w
7. Martens A, DeLucia M, Leyenaar JAK, Mallory LA. Foster caregiver experience of pediatric hospital-to-home transitions: A qualitative analysis. *Acad Pediatr*. 2018;18(8):928-934. doi: 10.1016/j.acap.2018.06.007
8. Desai AD, Jacob-Files EA, Lowry SJ, et al. Development of a Caregiver-Reported Experience Measure for Pediatric Hospital-to-Home Transitions. *Health Serv Res*. 2018;53:3084-3106. doi: 10.1111/1475-6773.12864
9. Desai AD, Zhou C, Simon TD, Mangione-Smith R, Britto MT. Validation of a parent-reported hospital-to-home transition experience measure. *Pediatrics*. 2020;145(2):20192150. doi: 10.1542/peds.2019-2150
10. Anthoine E, Moret L, Regnault A, Sbille V, Hardouin JB. Sample size used to validate a scale: a review of publications on newly-developed patient reported outcomes measures. *Health Qual Life Outcomes*. 2014;12:176.
11. Ulus B, Kubilay G. Turkish adaptation of the Pedsql health care parent satisfaction scale. *ACU Sağlık Bil Derg*. 2012;1(3):44-50.

12. Beaton D, Bombardier C, Guillemin F, Ferraz MB. Guidelines for the process of cross-cultural adaptation of self-report measures. *Spine (Phila Pa 1976)*. 2000;25(24):3186-3191.
13. Lawshe CH. A quantitative approach to content validity. *Pers Psychol*. 1975;28(4):563-575. doi: 10.1111/j.1744-6570.1975.tb01393.x
14. Hu LT, Bentler PM. Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. *Struct Equ Model*. 1999;6(1):1-55. doi: 10.1080/10705519909540118
15. MacCallum RC, Browne MW, Sugawara HM. Power analysis and determination of sample size for covariance structure modelling. *Psychol Methods*. 1996;1(2):130-149.
16. Forrest S, Lewis CA, Shevlin M. Examining the factor structure and differential functioning of the Eysenck personality questionnaire revised - Abbreviated. *Pers Individ Dif*. 2000;29(3):579-588. doi: 10.1016/S0191-8869(99)00220-2
17. Hooper D, Coughlan J, Mullen MR. Structural equation modelling: Guidelines for determining model fit. *Electron J Bus Res Methods*. 2008;6(1):53-60. doi: 10.21427/D79B73
18. Orcan F. Exploratory and confirmatory factor analysis: Which one to use first? *J Meas Eval Educ Psychol*. 2018;9(4):414-421. doi: 10.21031/epod.394323
19. DeVellis RF, Thorpe CT. *Scale Development: Theory and Applications*. Sage publications; 2021.
20. Clark LA, Watson D. Constructing validity: Basic issues in objective scale development. *Psychol Assess*. 1995;7(3):309-319. doi: 10.1037/1040-3590.7.3.309
21. Tavakol M, Dennick R. Making sense of Cronbach's alpha. *Int J Med Educ*. 2011;2:53-55. doi: 10.5116/ijme.4dfb.8dfd
22. Ravinder EB, Saraswathi AB. Literature review of cronbach alpha coefficient (a) and mcdonald's omega coefficient (Ω). *Eur J Mol Clin Med*. 2020;7(6):2943-2949.
23. Desai AD, Durkin LK, Jacob-Files EA, Mangione-Smith R. Caregiver perceptions of hospital to home transitions according to medical complexity: A qualitative study. *Acad Pediatr*. 2016;16(2):136-144. doi: 10.1016/j.acap.2015.08.003
24. Amar-Dolan LG, Horn MH, O'Connell B, et al. This is how hard it is" family experience of hospital-to-home transition with a tracheostomy. *Ann Am Thorac Soc*. 2020;17(7):860-868. doi: 10.1513/AnnalsATS.201910-780OC

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ORIGINAL PAPER

Prognostic significance of C-reactive protein/albumin and neutrophil/lymphocyte ratios in patients with COVID-19

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ABSTRACT

Introduction and aim. COVID-19 causes an uncontrolled and generalized inflammatory response of the host immune system. Early recognition of the disease and early prediction of the clinical course are of great importance. The aim of this study was to evaluate the predictive role of the C-reactive protein/albumin ratio (CAR) and the neutrophil/lymphocyte ratio (NLR) for mortality in patients hospitalized with the diagnosis of COVID-19.

Material and methods. The patients, who were hospitalized for COVID-19 and whose CRP, albumin, neutrophil, and lymphocyte levels were documented within the first 24 hours after admission, were analyzed retrospectively. Patients were divided into survivors and non-survivors; the groups were compared. Univariate and multivariate Cox regression models were developed to evaluate the CAR and the NLR as risk factors for mortality in COVID-19 patients.

Results. One hundred and thirty patients were included in this study. The mean age of the survivor group (n=114) was 60±16 and 52% were male. The mean age of the non-survivor group (n=16) was 75±13 and 56% were male. In the non-survivor group, the CAR detected at the time of admission to the hospital was significantly higher compared to patients in the survivor group (p=0.026).

Conclusion. As a result, the CAR, the NLR and LDH are independent risk factor indicators of mortality in hospitalized patients.

Keywords. COVID-19, C-reactive protein/albumin, neutrophil/lymphocyte

Introduction

The COVID-19 pandemic, caused by the SARS-CoV-2 virus that emerged in 2019, has spread globally and continues to be associated with mortality and morbidity despite vaccination.[1] The spectrum of disease caused by the coronavirus in humans can range from asymptomatic forms to severe viral pneumonia with severe acute respiratory failure, multi-organ dysfunction caused by sepsis and septic shock, and death.[2,3] While microbiological and radiological examinations are used for the diagnosis of COVID-19 infection, biochemical and hematological tests are used to grade disease risk and for disease follow-up and treatment.[4,5]

Biochemical and hematological alterations play important roles in the pathophysiology of COVID-19 infection and indicate the level of systemic inflammatory

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response. It has been stated that laboratory tests will be useful in the diagnosis of tissue and organ damage related to infection, identification of patients with low risk of severe disease, identification of patients with poor prognoses, and monitoring the course of the disease.[6] Complete blood count parameters are common, easy, and quick to measure and have been shown to be a prognostic marker in various disorders such as heart disease, tumors, sepsis, pneumonia and acute respiratory distress syndrome.[7,8] The value of the neutrophil/lymphocyte ratio (NLR), obtained by dividing the neutrophil count by the lymphocyte count, from hematological parameters, in the diagnosis and prognosis of COVID-19, has been demonstrated in many studies.[9-15]

The CRP/albumin ratio (CAR) represents a fraction of a positive acute phase inflammatory reactant versus a negative acute phase reactant and has the potential to simultaneously indicate the patient's inflammatory response and nutritional status.[16] In recent years, it has been shown that the CAR can be used as a prognostic biomarker in inflammatory disease, cancer and cardiovascular disease.[17,18] A recent study has shown that increased CRP levels and decreased albumin levels in COVID-19 patients may be associated with disease severity and mortality.[19] Biochemical and hematological biomarkers can play a crucial role in providing valuable insights into the severity and prognosis of COVID-19, guiding the determination of treatment strategies, and contributing to the clinical management of patients. In the current literature, there is a limited number of studies focusing on the clinical implications of such biochemical and hematological markers, and conflicting findings exist among the results of these studies.

Aim

The aim of this study is to show the prognostic significance of the CAR and the NLR detected at admission in patients diagnosed with COVID-19.

Material and methods

For this single-center, retrospective observational study, 130 patients who were hospitalized due to COVID-19 and were found to have a positive SARS-CoV-2 RT-PCR test between January and April 2021 were included.

Patients younger than 18 years of age, pregnant women, patients with negative or no SARS-CoV-2 RT-PCR test results, patients with respiratory distress due to a cause other than COVID-19, and patients with a positive SARS-CoV-2 RT-PCR test result while hospitalized with different diagnoses were excluded from the study. Additionally, patients with known hematological abnormalities and other inflammatory diseases were not included in the study (Fig. 1). The study was approved by the Internal Review Board (2021/170) of our center and was performed under the ethical standards of the Declaration of Helsinki.

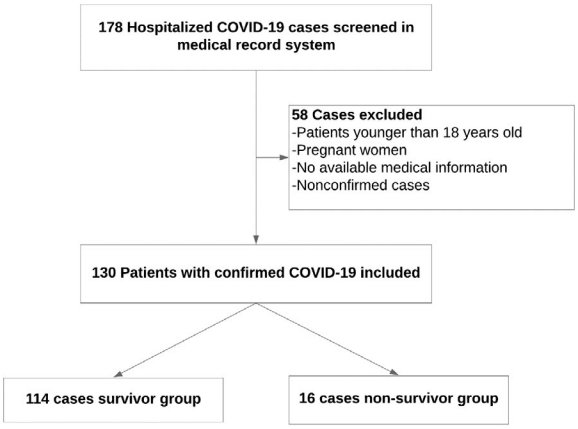

Fig. 1. Flowchart of the patient selection process

According to the WHO guideline (WHO interim guidance), the definitive diagnosis of COVID-19 is based on real-time reverse transcription-PCR (RT-PCR) testing. In our study, SARS-CoV-2 RNA was detected by the real-time RT-PCR method as determined in the Ministry of Health Public Health Microbiology Reference Laboratory. The current treatment guidelines prepared by the Ministry of Health were applied in the treatment of the patients.

Data collection and laboratory analyzes

The patients' demographic characteristics and clinical information were collected from the hospital's electronic information system. The existing diseases and diagnoses of the patients detected at the time of hospitalization were differentiated according to the 19 types of diagnosis groups in the Charlson Comorbidity Index (CCI) score system, and the sum of the scores corresponding to these diagnostic groups was determined as the CCI score. For each patient their individual data was entered, and the index was calculated online. The pulmonary involvement rate was evaluated by accessing the thorax computed tomography reports of the patients from the hospital registry system. The pulmonary infiltration rate was classified as either below 50% or above 50%. Laboratory results for each patient were recorded in the first 24 hours of hospitalization. Laboratory analyses were performed using a Mindray BC-6800 hematology analyzer (Mindray, Shenzhen, China) for hematological parameters and a Beckman AU5800 instrument (Beckman Coulter, Ireland Inc.) for biochemical parameters. Serum biochemical measurements were performed in the hospital clinical laboratory using routine automated techniques. Among the laboratory tests creatinine, Na, K, Ca, Mg, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), neutrophil, lymphocyte, platelet, ferritin, D-Dimer, fi-

brinogen, platelet crit (PCT), C-reactive protein (CRP) and HBA_1C results were recorded. The NLR was calculated by dividing the neutrophil count by the lymphocyte count, and the CAR was calculated by dividing the CRP level by the albumin level.

Patient groups were categorized into two distinct cohorts for comprehensive comparative analysis: Patient groups were compared as non-survivors and survivors. The primary focus of our investigation revolved around the critical endpoint of mortality, with a keen interest in unraveling the factors contributing to survival and those associated with unfortunate outcomes.

Statistical analysis

Data analysis was done using the SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) 22.0 program. Descriptive statistics were presented as mean ± standard deviation and median (IQR) for continuous variables, and as numbers and percentages for categorical variables. The distribution of continuous variables was evaluated and in cases where parametric conditions were met, the difference of continuous variables between the two groups was evaluated with the independent sample t-test, and the Mann Whitney U test. If parametric conditions were not met, the chi-square test or Fisher's exact test was used in the analysis of categorical variables. Log rank analyses were performed, and Kaplan-Meier survival plots were used. A univariate Cox regression analysis was used to determine hazard ratios for parameters. Then, a multivariate Cox regression analysis was performed using the Backward LR method with parameters having a p value of >0.2 to construct the final model. According to the model, proportional hazard ratios were used to evaluate the potential independent effects of the parameters after adjustment for oxygen saturation (SpO_2), age, and CCI. The CAR, which was found to be an independent risk factor for mortality, was analyzed via ROC curve analysis and the optimum cut-off value was determined by the Youden Index. Sensitivity and specificity values corresponding to the determined cut-off value were presented. A $p<0.05$ level was considered significant.

Results

The demographic, clinical and laboratory parameters of the 130 patients included in this study are shown in Table 1. The non-survivor group was older compared to the survivor group ($p<0.001$). While more than 50% pulmonary involvement was observed in 22% of the patients in the survivor group, it was observed in 31% of the patients in the non-survivor group. CCI score was higher and oxygen saturation was lower in the non-survivor group compared to the survivor group (p=0.004 and p=0.012, respectively). There was no difference between the groups in terms of length of hospital stay. PCT and creatinine levels were higher in the non-survivor group than in the survivor group ($p<0.001$ and p=0.007, respectively). In addition, CRP levels were higher in the non-survivor group, but albumin levels were lower, and so the CAR rate was also higher (p=0.022, p=0.048 and p=0.026, respectively). There was no difference between the groups in other laboratory parameters (Table 1).

Table 1. Demographic and clinical characteristics of patient survivors and non-survivors*

	Survivor (n=114)	Non-survivor (n=16)	*p*
Age (years)	60±16	75±13	<0.001
CCI	3±2	5±2	0.004
SpO_2 on admission, (%)	93±5.2	90±3.5	0.012
Hospital length of stay, (days)	11±6	18±14	0.099
Laboratory			
WBC, (/mm^3×10^3)	7.3±4.3	6.4±3.1	0.960
Hemoglobin, (g/dL)	12.4±2	11.6±2.4	0.136
Neutrophil, (/mm^3 ×10^3)	4.6 (3.3)	5.6 (4.6)	0.319
Lymphocyte, (/mm^3 ×10^3)	1.1±0.5	1.1±0.7	0.565
NLR	4.18 (5)	4.57 (7)	0.549
PLT, (/mm^3 ×10^3)	191.2±84.2	186.3±91.9	0.526
MPV, (fL)	10±1.3	10.2±1.3	0.409
PCT, (%)	0.12 (0.14)	0.54 (2.8)	<0.001
Glucose, (mg/dL)	158±79	144±60	0.461
AST, (U/L)	27 (18)	35 (69)	0.127
ALT, (U/L)	21 (17)	29 (41)	0.093
Creatinine, (mg/dL)	0.8 (0.4)	1.3 (1)	0.007
Sodium, (mEq/L)	136±4	138±6	0.076
Potassium, (mEq/L)	4.1±0.5	3.9±0.5	0.061
Magnesium, (mg/dL)	2.0±0.3	1.9±0.4	0.447
Calcium, (mg/dL)	9.1±0.5	9.1±0.5	0.650
HbA1c, (%)	7.6±1.5	7.2±0.3	0.607
Ferritin, (ng/mL)	283 (340)	415 (1535)	0.130
LDH, (U/L)	270±102	427±384	0.124
CRP, (mg/L)	42 (92)	104 (86)	0.022
Albumin, (g/dL)	3.7±0.6	3.4±0.7	0.048
CAR	13.16 (27)	39.05	0.026
D-dimer, (ng/mL)	0.68 (1)	0.98 (2)	0.065
Fibrinogen, (mg/dL)	469±147	519±174	0.224

* Values are given as mean ± SD; others given as median (IQR), CCI – Charlson comorbidity index, SpO_2 – oxygen saturation, WBC – white blood cell, NLR – neutrophil/lymphocyte ratio, PLT – platelets, MPV – mean platelet volume, PCT – platelet crit, LDH – lactate dehydrogenase, CRP – C-reactive protein, CAR – CRP/albumin ratio

Independent risk factors were evaluated with univariate and multivariate Cox regression analyses. The NLR, the CAR and LDH were found to be statistically significant independent risk factors of mortality. (NLR: HR:1.041 [95%CI:1.014–1.070], p=0.003; LDH: HR:1.003 [95%CI:1.000–1.005], p=0.031; CAR: HR:1.02 [95%CI:1.001–1.039], p=0.041) (Table 2).

The ROC of the CAR for the prediction of COVID-19 mortality is shown in Figure 2. A CAR of >17.7 was defined as the optimal cut-off point for determining COVID-19 mortality, exhibiting 75% sensitivity and 57% specificity. The area under the curve of

the CAR for the prediction of COVID-19 mortality was 0.672 (95%CI: 0.543-0.801; p = 0.026).

Table 2. Univariate and multivariate cox regression analyses for death of COVID-19

Variables	Univariate Analyses				Multivariate Analyses			
	p value	HR	95% CI for HR		*p* value	HR	95% CI for HR	
			Lower	Higher			Lower	Higher
Gender	0.919	0.947	0.33	2.715				
CCI	0.176	1.156	0.937	1.427	0.906	0.983	0.733	1.317
Age	0.073	1.038	0.997	1.082	0.324	1.024	0.977	1.074
PI	0.28	1.908	0.591	6.161				
D-dimer	0.453	1.115	0.839	1.482				
Fibrinogen	0.334	1.001	0.998	1.004				
WBC	0.968	1.000	1.000	1.000				
PLT	0.904	1.000	1.000	1.000				
MPV	0.253	1.231	0.862	1.757				
PCT	0.197	1.075	0.963	1.201				
Hemoglobin	0.459	0.908	0.704	1.172				
NLR	0.003	1.036	1.012	1.061	0.003	1.041	1.014	1.070
AST	0.862	1.000	0.998	1.002				
ALT	0.932	1.000	0.996	1.005				
Creatinine	0.993	0.999	0.758	1.317				
Sodium	0.108	1.087	0.982	1.202				
Magnesium	0.864	1.122	0.299	4.207				
Calcium	0.263	0.505	0.153	1.671				
Potassium	0.921	0.954	0.377	2.414				
LDH	0.008	1.002	1.001	1.004	0.031	1.003	1.000	1.005
Ferritin	0.057	1.000	1.000	1.001				
HbA1c	0.611	0.724	0.209	2.51				
SpO_2	0.02	0.902	0.828	0.984	0.663	0.972	0.854	1.106
CAR	0.001	1.025	1.010	1.041	0.041	1.02	1.001	1.039

* CCI – Charlson comorbidity index, PI – pulmonary involvement, WBC – white blood cell, PLT – platelets, MPV – mean platelet volume, PCT – platelet crit, NLR – neutrophil/lymphocyte ratio, LDH – lactate dehydrogenase, SpO_2 – oxygen saturation, CRP – C-reactive protein, CAR – CRP/albumin ratio

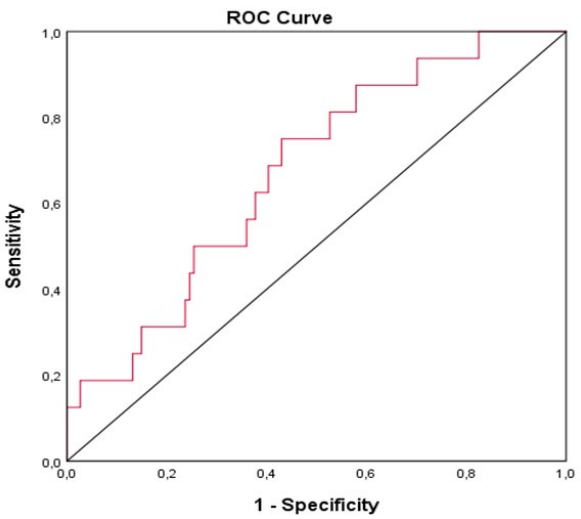

Fig. 2. Receiver operating characteristic curves for CAR for the mortality of COVID-19

A total of 16 patients died during hospitalization. All these patients had a CAR ≥17.7. Figure 3 show the Kaplan-Meier survival curve for the CAR according to this cut-off value. Patients with a CAR above the cut-off value had significantly higher mortality rates than those with a CAR below the cut-off value (log-rank test=4.972; p=0.026) (Fig. 3).

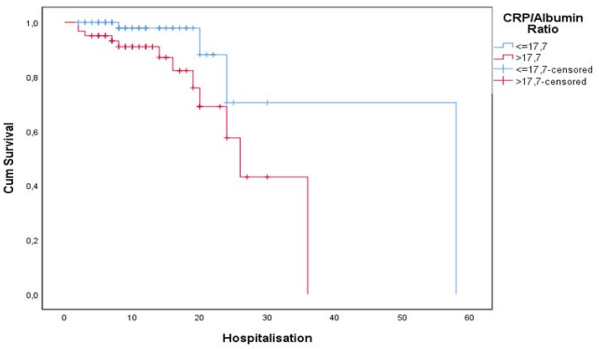

Fig. 3. The Kaplan-Meier survival curve for C-reactive protein/albumin ratio (CAR)

Discussion

In this study, we aimed to show the prognostic importance of the NLR, and the CAR detected at admission in patients diagnosed with COVID-19. The main results of our study are as follows. i) The CAR was observed to be higher in the non-survivor group than in the survivor group. ii) Multivariate analyses demonstrated that the NLR and the CAR are independent risk factors of mortality in COVID-19 patients. iii) A CAR of >17.7 was defined as the optimal cut-off point for determining COVID-19 mortality.

Since its emergence from Wuhan in 2019, COVID-19 has become a serious pandemic. Early detection of patients with poor prognoses is important. The systemic inflammatory response is responsible for the clinical course of the COVID-19, and one of the important causes of mortality is the cytokine storm caused by the excessive release of proinflammatory cytokines.[20]

The NLR, calculated by dividing the neutrophil count by the lymphocyte count, is one of the important markers of immune damage and inflammation, whose use has increased rapidly in recent years. Increase in the NLR is a risk factor for mortality in malignancy, acute coronary syndrome, intracerebral hemorrhage, polymyositis and dermatomyositis.[13] Numerous studies have investigated the impact of NLR on mortality in COVID-19 patients.[11,12,14]

The inflammatory response may cause an increase in neutrophil levels by stimulating the release of inflammatory cytokines such as TNF alpha and IL-6. Proinflammatory mediators such as catecholamines and cortisol can bind to the lymphocyte surface and initiate

lymphocytic apoptosis, leading to lymphopenia.[10] The NLR is considered a more reliable inflammatory marker because it reflects changes in both neutrophil and lymphocyte counts in conditions that correlate with the inflammatory response to a disease, such as COVID-19. Yang et al. demonstrated that the NLR is an independent biomarker to indicate poor clinical outcomes in COVID-19 patients.[15] Aksu et al. showed that the NLR values are inflammatory markers that can be used to show pulmonary involvement and disease severity in COVID-19 patients.[9] A meta-analysis by Sarkar et al. showed that the NLR is an independent predictor of disease severity and mortality in COVID-19.[14] In our study, the NLR was found to be an independent predictor of mortality, in line with previous studies.

CRP, a positive acute phase reactant, is known to increase in response to infections, inflammation and tissue damage.[21] It has been shown that CRP levels are high in COVID-19 patients and the magnitude of the increase correlates with the severity of the disease.[22] It is known that albumin, a negative acute phase reactant and the basic protein in the blood, synthesized by the liver, tends to decrease in response to acute conditions such as inflammation, trauma, surgery and burns.[23] A meta-analysis of 11 studies showed that hypoalbuminemia is associated with the severity of COVID-19.[24] In a study including 188 patients, it was revealed that the CAR levels are superior to the fibrinogen/albumin ratio (FAR) and the NLR in predicting disease severity in COVID-19 patients.[25] In a recent study, the CAR was shown to be an independent predictor of disease severity in hospitalized patients with COVID-19.[26] In a study of hypertensive patients with COVID-19, the CAR levels were shown to be an independent predictor of in-hospital mortality.[27] In a retrospective study of 2309 COVID-19 patients, the findings revealed that a high CAR rate was associated with respiratory impairment, the need for oxygen therapy and ventilation, bacteremia, and thrombosis. Moreover, four different prognostic categories of CAR ratios were identified and shown to be associated with 30-day survival.[28] In a study conducted to predict 30-day mortality in patients admitted to the emergency department due to COVID-19, it was shown that the BUN/albumin ratio and CAR ratio predicted 30-day mortality.[29] On the other hand, in a retrospective study of 75 COVID-19 patients, the CAR rate was identified as an indicator of disease severity, but its relationship with mortality was not demonstrated.[30] In our study, the CAR was found to be higher in the non-survivor group compared to the survivor group, and the CAR was found to be an independent risk factor of mortality.

Our findings are potentially clinically relevant for treatment options and follow-up for COVID-19 patients. Since it is more effective to start treatment in the early stages of the disease in COVID-19, earlier detection of high-risk COVID-19 patients using the NLR and the CAR values may be very important and effective in reducing mortality.

Our study does have some limitations. The number of patients is relatively small, and the analysis was retrospective. It is important to acknowledge the limitation of a relatively small sample size, particularly considering the global scale of the epidemic during the study period. The challenges posed by the sample size might impact the generalizability of the findings, and caution should be exercised in extrapolating these results to broader populations. It is important to acknowledge the limitation of a relatively small sample size, particularly considering the global scale of the epidemic during the study period. The challenges posed by the sample size might impact the generalizability of the findings, and caution should be exercised in extrapolating these results to broader populations. The NLR was measured only at the time of admission to hospital and follow-up NLR values were not determined. Some of the parameters are also affected by conditions such as body mass index, physical activity, smoking and alcohol consumption.[31]

Conclusion

In conclusion, both the CAR and NLR values, regarded as crucial indicators of the inflammatory response, offer valuable insights for clinicians in determining the disease trajectory. The ease and rapidity with which CAR and NLR can be measured in blood make them valuable tools for evaluating the prognosis of COVID-19 patients. However, it's essential to note that the AUC value for CAR ratio was found to be low for an ideal predictor in clinical decision-making. Therefore, we advocate for a cautious interpretation of the AUC, proposing its incorporation as supplementary information rather than a standalone determinant. Larger randomized controlled trials hold the potential to provide more conclusive evidence on the association between NLR and CAR values and in-hospital mortality among patients with COVID-19.

Declarations

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Author contributions

Conceptualization, H.B.P.; Methodology, M.A.; Software, M.A.; Validation, H.B.P., M.A. and A.E.; Formal Analysis, T.A.; Investigation, M.B.; Resources, F.B.Ç.; Data Curation, H.B.P.; Writing – Original Draft Preparation, Z.P.; Writing – Review & Editing, Z.P.; Visualization, H.B.P.; Supervision, A.E.; Project Administration, M.B.

Conflicts of interest

There is no conflict of interest between authors of this manuscript.

Data availability

Data available on request from the authors.

Ethics approval

This manuscript have been approved by the Internal Review Board of RTEÜ Hospital (2021/170).

References

1. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med.* 2020;382(8):727-733. doi: 10.1056/NEJMoa2001017
2. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med.* 2020;382(18):1708-1720. doi: 10.1056/NEJMoa2002032
3. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5
4. Asrani P, Eapen MS, Chia C, et al. Diagnostic approaches in COVID-19: clinical updates. *Expert Rev Respir Med*. 2021;15(2):197-212. doi: 10.1080/17476348.2021.1823833
5. Liao D, Zhou F, Luo L, et al. Haematological characteristics and risk factors in the classification and prognosis evaluation of COVID-19: a retrospective cohort study. *Lancet Haematol.* 2020;7(9):671-678. doi: 10.1016/S2352-3026(20)30217-9
6. Thompson S, Bohn MK, Mancini N, et al. IFCC interim guidelines on biochemical/hematological monitoring of COVID-19 patients. *Clin Chem Lab Med.* 2020;58(12):2009-2016. doi: 10.1515/cclm-2020-1414
7. Angkananard T, Anothaisintawee T, McEvoy M, et al. Neutrophil lymphocyte ratio and cardiovascular disease risk: a systematic review and meta-analysis. *Biomed Res Int*. 2018;2018:2703518. doi: 10.1155/2018/2703518
8. Huang Z, Fu Z, Huang W, et al. Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: A meta-analysis. *Am J Emerg Med.* 2020;38(3):641-647. doi: 10.1016/j.ajem.2019.10.023
9. Aksu Y, Uslu AU, Tarhan G, et al. Predictive value of platelet to lymphocyte ratio and neutrophil to lymphocyte ratio in evaluating both lung involvement and severity of patients with coronavirus disease 2019. *Saudi Med J.* 2021;42(11):1223-1228. doi: 10.15537/smj.2021.42.11.20210485
10. Alkhatip AAAMM, Kamel MG, Hamza MK, et al. The diagnostic and prognostic role of neutrophil-to-lymphocyte ratio in COVID-19: a systematic review and meta-analysis. *Expert Rev Mol Diagn*. 2021;21(5):505-514. doi: 10.1080/14737159.2021.1915773
11. Henry BM, De Oliveira MHS, Benoit S, et al. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clin Chem Lab Med.* 2020;58(7):1021-1028. doi: 10.1515/cclm-2020-0369
12. Imran MM, Ahmad U, Usman U, Ali M, Shaukat A, Gul N. Neutrophil/lymphocyte ratio-A marker of COVID-19 pneumonia severity. *Int J Clin Pract*. 2021;75(4):13698. doi: 10.1111/ijcp.13698
13. Liu Y, Du X, Chen J, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. *J Infect*. 2020;81(1):6-12. doi: 10.1016/j.jinf.2020.04.002
14. Sarkar S, Khanna P, Singh AK. The Impact of Neutrophil-Lymphocyte Count Ratio in COVID-19: A Systematic Review and Meta-Analysis. *J Intensive Care Med*. 2022;37(7):857-869. doi: 10.1177/08850666211045626
15. Yang A-P, Liu J-P, Tao W-Q, et al. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. *Int Immunopharmacol.* 2020;84:106504. doi: 10.1016/j.intimp.2020.106504
16. Gibson DJ, Hartery K, Doherty J, et al. CRP/Albumin Ratio. *J Clin Gastroenterol*. 2018;52(6):48-52. doi: 10.1097/MCG.0000000000000884
17. Devran Ö, Karakurt Z, Adıgüzel N, et al. C-reactive protein as a predictor of mortality in patients affected with severe sepsis in intensive care unit. *Multidiscip Respir Med*. 2012;7(1):47. doi: 10.1186/2049-6958-7-47
18. Karabağ Y, Çağdaş M, Rencuzogullari I, et al. Relationship between C-reactive protein/albumin ratio and coronary artery disease severity in patients with stable angina pectoris. *J Clin Lab Anal.* 2018;32(7):22457. doi: 10.1002/jcla.22457
19. Zhang J, Wang X, Jia X, et al. Risk factors for disease severity, unimprovement, and mortality in COVID-19 patients in Wuhan, China. *Clin Microbiol Infect.* 2020;26(6):767-772. doi: 10.1016/j.cmi.2020.04.012
20. Qin C, Zhou L, Hu Z, et al. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis.* 2020;71(15):762-768. doi: 10.1093/cid/ciaa248
21. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest.* 2003;111(12):1805-1812. doi: 10.1172/JCI18921
22. Luan YY, Yin CH, Yao YM. Update Advances on C--Reactive Protein in COVID-19 and Other Viral Infections. *Front Immunol.* 2021;12:720363. doi: 10.3389/fimmu.2021.720363
23. Yap F, Joynt G, Buckley T, et al. Association of serum albumin concentration and mortality risk in critically ill patients. *Anaesth Intensive Care.* 2002;30(2):202-207. doi: 10.1177/0310057X0203000213
24. Aziz M, Fatima R, Lee-Smith W, et al. The association of low serum albumin level with severe COVID-19: a systematic review and meta-analysis. *Crit Care.* 2020;24(1):255. doi: 10.1186/s13054-020-02995-3
25. Torun A, Çakırca TD, Çakırca G, et al. The value of C--reactive protein/albumin, fibrinogen/albumin, and neutrophil/lymphocyte ratios in predicting the severity of CoVID-19. *Rev Assoc Med Bras (1992)*. 2021;67(3):431-436. doi: 10.1590/1806-9282.20200883

26. Karakoyun I, Colak A, Turken M, et al. Diagnostic utility of C-reactive protein to albumin ratio as an early warning sign in hospitalized severe COVID-19 patients. *Int Immunopharmacol*. 2021;91:107285. doi: 10.1016/j.intimp.2020.107285
27. Saylik F, Akbulut T, Kaya S. Can C-Reactive Protein to Albumin Ratio Predict In-Hospital Death Rate Due to COVID-19 in Patients With Hypertension? *Angiology*. 2021;72(10):947-952. doi: 10.1177/00033197211012145
28. Lucijanić M, Stojić J, Atić A, et al. Clinical and prognostic significance of C-reactive protein to albumin ratio in hospitalized coronavirus disease 2019 (COVID-19) patients: Data on 2309 patients from a tertiary center and validation in an independent cohort. *Wien Klin Wochenschr*. 2022;134(9-10):377-384. doi: 10.1007/s00508-021-01999-5
29. Özdemir S, Altunok İ. Comparison of the Predictive Ability of the Blood Urea Nitrogen/Albumin, C-Reactive Protein/Albumin, and Lactate/Albumin Ratios for Short-Term Mortality in SARS-CoV-2-Infected Patients. *Avicenna J Med*. 2023;13(1):43-48. doi: 10.1055/s-0043-1761471
30. Kalabin A, Mani VR, Valdivieso SC, et al. Does C reactive protein/Albumin ratio have prognostic value in patients with COVID-19. *J Infect Dev Ctries*. 2021;15(8):1086-1093. doi: 10.3855/jidc.14826
31. Howard R, Scheiner A, Kanetsky PA, et al. Sociodemographic and lifestyle factors associated with the neutrophil-to-lymphocyte ratio. *Ann Epidemiol*. 2019;38:11-21.e6. doi: 10.1016/j.annepidem.2019.07.015

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ORIGINAL PAPER

Comparative study of pterygium excision with suture and sutureless conjunctival autograft

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ABSTRACT

Introduction and aim. This is a prospective interventional study to compare corneal astigmatism before and after pterygium excision with conjunctival autograft, to determine the difference in mean corneal curvatures before and after pterygium excision, to evaluate the safety and efficacy of autologous blood technique, and sutured conjunctival autograft for primary pterygium.

Material and methods. Patients were divided into group A – pterygium excision+sutured conjunctival limbal autograft and group B – pterygium excision+autologous conjunctival limbal autograft under local anesthesia. The preoperative and post operative K1 and K2 values and BCVA were noted.

Results. There was statistically significant reduction in mean astigmatism at each post operative follow up in group A at 1 week (p=0.0249), 2 weeks (p=0.004), 1 month (p=0.0031) and at 3 months (p=0.0009) and similarly in group B post operative follow up at 1 week (p=0.0011), 2 weeks (p=0.0021), 1 month (p=0.0009) and at 3 months (p=0.0003).

Conclusion. Pterygium surgery decreases the cylindrical error and reverts corneal curvatures to normal, thus improving subjective visual acuity. Astigmatism produced by the pterygium is reduced after surgery We found autologous conjunctival autograft is superior than sutural conjunctival autograft. Pterygium causes astigmatism depending upon the size of pterygium it should be surgically removed to prevent obstruction in the vision.

Keywords. astigmatism, autologous blood conjunctival autograft, pterygium

Introduction

Pterygium was derived from the Greek word 'Pterygos' which means wing. It is a triangular, wing-shaped, fibrovascular, degenerative, hyperplastic proliferative tissue growing from the conjunctival limbal area onto the cornea.[1] Pterygia usually develops in the interpalpebral space with a greater number occurring medially than temporally.[2] The pterygium is made up of cap, head, neck and body. The body extends from the limbus to the bulbar surface. The neck being the narrowest portion of tissue overlies the limbus and extends onto the peripheral cornea. The central extension of fibrovascular tissue is the head. A white grey avascular subepithelial cap is called as stocker line.[2] It results in cosmetic problems, decrease in visual acuity secondary to astigmatism and blockage of optical axis. When pterygium is small there is only slight irritation in the periods of inflammatory engorgement.[3] The progression of a pterygium onto the cornea can cause significant corneal distortion and corneal astigmatism.[4] When pterygium involves the pupillary aperture, obstruction of vision occurs in the peripheral field and later in the central. Advanced cases of pterygium which encroaches onto the cornea may cause visual loss secondary to:

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1. loss of corneal transparency in the visual axis,
2. irregular corneal astigmatism (localized flattening).

Induced astigmatism is due to pooling of tears over the pterygium apex or due to mechanical traction of the pterygium leading to tractional corneal flattening.[2] An advanced pterygium can produce changes in corneal curvature and refractive state before entering the optical zone, which can cause visual impairment.

Grades of pterygium:[5]

Grade-I: Crossing the limbus but not reaching the pupil margin (<2mm)

Grade-II: Crossing the limbus and reaching up to the pupil margin (2–4 mm)

Grade-III: Reaching beyond the pupil margin (involving the visual axis) (>4 mm)

Aim

The aim of the study was to determine the difference in mean corneal astigmatism before and after pterygium excision. Moreover, we evaluated the safety and efficacy of autologous blood technique and sutured conjunctival autograft for primary pterygium.

Material and methods

The prospective interventional study was done in Shri Shankaracharya Institute of Medical Sciences, Bhilai, C.G. The study period was June 2022 to March 2023. In this study total 58 patients with primary pterygium of age more than 30 years who attended the Ophthalmology Out Patient Department who were suffering from primary pterygium were taken after getting permission from ethical committee of college (SSIMS/EC/2022/21).

Inclusion criteria were as follows: primary pterygium and age more than 30 years.

Participants with recurrent pterygium, pseudo-pterygium, surface disorders, cases with serology positive for HIV and hepatitis B were excluded from the study.

All the patients with primary pterygium who attended ophthalmology department were selected. Written informed consent was taken. Thorough examination of the patient in terms of visual acuity, slit – lamp examination of anterior segment, posterior segment examination by direct and indirect ophthalmoscopy was done. Data was collected and patients were ruled out according to exclusion criteria. Before surgery the blood sample for random blood sugar, viral markers were sent.

The patients were randomly divided into two groups. All the surgeries were done by a single surgeon.

Group A – had undergone pterygium excision+sutured conjunctival limbal autograft under local anesthesia.

Group B – had undergone pterygium excision+autologous conjunctival limbal autograft under local anesthesia.

The preoperative and post operative K1 and K2 values and best corrected visual acuity (BCVA) was noted. Follow up was done on day 1 (D1), day 7 (D7), day (D14), 1 month, 3 months.

On each visits patients were examined for watering, foreign body sensation (Fb), pain and graft stability.

Operative procedure

Eye was painted with 5% povidone iodine and draped. Patient was anesthetized by infiltration of 2% xylocaine. Then the small conjunctival incision on neck of pterygium was made and dissected close to limbus separation of underlying subconjunctival growth was done. The tenon's capsule was dissected. Removal of the pterygium was done. A thin tenon's free conjunctival autograft was taken from superiotemporal quadrant. Gentian violet was used to outline the conjunctiva to be harvested measure by Castro Viejo caliper. The donor area should be 2 mm larger than the bare sclera as the larger graft takes care of graft shrinkage in post operative period.[6] The limbal edge of the graft should be denoted with specific marks in order to identify the edge. The conjunctival auto graft was then harvested by undermining and careful dissection with blunt Wescott scissors. The free conjunctival auto graft was positioned over the area of bare sclera. Maintaining the limbus to limbus and epithelial-side-up configuration keeping the distance of 0.5 mm to 1 mm from limbus to include the limbal stem cells.[7] The autograft was then sutured to the underlying sclera at the limbus and to the cut conjunctival edges with 10–0 Vicryl sutures in group A where as in group B the autograft was positioned over the bare sclera and it gets adhered by autologous blood. The graft harvest site does not require closure and will re-epithelialize on its own. Postoperatively, topical steroids and antibiotics were employed at the rate of 4 times a day, and tapering over 4 weeks depending on degree of inflammation and post-operative course.

Results

Total of 58 eyes were included in study among them 28 was in group A and 30 was in group B. Location of pterygium in each group in which majority of location is Nasal (left eye 8 to 10 o'clock and in right eye 2 to 4 o'clock approximately) with 96.4% and 96.7% in group A and group B respectively.

Table 1. Grading of pterygium

Grading of pterygium	Group A		Group B	
	Number of eyes	Percentage	Number of eyes	Percentage
I	4	14.3	5	16.7
II	18	64.3	21	70
III	6	21.4	4	13.3
Total	28	100	30	100

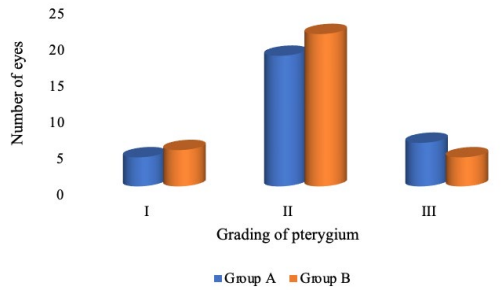

Fig. 1. Grading of pterygium

Table 1 shows the grading of pterygium in each group. The above table shows that 4 (14.3%) of the 28 eyes in group A and 5 (16.7%) of the 30 eyes in group B have grade I, 18 (64.3%) of the 28 eyes in group A and 21 out of 30 eyes in group B have grade II, and the remaining 6 eyes in group A and 4 eyes in group B have grade III (Fig. 1).

Table 2. Grading of pterygium and astigmatism

Grading of Pterygium	Astigmatism in diopter (Mean±SD)					
	Group A			Group B		
	Pre-operative	Post-operative	p	Pre-operative	Post-operative	p
I	2.34±2.2	0.96±0.16	0.002	2.45±0.91	0.94±0.31	0.0008
II	2.51±1.4	1.1±0.18	0.0001	2.81±0.55	1.02±0.25	0.0000
III	6.42±0.81	1.2±1.34	0.0000	6.58±1.73	1.19±1.3	0.0000

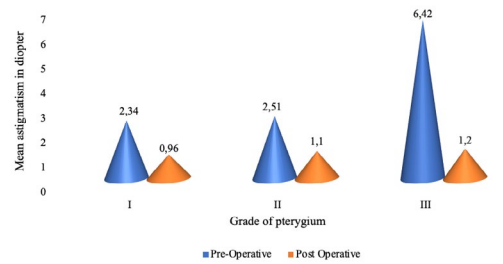

Fig. 2. Grading of pterygium and astigmatism for group A

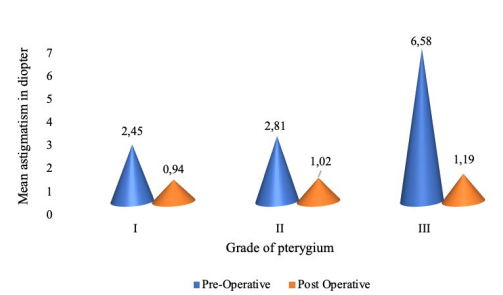

Fig. 3. Grading of pterygium and astigmatism for group B

Table 2 shows the mean astigmatism for each group. The table demonstrates that among patients in group A, the mean astigmatism pre-operatively is 2.34±2.2 diopter (D) and post-operatively 0.96±0.16 D in grade I, 2.51±1.4 D pre-operatively and post-operatively 1.1±0.18 in grade II, and 6.42±0.81 D pre-operatively and post-operatively 1.2±1.34 D in degree III. Pre-operative mean astigmatism in group B members is 2.45±0.31 D and post-operatively 0.94±0.31 D in grade I, 2.81±0.55 D pre-operatively and post-operatively 1.02±0.25 D in grade II, and 6.58±1.73 D pre-operatively and post-operatively 1.19±1.3 D in grade III. This suggests that astigmatism rises with pterygium grading in each group ($p<0.001$). However, for different grades of pterygium, the difference in mean astigmatism in diopters between group A and group B was statistically insignificant ($p>0.05$) (Fig. 2 and 3).

Table 3. Comparison of values of K1 and K2*

Follow Up		K1					K2				
		Group A		Group B		P-value	Group A		Group B		P
		Mean	±SD	Mean	±SD		Mean	±SD	Mean	±SD	
Pre-operative		42.55	1.78	43.45	1.89	0.0676	45.23	1.79	44.33	1.83	0.0631
Post- operative	1 week	43.81	1.63	44.19	1.35	0.3364	46.06	1.82	45.61	1.76	0.3426
	2 weeks	44.09	1.54	44.33	1.41	0.538	46.32	1.84	45.72	1.56	0.1822
	1 month	44.25	1.39	44.21	1.91	0.9281	46.42	1.72	46.78	1.39	0.1236
	3 months	44.51	1.25	44.27	1.12	0.351	46.55	1.66	46.81	1.45	0.5271

* K1 – curvature of cornea in vertical meridian,
K2 – curvature of cornea in horizontal meridian

Table 3 shows difference in vertical meridian between preoperative and post-operative values within each group. Between group A and group B, there was no statistically significant difference in the mean values of K1 at various follow-up times ($p>0.05$). However, in the postoperative follow-up, the values of K1 marginally increased over time.

K1 had pre-operative mean values of 42.55±1.78 for group A and 43.45±1.89 for group B, respectively. For groups A and B, the post-operative at 3 months mean of K1 values were 44.51±1.25 and 44.27±1.12, respectively. This indicates that both group A ($p=0.0001$) and group B ($p=0.0475$) experienced statistically significant steepening of the vertical meridian after surgery. It also shows difference in horizontal meridian between pre-operative and post-operative values within each group. Between group A and group B, there was no statistically significant difference in the mean values of K2 at various follow-up times ($p>0.05$). However, in the postoperative follow-up, the mean values of K2 marginally increased over time.

K2 had pre-operative mean values of 45.23±1.79 for group A and 44.33±1.83 for group B, respectively. For groups A and B, the post-operative at 3 months mean of K2 values was 46.55±1.66 and 46.81±1.45, respectively. This indicates that both group A ($p=0.0051$) and group B ($p=0.0001$) experienced statistically significant steepening of the horizontal meridian after surgery (Fig. 4).

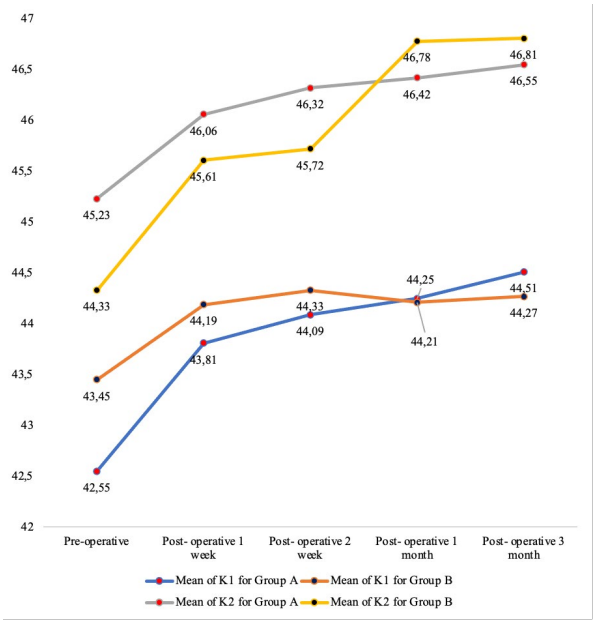

Fig. 4. Comparison of values of K1 and K2

Table 4. Change in mean astigmatism in group A

Follow Up	Number of patients (n=28)	Mean	SD±	p
Pre-operative	28	5	5.8	-
Post-operative 1 week	28	2.1	3.6	0.0249
Post-operative 2 week	26	1.71	1.5	0.0040
Post-operative 1 month	27	1.50	2.1	0.0031
Post-operative 3 month	26	1.18	1.3	0.0009

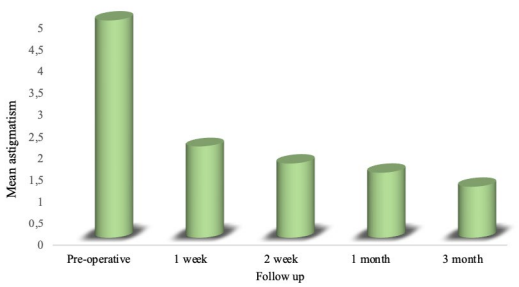

Fig. 5. Change in mean astigmatism in group A

Table 5. Change in mean astigmatism in group B

Follow Up	Number of patients (n=30)	Mean	SD	p
Pre-operative	30	5.11	5.5	-
Post-operative 1 week	30	2.09	3.2	0.0011
Post-operative 2 week	30	1.77	1.4	0.0021
Post-operative 1 month	27	1.33	2	0.0009
Post-operative 3 month	27	1.12	1.1	0.0003

Table 4 shows the change in mean astigmatism in group A after surgery. The Pre operative and post-operative at 3 months mean astigmatism were 5.00±5.8 and 1.18±1.3, respectively. There was statistically significant reduction in mean astigmatism at each post operative follow up at 1 week (p=0.0249), 2 weeks (p=0.004), 1 month (p=0.0031) and at 3 months (p=0.0009). This indicates that group A experienced statistically significant reduction of the mean astigmatism after surgery (Fig. 5).

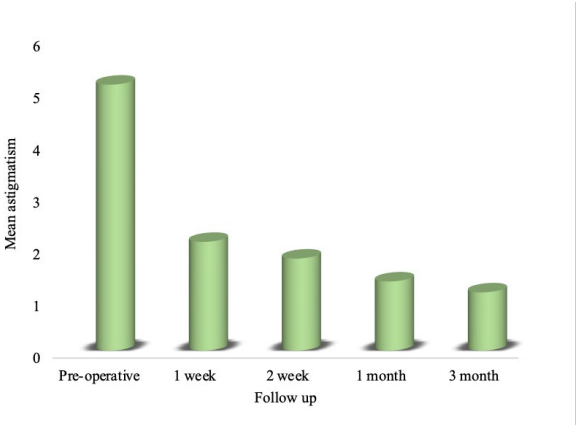

Fig. 6. Change in mean astigmatism in group B

Table 5 shows the change in mean astigmatism in group B after surgery. The Pre operative and post-operative at 3 months mean astigmatism were 5.11±5.5 and 1.12±1.1, respectively. There was statistically significant reduction in mean astigmatism at each post operative follow up at 1 week (p=0.0011), 2 weeks (p=0.0021), 1 month (p=0.0009) and at 3 months (p=0.0003). This indicates that group B experienced statistically significant reduction of the mean astigmatism after surgery (Fig. 6).

Table 6. Comparison of symptoms based on surgery

Symptoms		Pre-operative	Post-operative				Post-operative overall percentage
			1 Week	2 weeks	1 month	3 months	
Pain	Group A	25	20	10	4	1	0.35
	Group B	26	9	1	1	0	0.11
Watering	Group A	24	17	7	2	0	0.27
	Group B	19	6	4	0	0	0.13
FB Sensation	Group A	23	19	6	3	1	0.32
	Group B	22	4	1	1	0	0.069

Table 6 compares symptoms according to the type of surgery. The percentage of eyes that were watering, hurting, or had an FB feeling before surgery was roughly the same in groups A and B. There was a statistically significant difference in the percentage of eyes in groups A and B experiencing pain (p=0.041) and FB (0.034), while the difference in the percentage of eyes in groups A and B experiencing watering was insignificant (p=0.261). There was no graft displacement in group A while it was noticed in one eye of group B.

Figure 7 shows that the comparison of best corrected visual acuity (Log MAR) during follow up. In group A mean BCVA (Log MAR) pre-operatively was 0.24±0.2 while it was 0.18±0.1, 0.14±0.1, 0.11±0.14, and 0.09±0.06 at 1st week, 2nd week,1st month and 3rd

month. An ANOVA test of significance revealed a statistically significant improvement in mean visual acuity (p=0.034) in group A. In group B mean BCVA (Log MAR) pre-operatively was 0.23±0.18 while it was 0.15 ±0.14, 0.12±0.11, 0.1±0.09, and 0.08±0.07 at 1st week, 2ed week,1st month and 3rd month. An ANOVA test of significance revealed a statistically significant improvement in mean visual acuity in group B (p=0.039).

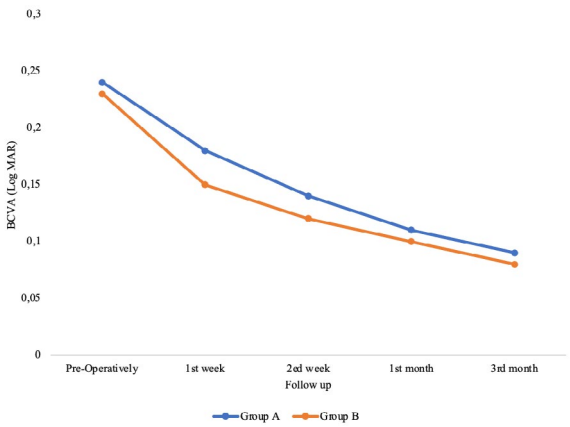

Fig. 7. Comparison of best corrected visual acuity (Log MAR) during follow up

We found that autologous conjunctival autograft has some advantages as compared to sutured conjunctival autograft which are as follows-Availability of patient's own blood, no extra cost, no risk of transmission of blood related diseases, no suture related complications. In comparison to sutured conjunctival autograft there was less watering, pain. Foreign body sensation in autologous conjunctival autograft. Graft stability was more in sutured conjunctival autograft as we found displacement of 1 graft in autologous conjunctival autograft.

Discussion

In our study we found females are more affected than males. Similar findings were found in Asokan et al. and by Tomidokoro et al. in which they stated that pterygium has positive correlation with females.[8,9]

The amount of astigmatism increases with grading of pterygium; the finding has positive correlation with the studies of Payman et al.[10]

Table 7. Amount of astigmatism and grading of pterygium

	Group A	Group B	Muhammad Imran Saleem et al.[11]	Sulman Jaffar et al.[12]
Grade 1	2.34±2.2 D	2.45±0.31 D	1.76±0.52 D	1.165±0.849 D
Grade 2	2.51±1.4 D	2.81±0.55 D	2.46±0.74 D	3.46±1.44 D
Grade 3	6.42±0.81 D	6.58±1.73 D	3.57±1.48 D	5.9±0.265 D

The number of astigmatic changes from pre-operative to post operative value is significant in both group A and group B. It has positive correlation with the following studies (Table 7 and 8).

Table 8. Changes in mean astigmatism pre and post operatively

Mean astigmatism±SD	Group A	Group B	Hetal Kumar Yagnik et al. study[13]	Sejal Maheshwari study[14]	Sejal Maheshwari study[15]	Mohd Yousuf study[16]
Pre-operative	5.0±5.8 D	5.11±5.5	4.23±3.4 D	4.6 ±2.2 D	4.40±3.64 D	4.32± 1.88 D
Post-operative	1.18±1.3 D	1.12±1.1	1.08±1 D	2.2±2.4 D	1.55±1.63 D	2.11±1.96 D

This verifies the statement that pterygium flattens the cornea and increases the horizontal corneal curvature.

There was significant steepening of horizontal and vertical curvature post-operatively in both Group A and group B. Similar findings were seen in the following studies (Table 9).

Table 9. Changes in horizontal and vertical curvature of cornea pre and post operatively

	Group A		Group B		Muhammad Imran Saleem Colleagues study[11]		Hetal Kumar Yagnik et al. study[13]	
	Horizontal meridian (K1)	Vertical meridian (K2)	Horizontal meridian (K1)	Vertical meridian (K2)	Horizontal meridian (K1)	Vertical meridian (K2)	Horizontal meridian (K1)	Vertical meridian (K2)
Pre-operative	42.55± 1.78 D	45.23± 1.79 D	43.45± 1.89 D	44.33± 1.83 D	43.71± 1.12 D	44.9± 1.41 D	42.15± 1.94 D	45.55± 1.62 D
Post-operative	44.51± 1.25 D	46.55± 1.66 D	44.27± 1.12 D	46.81± 1.45 D	44.45± 0.85 D	45.23± 0.78 D	44.41± 1.41 D	46.43± 1.08 D

A statistically significant improvement in mean visual acuity pre-operatively and post-operatively seen in group A and group B was found. Similar findings were seen in Bhandari et al., Zheleva et al., Amoah Kwadwo et al.[17-19] The limitation of this study was its relatively small sample size and short follow up period.

Conclusion

Pterygium surgery decreases the cylindrical error and reverts corneal curvatures to normal thus improving subjective visual acuity. These finding were noted in both sutured CAG and autologous CAG. Astigmatism produced by the pterygium is reduced after surgery. We found that autologous CAG is superior than sutural CAG.

Declarations

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Author contributions

Conceptualization, M.J.Y., R.K. and R.M.; Methodology, R.K.; Software, R.K.; Validation, M.J.Y., R.K. and R.M.; Formal Analysis, R.K.; Investigation, R.K.; Resources, T.; Data Curation, R.K.; Writing – Original Draft Preparation, R.K.; Writing – Review & Editing, R.K., M.J.Y. and A.D.; Visualization, R.K.; Supervision, M.J.Y. and

R.M.; Project Administration, A.D. and T.; Funding Acquisition, R.K.

Conflicts of interest

No potential conflict of interest was reported by the authors.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

Written permissions were obtained from the Ethical Committee of Shri Shankaracharya Institute of Medical Sciences (SSIMS/EC/2022/21). The participants were informed about the purpose, method, and plan of the study by the researchers, and their informed consent were obtained.

References

1. Ang LP, Chua JL, Tan DT. Current concepts and techniques in pterygium treatment. *Curr Opin Ophthalmol.* 2007;18(4):308-313. doi: 10.1097/icu.0b013e3281a7ecbb
2. Brightbill FS. *Corneal Surgery: Theory, Technique and Tissue*. Mosby: Mosby Elsevier; 2009.
3. Rokohl AC, Heindl LM, Cursiefen C. Pterygium: pathogenesis, diagnosis and treatment. *Ophthalmologe*. 2021;118(7):749-763. doi: 10.1007/s00347-021-01366-9
4. Lindsay RG, Sullivan L. Pterygium-induced corneal astigmatism. *Clin Exp Optom*. 2001;84(4):200-203. doi: 10.1111/j.1444-0938.2001.tb05026.x
5. Tan DTH. Effect of Pterygium Morphology on Pterygium Recurrence in a Controlled Trial Comparing Conjunctival Autografting With Bare Sclera Excision. *Arch Ophthalmol.* 1997;115(10):1235. doi: 10.1001/archopht.1997.01100160405001
6. Kirti Nath Jha. ResearchGate. https://www.researchgate.net/publication/275970613_Pterygium_Excision_and_Conjunctival-Limbal_Autograft_Transplantation_A_Simplified_Technique. Accessed September 5, 2023.
7. Shusko A, Schechter BA, Hovanesian JA. Pterygium Surgery Utilizing Limbal Conjunctival Autograft and Subconjunctival Amniotic Membrane Graft in High-Risk Populations. *Clin Ophthalmol.* 2020;14:2087-2090. doi: 10.2147/OPTH.S243584
8. Asokan R, Venkatasubbu RS, Velumuri L, Lingam V, George R. Prevalence and associated factors for pterygium and pinguecula in a South Indian population. *Ophthalmic Physiol Opt*. 2012;32(1):39-44. doi: 10.1111/j.1475--1313.2011.00882.x
9. Tomidokoro A. Effects of pterygium on corneal spherical power and astigmatism. *Ophthalmology.* 2000;107(8):1568-1571. doi: 10.1016/s0161-6420(00)00219-0
10. Mohammad-Salih PA-K, Sharif AFMD. Analysis of Pterygium Size and Induced Corneal Astigmatism. *Cornea.* 2008;27(4):434-438. doi: 10.1097/ico.0b013e3181656448
11. Saleem MI, Saleem Channar M, Saleem MF. Effects of Pterygium Excision on Corneal Curvatures. *Pak J Med Sci*. 2011;27(2):325-328.
12. Jaffar S, Dukht U, Rizvi F. Impact of Pterygium Size on Corneal Topography. *RMJ*. 2009;34(2):145-147.
13. Yagnik H K, Billore O P, Das S, et al. Corneal Topographical Changes after Pterygium Excision Surgery: A Reason to Smile. *All India Ophthalmic Conference Proceedings.* 2008:27-29.
14. Khanna RC, Honavar SG, Metla AL, Bhattacharya A, Maulik PK. Psychological impact of COVID-19 on ophthalmologists-in-training and practising ophthalmologists in India. *Indian J Ophthalmol*. 2020;68(6):994-998. doi: 10.4103/ijo.IJO_1458_20
15. Maheshwari S. Pterygium-induced corneal refractive changes. *Indian J Ophthalmol.* 2007;55(5):383-386. doi: 10.4103/0301-4738.33829
16. Yousuf M. ResearchGate. https://www.researchgate.net/publication/283137779_Role_of_pterygium_excision_in_pterygium_induced_astigmatism. Accessed September 25, 2023.
17. Bhandari V, Rao CL, Ganesh S, Brar S. Visual outcome and efficacy of conjunctival autograft, harvested from the body of pterygium in pterygium excision. *Clin Ophthalmol*. 2015;9:2285-2290. doi: 10.2147/opth.s93580
18. Zheleva V, Voynov L. Comparative study of astigmatic changes following pterygium excision with conjunctival autograft transplantation. *Biotechnology & Biotechnological Equipment*. 2018;32(2):433-436. doi: 10.1080/13102818.2017.1423516
19. Amoah K, Lartey SY, Mohammed AK, Ahmed AS, Arthur KAN, Kyei EN. Changes in Astigmatism and Visual Acuity after Pterygium Excision in the Ashanti Region of Ghana. *Annals of African Surgery.* 2022;19(2):86-93. doi: 10.4314/aas.v19i2.5

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ORIGINAL PAPER

The effect of thiocetam on the parameters of the nitric oxide system under the conditions of the experimental periodontitis and immobilization stress formation

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ABSTRACT

Introduction and aim. The aim of this work is to study the parameters of the nitric oxide (NO) system in the blood of guinea pigs under the conditions of the experimental periodontitis (EP) and immobilization stress (IS) formation and to evaluate the effectiveness of thiocetam use.

Material and methods. Experimental studies were performed on 50 guinea pigs (males, body weight 0.18–0.21 kg) which were divided into five groups (10 in each): the first group were intact animals as control; the second experimental group were animals with experimental periodontitis under conditions of immobilization stress (3rd day), the third group included guinea pigs with EP and IS on the 5th day of the combined model process, group IV – animals with EP and IS 15th day (without administration of thiocetam) and group V – animals on the 15th day of experiment with EP and IS after use of thiocetam.

Results. As a result of this research, changes in the activity of the NO system in the blood were observed, namely an increase in the level of stable metabolites and an increase in the activity of total NO-synthase, which is accompanied by a compensatory inhibition of the L-arginine activity, and these indicators were most pronounced in the late stages of EP and IS formation.

Conclusion. The use of thiocetam showed a corrective effect on the changed variables of NO metabolism in the peripheral blood of guinea pigs under the conditions of the EP and IS development.

Keywords. L-arginine, nitric oxide, periodontitis, stress, thiocetam

Introduction

Generalized periodontitis is a specific dystrophic inflammatory process (reflex neurovascular dystrophy of periodontal tissues) that occurs as a result of the various exogenous and endogenous factors interaction. It is characterized by progressive destruction of periodontal tissues with subsequent tooth loss. In particular, according to WHO data, periodontal disease is found in 80% of children and 95% of adults. It is a social problem that in the most able-bodied population aged 35–44, the preva-

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lence of periodontal lesions reaches 92–98%.[1,2] Progressive generalized periodontitis is one of the main causes of tooth loss among the adult population.[3]

When dystrophic inflammatory diseases of the periodontium occur, the interaction between conditionally pathogenic microorganisms of dental plaque (biofilm) and the patient's body (host) is important in its pathogenesis.[4] Its implementation is largely determined by the immune response of the host organism.[4,5] General somatic diseases, smoking, and psychological stress have a significant impact on this interaction.[6] The impact of stressful factors causes changes in the parameters of the functional activity of the body's physiological systems, including various adaptive changes in the immune system and periodontal tissues.[1,6,7] The degree of stressor tissue damage depends on the features of the neurohumoral mechanisms of tissue metabolism regulation.[6]

The relevance of the problem is due not only to a significant percentage of the periodontal diseases spread among the population worldwide, but also to the insufficiency of early diagnosis and the effective treatment of patients with this pathology.[7] For the early detection of periodontitis, a thorough examination is required, which includes collection of information, examination, probing the depth of the gingival pockets, as well as radiography. Considering the polyetiological nature and complex pathogenesis of generalized periodontitis, complex and multicomponent treatment regimens are used to treat patients.

In particular, Kuvaeva demonstrated the positive effect of the nitric oxide (NO) modulator in the form of arginine gel in the treatment of experimental periodontitis in rats.[8] Bedeniuk showed the corrective effect of a selective inhibitor and -NOS – aminoguanidine, which was used at a dose of 20 mg/kg in rats for 14 days together with the antioxidant lycopene at a dose of 100 mg/kg for generalized periodontitis.[9] Several scientists used L-arginine as a drug with a corrective effect on impaired levels of the nitric oxide system during adrenaline-induced myocardial damage and immobilization stress.[10]

Oleshchuk recommended the application of such modulators for the synthesis of NO:[11]

1. L-arginine, administered intraperitoneally at 25 mg/kg in the form of a 2.5% aqueous solution once a day for 7 days,

2. Glutargin is administered intraperitoneally at 45 mg/kg in an equimolar dose in terms of L-arginine once a day for 7 days,

3. N-nitro-L-arginine methyl ester was administered 10 mg/kg as a 1% solution intraperitoneally once a day for 7 days.

The full range of treatment measures is quite long, expensive and not always sufficiently effective,[4,5] therefore the issue of treatment regarding the expediency of using expert recommendations in the doctors' daily practice is extremely relevant and requires special research.

In recent years, researchers have paid considerable attention to studying the role of NO, which lies in providing it with optimal work of the whole organism. The important role of nitric oxide in the regulation of various body systems has been experimentally demonstrated, therefore it shows that most chronic diseases are directly related to the deterioration of the functional state of systems and organs responsible for the synthesis of NO in the body (blood vessels endothelium, nervous and connective tissue cells – neurons and macrophages).[12]

Bedeniuk established disorders in the microcirculation system in patients with chronic periodontitis with concomitant atrophic gastritis.[9] The direct cause of microcirculatory disorders is endothelial dysfunction, largely dependent on the bioavailability of nitric oxide produced by the endothelial form of NOS. In inflammatory lesions, hypersecretion of inducible NO usually occurs. It leads to the production of excess NO, which can play the role of an important effector in the development mechanisms of periodontal tissue inflammation. In addition, it has been shown that during periodontitis, so-called nitrooxidative stress develops, in which nitric oxide interacts with superoxide anion, resulting in the formation of peroxynitrite. The latter is associated with the altering effect of NO on biological macromolecules. Especially on proteins and lipids, which in turn causes an imbalance in the processes of inactivation of reactive oxygen forms, which leads to disruption of the structure and function of cell membranes and ends in cell death and the development of inflammation.[13]

Our previous studies also revealed the development of oxidative stress and an imbalance of cytokine status in experimental periodontitis (EP) and immobilization stress (IS). Savelieva showed that NO is a factor initiating and maintaining the development of inflammation in periodontal tissues in patients with chronic generalized periodontitis, and changes in the nitric oxide system play a significant role in the mechanisms of periodontitis formation.[14]

Thus, NO can be considered as one of the most important regulators of the body's general adaptive capabilities, which ensures its optimal adaptation to external influences of various character and, as a result, maintenance of the necessary level of health in general.[15] Almost every extreme state of the body and pathological process is directly or indirectly related to the multifunctional characteristics of NO. Therefore, further study of the nitric oxide effects in medicine and, through the process of research, obtaining new scientific facts about the peculiarities to the biosynthesis of this compound and how its molecular and biochemical effect will allow to develop a new strategy and tactics for correcting and treating pathologies of various genesis.[16]

It is known that the production of NO by macrophages under periodontal inflammation stimulates the phagocytosis reaction together with other radicals, that is, NO can also perform a useful function in periodontitis, acting as a non-specific factor of protection against bacteria.[17] At the same time, NO deficiency contributes to the reproduction of pathogens in the periodontal tissues, which leads to this pathological process developing into chronical. However, a sharp increase in the level of NO plays a negative role, as it initiates a number of free radical mechanisms that cause the destruction of tissues, including periodontal connective tissue.[18]

Proceeding from the above, it became necessary to carry out the pharmacological correction of the revealed disturbed metabolic processes concerning the nitric oxide system in EP and IS with the help of the drug thiocetam, which belongs to the group of cerebroactive agents, possessing nootropic, anti-ischemic, antioxidant, membrane-stabilizing properties, as well as improving the rheological properties of blood due to the activation of the fibrinolytic system, it stabilizes and reduces areas of necrosis and ischemia and eliminates the stress consequences.[19,20]

It is known that thiocetam has as its main active substance a morpholinium salt of tiazotic acid (1 mL of a solution containing morpholinium salt of tiazotic acid) in terms of 100% of the substance – 25 mg, which is equivalent to 16.6 mg of tiazotic acid, and piracetam – 100 mg. It should be emphasized that thiocetam contains two components: the first, piracetam, is an antioxidant, that stabilizes cell membranes, stimulates alternative metabolism pathways in hypoxia, and improves microcirculation. The second component is thiotriazolin, which has antioxidant, membrane-stabilizing, immunomodulating, cytokine-correcting, and anti-inflammatory properties, and is hepatoprotective and cardioprotective.

The positive antioxidant and immunocorrective effect of thiotriazolin was established by Shchepanskyi and Reheda in guinea pigs with bronchial asthma and experimental periodontitis and pneumonia.[21,22]

To date, the influence of thiocetam on disturbed indicators of the NO system in EP and IS is unknown. But taking into account the thiotriazolin component of this drug and its wide mechanism of action, which is covered in the article, it is possible to express an opinion about the mediated effect of thiocetam on the specified markers of the nitric oxide system in EP and IS.

The mechanisms underlying the stress impact on the periodontitis pathogenesis, the specifics of the combined pathology course, still require a more detailed study. In particular, the assessment of the nitrooxidative stress intensity, which to a certain extent determines the manifestation of resorptive processes in the connective tissue, becomes important both in studying the formation mechanisms and in correcting the periodontitis treatment.

Aim

The aim of the work is to study the parameters of the nitric oxide system in the blood of guinea pigs under the conditions of the EP and IS formation and to evaluate the effectiveness of the thiocetam use.

Material and methods

Experimental studies were performed on 50 guinea pigs (males), body weight 0.18–0.21 kg, which were divided into five groups (10 in each): the first were intact animals as control; the second (experimental) group were animals under the conditions of experimental periodontitis and immobilization stress development (3rd day), the third group included guinea pigs with EP and IS on the 5th day of the combined model process, group IV – animals with EP and IS 15th day (without using of thiocetam) and group V – animals on the 15th day of experiment with EP and IS after the use of thiocetam.

It is known that guinea pigs serve as a classic object for modeling inflammatory and allergic processes. Therefore, these animals were used to reproduce this experimental model of disease and stress.

Fixed days were chosen: the 3rd, 5^{th,} and 15th day for the experiment under conditions of the development of EP and IS separately and in their combination before and after treatment with thiocetam, which corresponded to the stages of the acute inflammatory response, which included the development of the disease (3rd), the height of the disease (5th), convalescence (15th day) and stages of stress: the 3rd day corresponded to the anxiety stage: the 5th day – the stage of resistance and the 15th day – the stage of exhaustion.

Experimental periodontitis was modeled by the method of Jogan, which consisted of animals that were on a diet that included 1 g of dry lyophilized cattle liver, 10 g of dry skimmed milk, and 20 g of crackers.[23] The diet is designed for one guinea pig per day. Morphologically, the presence of periodontitis in animals was not confirmed, since this is a long-known, tested experimental model.

Immobilization stress was reproduced by the method of Horizontov.[24] We selected fixed days (3$^{rd.}$, 5th and 15th) for studies that corresponded to the classic stages of acute inflammation. To correct disorders in group V, the drug thiocetam was administered at a rate of 250 mg/kg intramuscularly from the 6th day of the experiment for 10 days. The application of the specified dose of thiocetam was based on experimental scientific studies, which used thiocetam at 250 mg/kg of body weight once a day for the treatment of cranioskeletal trauma complicated by blood loss with functional and morphological disorders of the liver in rats with different resistance to hypoxia and established the effect and improvement of endogenous intoxication and cytolysis indicators, reduced dystrophic-necrotic processes in the liver.[19,20]

They also took into account the fact that thiocetam has the main active ingredient morpholinium salt of tiazotic acid, which is similar to that contained in thiotriazolin, and the latter is used in various doses of 100–250 mg/kg of body weight in various inflammatory and allergic processes.[21,22,25]

All experiments on laboratory animals carried out with following the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), Council Directive 2010/63/EU, the Law of Ukraine 3447- IV "Protection animals from the cruelty," the general ethics of animal experimentation adopted by the first national Congress on bioethics in Ukraine. The study protocol was approved by the Ethical Committee of Danylo Halytsky Lviv National Medical University (protocol No 35; 05.10.2022).

Activity of NO synthase (NOS) was detected by VV Sumbaev method.[26] The total activity of nitric oxide synthase was determined by the intensity of NADPH·H^+ use in a reaction medium containing 0.6 mL of 5 mM KH_2PO_4, 0.6 mL of 1 mM $MgCl_2$, 0.6 mL of 10 mM $CaCl_2$ in Tris-HCl buffer pH=7.4, 0.6 mL of 4 mM aqueous solution of L-arginine, 0.4 mL of 1.0 mM NADPH·H^+ solution. The reaction was started by adding 0.3 mL of experimental biopsy material (tissue homogenate, erythrocyte hemolysate) to the reaction mixture. The control tube contained a similar set of reagents, except for a solution of L-arginine, instead of which 0.6 mL of distilled water was added. The reaction was stopped by adding 8 mM $HClO_4$ solution to the reaction mixture.

A decrease in the absorbance of solutions was recorded at a wavelength of 340 nm. Nitric oxide synthase activity was expressed as nmol of NADPH·H^+, which was oxidized within 1 minute per 1 mg of protein.

L-arginine content in the blood serum was determined by the Aleinikov method.[27] Up 0.5 mL of a 5% trichloroacetic acid solution was added to 0.5 ml of blood serum and centrifuged for 10 minutes at 3000 rpm. 0.5 mL of the supernatant was taken and 1 mL of a 5% NaOH solution, 0.05 ml of a 0.02% alcohol solution of α-naphthol, 0.05 ml of a hypobromide reagent, and 0.2 mL of a 10% urea solution were added and made up with distilled water up to 4 mL. After 20 minutes, they were evaluated by spectrophotometry using at l=500 nm. The experimental sample and control were evaluated by spectrophotometry against distilled water. The control contained the same reagents as the experiment; instead of serum, distilled water was added. Arginine concentration was determined using a pre-constructed calibration graph.

Stable NO metabolites were determined by the Schmidt protocol.[28] The content of total NO products in the studied biological samples was determined using the Griess reagent, spectrophotometrically measuring the staining products at wavelengths l=550 nm. From the measured optical density values, the average value was found and the concentration of stable nitric oxide products was determined using a pre-constructed calibration curve.

This method was carried out as follows: 0.2 mL of the test sample was placed in a centrifuge tube, and 0.2 mL of 4% sodium hydroxide solution was added and incubated, stirring, in an ice bath for 10 minutes. After this, 0.4 mL of distilled water and 1.2 mL of a 4% solution of zinc sulfate were added and kept in a water bath with ice. In 10 min. centrifuged for 20 min. at a temperature of 0±4 °C at a speed of 15000 rpm. To 1.4 mL of the selected supernatant was added 1.4 mL of Griess reagent (1:1), which included: 0.1% N-(naphthyl)ethylenediamine hydrochloride and 1% sulfanilic acid, prepared in 5% orthophosphoric acid. The sample with the added reagent was placed for 15 min. in a dark place for color development, then absorbance was measured using a spectrophotometer at l=550 nm.

The control is an 8% protein solution, processed according to the experimental method. Recalculation was carried out according to the calibration graph obtained with standard solutions with a concentration of total nitric oxide metabolites from 1 to 250 μmol/L.

All numerical results were subjected to statistical processing using the arithmetic mean (M), error of the arithmetic mean (m), and Student's test. Calculations were performed using statistical and graphical analysis tools for Microsoft Excel spreadsheets in the Microsoft Office software package.

Results

When studying the activity of stable metabolites, total NOS (endothelial and inducible) and L-arginine in the blood of guinea pigs with EP and IS, it was recorded that at all stages of their formation there were likely changes in variables compared to the control group. Having compared the variables of nitric oxide metabolism in guinea pigs with EP and IS between different groups of animals, we also found shifts in all the indicators we had determined in different periods of its formation.

In the dynamics of EP and IS formation, it was noticed that stable metabolites grow practically linearly already at the initial stage and reach their maximum at the latest term of the experiment. When comparing the groups, we observe that the studied indicator is almost at the same level on the 5th day compared to the 3rd day of the experiment ($p_1 \leq 0.05$). Subsequently, the level of stable metabolites increases by 7.2% ($p_1 \leq 0.05$) on the 15th day of EP and IS compared to the second group (Table 1).

When studying the activity level of total nitric oxide synthase (endothelial and inducible) in the blood of guinea pigs with EP and IS, an increase in its level was found (on 3rd, 5th and 15th days), both in comparison with the control group and between different groups of animals. There is a significant increase in nitric oxide synthase in all studied days compared to the group II of animals: by

10% ($p_1 \leq 0.05$) and 11.8% ($p_1 \leq 0.05$) on the 5th and 15th days respectively, under EP and IS (Table 1, Fig. 1). These changes lead to excessive formation of nitric oxide and its active derivatives in the blood, which, in turn, causes inhibition of enzyme function, DNA damage, activation of free radical processes, i.e., in high concentrations, initiates the processes of oxidative and nitrosative stress.

Table 1. The parameters of the nitric oxide system in the blood of guinea pigs under the conditions of the EP and IS formation. (M ± m, n=40)[a]

Form of experiment	Duration of experiments in days	Number of animals	Stable metabolites NO mmol/l	Total activity NOS, nmol NADPH(H+) / (min/ml)	L-arginine mg/ml
Intact animals, control		10	17.1±2.4*	0.62±0.11*	41.3±5.0*
Guinea pigs with EP and IS	3	10	27.7±3.2*	1.1±0.02*	18.4±4.4*
	5	10	28.5±3.3*	1.21±0.03*	17.3±3.2*
	15	10	29.7±3.4*	1.23±0.03*	15.2±3.4*

[a] * – changes are likely with respect to the values of the control group (p≤0.05)

For a more complete assessment of the nitric oxide system parameters, the level of L-arginine activity was studied and its regression in the dynamics of EP and IS formation was found (Fig. 1).

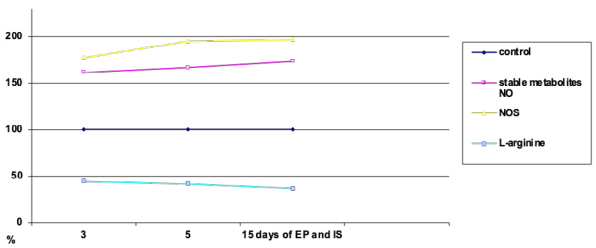

Fig. 1. The level of nitric oxide system indices in guinea pigs' blood in the EP and IS formation dynamics (% of control)

L-arginine is one of the most versatile metabolic amino acids which is a precursor to the synthesis of polyamines, proline, glutamate, creatine, agmatine, and urea and is an important component of the metabolic processes of maintaining optimal nitrogen balance in the body, as it is a precursor of NO and participates in transportation and detoxification excess NO in the body. The part of L-arginine that was not subject to metabolism is used as a substrate for NO production. Under physiological conditions, synthesis of NO from L-arginine occurs with the help of NO-synthase enzymes, and the main supplier of endogenous arginine is protein metabolism in the body. NO in the brain acts as a neurotransmitter, in the work of the immune system – as a mediator of the immune response.[16,29]

As a result of our work, it was noted that the level of the studied indicator decreased in comparison between groups of animals. Thus, on the 5th day of the development of the combined model process, L-arginine was reduced by only 6.0% ($p_1 \leq 0.05$) compared to the 3rd day of EP and IS. Later, on the 15th day, a similar pattern was noted: a gradual decline of L-arginine by 17.4% ($p_1 \leq 0.05$), respectively, against the second group of animals (Table 1, Fig.1).

Administration of thiocetam at the rate of 250 mg/kg intramuscularly from the 6th day of the experiment for 10 days led to a partial correction of the variables of the nitric oxide system, namely a decrease in the content of stable metabolites by 35.3% ($p_1 \leq 0.05$) and total activity NOS by 42.6% ($p_1 \leq 0.05$) and a rather significant increase in the content of L-arginine in the blood by 81.5% ($p_1 \leq 0.05$) compared to the group of guinea pigs with EP and IS before therapy (Fig. 2), which indicates a positive corrective effect of this medicinal product on the tested tests.

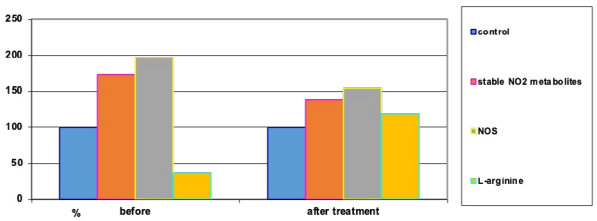

Fig. 2. The effect of thiocetam on the level of the parameters of the nitric oxide system in guinea pig blood under EP and IS formation dynamics

As a result of the research, changes in the activity of the NO system in the blood were observed, namely an increase in the level of stable metabolites and an increase in the activity of total NO-synthase, which is accompanied by a compensatory inhibition of the L-arginine activity, and these indicators were most pronounced in the late stages of the EP and IS formation.

Discussion

NO is a unique molecule for biological organisms, and has also very important effects on various pathologic and physiologic mechanisms. Many cell types can generate NO during different pathological and physiologic conditions. Recently, many authors have been reported the connections between NO and inflammatory diseases. Periodontal pathologies are characterized with specific periodontal inflammation and host tissues damage around the teeth, and additional factors such as force application on teeth. However, the production of NO and the role of NO in the development of periodontal pathologies are unclear. On the other hand, many evidences from previous studies indicates a close relationship between NO and periodontal pathologies.[30]

Inflammatory damage also usually involves overexpression of the NOS inducible form, which results in the production of excessive amounts of NO. This may play an important effector role in the inflammation mechanisms generated by bacterial endotoxin. Thus, the so-called nitrooxidative stress develops along with the inflammation.[9]

We have established that EP associated with IS causes such pathophysiological features of disorders of the nitric oxide system, which consist in a gradual progressive increase in the indicators of stable NO metabolites and total NOS activity against the background of a decrease in the L-arginine content, most pronounced on the 14th and 24th century, period of the experiment, corresponding to the stages of the inflammatory process (height and recovery) and the stage of stress (resistance and exhaustion) relative to the control group, which may indicate activation of the inflammatory process in the periodontium. The decrease in L-arginine content is explained by its excessive consumption for the synthesis of nitric oxide during EP and IS. In our opinion, changes in NO levels during periodontitis and stress are associated with impaired microcirculation in periodontal tissues, the influence of hypoxia and the inflammatory process, and the action of pro-inflammatory cytokines on them, which have been proven in several scientific studies.[9,21]

Thiocetam acts as an antioxidant and anti-ischemic agent, helps to improve microcirculation, normalizes bioenergetic processes, increases the body's resistance to hypoxia, inhibits the formation of reactive oxygen species, improves the rheological properties of blood by activating the fibrinolytic system, stabilizes and reduces areas of necrosis and improves cytolysis rates, endogenous intoxication; reduces degenerative processes in the liver during injury against the background of blood loss.[19,20]

This drug, by correcting the above pathological processes, can indirectly have a positive effect on impaired indicators of the NO system, since its use involves not only studying the effect on markers of the NO system but also elucidating its effect on immune indicators, lipid peroxidation processes, cytokine status, proteolysis processes in EP and IS.

Thiocetam was started to be administered from the 6th day of EP and IS, since during this period the disturbances of metabolic and immune processes were most pronounced, as established in our previous studies (development of oxidative stress, increase in the level of anti-inflammatory cytokines against the background of a decrease in anti-inflammatory cytokines, inhibition of cellular under conditions of stimulation humoral immunity, proteolysis processes against the background of inhibition of antiprotease potential), which dominated on the 5th, and especially on the 15th day of the experiment.

Conclusion

Under the conditions of the experimental periodontitis and immobilization stress development, a multidirectional vector of changes in the metabolism variables of the NO system is observed – an increase in the content of stable nitric oxide metabolites and the total activity of nitric oxide synthases against the background of the decrease in the L-arginine concentration, which were most pronounced on the 15h day of the experiment. This indicates the production of an excessive amount of NO, which can play the role of an important effector in the mechanisms of inflammation generated by bacterial endotoxin. Increased synthesis of NO can exhibit a cytotoxic effect associated with the formation of peroxynitrite, leading to necrosis or apoptosis, i.e. during inflammation, the so-called nitrooxidative stress develops, which will lead to the progression of the disease.

The use of thiocetam showed its corrective effect on the changed variables of NO metabolism in the peripheral blood of guinea pigs under the conditions of the EP and IS development.

Declarations

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Author contributions

Conceptualization, M.R. and P.O.; Methodology, M.R.-F.; Software, S. R.; Validation, S.R., P.O. and M.K.; Formal Analysis, L.F.; Investigation, P.O.; Resources, V.F.; Data Curation, M.R.-F.; Writing – Original Draft Preparation, S.R.; Writing – Review & Editing, P.O.; Visualization, M. K.; Supervision, M. R.; Project Administration, M.R.; Funding Acquisition, P.O., L.F., M.R.-F., M.K.

Conflicts of interest

The authors declare no competing interests.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

Ethics approval

The study protocol was approved by the Ethical Committee of Danylo Halytsky Lviv National Medical University (protocol No 35; 05.10.2022).

References

1. Kononova O. The effectiveness of the treatment of patients with exacerbative course of generalized periodontitis with manifestations of psychoemotional stress. *Actual Dentistry.* 2020;2:24-28. doi: 10.33295/1992-576X-2020-2-24
2. Kononen E, Gursoy M, Gursoy UK. Periodontitis: A Multifaceted Disease of Tooth-Supporting Tissues. *Journal Clin Med*. 2019;8(8):1135. doi: 10.3390/jcm8081135
3. Holde GE, Oscarson N, Trovik TA, Tillberg A, Jönsson B. Periodontitis prevalence and severity in adults: a cross-sectional study in Norwegian circumpolar communities. *J Periodontol.* 2017;88:1012-1022. doi: 10.1902/jop.2017.170164

4. Sanz M, Marco Del Castillo A, Jepsen S, et al. Periodontitis and cardiovascular diseases: consensus report. *J Clin Periodontol.* 2020;47:268-288. doi 10.1111/jcpe.13189
5. Ravidà A, Rodriguez MV, Saleh MHA, et al. The correlation between history of periodontitis according to staging and grading and the prevalence/severity of periimplantitis in patients enrolled in maintenance therapy. *J Periodontol.* 2021;92(11):1522-1535. doi: 10.1002/JPER.21-0012
6. Tarasenko LM, Bilets MV. Mechanisms of oral cavity tissues stress damages (a brief review of the literature and our own research). *Ukrainian Dental Almanac.* 2016;1(2)23:23-26.
7. Kononova O. Pathistological investigation of the influence of the adrenoblocator complex on regeneration of the mandible bone. *Actual Dentistry*. 2018;2:36-38. doi: 10.33295/1992-576X-2018-3-36-39
8. Borysenko AV, Kuvayev AS, Kononova OV, Palamarchuk SI. Correction of nitricoxide metabolism in the complex therapy of patients with generalized periodontitis. *Int J Med Dentistry*. 2016;6(2)6:84-87.
9. Bedeniuk OS, Korda MM. Role of oxidative and nitrooxidative stress in pathogenesis of periodontitis on the chronic gastritis background. *Medical and Clinical Chemistry.* 2016;18(4):11-15. doi: 10.11603/mcch.2410-681X.2016.v0.i4.7248
10. Lys OB, Reheda MS, Sementsiv NG, Reheda-Furdychko MM, Reheda SM. Peculiarities of disorders of the nitrogen oxide system in the blood at adrenalin-induced myocardial injury in conditions of immobilization stress and their correction by L-arginine. *ScienceRise: Pharmaceutical Science.* 2021;4(32):24-28. doi: 10.15587/2519-4852.2021.238320.
11. Oleschuk OM. The impact of modulators of nitric oxide synthesis on biochemical indices of liver in rats. *Fiziolohichnyi zhurnal*. 2014;60(2):57-62. doi: 10.15407/fz60.02.057
12. Yan Wang, Xiangdao Huang, Fuming He. Mechanism and role of nitric oxide signaling in periodontitis. *Exp Ther Med*. 2019;18(5):3929-3935. doi: 10.3892/etm.2019.8044
13. Zubachyk VM, Yarychkivska NV. The role of nitric oxide in periodontal tissue homeostasis (review of the references). *Bukovyna Med Bull.* 2016;20(2):194-198.
14. Saveleva N. Nitrogen oxide as factor initiating and keeping development of inflammation inparodonta fabrics at patients with chronic generalized periodontitis, severity level I-II in combination with parasitosis. *Exp Clin Med*. 2015;69(4):151-153.
15. Svyrydova NK, Zhhilova NO. Use of L-arginine in the treatment of comorbid pathology in neurological patients. *East European Journal of Neurology*. 2016;4-8. doi: 10.33444/2411-5797.2017.1(13).4-8
16. Sadlyak OV, Baida ML, Kolishetska MA, Musij-Sementsiv KhG, Sementsiv NG, Lesyk RB. Nitric oxide as the main multifunctional regulator of immunocompetent and endothelial cells. *Biopolymers and Cell*. 2017;33,5:323-334.
17. Regeda-Furdychko MM. The influence of thiotriazoline on the indicators of nitric oxide under the condition of experimental contact dermatitis and experimental pneumonia. *Journal of Education, Health and Sport.* 2020;10(2):35-40. doi: 10.12775/JEHS.2020.10.02.004
18. Arana C, Moreno-Fernández A M, Gómez-Moreno G, et al. Increased salivary oxidative stress parameters in patients with type 2 diabetes: relation with periodontal disease. *Endocrinol Diabetes Nutr.* 2017;64:258-264. doi: 10.1016/j.endinu.2017.03.005
19. Aslan T, Turer A, Joseph H. Pathogenesis of myocardial ischemia-reperfusion injury and rationale for therapy. *Am J Cardiol*. 2010;106(3):360-368. doi: 10.1016/j.amjcard.2010.03.032
20. Sikirynska DO, Hudyma AA, Pokhodun KA, Antonyshyn IV. Influence of cranioskeltal trauma, complicated with blood loss, on the bile-forming function of liver during the early period of traumatic disease among rats with different resistance to hypoxia and its correction. *Journal of Education, Health and Sport.* 2021;11(1):326-337. doi: 10.12775/JEHS. 2021.11.1.032
21. Reheda M, Shchepanskyi B. Changes of lipoperoxidation and antioxidant system in periodontal tissues in experimental bronchial asthma under conditions of chronic periodontitis and correction of these changes with thiotriazoline. *Journal of Education, Health and Sport*. 2018;8(4):470-478. doi: 10.5281/zenodo.1250463
22. Regeda SM. The role of disorders of immune processes in the pathogenesis of the formation of periodontitis on the background of experimental pneumonia and their correction tetrazolium. *Journal of Education, Health and Sport.* 2017;7(1):350-358. doi: 10.5281/zenodo.268001
23. Gorizontov PD, Belousov OI, Fedotov MI. Stress and blood system. *Medicine.* 1983:338.
24. Ozhogan Z.R. Improvement of orthopedic methods in the complex treatment of periodontal diseases. *Visnyk Stomatolohii.* 1995;1:41-42.
25. Regeda-Furdychko MM. The influence of thiotriazoline on the indicators of nitric oxide under the condition of experimental contact dermatitis and experimental pneumonia. *Journal of Education, Health and Sport*. 2020;10(2):35-40. doi: 10.12775/JEHS.2020.10.02.004
26. Sumbaev VV, Yasinskaya IM. Influence of DDT on activity of nitric oxide synthase in the rats' liver, lungs and brain. *Contemporary Problems of Toxicology*. 2000;3:3-7.
27. Aleinikova TL, Rubtsova GV, Pavlova NA. Guide to practical classes in biochemistry. *Medicine*. 2000;128.
28. Schmidt HHW. Determination of Nitrite and Nitrate in Culture Media. *Acta Biochemica*. 1995;2:323-327.
29. Kai Kang, Xiao-liang Shu, Jing-xia Zhong. Effect of L-arginine on immune function: a metaanalysis. *Asia Pac J Clin Nutr.* 2014;23(3):351-359. doi: 10.6133/apjcn.2014.23.3.09.
30. Balci N, Kurgan Ş, Çekici A, Çakır T, Serdar MA. Free amino acid composition of saliva in patients with healthy periodontium and periodontitis. *Clin Oral Investig*. 2021;25:4175-4183. doi: 10.1007/s00784-021-03977-7

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ORIGINAL PAPER

ChatGPT in hospital infection prevention and control – assessing knowledge of an AI model based on a validated questionnaire

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ABSTRACT

Introduction and aim. An AI model like ChatGPT is a good source of knowledge. We can explore the potential of AI models to complement the expertise of healthcare professionals by providing real-time, evidence-based information in infection prevention and control (IPC).

Material and methods. This study involved 110 queries related to IPC, validated by subject experts in IPC. The responses from ChatGPT were evaluated using Bloom's taxonomy by experienced microbiologists. The scores were divided as <3 as being a poor response, 3–4 as an average response, and >4 as a good response. Statistical analysis was done by correlation coefficient and Cohen's Kappa.

Results. The overall score was 4.33 (95% CI, q1 3.65–q3 4.64) indicating ChatGPT's substantial IPC knowledge. A good response (i.e.>4 score) was found in 70 (63.6%) questions, while in 10 (9%) questions, it showed a poor response. The poor response was seen in needle stick injury and personal protective equipment (PPE) doffing-related questions. The overall correlations were found to be significant. Cohen's Kappa confirmed moderate to substantial agreement between evaluators.

Conclusion. ChatGPT demonstrated a commendable understanding of IPC principles in various domains and the study identifies specific instances where the model may require further refinement especially in critical scenarios such as needlestick injuries and PPE doffing.

Keywords. artificial intelligence, ChatGPT, infection control, large language model, medical education

Introduction

According to a WHO 2022 report, out of every 100 patients in acute-care hospitals, seven patients in high-income countries and 15 patients in low- and middle-income countries acquire at least one healthcare-associated infection (HAI) during their hospital stay. On average, 1 in every 10 affected patients die from their HAI. Infection prevention and control measures play a vital role in maintaining patient safety within healthcare settings. Various studies have found knowledge gaps in healthcare workers regarding IPC.[1]

Many artificial intelligence (AI) models have come out as good knowledge models. AI models like ChatGPT, an advanced language model trained on a vast array of data, are capable of generating human-like responses to a wide range of queries. Several studies have been done to check knowledge of these AI models in various healthcare fields.[2-4]

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Aim

This study aimed to assess the knowledge of the AI model ChatGPT using a validated questionnaire in the context of hospital infection prevention and control. By evaluating its understanding and ability to provide accurate information related to IPC, we can explore the potential of AI models to complement the expertise of healthcare professionals by providing real-time, evidence-based information in infection prevention and control.

Material and methods

This cross-sectional study was conducted using the ChatGPT AI model (generation 3.5). A series of 110 higher-order reasoning queries were posed to ChatGPT, covering various learning objectives in IPC, such as hand hygiene, HAI prevention, injection safety, antimicrobial resistance, biomedical waste management, environmental cleaning, and disinfection, employee immunization status, high-risk areas, standard precautions, bundle care approach, and central sterile supply department (CSSD).

To ensure the validity and bias of the questionnaire, it was rigorously validated by four subject experts with extensive experience in IPC. The first response generated by ChatGPT for each question was collected and stored in an MS Word file for further analysis.

The collected responses were quantitatively evaluated by three authors of this study. Authors of the paper are experts in the field and intimately familiar with the study's objectives, methodologies, and context. This familiarity can contribute to a nuanced evaluation. Also, the authors have a deep understanding of the intricacies of the study, allowing for a more contextually informed evaluation. The authors were blind to each other's evaluation and to decrease further bias, correlation coefficient analysis was performed. The evaluators gave zero to five marks to each question. The scores obtained were stored in an MS Excel sheet for analysis.

In this study, the answers generated by ChatGPT were categorized into specific levels of Bloom's taxonomy. Bloom's taxonomy is a hierarchical model that classifies educational learning objectives based on complexity and specificity. Taxonomy comprises six domains ranging from lower to higher levels of cognitive processes: knowledge, comprehension, application, analysis, synthesis, and evaluation. The assessment determined the specific Bloom taxonomy group, to which the answers from ChatGPT belonged.

Statistical analysis

The data was analyzed using software Jamovi version 2.4.5, including numbers, means, medians, standard deviations, and quartiles. Correlation coefficient between different evaluators was measured. Cohen's Kappa was employed to validate the reliability of the evaluators' domain categorizations. $p<0.05$ was taken as significant.

Results

A total of 110 higher-order reasoning queries were posed to the ChatGPT model, all the questions were given marks on a scale from zero to five.

A section-wise score is given in Table 1.

Table 1. Section wise score

No.	Section name	Median score	Lower CI*	Upper CI
1	Hand hygiene	4.42	3.48	4.79
2	Standard and transmission-based precautions	4.58	3.88	4.78
3	Personal protective equipment (PPE)	4.17	3.52	4.41
4	Hospital acquired infection	4.25	3.37	4.79
5	CSSD	4.67	3.95	4.85
6	Injection safety needle stick management	4.33	3.05	4.52
7	Antimicrobial stewardship program	4.42	3.97	4.67
8	High risk areas	4.33	4	4.64
9	Infection control policy	4.17	3.68	4.45
10	Disinfection and environmental cleaning	4.08	3.65	4.59
11	Bundle care approach	4.25	3.64	4.39

*CI= Confidence interval

The overall score was found to be 4.33 (95% CI, q1 3.65–q3 4.64) out of five.

We divided the scores as <3 as poor a response, 3-4 as an average response and >4 as a good response. The result of 110 questions was:

Response	Number of questions
Poor	10
Average	30
Good	70

Table 2. Some questions asked to ChatGPT

Questions	Average marks	Answer domain
If a nurse gets a needle stick injury, what first aid she should take?	2.3	Knowledge
What types of tests are available to detect blood-borne viruses, list all?	4.5	Knowledge
Which parameters determine hepatitis B virus (HBV) infectivity?	3	Knowledge
How we improve hand hygiene compliance among nurses?	4.8	Knowledge
What are the infection control requirements to establish a 4-bed medical Intensive care unit (ICU)?	4.3	Application
How to prevent bacterial contamination of red blood cells (RBC) units in blood banks?	4.6	Application

In hand hygiene questions, such as "How can we improve hand hygiene compliance among doctors?" the provided answer included points such as education and training, leadership commitment, awareness campaigns, easy access to hand hygiene facilities, peer support and accountability, continuous quality improvement, celebrating success, and patient and family involvement. The score given to this answer is 4.8 (Table 2). In questions

related to standard and transmission-based precautions, like "What standards should be maintained in a negative pressure room?" the answer encompassed points such as airflow and pressure, per hour air exchange, filtration, room integrity, directional airflow, and monitoring and maintenance, earning a score of 4.6.

In the HAI section, the question asked was, "What is the infectivity period of measles?" The answer provided was, "The infectivity period of measles refers to the timeframe during which an individual with measles can transmit the virus to others. The infectivity period typically lasts for approximately 4 days before the rash develops until 4 days after the rash appears," resulting in a score of 4.2.

In the CSSD section, the question was, "How can we maintain the air quality of CSSD?" The answer outlined points such as the Heating, Ventilation, and Air Conditioning (HVAC) system, positive pressure, air filtration, segregated airflow, ventilation and exhaust, regular maintenance, monitoring and testing, staff training, and practices, receiving a score of 4.7.

In the injection safety section, a question was asked, "If a nurse experiences a needlestick injury, what first aid should she take?" The answer included points such as first aid, follow-up, and counseling, although the suggestion was given to squeeze the finger (score 2.3).

In the PPE section, a question was asked, "If PPE is visibly soiled while on duty, what should be done before doffing?" The answer suggested all points like hand hygiene, proper doffing, and disposing of contaminated PPE, although there was no mention of removing soiling with an alcohol swab before removing PPE (score 3).

A question regarding infection control policy was asked, "Where should a doctor sit in a respiratory outdoor department (OPD)?" The answer provided points such as physical distancing, proper ventilation, hand hygiene, and the availability of PPE facilities, receiving a score of 4.

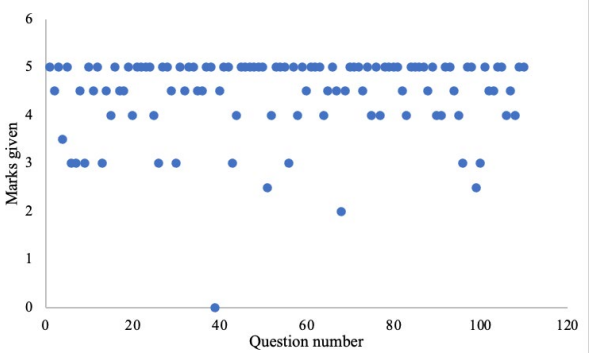

Fig. 1. Score distribution by evaluator 1

In Figure 1, 2 and 3, score distributions by evaluators are shown where, x axis shows question number and the y axis denotes marks given.

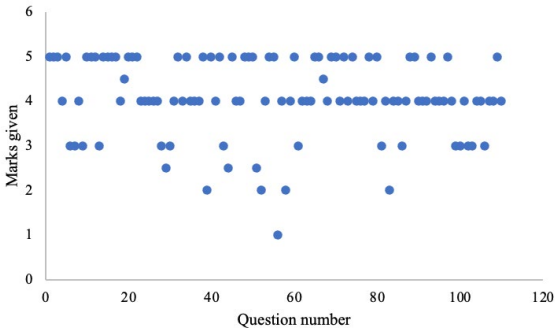

Fig. 2. Score distribution by evaluator 2

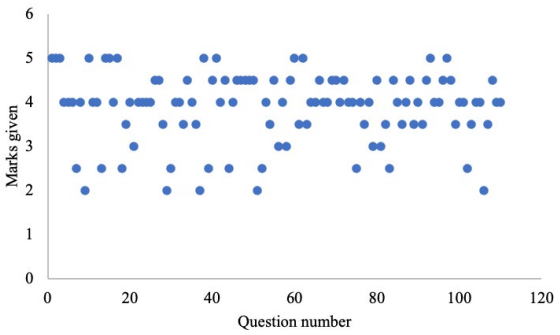

Fig. 3. Score distribution by evaluator 3

The marks given by the three evaluators were different, so to decrease bias and subjectivity, correlation coefficient was performed. The overall correlations between Evaluator 1 and Evaluator 2, Evaluator 2 and Evaluator 3, and Evaluator 3 and Evaluator 1 were found to be 0.55, 0.67, and 0.39, respectively (Fig. 4). These values provide an overview of the general agreement between each pair of evaluators across the entire set of questions.

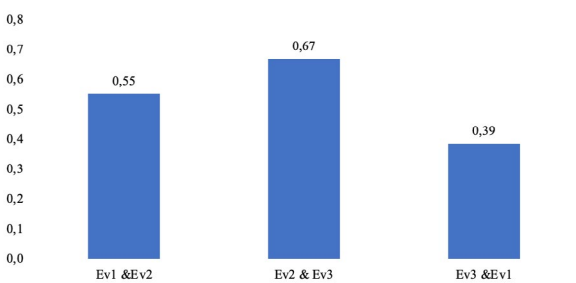

Fig. 4. Correlation coefficient between different evaluators (Ev)

To assess the consistency between different pairs of evaluators, correlations were calculated for all 11 sections of the questions (Fig. 5).

Overall, the highest correlation was observed between Evaluator 2 and Evaluator 3, indicating the closest agreement between these two evaluators. In contrast, the correlation between Evaluator 3 and Evaluator 1 was

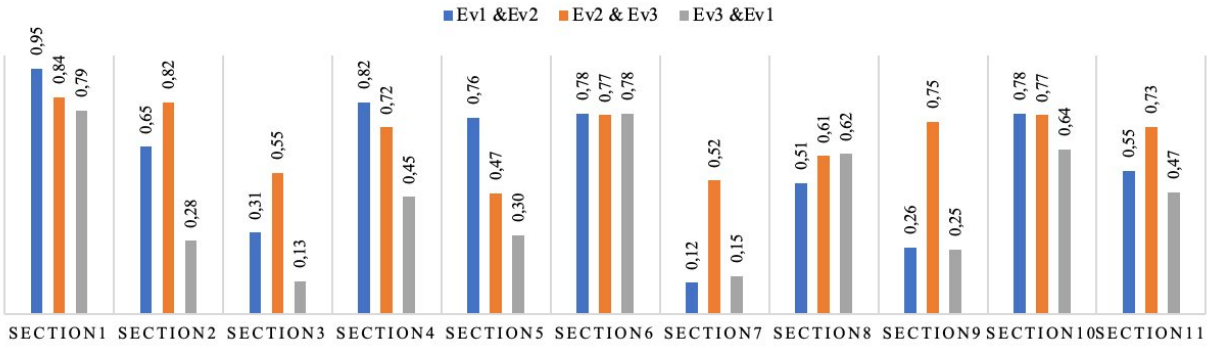

Fig. 5. Correlation coefficient between different evaluators for different sections (Ev – evaluator)

relatively lower. While some sections demonstrated strong consensus and agreement among all evaluators, others like the antimicrobial stewardship program (section 7) and infection control policy (section 9) showed mixed results with moderate to low agreement.

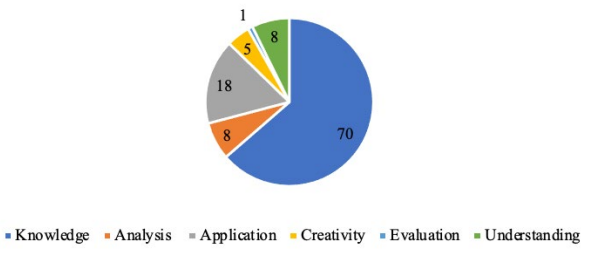

Fig. 6. Categorical analysis of answers

As shown in figure 6, out of 110, 70 answers were in the knowledge domain and 18 in the application domain. Rest questions showed creativity, understanding, analysis, and evaluation-based answers.

Table 3. Cohen's Kappa, to validate the reliability of the evaluators' domain categorizations*

Qualitative criteria	Kappa agreement				
Ev 1 & Ev 2					
Agreement	Expected agreement	Kappa	Std. Err.	Z	p>Z
67.27%	44.14%	0.4141	0.0549	7.55	0
Ev2 & Ev3					
Agreement	Expected agreement	Kappa	Std. Err.	Z	p>Z
76.36%	46.36%	0.5594	0.0563	9,93	0
Ev3 & Ev1					
Agreement	Expected agreement	Kappa	Std. Err.	Z	p>Z
59.09%	45.80%	0.2452	0.056	4.38	0

* Ev – evaluator, Std. Err. – standard error, Z – Z score

To validate the reliability of the evaluators' domain categorizations, Cohen's Kappa was employed. Cohen's kappa is a quantitative measure of reliability for two raters who are rating the same thing, correcting for how often the raters may agree by chance. These insights have practical implications for improving the validity of the evaluation process and enhancing the overall quality of the study's findings. For evaluator 1 and evaluator 2, the Kappa value of 0.41 indicated a moderate level of agreement beyond chance, with 67% of their domain categorizations matching. Similarly, for evaluator 2 and evaluator 3, the Kappa value of 0.55 signified substantial agreement beyond chance, with 76% of their domain categorizations matching. For evaluator 3 and evaluator 1, the Kappa value of 0.24 indicated a fair level of agreement beyond chance, with 59% of their domain categorizations matching (Table 3).

The significant (low p>Z) values of 0.00001 for all three pairs of evaluators underscored the statistical significance of the observed agreements, reinforcing the reliability of their domain categorizations.

Discussion

The results of this research study provide valuable insights into the knowledge base of ChatGPT in IPC, the overall score was found to be 4.33 (95% CI, q1 3.65–q3 4.64). The median score was almost similar in all the sections. A good response (i.e. >4 score) was seen in 70 (63.6%) questions while in 10 (9%) questions, it showed poor response.

ChatGPT consistently provided commendable responses to inquiries, with exemplary instances attached as annexures to this paper. Noteworthy is its comprehensive guidance when prompted for infection control measures to establish a 4-bedded medical ICU, where it emphasized adherence to "Universal precautions." These responses showcase the model's adeptness in offering practical and informed recommendations for healthcare scenarios. The model provided insightful and well-structured answers to questions related to

hand hygiene, standard and transmission-based precautions, HAI, CSSD, injection safety, PPE, and infection control policies.

In the context of hand hygiene, ChatGPT outlined a comprehensive strategy to improve compliance among doctors, covering crucial aspects such as education, training, leadership commitment, awareness campaigns, facility accessibility, peer support, accountability, continuous quality improvement, and patient and family involvement. In the section on HAI, ChatGPT accurately conveyed information regarding the infectivity period of measles, underlining the critical timeframe during which an individual with measles can transmit the virus.

However, the study identified a notable discrepancy in the injection safety section, where ChatGPT suggested squeezing the finger as part of first aid for a nurse experiencing a needlestick injury, contrary to established protocols. Similarly, in the PPE section, ChatGPT omitted a crucial step related to decontamination with an alcohol swab before removing visibly soiled PPE. This indicates a nuanced gap in the model's understanding of detailed protocols.

In studies done by Sinha et al. and Ghosh et al. in pathology and biochemistry respectively, the score was 4.08 and 4.0, which is similar to our score.[3,7] Studies have been done to solve image-based queries in pathology, ophthalmology, and dermatology using deep learning and convolutional neural networks (CNN).[8–10] With the help of technical expertise, we can also make deep learning networks to solve complex IPC problems.[11-13]

The overall correlations between different pairs of evaluators for the 110 questions were calculated, indicating agreement among the evaluators was on the positive side. While some sections demonstrated strong consensus and agreement among all evaluators, others like the antimicrobial stewardship program and infection control policy showed mixed results with moderate to low agreement. Although Sinha et al. and Ghosh et al. in their studies showed good inter-rater comparability which can be due to objective answer type questions.[3,7] In our study, the questions were based on routine healthcare activities, as well as real life scenarios, and evaluators were blind towards each other's evaluation.

While doing categorical analysis, most of the answers are in the knowledge domain followed by the application domain. This correlates with the fact that this is mainly a knowledge model which can show human-like responses.

To validate the reliability of the evaluators' domain categorizations, Cohen's Kappa was employed, yielding Kappa values of 0.41, 0.55, and 0.24 for the different pairs of evaluators. These Kappa values indicated moderate to substantial agreement beyond chance, with statistically significant results.

Study limitations

The study also highlights some limitations and areas for improvement. It was a questionnaire-based study which encompassed inquiries related to diverse facets of infection control. It's important to note that the nature of the queries could vary depending on the specific context of hospital settings. ChatGPT belongs to the category of large language models (LLMs), characterized by their capacity to update their knowledge base consistently. There were some other limitations like lengthy answers given for all types of questions and ChatGPT was unable to provide references, and guidelines for its source of information. Apart from that, we couldn't compare this model with other AI models, which can be done in further studies in this area.

Conclusion

AI models like ChatGPT are a good source of knowledge. Overall, ChatGPT demonstrated a commendable understanding of IPC principles in various domains like hand hygiene, standard precautions, hospital acquired infections, infection control policy, etc. The study identifies specific instances where the model may require further refinement to align consistently with established protocols, especially in critical scenarios such as needlestick injuries and PPE doffing. The findings underscore the importance of ongoing model training and validation to enhance its reliability in providing accurate and contextually appropriate information in healthcare settings. The integration of AI models like ChatGPT in IPC practices could enhance patient safety and overall outcomes by providing healthcare professionals with reliable and up-to-date information. It can complement the expertise of healthcare professionals and support decision-making processes in real time.

Declarations

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Author contributions

Conceptualization, A.J., A.R.B. and C.D ; Methodology, A.R.B. and A.J.; Software, M.H.U.R.; Validation, A.J., A.R.B. and C.D. ; Formal Analysis, A.J. and M.H.U.R.; Investigation, A.J. and A.R.B; Resources, A.J. and A.R.B; Data Curation, A.J. and A.R.B; Writing – Original Draft Preparation, A.J. and A.R.B., M.H.U.R.; Writing – Review & Editing, A.J., A.R.B. and M.H.U.R.; Visualization, A.J., A.R.B. and C.D Supervision, A.J. and A.R.B. Project Administration, A.J. and A.R.B.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The study was approved by Institutional Ethical Committee with No. MTMC/IEC/2023/11.

References

1. Alhumaid S, Al Mutair A, Al Alawi Z, et al. Knowledge of infection prevention and control among healthcare workers and factors influencing compliance: a systematic review. *Antimicrob Resist Infect Control*. 2021;10(1):86. doi: 10.1186/s13756-021-00957-0
2. Sallam M. ChatGPT Utility in Healthcare Education, Research, and Practice: Systematic Review on the Promising Perspectives and Valid Concerns. *Healthcare (Basel)*. 2023;11(6):887. doi: 10.3390/healthcare11060887
3. Sinha RK, Deb Roy A, Kumar N, Mondal H. Applicability of ChatGPT in Assisting to Solve Higher Order Problems in Pathology. *Cureus*. 2023;15(2):35237. doi:10.7759/cureus.35237
4. Lahat A, Shachar E, Avidan B, Shatz Z, Glicksberg BS, Klang E. Evaluating the use of large language model in identifying top research questions in gastroenterology. *Sci Rep*. 2023;13 (1):4164. doi: 10.1038/s41598-023-31412-2
5. Ponsford MJ, Ward TJC, Stoneham SM, et al. A Systematic Review and Meta-Analysis of Inpatient Mortality Associated with Nosocomial and Community COVID-19 Exposes the Vulnerability of Immunosuppressed Adults. *Front Immunol*. 2021;12:744696. doi: 10.3389/fimmu.2021.744696
6. Fitzpatrick F, Doherty A, Lacey G. Using Artificial Intelligence in Infection Prevention. *Curr Treat Options Infect Dis*. 2020;12(2):135-144. doi: 10.1007/s40506-020-00216-7
7. Ghosh A, Bir A. Evaluating ChatGPT's Ability to Solve Higher-Order Questions on the Competency-Based Medical Education Curriculum in Medical Biochemistry. *Cureus*. 2023;15(4):37023. doi: 10.7759/cureus.37023
8. Khosravi P, Kazemi E, Imielinski M, Elemento O, Hajirasouliha I. Deep Convolutional Neural Networks Enable Discrimination of Heterogeneous Digital Pathology Images. *EBioMedicine*. 2018;27:317-328. doi: 10.1016/j.ebiom.2017.12.026
9. Abràmoff MD, Lou Y, Erginay A, et al. Improved Automated Detection of Diabetic Retinopathy on a Publicly Available Dataset Through Integration of Deep Learning. *Invest Ophthalmol Vis Sci*. 2016;57(13):5200-5206. doi: 10.1167/iovs.16-19964
10. Esteva A, Kuprel B, Novoa RA, et al. Dermatologist-level classification of skin cancer with deep neural networks. *Nature*. 2017;542(7639):115-118. doi: 10.1038/nature21056
11. Classen DC, Longhurst C, Thomas EJ. Bending the patient safety curve: how much can AI help? *NPJ Digit Med*. 2023;6(1):2. doi: 10.1038/s41746-022-00731-5
12. Reverberi C, Rigon T, Solari A, et al. Experimental evidence of effective human-AI collaboration in medical decision-making. *Sci Rep*. 2022;12(1):14952. doi: 10.1038/s41598-022-18751-2
13. Khanna NN, Maindarkar MA, Viswanathan V, et al. Economics of Artificial Intelligence in Healthcare: Diagnosis vs. Treatment. *Healthcare (Basel)*. 2022;10(12):2493. doi: 10.3390/healthcare10122493

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ORIGINAL PAPER

Gifted children and their peers perceived parental attitudes, psychosocial problems and quality of life

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ABSTRACT

Introduction and aim. Gifted children are defined as extraordinary children due to their high level of intelligence and specific skills. This study aimed to examine gifted children and their peers perceived parental attitudes, quality of life (QoL), and psychosocial problems.

Material and methods. The study sample consisted of two groups: (1) gifted children (study group) and their parents and (2) peers (control group) and their parents. Data were collected using a descriptive characteristics form, the parental attitude scale (PAS), the pictorial pediatric symptom checklist (PPSC) and the pediatric quality of life inventory (PedsQL).

Results. Both groups were similar in terms of age, gender and grade level. It was mostly the mothers who completed the data collection forms. The study and control groups had a mean PAS score of 94.18±0.738 and 99.31±0.798, respectively and a mean PPSC score of 16.11±0.475 and 16.76±0.480, respectively. The study and control groups had a mean QLS score of 83.19±0.70 and 80.28±0.83, respectively. There was a weak positive correlation between the PAS and PPSC scores (r=0.92; p<0.166).

Conclusion. It is recommended that parental attitudes and their effects on children's psychosocial status and QoL be monitored and that parents be supported. Parental attitudes and a child's psychosocial and behavioral problems and QoL levels should be assessed to by health professionals be able to improve the well-being of both children and their parents.

Keywords. gifted children, parental attitudes, pediatric nursing, psychosocial problems and quality of life

Introduction

Although intelligence is a broad concept, it is a common component of talent. The concept of intelligence has evolved throughout history, but today it refers to only mental potential and academic achievement, and therefore, falls short of determining talent, which encompasses intelligence. In this study, we used the concept of "gifted" instead of "genius."[1,2]

Giftedness is a complicated and extensive concept, and therefore, there is no consensus on the definition of "gifted child." Although giftedness used to be defined from a one-parameter intelligence level, today, it is defined from the perspectives of talent, performance, and intelligence.[3]

Gifted children are defined as extraordinary children because they have high levels of intelligence and specific skills. Due to their distinctive comprehension, consideration, and perception capacities, they go through positive or negative experiences in the family, school, and society. Although they generally have no academic and linguistic problems, they experience emotional and social problems, such as challenging family authority, having difficulty communicating with peers, solitude, becoming easily bored, giving the impression of knowing it all, fear of making errors or failing, perfectionism, depression, anxiety, and social isolation.[4-7] They may also have difficulty communicating with their friends and have more

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problems than their peers due to their intuitive and sensitive nature.[8,9] The talents that gifted children have can affect the life heritage of children as well as their psychosocial problems. In the study conducted on the life heritage of gifted children, it was found that the quality of life of gifted children differed from their peers. Social and physical functionality, which are subcomponents of quality of life, were found to be worse in gifted children compared to their peers. In the study, it was stated that the quality of life of gifted children was significantly lower than that of their peers and that the abilities of children could negatively affect their quality of life.[10]

In the development of gifted children, parents' education levels, personality traits and communication styles can be among the important variables. Especially in early childhood, the positive attitude of parents, their support and involvement with their children can have a significant impact on their children's development by providing an atmosphere that encourages outstanding results. A kind, supportive, and respectful school, family and peer environment helps gifted children develop behavioral, emotional, and social skills and thrive academically, while parental conflict and rude and authoritative attitudes hinder their communication skills.[11-13] Unfortunately, when gifted people are unable to meet their own and their family's high standards, they may become disillusioned and dissatisfied with themselves and their environment, and may face numerous psychosocial problems.[14-16] Perceived negative parental attitudes may negatively impact gifted children's quality of life (QoL) and psychosocial status. QoL is a response to mental, physical, and social conditions. In other words, QoL refers to perceived well-being and value, which are the most fundamental subjective indicators of being at peace with oneself.[17,18]

As with all children, parents' attitudes play a vital role in the development of gifted children. Positive attitudes support psychosocial and behavioral development and improve the quality of life. Low psychosocial problems and high quality of life can have a significant impact on the individual and social development of children and can help them grow up as healthy adults. parents, teachers and nurses have important duties in the developmental processes of children. Pediatric nurses are responsible for protecting the physical, emotional and social health of children and play an important role in the development of gifted children. Therefore, pediatric nurses should monitor children and identify and solve their problems. Few studies have investigated the relationship between parents' attitudes and the psychosocial problems and quality of life of gifted children. Furthermore, there is little published research examining the impact of parents' support and family-centered care by pediatric nurses on children's psychosocial and behavioral development.

Aim

Therefore, this study analyzed the perceived parental attitudes, psychosocial problems and quality of life of gifted children and their peers. It is thought that the study can be pioneering by contributing to the field with the data obtained. In addition, in line with the findings, it is thought that it can be a resource in the content of training programs for parents.

Material and methods

This descriptive study was conducted to compare gifted children and their peers perceived parental attitudes, psychosocial problems, and QoL.

Research population and sample

The study was conducted in four science and art centers of a metropolitan city and at the elementary and secondary schools with the highest number of students in a major district. The study group consisted of 231 second- and fifth-grade gifted children and their parents. The first reason was that gifted children take an exam administered by the Ministry of National Education in the first, second, and third years, and therefore, they begin to attend science and art centers from the second year on. The second reason was that children reach preadolescence from sixth grade on, and therefore, may experience psychosocial problems.[19] The control group consisted of 249 second- to fourth- grade (elementary school) and fifth-grade (secondary school) children and their parents. Both groups were similar in terms of grade level, age, and gender.

Inclusion criteria for both groups

- Those with no attention deficit hyperactivity disorder (ADHD), autism, dyslexia, and any other chronic disease were included in the sample.
- Second- and fifth- graders were recruited.
- Not experiencing stress or trauma in the last 6 years
- Filling out data collection forms completely

Exclusion criteria for both groups

- Not going to 2nd or 5th grade
- Refusing to participate in the study
- Filling out data collection forms incompletely

Data were collected using a descriptive characteristics form, the parental attitude scale (PAS), the pictorial pediatric symptom checklist (PPSC), and the pediatric quality of life inventory (PedsQL). After data collection, grades were defined as second, third, fourth, and fifth to sample a control group. Sections for each grade were selected using simple random sampling. Power analysis was performed using GPower 3.1 to ascertain whether the sample was large enough (n=480) to detect significant differences. The power analysis revealed a power of 90% with an effect size of 0.1482 (α=0.05).

Data collection tools

The descriptive characteristics form consisted of 18 items on children's demographic characteristics (age, gender, grade level, school, birth order, etc.) and parent's demographic characteristics (age, gender, education, income, employment, etc.).

The parental attitude scale (PAS) is a 30-item scale developed by Kucukturan.[20] It consists of two subscales; responsibility/acceptance (15 items) and authority/supervision (15 items). The total score ranges from 15 to 75 in each subscale. Higher "responsibility/acceptance" scores indicate that parents accept, support, and trust their children more and give them more responsibility. Higher "authority/supervision" scores indicate that parents put more pressure on their children, inflict more punishment on them, have more psychological control over them, make them feel more guilty and embarrassed, and show them less affection. Kucukturan reported that PAS had a Cronbach's alpha of 0.79, while the responsibility/acceptance and authority/supervision subscales had a Cronbach's alpha of 0.88 and 0.85, respectively. PAS had a Cronbach's alpha of 0.78 in this study.[20]

The pictorial pediatric symptom checklist (PPSC) is a 35-item Likert-type scale developed by Leiner et al. The scale is completed by parents to early diagnose psychosocial problems in children between the ages of 6 and 16 years. Items 5, 6, 17, and 18 are removed when used for elementary school children aged 4 to 5 years. The cut-off point is 24 and over for minors. Ardıc and Barlas adapted the scale to Turkish for children aged 6 to 16 years.[21] They found the Cronbach's alpha of the Turkish version of the scale as 0.89, which was 0.84 in this study.

The pediatric quality of life inventory (PedsQL) is a 23-item scale developed by Varni et al. to measure the health-related QoL in children aged 5-7 and 8-12 years. The PedsQL consists of four subscales assessing physical functionality (eight items), emotional functionality (five items), social functionality (five items), and school functionality (three items for children 2–4 years of age and five items for other age groups). Scores are linearly transformed to a scale of 0 to 100 (0=100, 1=75, 2=50, 3=25, and 4=0). The "physical functionality" subscale score is linearly transformed and added and then divided by eight to obtain a physical health total score (PHTS), which is the sum of the "emotional functionality," "social functionality," and "school functionality" subscale scores before being divided by the total number of items (15) in those subscales. The total score is the sum of all item scores divided by the total number of items 23. Uneri and Memik et al. adapted the PedsQL to Turkish for children 8–12 years of age.[17] Uneri reported that the parent and children's form of the scale had a Cronbach's alpha of 0.84. and 0.86, both of which were 0.87 in this study.

Data collection

Data were collected in four science and art centers between 28 February–4 May 2018 and in primary and secondary schools between 7–25 May 2018. Permission was obtained from the Directorate of National Education and the directors of science and art centers. After the permissions were obtained, data were collected from children attending science and art centers between 28 February–4 May 2018. Before data collection, the study was explained to the classroom teachers and informed consent was obtained. The purpose of the study was explained to each participant at a level that they could understand, and then a consent form and an envelope containing the questionnaires were given. Participants who attended school on weekdays were asked to return the forms the next day, and those who attended school on weekends were asked to return them the following weekend. The researcher visited the schools daily to collect the forms and the forms were collected. The data obtained from the gifted children were then analyzed in detail. The data were then entered into the analysis program and analyzed for stratification. After data collection, participants were listed based on grade level, age, and gender to recruit a control group.

After collecting data from gifted children, primary and secondary schools were visited between the 7th and 25th of May. The number of students to be included in the control group was determined according to strata. School principals were informed about the content, purpose and procedure of the study. The grade levels to be included in the sample were determined using simple random sampling. After the students were informed about the procedure, envelopes were distributed to those who agreed to participate. They were asked to take the envelopes to their parents and return them to their teachers the next day or the day after. the schools were visited frequently between the specified dates and the envelopes left with the teachers were collected. after the data were collected, they were analyzed in detail and those who did not meet the inclusion criteria or did not participate in the study were examined in detail.

Data analysis

In the first stage of the study, data were analyzed using the Statistical Package for Social Sciences (SPSS, version 20, IBM, Armonk, NY, USA) at a significance level of 0.05. Percentage and frequency values were used for analysis. A chi-square test was used to determine the distributions of the participants and their parents' descriptive characteristics. The Kolmogorov-Smirnov test (analytical method) and histogram (visual method) were used for normality testing. Data were not normally distributed, and therefore, nonparametric tests (the Mann-Whitney U test and Spearman's correlation coefficients) were used for analysis. In the second stage of

the study, the relationships between parental attitudes, psychosocial problems and quality of life perceived by gifted children and their peers, which is the main topic of the study, were analyzed with structural equation model (SEM). Before the analysis, the assumptions of SEM were checked. For this purpose, normal distribution with skewness and kurtosis values and singularity with correlation coefficient were examined.

Ethical considerations

The study was approved by the Ethics Committee of the university (77082166-302.08.01), and written consent was obtained from the Provincial Directorate of National Education prior to data collection. Parents and children were informed about the purpose, procedure, and confidentiality of the study. Written consent was obtained from parents, and verbal consent was obtained from students.

Results

The study and control groups were homogeneous in age, gender, and grade level. It was mostly the mothers who completed the questionnaires. The groups did not differ by age, gender, income, number of children in the family, the stress level in the past six months, and the presence of a family member with a chronic disease (Table 1).

Table 1. Sociodemographic characteristics

Characteristics	Gifted children group (n=231)		Peer group (n=249)		x^2	p
	M±SD	Min–max	M±SD	Min–max	5.690	0.224
Age	8.86±0.65	7–11	9.04±0.62	7–11		
	n	%	n	%		
Gender						
Girl	120	51.9	129	51.8	0.001	0.975
Boy	111	48.1	120	48.2		
Grade level						
Second	73	31.6	75	30.1		
Third	92	39.8	95	38.2	3.185	0.364
Fourth	47	20.3	46	18.5		
Fifth	19	8.2	33	13.3		
Birth order						
First	137	59.3	133	53.4		
Second	75	32.5	85	34.2	3.804	0.283
≥ Third	19	8.2	31	12.4		
Stress over the last six months						
No	221	95.7	234	94.0	5.18	0.738
Yes*	10	4.3	15	6.0		
Acute health problems in the last six months						
No	217	93.9	234	94	21.997	0.055
Yes**	14	6.1	15	6		

Both groups had a high number of parents with a bachelor's degree. There was a significant difference in educational level between the two groups (X^2= 40.873; p<0.05). Gifted children's parents (70.7%) had a significantly higher employment rate than peer parents (X^2 = 15.912; p<0.05) (Table 1).

Table 2. PAS scores*

	Gifted children group (n=231)		Peer group (249)		U	p
Scale	M±SD	Min–max	M±SD	Min–max		
Responsibility acceptance	68.72±0.455	42–75	68.43±0.465	39–75	28541	0.885
Authority inspection	25.46±0.751	15–71	30.88±0.764	15–75	19849	<0.001
Scale total score	94.18±0.738	69–142	99.31±0.798	69–148	21031.5	<0.001

* Mann Whitney U analysis

There was no statistically significant difference in responsibility-acceptance subscale scores between the study and control groups. There was, however, a statistical significance in authority-supervision subscale and mean total scale scores between the two groups (Table 2).

Table 3. PPSC scores*

	Gifted children group (n=231)		Peer group (n=249)		U	p
Scale	M±SD	Min–max	M±SD	Min–max		
Scale total score	16.11±0.475	4–44	16.76±0.480	1–41	27123.000	0.281

* Mann Whitney U Analysis

There was no statistically significant difference in PPSC scores between the study and control groups (p>0.05) (Table 3).

Table 4. PedsQL scores*

	Gifted Children Group (n=231)		Peer Group (n=249)		U	p
Scale	M±SD	Min–max	M±SD	Min–max		
Physical functionality	80.76±0.91	43.75–100	78.82±1.13	2.50–100	28259.500	0.741
Emotional functionality	77.16±1.1	10–100	76.8±1.09	20–100	28398.000	0.811
Social functionality	89.03±0.95	15–100	86.04±1.09	20–100	26552.000	0.133
School functionality	86.67±0.91	10–100	80.38±1.01	30–100	21730.000	<0.001
Psychosocial functionality	84.48±0.76	41.67–100	81.06±0.6	40–100	24833.500	0.010
Scale total score	83.19±0.70	46.74–100	80.28±0.83	47.61–100	25706.500	0.044

* Mann Whitney U analysis

There was no statistically significant difference in PedsQL "physical functionality," "emotional functionality," and "social functionality" subscale scores between the study and control groups. However, there was a statistically significant difference in "school functionality" subscale and total scores between the two (p<0.05) (Table 4).

Table 5. Correlation between PAS and PPSC scores*

	PAS		PPSC			
	M±SD	Min–max	M±SD	Min–max	r	p
Gifted children group (n=231)	94.18±0.738	69–142	16.11±0.475	4–44	0.092	0.166
Peer group (n=249)	99.31±0.798	69–148	16.76±0.480	1–41	0.194	0.002

* Spearman correlation analysis

There was a weak positive correlation between PAS and PPSC scores (Table 5). Children's PAS and PedsQL scores were weakly negatively correlated, but parents PAS and PedsQL scores were not (r=-, 038; p>0.05) (Table 5).

Table 6. Correlation between PPSC and PedsQL scores*

	PPSA		PedsQL			
	M±SD	Min–max	M±SD	Min–max	r	p
Gifted children group (n=231)	16.11±.475	4–44	83.19±.70	46.74–100	-0.668	<0.001
Peer group (n=249)	16.76±.480	1–41	80.28±.83	47.61–100	-0.605	<0.001

* Spearman correlation analysis

Children's PPSC and PedsQL scores were weakly correlated (Table 6).

The diagram of the structural model is given below (Fig. 1).

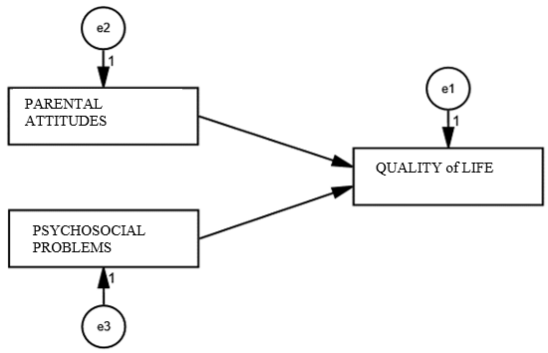

Fig. 1. Diagram of structural equation path analysis

The goodness of fit criteria for the structural model are given below (Table 7). Coefficients for the structural model hypotheses are given below (Table 8).

Table 7. Structural equation path analysis index values

Index	Normal value*	Acceptable value**	Gifted children	Peers
χ2/sd	<2	<5	3.88	4.36
GFI	>0.95	>0.90	0.98	0.96
AGFI	>0.95	>0.90	0.92	0.91
CFI	>0.95	>0.90	0.97	0.96
RMSEA	<0.05	<0.08	0.06	0.06
RMR	<0.05	<0.08	0.07	0.06

Table 8. Structural model path analysis coefficients

Gifted children	**β**	**Std. β**	**S.Hata**	**t**	**p**	**R^2**
Parental attitude	-0.028	-0.029	0.045	-0.619	0.536	0.498
Psychosocial problems	-1.046	-0.705	0.069	-15.082	***	
Dependent variable: Quality of life						
Theirs peers	**β**	**Std. β**	**S.Hata**	**t**	**p**	**R^2**
Parental attitude	0.091	0.087	0.053	1.735	0.083	0.377
Psychosocial problems	-1.063	-0.608	0.088	-12.136	***	
Dependent variable: Quality of life						

Parental attitude had no effect on quality of life in gifted children (p>0.05). Psychosocial problems decreased the quality of life (β=0.705; p<0.05). Parental attitude and psychosocial problems explained 49.8% of the total change in quality of life. Parental attitude had no effect on quality of life in peer children (p>0.05). Psychosocial problems decreased the quality of life (β=0.608; p<0.05). Parental attitude and psychosocial problems explained 37.7% of the total change in quality of life.

Discussion

This study investigated the relationship between gifted children's and their peers' perceptions of parental attitudes and their psychosocial problems and QoL. The results show that the parents of gifted children are more democratic and less authoritarian than those of their peers. Rudasill et al. also reported that the parents of gifted children (332 fifth and eleventh graders) were more tolerant and democratic, while those of peers were more permissive and authoritarian.[22] A study concluded that parents of gifted children adopt democratic and protective attitudes and avoid permissive and authoritarian attitudes.[23] Yazdani and Daryei found that the parents of gifted children (sixth and ninth graders) were less authoritarian than those of peers.[24] The parents of gifted children seem to be more democratic and tolerant towards their children, probably because they feel happy and proud of their children's academic achievement.[25,26] Unlike other studies, Olgun-Kaval found that gifted children perceived more authoritarian parental attitude, parental rejection, indifference and neglect compared to the normally developing group.[27] Democratic parental attitudes encourage children to develop skills and improve their QoL.

There was no statistically significant difference in PPSC scores between the study and control groups. Yazdani and Daryei reported that gifted children had more self-respect, social competence, and cooperation than their peers but that the two groups did not differ significantly in depression and anxiety levels.[24] We also observed that the study and control groups had similar psychosocial and behavioral problems. Kroesbergen et al. found that first- and second-grade gifted children (n=35) had less self-respect and more difficulty adapt-

ing to social norms and expectations than their peers.[28] Children's psychosocial and behavioral problems may depend on the place and time of residence and cultural norms. Therefore, the lack of a significant difference in PPSC scores between the study and control groups may be because they have similar sociocultural backgrounds.

There was no statistically significant difference in the PedsQL "physical functionality," "emotional functionality," and "social functionality" subscale scores between the study and control groups. However, the study group had significantly higher school and psychosocial functionality scores than the control group. In contrast, Eren et al. reported that gifted children had significantly lower physical and social functionality scores than their peers, suggesting that special skills may negatively affect gifted children' QoL.[29] Kaya et al. determined that gifted children had lower quality of school life than their peers.[30] The gifted children in our study have higher QoL probably because they have fewer problems in school thanks to the training offered by the science and art centers and the education provided by their schools.

Our results also show that democratic parental attitudes reduce psychosocial and behavioral problems in children. Research shows that authoritarian parental attitudes increase the rate of psychosocial and behavioral problems in children.[31-33] Democratic and kind parental attitudes help establish healthy communication, support children, and provide them with a safe environment to express themselves. Research also shows that children who share their problems with their parents are likely to have better behavioral development, more social and problem-solving skills, and higher self-respect than those who do not.[11,34,35] Furthermore, in the study conducted by Topuz and Çankaya with 219 students, it was revealed that external protective factors such as acceptance/affection from parents and peer relationships explained the psychological resilience levels of gifted students more strongly.[36] Kim states that the support gifted students receive from their families is a key protective factor for psychological resilience.[37] On the other hand, perfectionist and authoritarian parental attitudes lead to a fear of failure and disappointment in children.[38,39] In one of the studies showing that the families of gifted children were not always supportive, it was reported that the families of gifted children were insistent on success, critical of mistakes, and that the parents' high expectations caused anxiety in children.[40] Studies examining parental attitudes in gifted children have revealed that parental attitudes are effective on the social emotional characteristics of gifted children, which is consistent with the results of the present study.[41,42]

There was a negative correlation between PedsQL and PAS scores in the study group, indicating that more democratic parental attitudes result in higher QoL in their gifted children. There was also a negative correlation between PedsQL and PAS scores in the control group, indicating that more authoritarian parental attitudes result in lower QoL in their children. There is no published research examining the effect of parental attitudes on gifted children's QoL. Therefore, we focused on studies on peer groups to make a comparative analysis. Aytekin, Arslan, and Kucukoglu (2014) reported no effect of parental attitudes on QoL in children 3-6 years of age.[43]

There was a negative correlation between PedsQL and PPSC scores in both study and control groups. There is no research investigating gifted children's psychosocial and behavioral problems and QoL levels. Therefore, we addressed studies on adolescent and adult peer groups to make a comparative analysis. Ates and Akbas reported that adolescents with fewer problematic, risky, and criminal behaviors had higher QoL, probably because such behaviors are associated with psychosocial and behavioral problems. Therefore, low rates of such behaviors may lead to high QoL.[44] Our results also point to a negative correlation between problematic behaviors and QoL. We think that developing various skills early and attending science and art centers reduces the likelihood of gifted children experiencing psychosocial problems. According to our results, democratic parental attitudes do not affect the prevalence of psychosocial problems in gifted children, but authoritarian parental attitudes affect the prevalence of psychosocial problems in their peers. Our results also show that psychosocial and behavioral problems affect the QoL in gifted children and their peers.

As a result of the structural equation model analysis, one of the advanced statistics conducted in our research; It has been determined that parental attitudes have no effect on the quality of life of gifted children and their peers, while psychosocial problems have a significant effect on the quality of life. Since there are not enough studies on the impact of psychosocial problems on the quality of life of gifted children, data from studies conducted from different groups and topic were discussed. In a cross-sectional study conducted with 2703 children aged 8–12, psychosocial problems were found to be common, especially in boys, and children's quality of life was found to be low. It has been determined that the high number of psychosocial problems experienced by children causes their quality of life to decrease.[45] Cyberbullying is associated with internalizing and externalizing problems, as well as emotional and psychosocial problems such as depression, stress and anxiety. Children exposed to bullying may experience various psychosocial problems. In a study examining the quality of life of gifted children who were exposed to cyberbullying, it was stated that gifted children experience more psychosocial problems such as stress and depression and their quality of life and life satisfaction are lower.[46] Situations such as long-term persistence of psychosocial problems and failure to develop appropriate programs for children may affect children's quality of life.

Conclusion

Gifted children define their parents' attitudes as more democratic and have higher QoL than their peers. However, gifted children and their peers have similar psychosocial and behavioral problems. Democratic parental attitudes help children develop psychosocial skills and resources to cope with psychosocial problems and improve their QoL. Therefore, pediatric nurses should assess parental attitudes and their children's psychosocial and behavioral problems and QoL levels to improve their well-being. Support programs should be developed for children and their parents based on nurse evaluations. Parental attitudes should be regularly assessed, and parents should be educated to raise their awareness of the effect of their behavior on their children. Factors improving physical, social, emotional, and school functionalities should be determined to improve the QoL of gifted children and their peers. Different research instruments should be used to evaluate parental attitudes and children's psychosocial problems and QoL levels. Both children and parents should be educated about the subject matter.

This study has several limitations. The sample consisted only of second and fifth grade students and therefore the results cannot be generalized. The same questionnaires were used at all grade levels. Different questionnaires could have produced different results. The study may not contain the same results for all children because it was collected in a certain region and in groups with socio-cultural proximity. It is thought that the fact that parents were not directly interviewed in the data collection forms may create deficiencies in understanding the purpose of the study.

Declarations

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Author contributions

Conceptualization, S.Y. and N.A.; Methodology, S.Y. and N.A; Software, S.Y; Validation, S.Y. and N.A.; Formal Analysis, S.Y. and N.A.; Investigation, S.Y.; Resources, S.Y.; Data Curation, S.Y.; Writing – Original Draft Preparation, S.Y. and N.A; Writing – Review & Editing, N.A; Visualization, S.Y.; Supervision, S.Y.; Project Administration, N.A; Funding Acquisition, S.Y.

Conflicts of interest

No potential conflicts of interest were declared with respect to the research, authorship, and/or publication of this article.

Data availability

The data are kept by the researchers. New analyses are available when necessary.

Ethics approval

The study was approved by the Ethics Committee of the university (77082166-302.08.01).

References

1. Kaya F, Juntune J, Stough L. Intelligence and Its Relationship to Achievement. *Elementary Education Online.* 2015;14(3):1060-1078. doi: 10.17051/io.2015.25436
2. Lozano-Blasco R, Quílez-Robres A, Usán P, Salavera C, Casanovas-López R. Types of Intelligence and Academic Performance: A Systematic Review and Meta-Analysis. *J Intell.* 2022;10(4):123. doi: 10.3390/jintelligence10040123
3. Smedsrud J. Explaining the Variations of Definitions in Gifted Education. *Nordic Studies in Education.* 2020;40(1):79-97. doi: 10.23865/nse.v40.2129
4. Harrison G, Haneghan JPV. The Gifted and The Shadow of The Night: Dabrowski's Overexcitabilities and Their Correlation to Insomnia, Death Anxiety, and Fear of The Unknown. *Journal for the Education of the Gifted.* 2011;34(4):669-697. doi: 10.1177/016235321103400407
5. Renati R, Bonfiglio NS, Dilda M, Mascia ML, Penna MP. Gifted Children through the Eyes of Their Parents:Talents, Social-Emotional Challenges, and Educational Strategies from Preschool through Middle School. *Children*. 2022;10(1):42. doi: 10.3390/children10010042
6. Blaas S. The Relationship Between Social–Emotional Difficulties and Underachievement of Gifted Students. *J Psychol Couns Sch*. 2014;24(2):243-55. doi: 10.1017/jgc.2014.1
7. Cross JR, Cross TL. Clinical and Mental Health Issues ın Counseling The Gifted Individual. *Journal of Counseling and Development*. 2015;93(2):163-172. doi: 10.1002/j.1556-6676.2015.00192.x
8. Morawska A, Sanders M. Parenting Gifted and Talented Children: What Are The Key Child Behaviour and Parenting Issues? *Australian and New Zealand Journal of Psychiatry*. 2008;42(9):819-827. doi: 10.1080/00048670802277271
9. Stuart T, Beste A. I knew I was different: being able to understand the gifted. *Ankara Root Publishing*; Ankara, 2008;15-50.
10. Senol FB, KocaS, Erbasan O. Gifted Children's Social Problem Solving Skills, Social Competence and School Adjustment. *Journal of Gifted Education and Creativity.* 2023;10(1):33-43.
11. Olszewski-Kubilius P, Lee SY, Thomson D. Family Environment and Social Development ın Gifted Students. *Gifted Child Quarterly*. 2014;58(3):199-216.
12. Keli G, Gabrijelčič Mojca K. Parents Nominating Gifted Children in Their Early Years-The Case of Slovenia. *The New Educational Review*. 2021;66:170-181.
13. Huey L, Sayler F, Rinn A. Effects of Family Functioning and Parenting Style on Early Entrants' Academic Performance And Program Completion. *Journal for the Education of the Gifted.* 2013;36(4):418-432. doi: 10.1177/0162353213506066

14. Sękowski A, Siekańska M. National academic award winners over time: Their family situation, education and interpersonal relations. *High Ability Studies.*2008;19(2):155-171.
15. Sekowski A, Łubianka B. Education of Gifted Students – An Axiological Perspective. *Gifted Education International.* 2014;30(1):58-73. doi: 10.1177/0261429413480423
16. Papadopoulos D. Parenting the Exceptional Social-Emotional Needs of Gifted and Talented Children: What Do We Know? *Children*. 2021;8(11):953. doi: 10.3390/children8110953
17. Uneri O, Memik N. Concept of Quality of Life ın Children and Review Inventories about Quality of Life. *Turkish Journal of Child and Adolescent Mental Health*. 2007;14(1):48-56.
18. Eser E, Yuksel H, Baydur, H. Psychometric properties of the Turkish version of the general purpose health-related Quality of Life Scale for Children (Kid-KINDL). *Turkish Journal of Psychiatry*. 2008;19(4):409-417.
19. Miller-Slough R, Dunsmore J. Longitudinal Patterns in Parent and Friend Emotion Socialization: Associations with Adolescent Emotion Regulation. *Journal of Research on Adolescence.* 2018;1-14. doi: 10.1111/jora.12434
20. Kucukturan G. Parent Attitude Scale. *Eurasian Journal of Educational Research (EJER)*. 2005;19:238-50.
21. Ardıc E, Unsal G, Bayram S. Validity and Reliability of the Pictorial Pediatric Symptom Checklist. *Journal of Psychiatric Nursing.* 2020;11(1):20-27. doi: 10.14744/phd.2019.56933
22. Rudasill K, Adelson L, Callahan M, Houlihan V, Keizer M. Gifted Students' Perceptions of Parenting Styles: Associations with Cognitive Ability, Sex, Race, And Age. *Gifted Child Quarterly.* 2013;57(1):15-24. doi:10.1177/0016986212460886
23. Ogurlu U. The effect of training programme for families with gifted children on parents' parental self-efficacy and awareness. *Journal of National Education.* 2016;45(209):144-159.
24. Yazdani S, Daryei G. Parenting Styles and Psychosocial Adjustment of Gifted and Normal Adolescents. *Pacific Science Review B: Humanities and Social Sciences*. 2016;2(3):100-105. doi: 10.1016/j.psrb.2016.09.019
25. Bozoglan B. A Systemic Intervention Model for The Parents of Gifted Children. *Current Psychology.* 2023;42(6):4821-4829.
26. Mehdiyev G. Psychology of Gifted Children: Analysing The Book from Theory to Practice. *Journal of Bayburt Faculty of Education.* 2017;12(24):907-912.
27. Khaleque A, Rohner P. Perceived Parental Acceptance-Rejection and Psychological Adjustment: A Meta-Analysis of Cross-Cultural and Intercultural Studies. *Journal of Marriage and the Family.* 2002;64(1):54-64.
28. Kroesbergen H, van Hooijdonk, Van Viersen S, Middel-Lalleman M, Reijnders J. The Psychological Well-Being of Early Identified Gifted Children. *Gifted Child Quarterly*. 2016;60(1):16-30. doi: 10.1177/0016986215609113
29. Eren F, Cete Ö, Avcil S, Baykara B. Emotional and Behavioural Characteristics in Gifted Children and Their Families. *Archives of Neuropsychiatry.* 2018;55(2):105-112. doi: 10.5152/npa.2017.12731
30. Kaya F, Erdogan R, Caglayan Y. A Comparison of Gifted and Non-Gifted Students' School Life Quality and Peer Relations. *Turkish Journal of Superior Intelligence and Education.* 2014;4(2):107-125.
31. Thompson A, Hollis C, Richards D. Authoritarian Parenting Attitudes as A Risk for Conduct Problems. *European Child & Adolescent Psychiatry*. 2003;12(2):84-91. doi: 10.1007/s00787-003-0324-4
32. Eldeklioglu J. The Relatıonshıps Between Aggressıveness, Peer Pressure and Parental Attıtudes among Turkısh Hıgh School Students. *Social Behavior & Personality: An İnternational Journal.* 2007;35(7):975. doi: 10.2224/sbp.2007.35.7.975
33. Williams R, Degnan A, Perez-Edgar E. A. Impact of Behavioral Inhibition and Parenting Style on Internalizing and Externalizing Problems from Early Childhood through Adolescence. *Journal of Abnormal Child Psychology.* 2009;37(8):1063-1075. doi: 10.1007/s10802-009-9331-3
34. Morawska A, Sanders R. Parenting Gifted and Talented Children: Conceptual and Empirical Foundations. *Gifted Child Quarterly.* 2009;53(3):163-73. doi: 10.1177/0016986209334962
35. Koshy V, Smith P, Brown J. Parenting Gifted and Talented Children in Urban Areas: Parents' Voices. *Gifted Education International.* 2017;33(1):3-17. doi: 10.1177/0261429414535426
36. Topuz I, Cihangir-Cankaya Z, Predictors of Psychological Resilience of Gifted Students: Self-Esteem, Peer Relationships and Parental Attitudes. Ankara University Faculty of Educational Sciences *Journal of Special Education.* 2022;23(2):271-294. doi: 10.21565/ozelegitimdergisi.742426
37. Kim M. Enhancing Resilience of Gifted Students. *Texas Association for the Gifted & Talented Article.* 2015;36(3):17-21.
38. Neumeister S. Factors Influencing The Development of Perfectionism in Gifted College Students. *Gifted Child Quarterly.* 2004;48(4):259-274. doi: 10.1177/001698620404800402
39. Neumeister L, Finch, H. Perfectionism in High-Ability Students: Relational Precursors and Influences on Achievement Motivation. *Gifted Child Quarterly.* 2006;50(3):238-253. doi: 10.1177/001698620605000304
40. Pilarinos V, Solomon R. Parenting Styles and Adjustment in Gifted Children. *Gifted Child Quarterly.* 2017;61(1):87-98. doi: 10.1177/0016986216675351
41. Guilamo-Ramos V, Jaccard J, Dittus P, Bouris M. Parental Expertise, Trustworthiness, and Accessibility: Parent-Adolescent Communication and Adolescent Risk Behavior. *Journal of Marriage and Family.* 2006;68(5):1229-1246. doi: 10.1111/j.1741-3737.2006.00325

42. Sumer N, Gundogdu-Akturk E, Helvacı E. Psychological Effects of Parental Attitudes and Behaviours: A Review of Studies in Türkiye. *Turkish Psychological Writings*. 2010;13(25):42-59.
43. Aytekin A, Arslan N, Kucukoglu S. Investigation of Nurses' Quality of Life and Self-Care Skills of Children Aged 3-6 years. *Cumhuriyet Journal of Nursing*. 2014;3(2):8-15.
44. Ates B, Akbas T. Investigation of Irregular Behaviours in Adolescents in Terms of Family Functioning and Quality of Life. *Journal of the Cukurova University Institute of Social Sciences*. 2012;21(2):337-352.
45. Bot M, Den Bouter L, Adriaanse C. Prevalence of Psychosocial Problems in Dutch Children Aged 8–12 Years and Its Association with Risk Factors and Quality of Life. *Epidemiology and Psychiatric Sciences*. 2011;20(4):357-365. doi: 10.1017/S2045796011000540
46. González-Cabrera J, Tourón J, Machimbarrena M, Gutiérrez-Ortega M, Álvarez-Bardón A, Garaigordobil M. Cyberbullying in Gifted Students: Prevalence and Psychological Well-Being in A Spanish Sample. *Int J Environ Res Public Health*. 2019;16(12):2173. doi: 10.3390/ijerph16122173

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ORIGINAL PAPER

The relationship between psychological well-being with levels of anxiety, COVID-19 fear and depression in individuals hospitalized with COVID-19

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ABSTRACT

Introduction and aim. The COVID-19 pandemic negatively affected both the physical and mental health of societies. The present study was conducted to determine the relationship between psychological well-being with levels of anxiety, COVID-19 fear, and depression in individuals hospitalized with COVID-19.

Material and methods. This descriptive correlative study was conducted with 685 individuals diagnosed with COVID-19 treated in the clinics of a pandemic hospital. The data of the study were collected with the information form, psychological well-being scale (PWBS), COVID-19 fear scale (FCV-19S), and hospital anxiety and depression scale (HADS).

Results. The mean PWBS score of the participants was 37.21±14.3, the FCV-19S score was 20.10±10.41, the HADS-anxiety score was 9.07±7.29, and the depression score was 10.74±7.35. The PWBS scores with FCV-19S scores of the participants, HADS-anxiety, and HADS-depression scores were found to be negatively correlated ($p<0.001$).

Conclusion. In conclusion, as the anxiety, fear, and depression levels experienced by individuals receiving inpatient treatment in a pandemic hospital due to COVID-19 increase, their psychological well-being levels decrease at a statistically significant level. It may be recommended to intervene in the anxiety, fear, and depressive symptoms of individuals receiving inpatient treatment due to a diagnosis of COVID-19 to increase their psychological well-being.

Keywords. anxiety, COVID-19, depression, fear, psychological well-being

Introduction

The novel coronavirus disease (COVID-19) affected both the physical and mental health of individuals and communities negatively.[1,2] The pandemic spread rapidly all over the world, causing intense psychological pressure with the risk of death.[3] Besides the physical manifestations of infectious diseases e.g., COVID-19.[4,5] It is reported that it can also cause psychiatric problems such as anxiety, fear, depression, panic attacks, somatic symptoms, sleep problems, post-traumatic stress disorder, and even suicidal tendencies.[6,7] This conclusion was shared that during 2020, the pandemic led to a 27.6% increase in major depressive disorder cases and a 25.6% increase in anxiety disorder cases worldwide.[8]

It was reported that practices such as quarantine, isolation, and social distance cause psychological problems such as depression, loneliness, and anxiety.[9,10] It was reported that patients who were quarantined were

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more likely to experience depression and anxiety than those who were not.[11]

Another finding caused by the COVID-19 pandemic is fear.[12,13] People who were not infected with COVID-19 reported fear of coming into contact with people infected with COVID-19.[14] This caused the stigmatization and social exclusion of individuals who were diagnosed with or recovered from COVID-19. It was reported that this may increase the risk of developing mental health problems such as adjustment disorder and depression.[15]

It was found that studies conducted to determine the psychosocial impact of COVID-19 are mostly conducted with healthcare workers.[16-18] In a study in which the COVID-19 pandemic and mental health outcomes were systematically examined, it was reported that the sample of only two of the 43 studies evaluated was individuals with a diagnosis of COVID-19. It was also stated in the same study that individuals with a diagnosis of COVID-19 experienced high levels of post-traumatic stress symptoms and significantly higher depressive symptoms than other individuals.[19]

Current global disasters and pandemics affect the mental health of societies. It is important to keep the issue on the agenda to be prepared for disasters and pandemics. Studies evaluating the direct neuropsychiatric consequences of COVID-19 and its indirect effects on mental health are greatly needed for mental health care planning and preventive measures during possible subsequent pandemics.[19] In this respect, it is considered that studies evaluating the psychological well-being of individuals diagnosed with COVID-19 and their psychological symptoms such as anxiety, depression, and fear are needed. It is thought that being treated, especially in a hospital environment, may have a more negative impact on the mental health of individuals diagnosed with COVID-19. As a matter of fact, hospitalization due to COVID-19 has been found to increase the risk of new-onset mental disorders compared to the general population not hospitalized due to COVID-19.[20] In this way, psychological interventions can be developed to cope with the psychological effects caused by the epidemic. It is thought that the results of this study can be used in planning psychosocial interventions for patients hospitalized in pandemic hospitals under quarantine conditions.

Aim

The present study aimed to determine the relationship between the psychological well-being of individuals receiving treatment in the COVID-19 clinic of a pandemic hospital and their anxiety, fear of COVID-19, and depression levels.

Material and methods

Study design and participants

The study had a descriptive and correlational design. The data of the study were collected in a hospital in the Central Anatolia Region of Türkiye, affiliated with the Ministry of Health, serving as the largest pandemic hospital in the region.

Convenience sampling was used as the sample selection method in the study.[21] In July and August 2020, when the study was conducted, a total of 693 participants were included in the study, and the data collection process of the study was terminated with 685 individuals diagnosed with COVID-19 who met the inclusion criteria.

Inclusion criteria

- Receiving inpatient treatment at the hospital where the study was conducted
- PCR test (+)
- Ability to communicate verbally
- Being over 18 years old
- Volunteering to participate in the study

Exclusion criteria

- PCR test (-)
- Having a physical or mental illness that prevents communication
- The need for intense oxygen

Two patients were excluded from the study during the data collection process because of the diagnosis of dementia, 1 patient requiring intense oxygen and 5 patients not accepting to participate in the study.

Data collection

The data were collected by the first researcher between July and August 2020. Because of the risk of COVID-19 transmission, the telephone interview method was used to collect the data. The patient list and the internal phone numbers of the rooms where the patients were located were obtained from the responsible health personnel by visiting the pandemic hospital every morning during the data collection process. Preliminary information about the patients was obtained from the responsible health staff and patients who did not meet the inclusion criteria were not included in the study. The patients were called by the first researcher and brief information about the study was given. Then, the patient was asked whether he or she would volunteer to participate in the study. Due to pandemic conditions, the researchers did not contact the participants. Two telephone interviews were held with the participants. During the first phone call, participants were informed about the study and their verbal consent was obtained. Written consent was then obtained by the responsible healthcare personnel in accordance with the pandemic conditions.

A survey was conducted by the researcher during the second phone call. The questions included in the data collection tools were asked of the patients who agreed to participate in the study. The interview with each patient lasted an average of 15–20 minutes. A preliminary application was conducted with 10 individuals to evaluate the data collection tools and data collection method. After pre-application individuals who were pre-implemented as no changes were made were included in the study.

Data collection tools

The data of the study were collected with the information form, psychological well-being scale, COVID-19 fear scale, and hospital anxiety and depression scale.

Information form

This form was prepared by researchers according to the literature data.[18,22] The form included sociodemographic characteristics. Also, the participants' COVID-19 symptoms, taking protective measures against COVID-19, having COVID-19 + in their relatives, mental and/or physical illness, and continuous use of drugs were evaluated in this form. There are twenty questions in the information form

Psychological well-being scale (PWBS)

Psychological well-being includes important elements from positive relationships that support human well-being to having a purposeful life. It was developed by Diener in 2010.[23] Turkish validity and reliability was made by Telef in 2013, and was found to be 0.80. It is a 7-point Likert-style one-dimensional scale that consisted of eight positive items. The score obtained from the scale varies between 8–56. A high score indicates that the person has many psychological resources and power.[24] In this study, Cronbach's alpha value of the scale was found to be 0.971.

COVID-19 fear scale (FCV-19S)

The scale was developed by Ahorsu et al. and its Turkish adaptation was conducted by Satıcı et al. as a 7-item, 5-point Likert style scale.[12,25] The total score obtained from the scale varies between 7 and 35. A high score on the scale indicates a high level of fear of COVID-19. The scale was shown to have strong psychometric properties, including high internal consistency (α=0.82).[12] In this study, Cronbach's alpha value of the scale was found to be 0.973.

Hospital anxiety and depression scale (HADS)

The scale was developed by Zigmond et al. in 1983 and the validity and reliability study was carried out by Aydemir et al.[26,27] The scale aims to determine the risk group by screening for anxiety and depression in a short time in patients with physical illness. Depression and anxiety are evaluated with the help of two subscales. HADS, which consists of 14 items in total, consists of 7 items of HADS-Anxiety and 7 items of HADS-depression subscales. The lowest score for each sub-dimension is 0 and the highest score is 21. The cut-off point of the anxiety subscale of the HADS Turkish version is 10, and the cut-off point for the depression subscale is 7. Patients who have scores above these points are considered a risk group.[27]

The reliability coefficients of the anxiety and depression sub-dimensions of the HADS scale for the Turkish patient group were determined as 0.85 and 0.78, respectively.[27] In this study, Cronbach's alpha values were found to be 0.965 for the HADS-Anxiety subscale and 0.962 for the HADS-Depression subscale.

Ethics approval

After the approval of the Ministry of Health (Approval No: 2020-05-28T23-23-45) and the permission of the pandemic hospital where the study was conducted, and approval from the Nevşehir Hacı Bektaşi Veli University Non-Interventional Clinical Research Ethics Committee (Approval No: 2020.15.180.) the study was initiated. Verbal informed consent was obtained from the participants in the study, adhering to the principles of the Declaration of Helsinki. In addition, permission was obtained from the authors for the scales used in this study.

Analysis of data

The data analysis was performed by using the SPSS 22.0 (IBM, Armonk, NY, USA) statistical package program. Descriptive data were evaluated by using percentage calculation, mean, standard deviation, and minimum and maximum values. The Shapiro-Wilk test was used to test normality. Parametric tests were used as the data were following the normal distribution. The Independent Samples T-test was used in paired groups to compare the data. The One-Wav ANOVA Test was used in more than two groups and Hochberg's GT2 post hoc test was used to determine the group that determined the difference between groups. The relationship between the scores of the PWBS and the FCV-19S, HADS-anxiety, and HADS-depression subscales was evaluated with the Pearson correlation analysis, and $p<0.05$ was accepted as a statistical significance level.

Results

The mean age of the participants (n=685) was found to be 52.88±14.22, 52.1% were male, 87.4% were married, and 54.2% were primary school graduates. 39.6% of the participants worked in an income-generating job and 77.7% of them had a medium income. The income level of 33.3% of them decreased during the pandemic period. The rate of those who had to work during the quarantine period was 19% (Table 1).

Table 1. Comparison of participants' personal characteristics and PWBS total scores*

Personal characteristics	n	%	PWBS Mean±SD	Statistical analysis	Significant difference (post hoc)[a]
Age group					
45 and below	212	30.9	41.41±12.48	F=57.290	1–3 (p<0.001)
46-65	324	50.7	39.24±13.63	p<0.001	2–3 (p<0.001)
66 and over	149	18.4	27.10±13.5		
Age (year) mean±SD (min–max)			**52.88±14.22 (19–91)**		
Gender					
Female	328	47.9	31.55±13.25	t=-10.720	-
Male	357	52.1	42.41±13.24	p<0.001	-
Marital status					
Married	599	87.4	37.27±14.35	t=0.317	-
Single	86	12.6	36.75±14.09	p=0.752	-
Education					
Literate	35	5.1	28.80±14.5	F=28.793	1–3 (p<0.001)
Primary school	371	54.2	33.83±14.2	p<0.001	1–4 (p<0.001)
High school	129	18.8	41.35±12.8		2–3 (p<0.001)
University and over	150	21.9	43.96±11.8		2–4 (p<0.001)
Working					
Yes	271	39.6	45.08±11.33	t=13.520	-
No	414	60.4	32.06±13.70	p<0.001	-
Income					
Good	73	10.7	44.41±12.91	F=10.636	1–2 (p<0.001)
Middle	532	77.7	36.32±14.25	p<0.001	1–3 (p=0.002)
Bad	80	11.7	36.55±14.16		
Have to work in quarantine					
Yes	130	19.0	42.16±11.66	t=5.104	-
No	555	81.0	36.05±14.63	p<0.001	-
Income change during the pandemic					
No	427	62.3	37.07±14.52		
İncreased	30	4.4	32.83±14.67	F=1.823	-
Decreased	228	33.3	38.05±13.79	p=0.162	-

* PWBS – psychological well-being scale, t – independent samples T-test, F – the one-way ANOVA, a – post hoc analysis Hochberg'S GT2

The participants who were 66 years of age and older had a significantly lower PWBS score (27.1±13.5) when compared to other age groups (p<0.001). Women's (31.55±13.25) PWBS scores were significantly lower than men's (42.41±13.24) (p<0.001). Those who were literate (28.8±14.5) and primary school graduates (33.83±14.2) had a significantly lower PWBS score than those who graduated from high school and university and above (p<0.001). Those who did not work (32.06±13.7), whose income was moderate (36.32±14.25) and poor (36.55±14.16), and who did not have to work during the quarantine period (36.05±14.63) had a significantly lower total PWBS score than the others (p<0.001). There were no significant differences between the change in the income status of the participants during the pandemic period and the participants' PWBS scores (p>0.05) (Table 1).

Table 2. Comparison of participants' personal and clinical characteristics with PWBS score*

Characteristics	n	%	PWBS Mean±SD	Statistical analysis
Protective measures against COVID-19				
Yes	575	83.9	37.86±14.07	t=2.729
No	110	16.1	33.81±15.1	p=0.007
COVID-19+ in relatives				
Yes	461	67.3	35.08±14.49	t=-5.943
No	224	32.7	41.58±12.88	p<0.001
Diagnosed mental illness				
Yes	26	3.8	27.88±13.04	t=-3.415
No	659	96.2	37.58±14.24	p<0.001
Diagnosed chronic physical illness[a]				
Yes	242	35.3	29.72±13.39	t=-10.973
No	443	64.7	41.30±13.09	p<0.001
Diabetes				
No	536	78.2	39.73±13.78	t= 9.856
Yes	149	21.8	28.13±12.39	p<0.001
Hypertension				
No	546	79.7	39.15±13.7	t=7.306
Yes	139	20.3	29.58±14.14	p<0.001
COPD or asthma				
No	665	97.1	37.44±14.25	t=2.439
Yes	20	2.9	29.55±14.23	p=0.015
Continuous drug use				
Yes	237	34.6	29.78±13.5	t=-10.658
No	448	65.4	41.14±13.13	p=0.001
HADS-anxiety subscale				
Non anxiety	374	54.6	47.60±8.19	t=34.498
Anxiety	311	45.4	24.71±9.16	p=0.001
HADS-depression subscale				
Non depression	307	44.8	49.98±6.28	t=35.417
Depression	378	55.2	26.84±9.94	p=0.001

* [a] – there are individuals with more than one chronic physical illness, t – independent samples T-test

A total of 83.9% of the participants said that they took protective measures against COVID-19 (Mask, distance, hygiene) 67.3% reported that their relatives had COVID-19+ individuals and 3.8% of them reported that they had a diagnosis of a mental illness, 35.3% of them had a diagnosis of chronic physical disease (diabetes mellitus: 21.8%, hypertension 20.3%, COPD or asthma: 2.3%) and 34.6% of them said that they had at least one drug that they used constantly. It was determined that 45.4% of the participants experienced anxiety and 55.2% experienced depression (Table 2).

The participants who did not take protective measures against COVID-19 (33.81±15.1; p=0.007) and those who had COVID-19+ in their relatives (35.08±14.49; p<0.001) had significantly lower PWBS scores. Those with a current diagnosis of mental and/or physical illness had a significantly lower PWBS total score (p<0.001) and those who currently had diabetes, hypertension, COPD, or asthma and who used continuous medication had a significantly lower total PWBS score (p<0.001). According to HADS, participants who experienced anxiety (24.71±9.16, p<0.001) and depression (26.84±9.94, p<0.001) had significantly lower PWBS scores (Table 2).

A total of 40.9% of the participants had a cough, 33.7% had respiratory distress, 25% had a fever, and 21% had joint and muscle pain symptoms. Participants who experienced cough, respiratory distress, fever, and joint and muscle pain symptoms had a significantly lower PWBS total score than those who did not (p<0.05) (Table 3).

Table 3. Comparison of participants' COVID-19 symptoms with PWBS total scores*

COVID-19 Symptoms	n	%	PWBS Mean±SD	Statistical analysis
Respiratory distress				
No	454	66.3	40.04±13.94	t=7.556
Yes	231	33.7	31.64±13.37	p<0.001
Cough				
No	405	59.1	39.83±13.86	t=5.919
Yes	280	40.9	33.41±14.10	p<0.001
Fever				
No	514	75	38.29±14.48	t=3.605
Yes	171	25	33.96±13.29	p=0.001
Joint and muscle pain				
No	541	79	37.89±14.11	t=2.411
Yes	144	21	34.66±14.78	p=0.016

* t – independent samples T-test

The mean PWBS score of the participants was 37.21±14.3, the FCV-19S total score was 20.1±10.41, the HADS-anxiety subscale total score was 9.07±7.29, and the HADS-depression subscale total score was 10.74±7.35 (Table 4).

Table 4. Participants' PWBS, FCV-19S, HADS scores*

Scales	Mean±SD	Min-max
PWBS	37.21±14.3	8–56
FCV-19S	20.10±10.41	7–35
HADS-anxiety subscale	9.07±7.29	0–21
HADS-depression subscale	10.74±7.35	0–21

* PWBS – psychological well-being scale, FCV-19S – COVID-19 fear scale, HADS – hospital anxiety and depression scale

A significant relationship was detected between the participants' PWBS scores and their FCV-19S, HADS-Anxiety subscale, and HADS-Depression subscale scores. There was a significant and negative correlation between the participants' PWBS and FCV-19S scores (r=-0.883, p<0.001). A significant and negative correlation was detected between the participants' PWBS scores and HADS-Anxiety subscale (r=-0.878, p<0.001) and HADS-Depression subscale (r=-0.874, p<0.001) scores (Table 5).

Table 5. The relationship between participants' FCV-19S, PWBS, HADS scores*

Scales	1	2	3	4
1 FCV-19S	1			
2 HADS-anxiety subscale	r=0.957[a] p<0.001	1		
3 HADS-depression 4 subscale	r=0.945[a] p<0.001	r=0.957[a] p<0.001	1	
PWBS	r=-0.883[a] p<0.001	r=-0.878[a] p<0.001	r=-0.874[a] p<0.001	1

* [a] – Pearson correlation analysis

Discussion

In the present study, which was conducted to determine the relationship between the psychological well-being of individuals receiving treatment in the COVID-19 clinic and the levels of anxiety, fear of COVID-19, and depression.

The psychological well-being of COVID-19+ patients aged 66 and over, female, with low education and income level was found to be significantly lower than the other groups. In a previous study, it was reported that especially women and individuals with low education levels have more psychological problems and suffer from insomnia in risky situations.[28] In other studies, depression and anxiety were found to be significantly higher in COVID-19+ women.[29-32] It can be said that women are more affected by COVID-19 psychologically.[33] Advanced age is an important factor in coping with COVID-19. In the study evaluating the fear of COVID-19 infection in the elderly population and its relationship with depressive and anxiety symptoms. It was determined that individuals who are more concerned about having the disease develop more anxiety and depression symptoms during the COVID-19 epidemic.[34] Similar to the literature, it can be said that the psychological well-being of elderly individuals is negatively affected by COVID-19. Another reason for this may be the chronic diseases of elderly individuals. In this study, it was determined that the psychological well-being of the participants with a current diagnosis of mental and/or physical disease and those who currently have diabetes, hypertension, COPD, or asthma and who use drugs continuously are significantly lower.

Studies have shown that the rate of depression and anxiety is high in patients with chronic heart disease and diabetes.[35-37] These results are consistent with our study findings. Also, COVID-19 may have caused anxiety and depression as a result of fear of death, especially in individuals with chronic diseases. Also, individuals who do not pay attention to protective measures and because of the risk of infecting their relatives with COVID-19 may be adversely affected psychologically. In support of this view, it was determined that the psychological well-being of the participants who did not take protective measures against COVID-19 and whose relatives had COVID-19+ was significantly lower. The difficulty experienced by COVID-19 symptoms may be another reason why participants feel psychologically negative during the disease period. The psychological well-being of participants who did not experience symptoms of cough, respiratory distress, fever, joint and muscle pain was better ($p<0.05$).

Participants who experienced anxiety and depression and those who experienced cough, respiratory distress, fever, and joint and muscle pain symptoms had significantly lower PWBS scores than those who did not. Since these findings will create the perception that the disease is getting worse, it may cause the individual to feel worse. Also, individuals who are not economically well may have experienced anxiety because of the financial burden of the treatment process of the disease. In a previous study, it was reported that uncertainty, social discrimination, and poor economic situation caused anxiety in patients.[38]

It is a fact that the COVID-19 pandemic affects individuals and societies in many ways. It is stated that new cases of anxiety and depressive disorders have been added with the pandemic. It was reported that 53.2 million additional major depressive disorder cases and 76.2 million additional anxiety disorder cases are added worldwide.[8,22,39] In the present study, anxiety and depressive symptoms were detected in approximately half of the participants. It was particularly noteworthy that the mean score of the HADS-Depression subscale was higher than the cut-off score. In previous studies conducted with individuals diagnosed with COVID-19 in 5 different countries, it was determined that 7.7-34.72% of patients experienced anxiety symptoms and 8.0-40.0% depression symptoms.[29,30,32,33,40-42] It can be reported that the anxiety and depressive symptoms experienced by the individuals in this study were relatively higher than in other studies. In this study, it is considered that the psychological well-being of individuals and many factors that will affect this may be related to anxiety and depressive symptoms. However, it is considered in this study that the sample is individuals in the acute phase of the disease receiving treatment in the pandemic hospital and the fear caused by COVID-19. As a matter of fact, when the relationship between fear of COVID-19 and other psychological variables in this study is evaluated, the result supports this view.

It was determined in this study that there was a negative significant relationship between the participants' PWBS scores and their FCV-19S, HADS-anxiety, and HADS-depression scores. In other words, as the psychological well-being of the participants decreases, the fear of COVID-19, anxiety, and depression levels increase. In a study conducted previously on the subject, a positive and significant relationship was found between fear of COVID-19 and anxiety and depression, similar to the findings of our study.[43] It was found in another study that while fear and anxiety were very high in the period before catching COVID-19 and during the COVID-19+ period, these feelings gradually decreased when the recovery period was entered.[44] When patients feel good, their fear of Covid-19 also decreases. However, there is a decrease in anxiety and depression levels.[38] In this study, as the fear of COVID-19, anxiety, and depression levels of the participants decreased, the increase in the level of psychological well-being supports this view. Individuals diagnosed with COVID-19 were affected psychologically for many reasons such as isolation and uncertainty in the healing process. Signs of depression were detected especially in those who had to stay in the hospital for a long time for treatment.[45] In a study, it was reported that COVID-19+ patients may experience depression and anxiety symptoms up to 12 months after discharge.[46]

Study limitations

This study is limited to the experiences of patients receiving inpatient treatment in pandemic clinics of a pandemic hospital.

Conclusion

According to the results obtained in this study, as anxiety, fear of COVID-19, and depression levels increase in individuals receiving hospital treatment because of the diagnosis of COVID-19, their psychological well-being levels decrease significantly. Also, advanced age, being a woman, low education level, not working and having a middle/low-income level, and being obliged to work during the quarantine period are the sociodemographic characteristics of individuals whose psychological well-being is adversely affected. Not taking preventive measures against COVID-19, having individuals diagnosed with COVID-19 in their relatives, being diagnosed with chronic mental and physical diseases, and using regular medication affect psychological well-being negatively.

Psychosocial interventions are recommended for anxiety, fear of COVID-19, and depressive symptoms in order to increase the psychological well-being levels of individuals receiving inpatient treatment due to a diagnosis of COVID-19.

Declarations

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Author contributions

Conceptualization, N.D., S.A.Ç. and Ö.B.K.; Methodology, N.D. and S.A.Ç.; Software, N.D. and S.A.Ç.; Validation, N.D.; Formal Analysis, S.A.Ç.; Investigation, N.D. and S.A.Ç.; Resources, N.D., S.A.Ç. and Ö.B.K.; Writing – Original Draft Preparation, N.D. and S.A.Ç.; Visualization, N.D. and S.A.Ç.; Supervision, N.D., S.A.Ç. and Ö.B.K.; Project Administration, N.D., S.A.Ç. and Ö.B.K.; Funding Acquisition, N.D. and S.A.Ç.

Conflicts of interest

The author declares no conflicts of interest.

Data availability

Data will be made available on request.

Ethics approval

After the approval of the Ministry of Health (Approval No: 2020-05-28T23-23-45) and the permission of the pandemic hospital where the study was conducted, and approval from the Nevşehir Hacı Bektaşi Veli University Non-Interventional Clinical Research Ethics Committee (Approval No: 2020.15.180.) the study was initiated.

References

1. Bao Y, Sun Y, Meng S, Shi J, Lu L. 2019-nCoV Epidemic: Address mental health care to empower society. *Lancet*. 2020;395(10224):37-38. doi: 10.1016/S0140-6736(20)30309-3
2. Rajkumar RP. COVID-19 and mental health: A Review of the existing literature. *Asian J Psychiatr.* 2020;10(52):102066. doi: 10.1016/j.ajp.2020.102066
3. Xiao CA. Novel approach of consultation on 2019 Novel Coronavirus (COVID-19)-related psychological and mental problems: Structured letter therapy. *Psychiatry Investigation*. 2020;17(2):175-176. doi: 10.30773/pi.2020.0047
4. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395:497-506. doi: 10.1016/S0140-6736(20)30183-5
5. Su S, Wong G, Shi W, et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. *Trends in Microbiology*. 2016;24:490-502. doi: 10.1016/j.tim.2016.03.003
6. Tucci V, Moukaddam N, Meadows J, Shah S, Galwankar SC, Kapur GB. The forgotten plague: psychiatric manifestations of Ebola, Zika, and emerging infectious diseases. *J Glob Infect Dis.* 2017;9:151-156.
7. Torales J, O'Higgins M, Castaldelli-Maia JM, Ventriglio A. The outbreak of COVID-19 coronavirus and its impact on global mental health. *International Journal of Social Psychiatry.* 2020;66(4):317-320. doi: 10.1177/0020764020915212
8. Santomauro DF, Herrera M, Shadid J, et al. Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. *Lancet.* 2020;398(10312):1700-1712. 10.1016/S0140-6736(21)02143-7
9. JAMA Network. Coronavirus disease 2019 (COVID-19). https://jamanetwork.com/journals/jama/pages/coronavirus-alert. Accessed November 5, 2020.
10. Mazza C, Ricci, E, Biondi S, et al. A Nationwide survey of psychological distress among Italian people during the COVID-19 pandemic: immediate psychological responses and associated factors. *Int J Environ Res Public Health.* 2020;17(9):3165. doi: 10.3390/ijerph17093165
11. Lei L, Huang X, Zhang S, Yang J, Yang L, Xu M. Comparison of prevalence and associated factors of anxiety and depression among people affected by versus people unaffected by quarantine during the COVID-19 Epidemic in Southwestern China. *Med Sci Monit.* 2020;26:924609. doi: 10.12659/MSM.924609
12. Ahorsu DK, Lin CY, Imani V, Saffari M, Griffiths MD, Pakpour AH. The Fear of COVID-19 Scale: development and initial validation. *Int J Ment Health Addict.* 2022;20:1537-1545. doi: 10.1007/s11469-020-00270-8
13. Xiang YT. Timely mental health care for the 2019 novel coronavirus outbreak is urgently needed. *Lancet Psychiatry*. 2020;7:228-229. doi: 10.1016/S2215-0366(20)30046-8
14. Lin CY. Social reaction to ward the 2019 novel coronavirus (COVID-19). *Social Health and Behavior.* 2020;3(1):1-2. doi: 10.4103/SHB.SHB_11_20
15. Zhang J, Wu W, Zhao X, Zhang W. Recommended psychological crisis intervention response to the 2019 novel coronavirus pneumonia outbreak in China: a model of West China Hospital. *Precision Clinical Medicine.* 2020;3(1):3-8.
16. Blake H, Bermingham F, Johnson G, Tabner A. Mitigating the psychological impact of COVID-19 on healthcare workers: a digital learning package. *Int J Environ Res Public Health*. 2020;17:2997. doi: 10.3390/ijerph17092997
17. Salari N, Hosseinian-Far A, Jalali R, et al. Prevalence of stress, anxiety, depression among the general population during the COVID-19 pandemic: a systematic review and meta-analysis. *Globalization and Health.* 2020;16(1):57. doi: 10.1186/s12992-020-00589-w
18. Wang C, Pan R, Wan X, et al. Immediate psychological responses and associated factors during the initial stage of the 2019 Coronavirus Disease (COVID-19) epidemic among the general population in China. *Int J Environ Res Public Health.* 2020;17(5):1729. doi: 10.3390/ijerph17051729
19. Vindegaard N, Benros ME. COVID-19 pandemic and mental health consequences: Systematic review of the current evidence. *Brain, behavior, and immunity*. 2020;89:531-542. doi: 10.1016/j.bbi.2020.05.048

20. Nersesjan V, Christensen RHB, Kondziella D, Benros ME. COVID-19 and risk for mental disorders among adults in Denmark. *JAMA Psychiatry.* 2023;80(8):778-786. doi: 10.1001/jamapsychiatry.2023.1265
21. Kılıç S. Sampling methods. *Journal of Mood Disorders*. 2013;3(1):44-46. doi: 10.5455/jmood.20130325011730
22. Ettman CK, Abdalla SM, Cohen GH, Sampson L, Vivier PM, Galea S. Prevalence of depression symptoms in US adults before and during the COVID-19 pandemic. *JAMA Network Open*. 2020;3(9):2019686. doi: 10.1001/jamanetworkopen.2020.19686
23. Diener E, Wirtz D, Tov W, et al. New well-being measures: Short scales to assess flourishing and positive and negative feelings. *Soc Indic Res.* 2010;97(2):143-156.
24. Telef BB. Psychological well-being scale: Adaptation to Turkish, validity and reliability study. *Hacettepe Üniversitesi Eğitim Fakültesi Dergisi.* 2013;28(3):374-384.
25. Satici B, Gocet-Tekin E, Deniz ME, Satici SA. Adaptation of the Fear of COVID-19 Scale: Its association with psychological distress and life satisfaction in Türkiye. *Int J Ment Health Addict.* 2021;19(6):1980-1988. doi: 10.1007/s11469-020-00294-0
26. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scandinavica.* 1983;67(6):361-370. doi: 10.1111/j.1600-0447.1983.tb09716.x
27. Aydemir Ö, Güvenir T, Küey L. Validity and realibility of Turkish version of Hospital Anxiety and Depression Scale. *Turkish Journal of Psychiatry*. 1997;8:280-287.
28. Guandalini LS, da Silva EF, Lopes JDL, Santos VB, Lopes CT, de Barros ALBL. Analysis of the evidence of related factors, associated conditions and at-risk populations of the NANDA-I nursing diagnosis insomnia. *Int J Nurs Sci.* 2020;7(4):466-476. doi: 10.1016/j.ijnss.2020.09.003
29. Alqurashi E, Aldobyany A, Touman, A, et al. Prevalence of depression and its associated factors among patients with confirmed COVID-19 in Makkah, Saudi Arabia. *Front Psychiatry.* 2022;30(13):863215. doi: 10.3389/fpsyt.2022.863215
30. Chakrabarti S. Mental health in hospitalised COVID-19 patients in Quarantine during second wave in a South Indian Private Teaching Hospital. *J Multidiscip Healthc*. 2021;14:2777-2789. doi: 10.2147/JMDH.S330812
31. He Y, Huang L, Chen J, et al. Mental health status and related influencing factors in patients with COVID-19. *BMC Psychol.* 2023;11(1):225. doi: 10.1186/s40359-023-01254-8
32. Argüder E, Kılıç H, Civak M, et al. Anxiety and depression levels in hospitalized patients due to COVID-19 infection. *Ankara Med J.* 2020;(4):971-981. doi: 10.5505/amj.2020.80775
33. Kesgin TM, Hançer Tok H, Uzun LN, Pehlivan Ş. Comparison of anxiety levels of hospitalized COVID-19 patients, individuals under quarantine, and individuals in society. *Perspect Psychiatr Care.* 2022;58(1):149-158. doi: 10.1111/ppc.12857
34. Khalaf OO, Abdalgeleel AS, Mostafa N. Fear of COVID-19 infection and its relation to depressive and anxiety symptoms among elderly population: online survey. *Middle East Current Psychiatry.* 2022;29:7. doi: 10.1186/s43045-022-00177-1
35. Doerfler LA, Paraskos JA. Anxiety, posttraumatic stress disorder, and depression in patients with coronary heart disease: a practical review for cardiac rehabilitation professionals. *J Cardiopulm Rehabil.* 2004;24:414-421. doi: 10.1097/00008483-200411000-00009
36. Shuhaida MHN, Suhaila MYS, Azidah KA, Norhayati NM, Nani D, Juliawati M. Depression, anxiety, stress and socio-demographic factors for poor glycaemic control in patients with type II diabetes. *J Taibah Univ Med Sci.* 2019;14:268-276. doi: 10.1016/j.jtumed.2019.03.002
37. Minoura A, Sugiyama T, Koyama T, Yoshioka, T, Tabuchi T. Structural equation modeling of the effects of psychological distress and a fear of coronavirus disease 2019 on diabetes care in Japan: a cross-sectional study. *Sci Rep.* 2022;12(1):16142. doi: 10.1038/s41598-022-20716-4
38. Li T, Hu Y, Xia L, et al. Psychological experience of patients with confirmed COVID-19 at the initial stage of pandemic in Wuhan, China: A qualitative study. *BMC Public Health.* 2021;21:2257. doi: 10.1186/s12889-021-12277-4
39. Arora T, Grey I, Östlundh L, Lam KBH, Omar OM, Arnone D. The prevalence of psychological consequences of COVID-19: a systematic review and meta-analysis of observational studies. *J Health Psychol.* 2022;27(4):805-824. doi: 10.1177/1359105320966639
40. Adhikari B, Poudel L, Thapa TB, et al. Prevalence and factors associated with depression, anxiety, and stress symptoms among home isolated COVID-19 patients in Western Nepal. *Dialogues Health*. 2023;2:100090. doi: 10.1016/j.dialog.2022.100090
41. Kong X, Zheng K, Tang M, et al. Prevalence and factors associated with depression and anxiety of hospitalized patients with COVID-19. *medRxiv*. 2020. doi: 10.1101/2020.03.24.20043075
42. Yilmaz Bulut T, Cekic Y, Altay B. Examination of fear, anxiety, depression and hope levels of COVID-19 patients treated in hospital. *International Journal of Caring Sciences.* 2023;16(2):603-614.
43. Uka V, Retkoceri G, Beka D, Karameta M. The association between Fear of COVID-19 and the Level of Stress, Anxiety and Depression. *J Acta Psychopathol.* 2021;7(9):9736.
44. Sun N, Wei L, Wang H, et al. Qualitative study of the psychological experience of COVID-19 patients during hospitalization. *J Affect Disord.* 2021;278:15-22. doi: 10.1016/j.jad.2020.08.040
45. Villa S, Jaramillo E, Mangioni D, Bandera A, Gori A, Raviglione MC. Stigma at the time of the COVID-19 pandemic. *Clin Microbiol Infect.* 2020;26(11):1450-1452. doi: 10.1016/j.cmi.2020.08.001
46. Liu Z, Qiao D, Xu Y, et al. The efficacy of computerized cognitive behavioral therapy for depressive and anxiety symptoms in patients with COVID-19: Randomized Controlled Trial. *J Med Internet Res*. 2021;23(5):26883. doi: 10.2196/26883

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ORIGINAL PAPER

A study of etiological spectrum in 106 cases of pancytopenia

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ABSTRACT

Introduction and aim. Pancytopenia is the simultaneous presence of anemia, leucopenia and thrombocytopenia. The aim of this work was to study the etiological spectrum of pancytopenia in the National capital region of India, and evaluate the relationship of pancytopenia with serum vitamin B12 levels.

Material and methods. This study is of a prospective and analytical type conducted on patients attending the outpatient and inpatient department of Santosh Medical College and the Saraswathi Institute of Medical Sciences tertiary care centers in NCR. Complete blood counts and peripheral blood smear examination was used for morphological classification and typing of anemia. Bone marrow aspiration and vitamin B12 estimation was performed where required.

Results. The maximum number of pancytopenia cases were etiologically attributed to megaloblastic anemia (64.2%) followed by aplastic anemia (19.8%). Malaria was attributed to 6.6% cases of pancytopenia. Iron deficiency anemia and tuberculosis both accounted for 1.9% of cases. each. A history of drug intake and mixed nutritional anemia each contributed to 2.8% of cases. Serum vitamin B12 levels showed a significant relationship with pancytopenic cases.

Conclusion. In our study, the main cause of pancytopenia is megaloblastic anemia which responds very well to treatment if diagnosed correctly in time. A detailed hematological assessment along with vitamin B12 levels should be evaluated in all cases of pancytopenia irrespective of the etiological categorization.

Keywords. anemia, aplastic, leucopenia, megaloblastic, pancytopenia, thrombocytopenia

Introduction

Pancytopenia is the simultaneous presence of anemia, leukopenia as well as thrombocytopenia. The cause is usually a decrease in the production of hematopoietic cells in the bone marrow due to infections, toxins, infiltration of malignant cells, chemotherapy or radiation. For effective management of pancytopenia, its identification is very important. The clinical symptoms of pancytopenia are variable.[1]

Marrow cellularity and composition vary based on the underlying pathological condition. The marrow is usually hypocellular when pancytopenia occurs due to a primary production defect. Normocellular or hypercellular marrow is seen when cytopenia occurs because of ineffective hematopoiesis, invasive bone marrow procedures, and increased peripheral utilization or destruction of cells. Pancytopenia was not known as a discrete hematological entity before 1919. This term was used as a synonym for aplastic anemia, which is a life-threatening disorder leading to failure of bone marrow, and is associated with a high mortality if left untreated. Aplastic anemia is two to three times more common in Asia than in Europe. Its exact incidence is not clear in India due to the dearth of epidemiological studies. However, in an ep-

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idemiological study of children in and around Lucknow, India, the statistics show that the annual incidence of aplastic anemia is around 6.8 cases per million.[2]

Peripheral cytopenia results when there is a reduction in any of the cellular elements of the blood, i.e. red blood cells, white blood cells or platelets. Bicytopenia is the reduction of any of the two cellular elements, the symptoms attributable to anemia or thrombocytopenia. Pancytopenia is reduction of all the three types of cells. In adults with pancytopenia, hemoglobin levels fall below 13.5 g/dL in males and 11.5 g/dL in females, the leucocyte count is less than 4×10^9/L (or absolute neutrophil count of less than 1800 per mL), and the platelet count less than 100×10^9/L. Leukopenia is primarily seen as neutropenia since neutrophils constitute the majority of the leukocytes.[2,3]

This reduction in the number of cells occurs due to increased destruction, reduced production, or increased pooling in the spleen or other organs. Primary or secondary involvement of bone marrow is seen in most of the cases. There are various neoplastic and non-neoplastic causes of pancytopenia. In most cases, pancytopenia occurs due to nutritional deficiencies, thus it can be treated and is reversible. Proper diagnostic evaluation of pancytopenia needs detailed clinical history, physical examination and hematological assessment including precise peripheral blood smear examination and if needs, bone marrow evaluation.[3]

The underlying pathophysiology depends on the cause of pancytopenia. In aplastic anemia, the pathophysiology is an autoimmune-mediated T cell activation which leads to destruction of the hematopoietic stem cells. Bone marrow suppression is also caused by direct cytotoxic effects of medications such as methotrexate, anticonvulsants, and chemotherapeutic agents. Ineffective hematopoiesis is observed in the bone marrow of myelodysplastic syndrome.

The clinical presentation varies widely from mild pancytopenia which may be asymptomatic to severe pancytopenia which may lead to life-threatening emergencies.[4] Patients can manifest any of the decreased cell lines. Anemia presents as shortness of breath, fatigue and chest pain. Leukopenia manifests as increased infections, while thrombocytopenia presents with bruising, petechiae, and propensity for bleeding. Patients with severe neutropenia suffer from severe infections. Patients with underlying liver disease can present with anorexia, nausea, or lethargy. Patients with splenic sequestration can present with left upper quadrant pain. Constitutional symptoms are seen in patients with underlying autoimmune disorders or malignancies.[5]

Medical history is of utmost importance for an effective evaluation of pancytopenia. It must include investigating the symptoms of autoimmune conditions, malignancies, recent infections, medications, chemotherapy, or radiation therapy. A detailed history of nutritional status should be taken. For inherited aplastic anemia, family history should also be taken into account.

Physical examination may show pallor, petechiae, ulcers and rashes. Signs of underlying liver disease may be seen in patients with cirrhosis. Splenomegaly may be seen in patients with splenic sequestration. Lymphadenopathy can be seen in patients with infections and lymphoma. In patients with eating disorders and alcoholism, the subtle signs of nutritional deficiencies must be evaluated. The neurological examination is a must as it may highlight any impairment of proprioception with a positive Romberg test and ataxia, suggesting subacute combined degeneration of the spinal cord secondary to vitamin B12 (cobalamin) deficiency and macrocytic anemia.[2,5]

Preliminary investigations include a complete blood count and reticulocyte count to determine whether pancytopenia is secondary to decreased production or not. The mean corpuscular volume will indicate megaloblastic anemia. A peripheral blood smear may show abnormal cells such as blasts, dysplastic leukocytes, and immature cells. The investigations should also include assessment of vitamin B12 and folate levels, liver function tests, and lactate dehydrogenase levels. Infections shall also be considered because pancytopenia can be associated with infections such as HIV, malaria, and tuberculosis.[6]

In pancytopenia cases secondary to an acute viral infection, no further tests are required as these infections get cured on their own speedily. In cases of severe infections with sepsis, the termination of the infection and sepsis will also automatically correct.

Bone marrow aspiration and biopsy must be done to evaluate the status of the bone marrow stem cells if no specific etiology is found. The bone marrow aspiration can establish the diagnosis for pancytopenia in 75% of cases.[7] Pathological examination of the bone marrow biopsy is helpful in malignant etiologies. It can show a clonal population of cells, primary/secondary malignant cells, acellular marrow, fibroblasts, granulomas from tuberculosis, sarcoidosis, or fungal infections.[7]

Treatment is designed on the basis of the underlying etiology for pancytopenia. Nutritional deficiencies, if any, should be corrected. Hematologic consequences include macrocytosis, hypersegmented neutrophils, leukopenia, thrombocytopenia, and rarely, pancytopenia. In fact, pancytopenia, in which all blood cell lines are decreased, is found in only 5% of patients with a known B12 deficiency.[9] Any drug that may have precipitated the disease should be discontinued with immediate effect. Treatment of infections such as HIV or tuberculosis should be started immediately. If an autoimmune condition or malignancy is diagnosed, it should be treated. Aplastic anemia secondary to viral infections

such as parvovirus is temporary and symptomatic treatment is sufficient. For patients with severe aplastic anemia, treatment options include hematopoietic stem cell transplant and immunosuppression.[8,9]

Various studies have been done to evaluate the causes of pancytopenia, both benign and malignant. However, very few studies enumerate the specific causes related to pancytopenia encountered in daily hospital practices. The bone marrow correlation is also not emphasized in most studies.

Aim

Our study was targeted to illustrate the causative factors of pancytopenias in hospital patients who were sometimes discovered even accidentally after being admitted with epistaxis or breathlessness. Vitamin B12 deficiency in the absence of megaloblastosis leading to pancytopenia was the most important finding of our study.

Material and methods

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the protocol approved by the Ethical Committee of Santosh Medical College, Santosh Deemed to be University, NCR, India (811/2020/SMC).

The subjects of this prospective study included all adult patients presenting with pancytopenia in the age group between 18 to 60 years who were treated either at the Hematology Department of Santosh Hospital or Saraswathi Institute of Medical Sciences over a period of 2 years from March 2020 to December 2022.

Patients on myelotoxic chemotherapy, who were uncooperative or did not give consent and in whom bone marrow examination was contraindicated were excluded from the study. Diagnosed cases of malignancy, including leukemia, receiving chemotherapy or radiotherapy, and patients taking vitamin B12 and Folid acid supplements were also excluded from the study. Out of 118 patients presented to us with pancytopenia, 12 meet exclusion criteria. So, finally 106 patients were included in our study.

Diagnostic criteria for pancytopenia is:

1. hemoglobin less than 10 gm/dL,
2. total leucocyte count <4000/mm^3,
3. platelet count less than 1,000,000/mm^3.[10,11]

Detailed clinical history regarding generalized weakness, fever, bleeding tendencies and other symptoms was taken. An examination was performed, pallor, hepato-splenomegaly, lymphadenopathy, and petechiae were assessed.

Blood was withdrawn in EDTA vials for Complete blood counts and peripheral blood smear examination. Hemoglobin (Hb), total leucocyte count (TLC), differential leucocyte count (DLC), platelet count (PC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were assessed. Complete blood counts were carried out using Nihon Coden Automated Hematology Analyzer based on the principle of impedance. Peripheral smear were stained using Leishman Giemsa stain for all the cases and examined in detail. Peripheral smear was used for morphological classification and typing of anemia.

Bone marrow aspiration from iliac crest was done in 28 cases where needed using 16G needle and smears fixed in methanol and stained with MGG. The smears were assessed in detail.

Data was recorded in MS Excel and imported in SPSS v20.0 software (IBM, Armonk, NY, USA) to analyse the variables like mean, median and standard deviation.

Chi-square test and Fisher's Exact test were employed to compare the findings.

Results

In this study, out of the total 106 patients , there were 75 males (70.75%) and 31 females (29.25%) patients and male to female ratio was found to be 2.4:1.

The age distribution shows maximum number of patients in the age group of 20 to 30 years (30.2%) followed by 30 to 40 year group (23%). The minimum number of pancytopenia cases fall in the 50 to 60 year category (9%) (Table 1).

Table 1. Age wise distribution of cases

Age (years)	Number of cases	Percentage
12–20	15	14.2
20–30	32	30.2
30–40	23	21.7
40–50	17	16
50–60	9	8.5
>60	10	9.4

The maximum number of pancytopenia cases were etiologically attributed to megaloblastic anemia (64.2%) followed by aplastic anemia (19.8%). 6.6% cases of pancytopenia resulted due to malaria. Iron deficiency anemia and tuberculosis resulted in 1.9% cases each. 3 cases (2.8%) each were attributed to history of drug intake and mixed nutritional anemia (Fig. 1).

Peripheral smears revealed anisocytosis in 90% cases. Predominant macrocytic picture (cases of megaloblastic anemia) was seen in 60 cases, hyper-segmented neutrophils in 50 cases, normocytic picture in 24 cases, dimorphic in 15 cases and microcytic in 7 cases. Relative lymphocytosis and plasma cells were seen in peripheral smear in 21 cases of aplastic anemia. A few immature cells were discovered in the cases of megaloblastic anemia (Table 2).

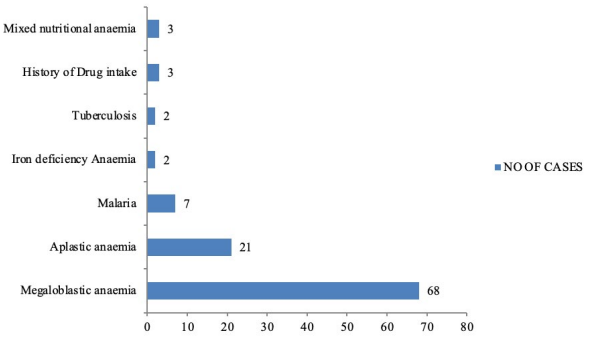

Fig. 1. Etiological distribution of pancytopenia cases

Table 2. Peripheral blood findings in 106 cases of pancytopenia

Peripheral Blood Findings	No of cases	Percentage (%)
Normocytic	24	22.6
Macrocytic	60	56.6
Microcytic	7	6.6
Dimorphic	15	14.2
Anisocytosis	90	84.9
Hypersegmented polymorphs	50	47.2
Lymphocytosis	21	19.8
Plasma cells	21	19.8
Immature cells	5	4.7

Vitamin B12 estimation was done in all the patients and it was found that a total of 80 patients were vitamin B12 deficient, i.e. <200 pg/mL. 60 out of 68 patients of megaloblastic anemia showed vitamin B12 deficiency. 15 out of 21 aplastic anemia patients had vitamin B12 levels below 200 pg/mL. This pointed out to the fact that vitamin B12 deficiency has a direct significant association with Pancytopenia. Also, in cases of severe deficiency, an aplastic blood picture can manifest itself. The p value derived by Pearson Chi-Square test was found to be <0.05, i.e significant and conclusive of the fact that vitamin B12 deficiency is linked closely with pancytopenia.

Table 3. Chi Square p value – vitamin B12 deficiency associated with causes of pancytopenia

		B12 deficiency <200 pg/mL	
		No	**Yes**
Causes	Aplastic anemia	6	15
	Drug intake	3	0
	Iron deficiency anemia	2	0
	Malaria	6	1
	Megaloblastic A	8	60
	MNA	0	3
	Tuberculosis	1	1
Total		26	80
Chi-Square Tests			
	Value	df	p
Pearson Chi-Square	37.386	6	<0.05
Likelihood Ratio	35.202	6	<0.05
Fisher-Freeman-Halton Exact Test	31.717		**Yes**

Discussion

In this study of pancytopenia patients, the highest incidence of 30.2% was in the age group of 20–30 followed by 21.7% in 30–40 age group, with a mean age of 35.53±1.467 years. Similarly, Khodke et al. found the maximum number of pancytopenia in the age group of 12–30 years.[4] About 10% of the cases were above 50 years. Jha et al. and Ishtiaq et al. in their studies found mean ages to be 30 years and 36.7 years, respectively.[14,15] Niazi and Raziq in their study found most common age group of pancytopenia in the range from 21 to 30 years.[16] The common age of 20-30 years clearly shows the increased demand and comparative reduced intake of nutritious food in this age. The high dependence on vegetarian food and incorporation of junk food in diet of young individuals make them prone to vitamin B12 deficiency causing increased incidence of pancytopenia.

In this study, out of the total 106 patients, there were 75 males (70.75%) and 31 female (29.25%) patients and male to female ratio was found to be 2.4:1.The male to female ratio in the study by Jha et al. was 1.43:1.[14] The ratio was 1.3:1 in the study by Khodke et al.[4] The study by Niazi and Raziq has given the male to female ratio of 2:1.[17] The higher number of male patients seeking medical advice accounts for the male to female ratio being 2:1.

In the present study, 64.2% cases were diagnosed as megaloblastic anemia thereby being the most common cause of pancytopenia while in other similar studies it varied from 0.8% to 80%. The high prevalence of nutritional anemia in India has been cited for increase in frequency of megaloblastic anemia. The age of the patients in this study varied from 12 to 80 years with mean age of 39.14±3.93 years. The male to female ratio was 1.6:1 clearly pointing to the fact that female health is neglected for a long time making the situation worse and the prognosis compromised.

In the present study most common presentation was pallor in 104 cases and early fatigue in 91 cases followed by dyspnea in 54 patients. Fever was the next common presentation in 53 patients followed by splenomegaly in 32 patients and bleeding in 26 cases. Hepatomegaly was seen in 17 cases. Memon et al. in their study have described the presenting features of megaloblastic anemia with pancytopenia as pallor with varying degree of skin and mucosal bleedings.[5] Bleeding manifestations were seen in 24.5% cases in the present study. Pallor is found to be associated with low hemoglobin seen in all anemics, thereby making it the commonest presentation. Reduced platelet counts lead to bleedings from various sites. Hepatomegaly and splenomegaly can be associated with extramedullary hematopoiesis and malaria.[13]

Megaloblastic anemia is characterized by ineffective erythropoiesis leading to macrocytes which are sequestrated in the spleen leading to mild to moderate sple-

nomegaly.[18] Hepatomegaly on the other hand results from *extramedullary hematopoiesis*.[13,14] In our study, 30.19% cases of megaloblastic anemia presented with splenomegaly and 16% with hepatomegaly. Ishtiaq et al. in their study found 15.4% and 17.9% of megaloblastic anemia with splenomegaly and hepatomegaly respectively.[9] Hepatomegaly (66%) and splenomegaly (21%) were seen in the study done by Keisu et al. in patients with megaloblastic anemia.[19]

In the present study, 68 cases of megaloblastic anemia patients had macrocytic RBCs and 60 cases had hyper-segmented neutrophils, which are the diagnostic features. Increased MCV was found in 62 patients; normal MCV was found in 6 patients and decreased MCV found in 3 patients with mixed nutritional deficiency having both megaloblastic anemia and iron deficiency. MCV is usually increased in severe megaloblastic anemia. Reticulocytes counts below 2% were seen in all 68 patients with megaloblastic anemia. This may be due to the abnormal maturation process. The cellularity of bone marrow ranged from 75% to 95%. Erythroid hyperplasia with predominance of precursors were also noted in the bone marrow aspiration of the megaloblastic anemia. Giant metamyelocytes, hypersegmented neutrophils and an abnormal proliferation and maturation in the erythroid precursors with large megaloblastic erythroblasts were present in bone marrow of all patients of megaloblastic anemia.[18-20]

In this study, 19.4% cases were diagnosed as aplastic anemia, age of the patients varied from 15 to 74 years with the mean age of 34±2.96 years. The male to female ratio was 6:1.3 and patients in this group were farmers and 2 were painters by profession who were exposed to insecticides and chemicals like benzene. These should be considered as possibilities for causing aplastic anemia. In this study all the cases diagnosed as aplastic anemia had reticulocytes counts below 1%.

In the present study most common presentation was pallor in 104 cases (98.1%) and early fatigue in 91 cases (85.8%) followed by dyspnea in 54 patients (50.9%). Fever was the next common presentation in 53 patients (50%) followed by splenomegaly in 32 (30.19%) patients and bleeding in 26 cases (24.5%). Hepatomegaly was seen in 17 cases (16.03%). These findings were similar to the findings of Memon et al. and Khodke et al.[5,7] Fever (47.7%) and bleeding (33.7%) were present in the patients in the study by Niazi and Raziq.[4] In the present study, splenomegaly was seen in 30.19% and hepatomegaly in 16.03% cases. The frequencies of splenomegaly and hepatomegaly were similar in various studies by Niazi and Raziq and Khodke et al.[4,7]

In this study, the disease processes resulting in pancytopenia in the peripheral blood in order of decreasing frequency were megaloblastic anemia (64.2%), aplastic anemia (19.8%), malaria (6.6%), history of drug intake (2.8%), mixed nutritional anemia (2.8%), iron deficiency (1.9%) and tuberculosis (1.9%).

The findings of our study corresponds with the findings of the study done by Khodke et al and Khunger et al. and Sweta et al. who found megaloblastic anemia 44%,74% and 66% respectively as the most common cause of pancytopenia, followed by aplastic anemia 14%, 14% and 18%.[7,6,15]

In the present study, megaloblastic anemia (64.2%) was the most common cause of pancytopenia. Incidence of megaloblastic anemia varies from 0.8% to 68% in different studies. In our country, high incidence of megaloblastic anemia may be due to high prevalence of nutritional deficiencies of vitamin B12, folic acid or both.[13,14]

Malaria (6.6%) was third most common cause of pancytopenia in this study. Similarly, Tilak and Jain in their study also described malaria as the third most common cause of pancytopenia.[7] In the study by Kumar et al. 3% of pancytopenia was due to malaria.[12]

Vitamin B12 deficiency (<200 pg/mL) was found to be a significant cause of pancytopenia even in patients who did not present with megaloblastic anemia on morphological assessment. The Chi Square test showed a significant p value of <0.05 amongst all the 106 cases irrespective of etiology.

A few limitations of the study include limited number of patients. A larger sample size will give a more comprehensive result for this study. Pancytopenia related to malignant disorders is not included in the study to keep it more focused on everyday clinical admissions.

Conclusion

Megaloblastic anemia was the most common cause of pancytopenia in this study followed by aplastic anemia among the non-malignant disorders. Mixed nutritional deficiency, malaria, tuberculosis and iron deficiency were found to be significant causes of pancytopenia. A comprehensive clinical and hematological workup helps in evaluating the etiology of pancytopenia. In addition, vitamin B12 deficiency is found to be a significant cause of pancytopenia.

Variation in the frequency of disorders causing pancytopenia has been ascribed to differences in methodology, stringency of diagnostic criteria, geographic area, period of observations and genetic differences, after analyzing the observations noted in the present study. Maximum diagnostic yield can be achieved by correlation with clinical findings, peripheral blood findings and with other laboratory and radiological parameters.

Declarations

Funding

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Author contributions

Conceptualization, P.S.; Methodology, P.S.; Software, R.D.; Validation, R.D.; Formal Analysis, R.D.; Investigation, P.S.; Resources, P.S.; Data Curation, P.S.; Writing – Original Draft Preparation, P.S.; Writing – Review & Editing, P.S. and A.M.; Visualization, A.M.; Supervision, A.M.; Project Administration, A.M.

Conflicts of interest

The authors of the given original work declare that there are no conflicts of interest.

Data availability

The data sets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the protocol approved by the Ethics Committee of Santosh Medical College, Santosh Deemed to be University, NCR, India (811/2020/SMC).

References

1. Firkin F, Chesterman C, Penington D, Rush B. de Gruchy's Clinical Hematology in Medical Practice. 6th ed. Wiley:2013.
2. Aggarwal M, Mahapatra M. Epidemiology of Clinico-Haematological Profile and Management of Aplastic Anaemia AIIMS Experience. *Assoc Physicians India*. 2015;63(3):30-35.
3. Chandra K, Kumar P. Morphological Spectrum of Bone Marrow in Pancytopenia – a retrospective study in a Tertiary Care Centre. *JEMDS*. 2014;3(4):1056-1064.
4. Khodke K, Marwah S, Buxi G, Yadav RB, Chaturvedi NK. Bone Marrow Examination in cases of pancytopenia. *J Academy Clin Med*. 2001;2(1,2):55-59.
5. Tilak V, Jain R. Pancytopenia-A Clinco-hematologic analysis of 77 cases. *Indian J Pathol Microbiol*. 1999;42(4):399-404.
6. Sweta, Barik S, Chandoke RK, Verma AK. A Prospective Clinico-haematological study in 100 cases of Pancytopenia in capital city of India. *J Appl Hematol*. 2014;5(2):45-50. doi: 10.4103/1658-5127.137139
7. Dasgupta S, Mandal PK, Chakrabarti S. Etiology of Pancytopenia: An Observation from a Referral Medical Institution of Eastern Region of India. *J Lab Physicians*. 2015;7(2):90-95.
8. Khunger JM, Arulselvi S, Sharma U, Ranga S, Talib VH. Pancytopenia – a clinico haematological study of 200 cases. *Indian J Pathol Microbiol*. 2002;45:375-379.
9. Bagheri Z, Labbani-Motlagh Z, Mirjalili M, Karimzadeh I, Khalili H. Types and outcomes of cytopenia in critically ill patients. *J Comp Eff Res*. 2022;9(9):627-637. doi: 10.2217/cer-2020-0044
10. Sasi S, Yassin MA. A Rare Case of Acquired Hemolytic Anemia and Pancytopenia Secondary to Pernicious Anemia. *Case Rep Oncol*. 2020;13(2):783-788. doi: 10.1159/000507981
11. Javalgi AP, Dombale VD. Clinico–Hematological Analysis of Pancytopenia:A Bone Marrow Study. *NJLM*. 2013;2(4):12-17.
12. Kumar R, Kalra SP, Kumar H, Anand AC, Madan H. Pancytopenia-A six year study. *J Assoc Physicians India*. 2001;49:1078-1081.
13. Khanduri U, Sharma A. Megaloblastic anaemia: Prevalence and causative factors. *Natl Med J India*. 2007;20(4):172-175.
14. Desalphine M, Bagga PK, Gupta PK, Kataria AS. To Evaluate the Role of Bone Marrow Aspiration and Bone Marrow Biopsy in Pancytopenia. *JCDR*. 2014;8(11):11-15. doi: 10.7860/JCDR/2014/9042.5169
15. Jha A, Sayami G, Adhikari RC, Panta AD, Jha R. Bone marrow examination in cases of pancytopenia. *JNMA J Nepal Med Assoc*. 2008;47:12-17.
16. Vargas-Carretero CJ, Fernandez-Vargas OE, Ron-Magaña AL, et al. Etiology and clinico- hematological profile of pancytopenia: experience of a Mexican Tertiary Care Center and review of the literature. *Hematology*. 2019;24(1):399-404. doi: 10.1080/16078454.2019.1590961
17. Ishtiaq O, Baqai HZ, Anwer F, Hussain N. Patterns of pancytopenia patients in a general medical ward and a proposed diagnostic approach. *J Ayub Med Coll Abbottabad*. 2004;16:8-13.
18. Rehman HU, Fazil M, Khan FM. The etiological pattern of pancytopenia in children under 15 years. *Pak Armed Forces Med J*. 2003;53:183-187.
19. Niazi M, Raziq F. The incidence of underlying pathology in pancytopenia. *J Postgrad Med Inst.* 2004;18:76-79.
20. Memon S, Shaikh S, Nizamani MA. Etiological spectrum of pancytopenia based on bone marrow examination in children. *J Coll Physicians Surg Pak*. 2008;18:163-167.

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ORIGINAL PAPER

Synthesis, characterization of isoxazole derivatives and evaluation of their antibacterial, antioxidant and anticancer activity

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ABSTRACT

Introduction and aim. The synthesis of heterocyclic compounds containing oxygen and nitrogen is profoundly intriguing due to their mechanistic implications in both research and development within organic chemistry and drug discovery. The primary aim of this study is to fabricate a range of pharmacologically active drugs containing the isoxazole moiety.

Material and methods. The synthesis of new derivatives of isoxazole was achieved through a one-pot condensation reaction of 2-[(Substituted phenyl)hydrazono]malononitrile (1) and 3-[(Substituted phenyl)azo]-2,4-Pentanedione (2) with sodium acetate and hydroxylamine hydrochloride (1:1) in ethanol. All the compounds were screened for their *in vitro* antibacterial activity, in vitro antioxidant and anticancer activity. The synthesized compounds underwent characterization through FTIR, ^{1}H NMR, and ^{13}C NMR analyses, supported by mass spectral data and elemental analysis.

Results. A set of novel isoxazole derivatives was synthesized with a favorable yield. Among compounds 1d, 1e, 2c, 2d, and 2e exhibited notable antioxidant activities. Compounds 1a, 1b, and 1c demonstrated significant anticancer potential against prostate cancer [PC3] cell lines compared to normal HEK cell lines, while 2a displayed the highest inhibitory zone against *Escherichia coli*.

Conclusion. Novel compounds with multifaceted biological activities have been successfully designed, and a synthetic route to create isoxazole derivatives has been devised and verified.

Keywords. antibacterial, antioxidant activity and anticancer potential, isoxazole, pathogenic bacterial

Introduction

For researchers worldwide, addressing bacterial infectious diseases remains an extremely vital and challenging issue.[1] Despite the invention of numerous novel antimicrobial drugs, their clinical efficacy remains limited in treating a growing number of life-threatening viral infections. This limitation arises from their high toxicity risk and the potential for developing drug resistance through gene sequence alterations.[2-3]

Isoxazole, along with numerous other heterocyclic compounds, holds a broad spectrum of pharmaceutical applications (Fig. 1).

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Vashisht K, Sethi P, Bansal A, Singh T, Kumar R, Tuli HS, Saini S. Synthesis, characterization of isoxazole derivatives and evaluation of their antibacterial, antioxidant and anticancer activity. *Eur J Clin Exp Med.* 2024;22(2):376–387. doi: 10.15584/ejcem.2024.2.25.

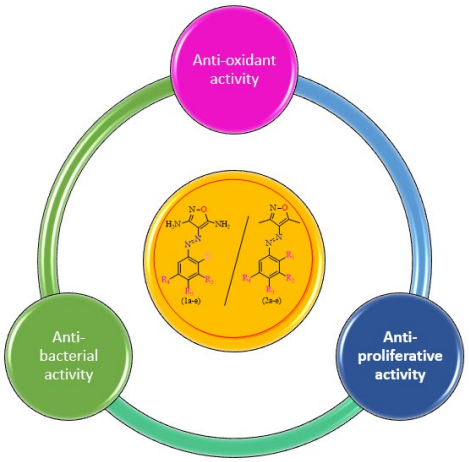


Fig. 1. Graphical abstract depicting the activity of isoxazole compounds

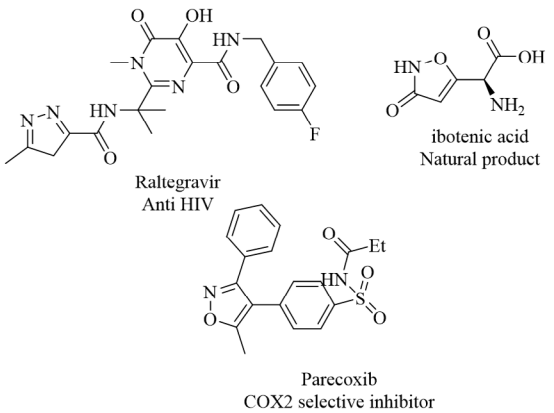


Fig. 2. Isoxazole contained drug

Exploring techniques for the development and synthesis of various heterocyclic-containing isoxazole scaffolds and their applications holds great importance in the realm of medicinal chemistry. Heterocyclic compounds, including isoxazole, are meticulously examined for their pharmacological actions and have emerged as significant pharmacophores.[4-6] These compounds exhibit a wide range of therapeutic uses, including anti-inflammatory effects, CNS depressant properties, antimicrobial activity, analgesic effects, anti-cancer properties, antioxidant properties, anti-tubercular activity, and various other biological activities such as GABA (γ-aminobutyric acid) agonistic activity, antihypertensive activity, and inhibitory activity.[7-23] Isoxazole plays a crucial role in synthesizing numerous natural and artificial compounds. Additionally, researchers have demonstrated various biological activities of different types of azole-based heterocyclic compounds, underscoring their medicinal significance (Fig. 2). The presence of diverse functional groups, such as amides, azoles, and alkyl groups, linked to the basic pharmacophoric unit structure, results in different modes of action that may be beneficial for treating microbial infections.[24]

Based on these observations and our work related to isoxazole synthesis, spectroscopy, and biological studies, this study aimed to evaluate the antioxidant, anti-cancer, and antibacterial activities of a series of novel isoxazole compounds.

Aim

In this research article, we conducted the synthesis of isoxazole derivatives and evaluated the antibacterial, antioxidant and anticancer activities of a series of novel isoxazole compounds.

Material and methods

Thin layer chromatography (TLC) was employed to ensure the completeness and purity of the reactions. Infrared (IR) spectra were acquired using a DRS probe on a SHIMADZU-FTIR-8400 spectrophotometer, covering frequencies ranging from 4000 to 400 cm^{-1}. NMR spectra were recorded on a BRUKER AVANCE II spectrometer at 500 MHz for both ^{1}H and ^{13}C nuclei, with DMSO-d6 serving as the solvent and TMS as the internal standard. Mass spectra were obtained using a direct intake probe on the mass spectrometer. Compounds utilized in this study were provided by Merck and Spectrochem Chemical companies, and all obtained compounds were of reagent grade. Additionally, all solvents were freshly distilled prior to use.

Experimental section

General protocol for the synthesis of hydrazone 2-[(Substituted Phenyl)hydrazono]malononitrile (1) (SMN) and 3-(substituted phenylazo)-2,4-pentanedione (2) (SPD)

The diazotization of various *-o*, *-m*, *-p*-substituted anilines is followed by *in situ* condensation with malononitrile and acetylacetone in the presence of sodium acetate, yielding the intermediates 2-[(substituted phenyl)hydrazono]malononitrile (1) (SMN) and 3-(substituted phenylazo)-2,4-pentanedione (2) (SPD). Diazotization is a process wherein an amine group ($-NH_2$) is converted into a diazonium salt by treatment with sodium nitrite ($NaNO_2$). After diazotization, the diazonium salt reacts with malononitrile (cyanoacetic acid) within the same reaction vessel. In a separate step, the diazonium salt reacts with acetylacetone (also known as 2,4-pentanedione). Both end products, 2-[(substituted phenyl) hydrazono]malononitrile (1) (SMN) and 3-(substituted phenylazo)-2,4-pentanedione (2) (SPD), serve as versatile intermediates that can be utilized in other synthetic transformations to generate alternative compounds with desired properties.[25]

General process for the synthesis of isoxazole (1a-e) and (2a-e)

Furthermore, treatment of 2-[(substituted phenyl)hydrazono]malononitrile (SMN) (1) (0.94 g, 5 mmol) and

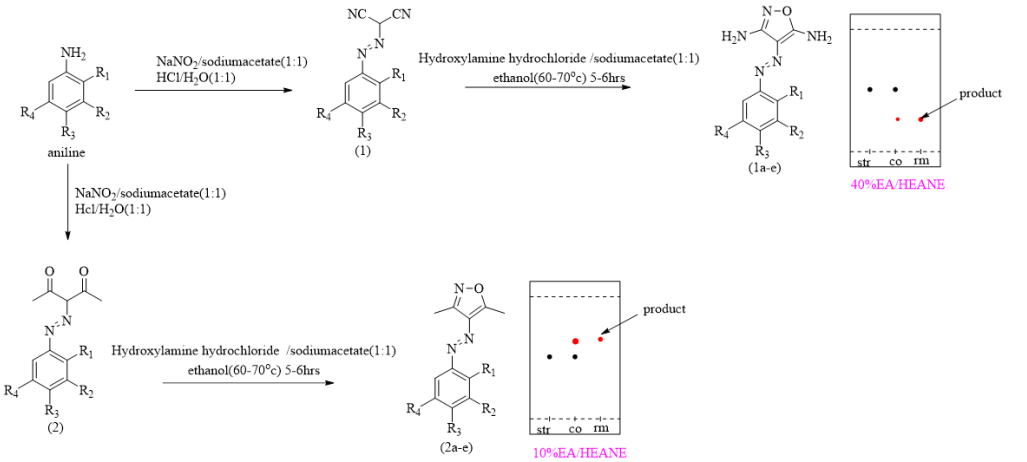


Fig. 3. Synthesis of compound (1a-e) and (2a-e)

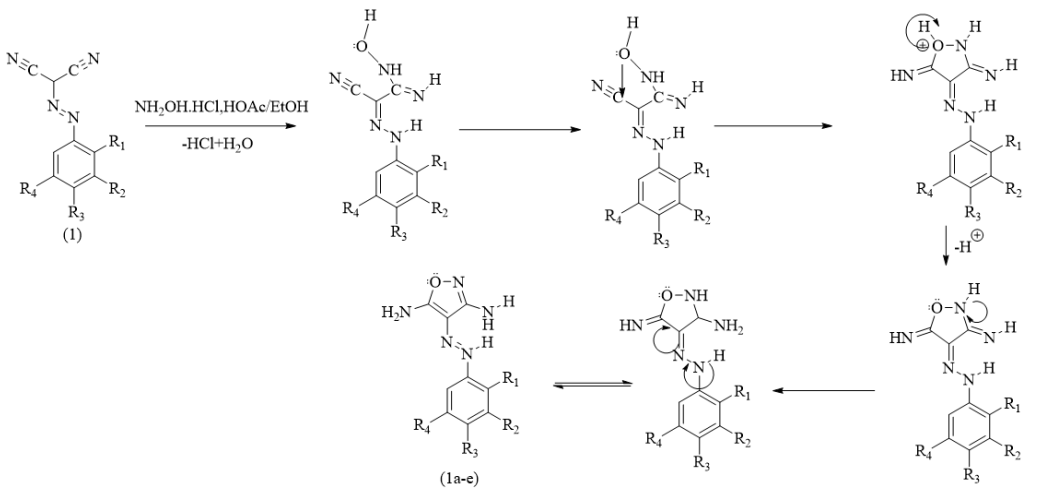


Fig. 4. Mechanism of synthetic path of compounds (1a-e)

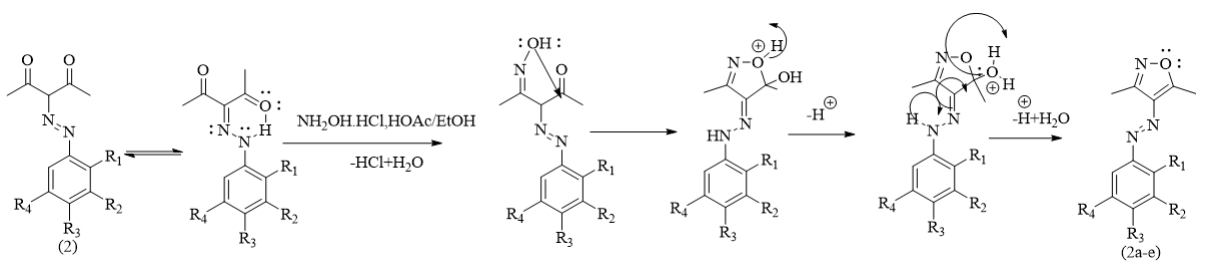


Fig. 5. Mechanism of synthetic path of compounds (2a-e)

3-(substituted phenylazo)-2,4-pentanedione (SPD) (2) (5 mmol) with hydroxylamine hydrochloride and sodium acetate (1:1) in refluxing ethanol yielded compounds (1a-e) and (2a-e) as exclusive products (Fig. 3). The reaction mixture was stirred for 5-6 hours at 60–70°C and monitored by TLC. Upon completion, the reaction was quenched with ice-cold water. The crude product (15 mL) was then extracted with chloroform, and the organic layer was washed with water before being evaporated in a rotary evaporator to obtain the solid component. Subsequently, the product underwent recrystallization in ethanol for purification. The physicochemical characterization is presented in Table 1, and Figure 4 and 5 illustrate the synthesis mechanisms.

3,5-Diamino-4-(4'-bromophenylazo) isoxazole (1a) (Fig. 6)

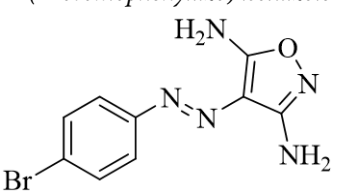


Fig. 6. 3,5-Diamino-4-(4'-bromophenylazo) isoxazole (1a)

Yield: 81%; IR (ν_{max},cm^{-1}): 3420, 3234(NH_2), 1610(C=C), 1391(N=N), 616(C-Br); (IR spectrum of compound in Figure S1); ^{1}H NMR (500 MHz, DMSO-d6) δ 7.61(d, 2H), 7.72 (d,2H), 6.15(s, 2H, D_2O exchangeable), 8.32(s, 2H, D_2O exchangeable), ^{13}C NMR (500 MHz, DMSO) δ 105.11, 120.25, 122.66, 131.14, 131.70, 150.12, 151.86, MS (EI): $[M+1]^+$ and $[M+1+2]^+$ 283.96, 284.96. Anal. Calcd. For $C_9H_8BrN_5O$: C, 38.32; H, 2.86; N, 24.83; Found: C, 38.31; H, 2.85; N, 24.79. (^{1}H-NMR data is shown in Figure S11, ^{13}C- NMR data is shown in Figure S21, D_2O exchange data shown in Figure S26, mass spectrum in Figure S28 and elemental analysis shown in Figure S33).

3,5-Diamino-4-(3'-chlorophenylazo) isoxazole (1b) (Fig. 7)

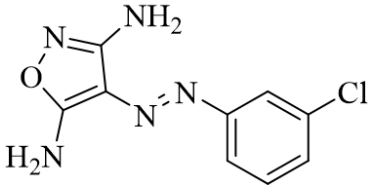


Fig. 7. 3,5-Diamino-4-(3'-chlorophenylazo) isoxazole (1b)

Yield: 85%; IR (ν_{max},cm^{-1}): 3408, 3242(NH_2), 1625(C=C), 1370(N=N), 631(C-Cl); (IR spectrum of compound in Figure S2); ^{1}H NMR (500 MHz, DMSO-d6) δ 7.87(s,1H), 7.72 (d,1H), 7.48(d,1H), 7.3(dd,1H), 6.25(s, 2H, D_2O exchangeable), 8.41(s, 2H, D_2O exchangeable), ^{13}C NMR(500 MHz, DMSO) δ 107.14, 126.92, 128.81, 129.11, 130.08, 130.24, 132.24, 155.12, 161.9, MS(EI): $[M+1]^+$ and $[M+1+2]^+$, m/z, 238.95, 239.95. Anal. Calcd. For $C_9H_8ClN_5O$: C, 45.49; H, 3.39; N, 29.47; Found: C, 46.12; H, 3.05; N, 29.14 (^{1}H-NMR data is shown in Figure S12).

3,5-Diamino-4-(4'-fluorophenylazo) isoxazole (1c) (Fig. 8)

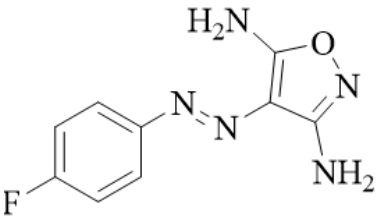


Fig. 8. 3,5-Diamino-4-(4'-fluorophenylazo) isoxazole (1c)

Yield: 82%; IR (ν_{max},cm^{-1}): 3420, 3242(NH_2), 1610(C=C), 1395(N=N), 609(C-F); (IR spectrum of compound in Figure S3); ^{1}H NMR(500 MHz, DMSO-d6) δ 7.80(d, 2H), 7.26 (d,2H), 6.11(s, 2H, D_2O exchangeable), 8.29(s, 2H, D_2O exchangeable), ^{13}C NMR (500 MHz, DMSO) δ 107.62, 115.58, 116.10, 116.27, 122.48, 149.55, 162.48 MS(EI):$[M+1]^+$, m/z, 221.09. Anal. Calcd. For $C_9H_8FN_5O$: C, 48.87; H, 3.65; N, 31.66; Found: C, 48.42; H, 3.55; N, 30.92. (^{1}H-NMR data is shown in Figure S13, ^{13}C- NMR data is shown in Figure S22, D_2O exchange data shown in Figure S27, mass spectrum in Figure S29).

3,5-Diamino-4-(2'-bromophenylazo) isoxazole (1d) (Fig. 9)

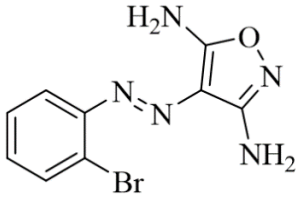


Fig. 9. 3,5-Diamino-4-(2'-bromophenylazo) isoxazole (1d)

Yield: 78%; IR (ν_{max},cm^{-1}): 3397, 3234 (NH_2), 1618 (C=C), 1469(N=N), 624(C-Br); (IR spectrum of compound in Figure S4); ^{1}H NMR(500 MHz, DMSO-d6) δ 7.81(d, 1H), 7.72 (dd,1H), 7.58(dd,1H), 7.20(d,1H), 6.45(s, 2H, D_2O exchangeable), 8.51(s, 2H, D_2O exchangeable), ^{13}C NMR (500 MHz, DMSO) δ 102.52, 117.41, 127.71, 128.12, 131.0, 131.6, 131.9, 150.21, 158.9, MS (EI): $[M+1]^+$ and $[M+1+2]^+$, 283.96, 284.96. Anal. Calcd. For $C_9H_8BrN_5O$: C, 38.32; H, 2.86; N, 24.83; Found: C, 38.24; H, 2.91; N, 24.72. (^{1}H-NMR data is shown in Figure S14 and elemental analysis shown in Figure S34).

3,5-Diamino-4-(2'-chlorophenylazo) isoxazole (1e) (Fig. 10)

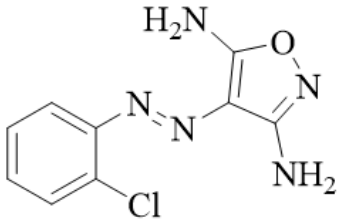


Fig. 10. 3,5-Diamino-4-(2'-chlorophenylazo) isoxazole (1e)

Yield: 81%; IR (ν_{max},cm^{-1}): 3412, 3264 (NH_2), 1632 (C=C), 1506(N=N), 602(C-Cl); (IR spectrum of compound in Figure S5); ^{1}H NMR(500 MHz, DMSO-d6) δ 7.81(d,1H), 7.72(dd,1H), 7.57(dd,1H), 7.28(d,1H), 6.43(s, 2H, D_2Oexchangeable), 8.50(s, 2H, D_2O exchangeable), ^{13}C NMR(500 MHz, DMSO) δ 105.12, 126.81, 128.47, 128.88, 130.29, 130.34, 134.37, 152.21, 158.91, MS(EI): $[M+1]^+$and $[M+1+2]^+$, m/z, 238.95, 239.95. Anal. Calcd. For $C_9H_8ClN_5O$: C, 45.49; H, 3.39; N, 29.47; Found: C, 45.92; H, 3.35; N, 29.24. (^{1}H-NMR data is shown in Figure S15).

3,5-Dimethyl-4-(2'-chloro-4'-nitrophenylazo) isoxazole (2a) (Fig. 11)

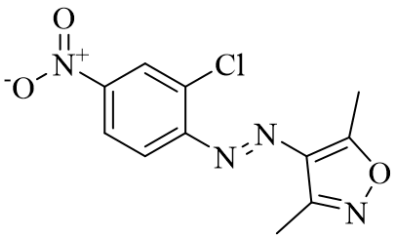


Fig. 11. 3,5-Dimethyl-4-(2'-chloro-4'-nitrophenylazo) isoxazole (2a)

Yield: 91%; IR (ν_{max},cm^{-1}): 2915, 2896(C-H), 1610 (N=N), 1545(C=C), 1403(N-O), 672(C-Cl); (IR spectrum of compound in Figure S6); ^{1}H NMR(500 MHz, DMSO-d6) δ 2.51 (s, 3H), 2.84(s,3H), 7.88(d,1H), 8.31(d,1H), 8.53(s,1H); ^{13}C NMR(500 MHz, DMSO) δ 11.47, 11.64, 118.05, 123.41, 125.80, 133.28, 133.52, 148.35, 151.50, 152.49, 173.31, MS(EI): $[M+1]^+$ and $[M+1+2]^+$, m/z, 281.04, 282.04. Anal. Calcd. For $C_{11}H_9ClN_4O_3$: C, 47.07; H, 3.23; N, 19.96; Found: C, 47.11; H, 3.29; N, 19.94. (^{1}H-NMR data is shown in Figure S16, ^{13}C- NMR data is shown in Figure S23, and elemental analysis shown in Figure S35).

3,5-Dimethyl-4-(3'-nitrophenylazo) isoxazole (2b) (Fig. 12)

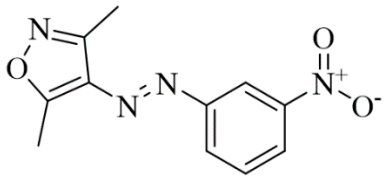


Fig. 12. 3,5-Dimethyl-4-(3′-nitrophenylazo) isoxazole

Yield: 86%; IR (ν_{max},cm^{-1}): 2923, 2856(C-H), 1603(N=N), 1491(C=C), 1405(N-O); (IR spectrum of compound in Figure S7); ^{1}H NMR(500 MHz, DMSO-d6), δ 2.50(s, 1H), 2.82 (s,3H), 7.89(dd,1H), 8.26(d,1H), 8.39(d,1H), 8.52(s,1H); ^{13}C NMR(500 MHz, DMSO) 11.14, 11.43, 112.25, 123.21, 128.12, 131.24, 134.92, 148.32, 155.50, 156.39, 163.31, MS(EI):$[M+1]^+$, m/z, 247.05. Anal. Calcd. For $C_{11}H_{10}N_4O_3$: C, 53.66; H, 4.09; N, 22.75; Found: C, 54.19; H, 4.91; N, 22.12. (^{1}H-NMR data is shown in figure-S17, mass spectrum in Figure S30).

3,5-Dimethyl-4-(2'-fluoro-4'-methylphenylazo) isoxazole (2c) (Fig. 13)

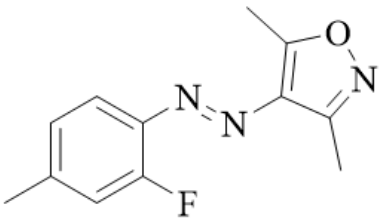


Fig. 13. 3,5-Dimethyl-4-(2′-fluoro-4′-methylphenylazo) isoxazole (2c)

Yield: 83%; IR (ν_{max},cm^{-1}): 2915, 2856(C-H), 1640(N=N), 1503(C=C), 615(C-F); (IR spectrum of compound in Figure S8); ^{1}H NMR(500 MHz, DMSO-d6) δ2.40(s,3H), 2.50(s,3H), 2.75 (s,3H), 7.10(d,1H), 7.30(d,1H), 7.6(s,1H); ^{13}C NMR(500 MHz, DMSO) δ 11.16, 11.43, 117.10, 117.25, 125.43, 132.21, 143.99, 152.72, 157.80, 159.83, 170.08; MS(EI):$[M+1]^+$, m/z, 234.12. Anal. Calcd. For $C_{12}H_{12}FN_3O$: C, 61.79; H, 5.19; N, 18.02; Found: C, 61.19; H, 4.91; N, 18.11. (^{1}H-NMR data is shown in figure-S18, ^{13}C- NMR data is shown in Figure S24, mass spectrum in Figure S31 and elemental analysis shown in Figure S36).

3,5-Dimethyl-4-(2'-fluoro-3'-chlorophenylazo) isoxazole (2d) (Fig. 14)

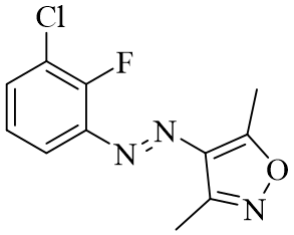


Fig. 14. 3,5-Dimethyl-4-(2′-fluoro-3′-chlorophenylazo) isoxazole (2d)

Yield: 82%; IR (ν_{max},cm^{-1}): 2976, 2861(C-H), 1570 (N=N), 1488(C=C), 608(C-F), 561(C-Cl); (IR spectrum of compound in Figure S9); ^{1}H NMR(500 MHz, DMSO-d6) δ2.45 (s, 3H), 2.78(s,3H), 7.35(d,1H), 7.65(dd,1H), 7.75(d,1H); ^{13}C NMR (500 MHz, DMSO) δ 11.35, 11.52, 117.48, 120.71, 122.91, 131.62, 149.11, 152.62, 157.14, 159.14, 170.82; MS(EI):$[M+1]^+$, $[M+1+2]^+$ m/z, 254.06, 256. 06. Anal. Calcd. For $C_{11}H_9ClFN_3O$: C, 52.09; H, 3.58; N, 16.57; Found: C, 51.90; H, 4.11; N, 16.12. (^{1}H-NMR data is shown in Figure S19, mass spectrum in Figure S32).

3,5-Dimethyl-4-(3'-chloro-4'-fluorophenylazo) isoxazole (2e) (Fig. 15)

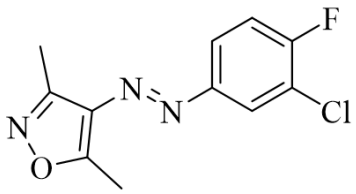


Fig. 15. 3,5-Dimethyl-4-(3′-chloro-4′-fluorophenylazo) isoxazole (2e)

Yield: 88%; IR (ν_{max},cm^{-1}): 2923, 2841(C-H), 1598 (N=N), 1496(C=C), 615(C-F), 578(C-Cl); (IR spectrum of compound in Figure S10); ^{1}H NMR(500 MHz, DMSO-d6) δ2.47(s, 3H), 2.78(s,3H), 7.60(dd,1H), 7.85(dd,1H), 8.0(dd,1H); ^{13}C NMR(500 MHz, DMSO) δ 11.25, 11.54, 117.68, 120.86, 123.64, 131.62, 149.08, 152.72, 157.34, 159.34, 170.90; MS(EI):$[M+1]^+$, $[M+1+2]^+$ m/z, 254.06, 256. 06. Anal. Calcd. For $C_{11}H_9ClFN_3O$: C, 52.09; H, 3.58; N, 16.57; Found: C, 52.12; H, 3.51; N, 17.02. (^{1}H-NMR data is shown in Figure S20, ^{13}C- NMR data is shown in Figure S25 and elemental analysis shown in Figure S37).

Biological evaluation

Anti-bacterial activity

The agar-well diffusion technique was used to evaluate the antimicrobial activity.[26] The agar plates were used to test the antibacterial activity of isolated compounds against the tested microorganisms. By using

a sterile cork borer, a plate was punched with a diameter of 6 to 8mm. Four pathogenic bacterial strains—two Gram-positive [*Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 121)] and two Gram-negative [*Escherichia coli* (MTCC 1652) and *Pseudomonas fluorescens* (MTCC 741)]—with a volume of 100µl inoculum were spread over the Petri plates. The diluted compound, having a concentration of up to 20 to 100 µM, was suspended inside the wells. In a BOD incubator, the plates were incubated for 24 hours at 37°C. Antibacterial activity was interpreted based on the diameter of the zone of inhibition, which was measured in millimeters (mm). The reference antibiotics used were bacitracin and chloramphenicol.

Table 1. Physiochemical properties of compound (1a-e) and (2a-e)

Code	R_1	R_2	R_3	R_4	Molecular formula	Molecular weight	Melting point	Color	Physical State
1a	-H	-H	-Br	-H	$C_9H_8BrN_5O$	282.10	152°C	brown	solid
1b	-H	-Cl	-H	-H	$C_9H_8ClN_5O$	237.65	158°C	light brown	solid
1c	-H	-H	-F	-H	$C_9H_8FN_5O$	221.20	156°C	light brown	solid
1d	-Br	-H	-H	-H	$C_9H_8BrN_5O$	282.10	148°C	yellow	solid
1e	-Cl	-H	-H	-H	$C_9H_8ClN_5O$	237.65	159°C	yellow	solid
2a	-Cl	-H	$-NO_2$	-H	$C_{11}H_9ClN_4O_3$	280.67	162°C	yellow	solid
2b	-H	$-NO_2$	-H	-H	$C_{11}H_{10}N_4O_3$	246.23	168°C	yellow	solid
2c	-F	-H	$-CH_3$	-H	$C_{12}H_{12}FN_3O$	233.25	172°C	yellow	solid
2d	-F	-Cl	-H	-H	$C_{11}H_9ClFN_3O$	253.66	178°C	yellow	solid
2e	-H	-Cl	-F	-H	$C_{11}H_9ClFN_3O$	253.66	170°C	yellow	solid

Anti-oxidant activity by DPPH radical scavenging assay

The *in vitro* antioxidant potential of synthesized compounds was evaluated using the 1,1-diphenyl-2-picryl hydrazide (DPPH) radical scavenging method.[27] For the stock solution, a total of 24 mg of DPPH was dissolved in 100 ml of ethanol. Ethanol filtering of the DPPH stock solution produced an effective combination with an absorbance of about 0.973 at 517 nm. Used a 3 mL DPPH solution, and introduced 100 µl of varying concentrations of the test compound (20, 40, 60, 80, and 100 µM) in ethanol. After 30 minutes of rt incubation, the absorbance was measured at 517 nm against a blank.

The percentage inhibitions were calculated using the following formula: DPPH scavenging activity (%)

$$= \frac{[(A_{control}\text{-}A)] \times 100}{A_{control}}$$

where A is the absorbance of the test compound and $A_{control}$ is the absorbance of the control reaction (which contains all the reagents save the test compound).

Anti-cancer activity

Materials

Fetal Bovine Serum (FBS), Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute (RPMI)-1640 medium, and antibiotic solutions (100 units/ml penicillin and 10 µM streptomycin) were provided by HiMedia Laboratories Pvt. Ltd., Mumbai, India. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] and all other chemical and reagent with analytical grade were used in different experiments.

Cell culture

The PC3 cell line (derived from a metastatic site: bone) representing human prostate cancer and human embryonic kidney (HEK-293) cells were procured from the National Centre for Cell Science (NCCS) located in Pune, India. PC-3 cells were nurtured in RPMI-1640 medium, while HEK-293 cells were cultivated in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin antibiotic solution. Cells were grown at 37°C in a 5% CO_2 culture condition.

Drug treatment

The stock solutions of various compounds at a concentration of 1M were prepared using DMSO as the solvent. The initial stock was further diluted into millimolar concentrations, and the ultimately specified final concentration for cell treatment was directly diluted into the culture media. For vehicle control, 0.1% DMSO in media was used.

MTT [3-(4,5-Methylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] assay

The cytotoxicity of different substances on normal human embryonic kidney (HEK) and prostate cancer (PC3) cells was assessed using the MTT assay. In 96-well plates, cells were seeded at a density of 2.5×10^3 for 24 hours and then left to re attach for another 24 hours. Different chemical concentrations, ranging from 5 µM to 640 µM, were applied to the cells. The media was changed after the treatment period of 24 hours to new media containing 100 µg of MTT per well, and it was incubated for 4 hours at 37°C.

After the removal of the media, to dissolve the purple formazan crystal, 100 µL of DMSO was added to each well. The absorbance was measured at 595 nm using an ELISA reader (Bio-Rad). Cell viability was calculated by using the following formula:

$$\textbf{\% cell viability} = \frac{\mathbf{A_{treated}\text{-}A_{blank}}}{\mathbf{A_{control}\text{-}A_{blank}}} \times \mathbf{100}$$

Statistical analysis

GraphPad prism 8 [GraphPad software, Inc., La Jolla. GA] was used for statistical analysis. Ordinary One-way ANOVA [Analysis for variance] was employed

for checking the statistical significance of the results. All sample were analyzed in triplicate and expressed as mean± SD for n=3. The p value determined the level of significance at different. The p<0.0001 (****) were regarded as highly significant.

Results and discussion

Chemistry

The synthetic pathways selected for the preparation of key intermediates, 2-[(substituted phenyl)hydrazono]malononitrile (1) and 3-[(substituted phenyl) azo]-2,4-pentanedione (2), as well as the target compounds (1a-e) and (2a-e), are outlined in Figure 3, with Figures 4 and 5 illustrating the mechanisms of the synthetic paths. In Figure 3, the reaction of 2-[(substituted phenyl)hydrazono]malononitrile (1) and 3-[(substituted phenyl)azo]-2,4-pentanedione (2) with hydroxylamine hydrochloride and sodium acetate in a 1:1 ratio yielded isoxazoles (1a-e) and (2a-e) in good yields (75-91%). The infrared (IR) spectrum of the novel isoxazole analogues (1a-e) and (2a-e) revealed distinctive absorption bands indicative of specific functional groups. Notably, the NH_2 groups exhibited symmetric stretching at 3397 cm^{-1} and asymmetric stretching at 3264 cm^{-1}, while the CH_3 groups demonstrated symmetric stretching at 2915 cm^{-1} and asymmetric stretching at 2896 cm^{-1}. These absorption peaks provided valuable insights into the molecular composition and structural characteristics of the compounds under investigation. The stretching frequency resulting from the azo group gave rise to a prominent absorption band in the range of 1410 to 1490 cm^{-1}, while absorption bands associated with C=C stretching manifested within the range of 1610 to 1632 cm^{-1}.

The disappearance of two singlet peaks in compounds (1a-e) and the emergence of two singlet peaks at δ 6.12-8.51 ppm, corresponding to two NH_2 protons, were confirmed by the ^1H NMR and D_2O exchangeable spectra of the respective isoxazoles. In the new isoxazole ring (2a-e), two substituted methyl groups appeared as singlet peaks, each representing three protons with intensity at δ 2.45-2.84 ppm in the ^1H NMR spectra. All compounds exhibited a proton signal in the range of δ 7.11 to 8.52 ppm for the substituted phenyl ring with various functional groups. Additionally, the ^{13}C NMR spectra of (1a-e) and (2a-e) were observed in the range of δ 105.12-173.12, with methyl carbon in (2a-e) appearing at δ 11.16-11.64 ppm.

Antibacterial activity assessment

The results revealed that all the compounds exhibited a moderate level of antibacterial activity against the four tested pathogenic bacterial strains *B. subtilis* (MTCC 121) and *S. aureus* (MTCC 96), *P. fluorescens* (MTCC 741) and *E. coli* (MTCC 1652). Compound 2a demonstrated the highest level of activity against *E. coli* (20 mm), compound 1d demonstrated the lowest level of activity against *P. fluorescens* (11 mm), and compound 1e demonstrated the least amount of activity against *B. subtilis*. There was no evidence of inhibition observed in the negative control (DMSO). All the compounds showed significant inhibition of growth in the tested microorganisms at a concentration of 100 µM. The potency of all compounds was compared with the reference drugs bacitracin and chloramphenicol. Bacitracin exhibited the largest zone of inhibition at 28 mm against both *P. fluorescens* and *E. coli*. Meanwhile, chloramphenicol demonstrated the highest zone of inhibition at 30 mm, particularly against *E. coli*. Table 2 indicates the zone of inhibition, while Figure 16 illustrates the antibacterial activity graph of compounds (1a-e) and (2a-e).

Table 2. Zone of inhibition of antibacterial activity of compound (1a-e) and (2a-e)

	Gram positive bacteria		Gram negative bacteria	
	B. subtilis	*S. aureus*	*P. fluorescens*	*E. coli*
Compound	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)
1a	14.14±0.16	18.11±0.16	12.25±0.34	17.14±0.16
1b	13.15±0.19	13.14±0.16	16.22±0.22	12.16±0.20
1c	16.22±0.24	16.18±0.21	12.36±0.34	17.16±0.24
1d	12.17±0.22	14.16±0.19	11.33±0.40	18.21±0.26
1e	11.23±0.27	14.16±0.21	12.25±0.29	16.17±0.26
2a	16.26±0.29	18.20±0.31	14.32±0.29	20.14±0.17
2b	19.24±0.27	15.25±0.29	15.32±0.34	16.22±0.21
2c	15.17±0.19	14.18±0.19	12.43±0.48	15.14±0.16
2d	14.2±0.25	15.22±.29	14.22±0.29	18.13±0.14
2e	16.21±0.21	14.26±0.29	16.43±0.38	19.15±0.18
Bacitracin	25.16±0.20	26.18±.24	28.34±0.35	28.18±0.26
Chloramphenicol	26.13±0.16	23.25±0.37	27.44±0.44	30.18±0.19

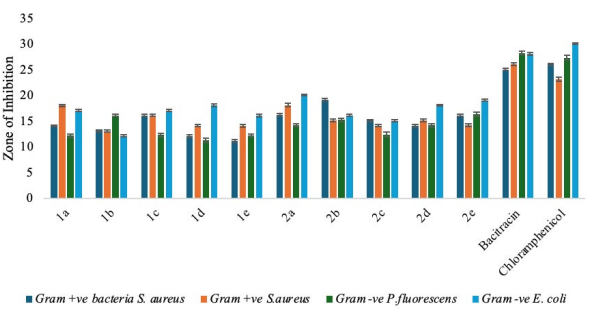


Fig. 16. Antibacterial activity graph of isoxazole derivative (1a-e) & (2a-e). Data are means ± SD from three independent experiments

Antioxidant activity

The newly synthesized compounds were also evaluated for their antioxidant properties using 1,1-diphenyl-2-picryl hydrazide (DPPH). All the compounds exhibited good radical-scavenging activity, but compounds 1d, 1e, 2c, 2d, and 2e demonstrated a particularly strong range of radical scavenging activity at the

concentration of 20, 40, 60, 80,100 µM compared to the others (Fig. 17). The percentage of DPPH activity, calculated using the reported formula, was 33.19%, 22.23%, 36.51%, 28.22%, and 36.04%, respectively. Color variations were observed in the tested compounds after a 30-minute incubation with a DPPH-containing solution. Figure 17 illustrates the antioxidant activity graph of the isoxazole derivatives (1a-e) and (2a-e).

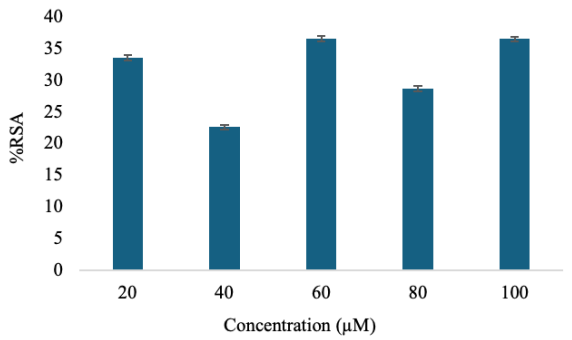
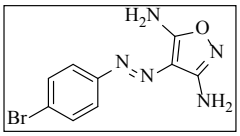
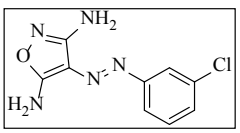
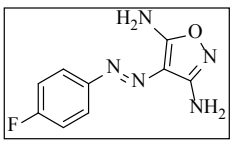
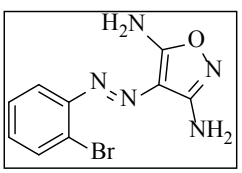


Fig. 17. Anti-oxidant activity graph of isoxazole derivative (1a-e) & (2a-e). Data are means ± SD from three independent experiments

Anticancer activity

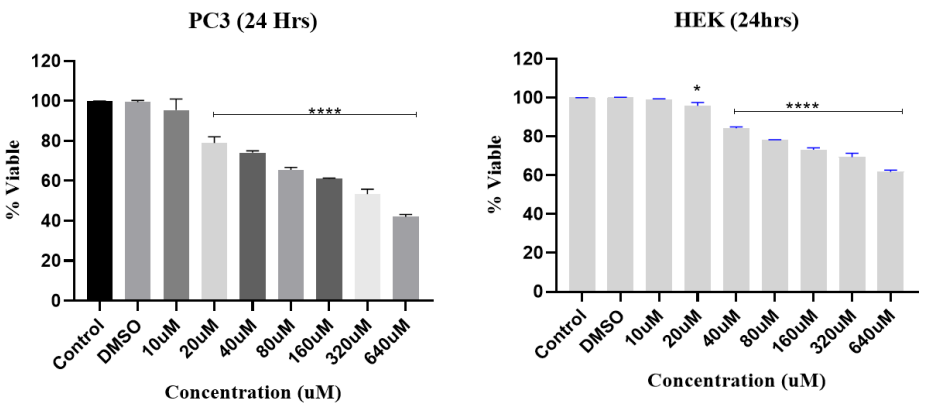
Compounds 1a, 1b, 1c, and 1d were assessed for their anticancer activity against PC3 and HEK cell lines at various concentrations ranging from 10 µM to 640 µM, among all the synthesized compounds. Across different dosages of drug treatments, these tested compounds consistently demonstrated greater inhibition of cancer cell lines compared to normal cells (Figures 18-21). Notably, these compounds exhibited their maximum inhibitory effect on normal cells only at higher doses, specifically at 640 µM, while demonstrating an effect on cancer cell lines at lower doses. The IC_{50} values of compounds 1a, 1b, 1c, and 1d are presented in Table 3.

Table 3. The IC_{50} value of compound 1a, 1b, 1c, and 1d

Compound	IC_{50} (µM)	
	PC3	HEK
(1a) 3, 5-Diamino-4-(4′-bromophenylazo) isoxazole 	53.96±1.732	41.24±1.881
(1b) 3, 5-Diamino-4-(3′-chlorophenylazo) isoxazole 	47.27±1.675	42.10±1.46
(1c) 3, 5-Diamino-4-(4′-fluorophenylazo) isoxazole 	147.9±2.170	66.13±2.073
(1d) 3,5-Diamino-4-(2′-bromophenylazo) isoxazole 	38.63±1.587	103.1±1.900
Doxorubicin	0.09±0.014	171.65±2.65

SAR analysis

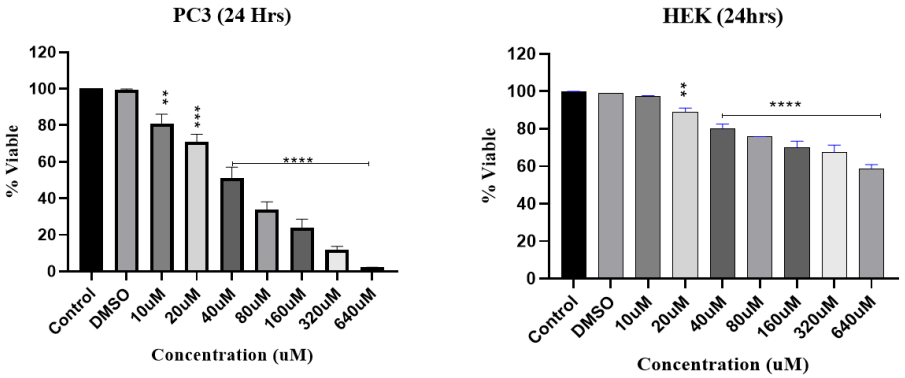
The isoxazole heterocycle exhibits significant pharmacological activities, particularly as an anticancer agent, as highlighted in recent research findings concerning this fundamental structure.[28] This observation is corroborated by evidence that the isoxazole heterocycle serves as a crucial pharmacophore for antiproliferative activities. Furthermore, the effectiveness of these activities is notably enhanced when the phenyl ring is substituted with halogens, specifically fluorine (F), bro-



MTT assay showing dose-dependent decrease in cell viability of PC3 cells after 24hrs of treatment. Data are means ± SD from two independent experiments, (****p<0.0001) with respect to untreated control. Differences between groups were analysed by one way ANOVA.

MTT assay showing dose-dependent decrease in cell viability at higher doses on HEK cells after 24hrs of treatment. Data are means ± SD from two independent experiments, (*p=0.0161, ****p<0.0001)) with respect to untreated control. Differences between groups were analysed by one way ANOVA.

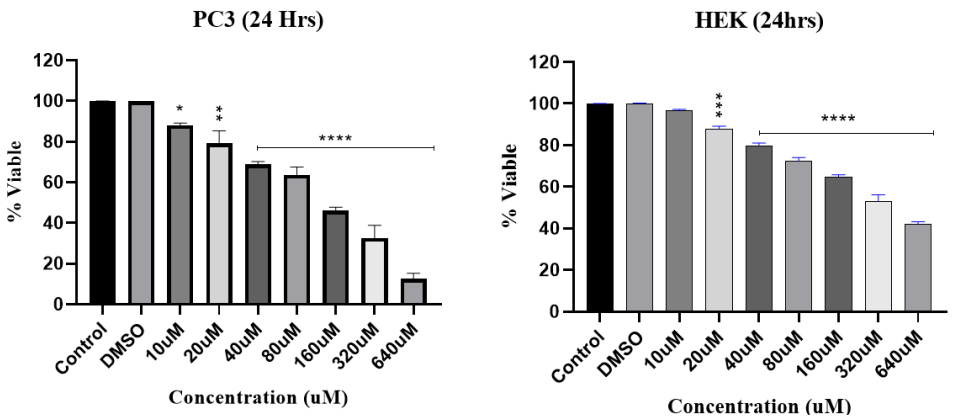
Fig. 18. Effect of different doses of 1a compound on cell viability of prostate cancer; and effect of different doses of 1a compound on cell viability of normal human embryonic kidney cells (HEK)



MTT assay showing dose-dependent decrease in cell viability of PC3 cells after 24hrs of treatment. Data are means ± SD from two independent experiments, (**p=0.0030, ***P=0.0001 ****p<0.0001) with respect to untreated control. Differences between groups were analysed by one way ANOVA.

MTT assay showing dose-dependent decrease in cell viability at higher doses on HEK cells after 24hrs of treatment. Data are means ± SD from two independent experiments, (**p=0.0042, ****≤p=0.0001) with respect to untreated control. Differences between groups were analysed by one way ANOVA.

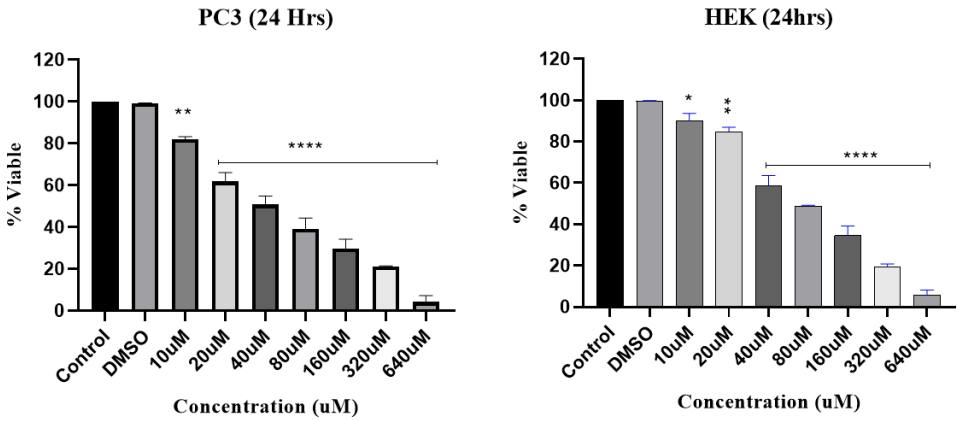
Fig. 19. Effect of different doses of 1b compound on cell viability of prostate cancer; and effect of different doses of 1b compound on cell viability of normal human embryonic kidney cells (HEK)



MTT assay showing dose-dependent decrease in cell viability of PC3 cells after 24hrs of treatment. Data are means ± SD from two independent experiments, (*P=0.0372 **P=0.0011, ****p<0.0001) with respect to untreated control. Differences between groups were analysed by one way ANOVA.

MTT assay showing dose-dependent decrease in cell viability at higher doses on HEK cells after 24hrs of treatment. Data are means ± SD from two independent experiments, (***P=0.0001, ****p<0.0001) with respect to untreated control. Differences between groups were analysed by one way ANOVA.

Fig. 20. Effect of different doses of 1c compound on cell viability of prostate cancer; and effect of different doses of 1c compound on cell viability of normal human embryonic kidney cells (HEK)



MTT assay showing dose-dependent decrease in cell viability of PC3 cells after 24hrs of treatment. Data are means ± SD from two independent experiments, (**p=0.0019, ****p<0.0001) with respect to untreated control. Differences between groups were analysed by one way ANOVA.

MTT assay showing dose-dependent decrease in cell viability at higher doses on HEK cells after 24hrs of treatment. Data are means ± SD from two independent experiments, (*P=0.0295, **P=0.0022, ****p<0.0001) with respect to untreated control. Differences between groups were analysed by one way ANOVA.

Fig. 21. Effect of different doses of 1d compound on cell viability of prostate cancer; and effect of different doses of 1d compound on cell viability of normal human embryonic kidney cells (HEK)

mine (Br), and/or chlorine (Cl).[29-30] In our study, all analogues with electron withdrawing groups such as -F, -Cl, and -Br demonstrated anticancer effects against experimental cancer cell lines. Among these, in the PC3 cell line, the ortho-substituted analogue 1d exhibited the most potent cytotoxic effects, followed by the meta-substituted 1b and then the para-substituted 1a and 1c. Among the para-substituted analogs, 1a and 1c, the bromine-substituted analogue showed greater cytotoxic effects compared to the fluorine-substituted analogue. In the HEK cell line, the para-substituted analogue 1a showed superior anticancer effects compared to the meta-substituted 1b, followed by the ortho-substituted 1d. However, among the para-substituted analogs, 1a and 1c, the bromine-substituted analogue demonstrated greater cytotoxic effects compared to the fluorine-substituted analogue, similar to what was observed in the PC3 cell line. It appears that the size, position, and electronic effects of the substituents play an important role in determining activity.

Conclusion

In the pursuit of novel compounds with multifaceted biological activity, we have successfully devised and validated a synthetic route to produce isoxazole derivatives (1a-e) and (2a-e). Rigorously scrutinized through diverse spectral analyses, this synthetic pathway has been validated. Moving beyond synthesis, we embarked on a comprehensive journey to unravel the potential biological attributes of these compounds. Our investigations encompassed a holistic evaluation of antibacterial, antioxidant, and anticancer properties. In terms of antibacterial prowess, all compounds emerged as robust contenders against both Gram-positive and Gram-negative bacterial strains. Notably, compound 2a exhibited maximum activity against *E. coli*. Compounds 1d, 1e, 2c, 2d, and 2e demonstrated a good range of radical scavenging activity compared to other compounds. The percentage of DPPH activity calculated using the reported formula was 33.19%, 22.23%, 36.51%, 28.22%, and 36.04%, respectively. Compounds 1b, 1c, and 1d exhibited greater anticancer potential against PC3 cell lines at a dose of 640 μM of drug treatment, with 1d also demonstrating excellent potential and effect against the cell viability of HEK cell lines. The synthetic route can be further optimized to enhance efficiency, reduce costs, or explore variations in the chemical structure to improve the overall synthetic process. Furthermore, we can delve deeper into the structure-activity relationship of these compounds to understand the specific structural features responsible for their biological activities. This knowledge can guide the design of new derivatives with improved efficacy.

The potential of isoxazole derivatives is rich with promise, spanning a diverse array of therapeutic applications within medicine and beyond. The future prospects of isoxazole in the development of antibacterial and anticancer drugs entail further exploration of their mechanisms of action, optimization of their pharmacokinetic properties, and rigorous preclinical and clinical evaluations. Given the global health threat posed by multidrug-resistant bacteria, isoxazole derivatives could provide a new avenue for combating bacterial infections. Additionally, their potential as anticancer agents open doors to innovative and targeted cancer treatments, instilling hope for improved therapeutic outcomes in the battle against both infectious diseases and cancer.

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Declarations

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Author contributions

Conceptualization, P.S. and A.B.; Methodology, K.V.; Software, T.S.; Validation, T.S., R.K. and H.T.; Formal Analysis, K.V.; Investigation, P.S.; Resources, P.S.; Data Curation, K.V.; Writing – Original Draft Preparation, K.V.; Writing – Review & Editing, P.S.; Visualization, A.B.; Supervision, P.S.

Conflict of interest

The authors state, there are no conflict of interest about the publication of this research work.

Data availability

The dataset generated during research work is available from corresponding author on request.

Ethics approval

Not applicable.

References

1. Doron S, Gorbach SL. Bacterial infections: overview. *International Encyclopedia of Public Health*. 2008:273. doi: 10.1016/B978-012373960-5.00596-7
2. Ma M, Cheng Y, Xu Z, et al. Evaluation of polyamidoamine (PAMAM) dendrimers as drug carriers of anti-bacterial drugs using sulfamethoxazole (SMZ) as a model

drug. *Eur J Med Chem*. 2007;42(1):93-98. doi: 10.1016/j.ejmech.2006.07.015
3. Yanagihara K. Design of anti-bacterial drug and anti-mycobacterial drug for drug delivery system. *Curr Pharm Des*. 2002;8(6):475-482. doi: 10.2174/1381612023395808
4. Diana P, Carbone A, Barraja P, Kelter G, Fiebig H, Cirrincione G. Bioorganic & Medicinal Chemistry and 3 , 5-bis (3 0 -indolyl) -isoxazoles , nortopsentin analogues. *Bioorg Med Chem*. 2010;18(12):4524-4529. doi: 10.1016/j.bmc.2010.04.061
5. Sethi P, Khare R, Choudhary R. Complexes of pyrimidine thiones: Mechanochemical synthesis and biological evaluation. *Asian J Chem*. 2020;32(10):2594-2600. doi: 10.14233/ajchem.2020.22813
6. Pooja S, Pernita D, Gupta GK, Mostafa Sahar I, Simrat K. Synthesis, characterization, anti-bacterial and DNA nicking activity of new complexes of 1-(2,4-dinitrophenylamino)- 4, 4, 6-trimethyl-3, 4-dihydropyrimidine-2-(1H)--thione. *Res J Chem Environ*. 2018;22(11):73-88.
7. Joseph L, George M. Evaluation of in vivo and in vitro anti-inflammatory activity of novel isoxazole series. *Eur Int J Sci Technol*. 2016;5(3):35-42.
8. Mączyński M, Artym J, Kocięba M, Kochanowska I, Ryng S, Zimecki M. Anti-inflammatory properties of an isoxazole derivative - MZO-2. *Pharmacol Reports*. 2016;68(5):894-902. doi: 10.1016/j.pharep.2016.04.017
9. Huang X, Dong S, Liu H, et al. Design, Synthesis, and Evaluation of Novel Benzo [d] isoxazole Derivatives as Anticonvulsants by Selectively Blocking the Voltage--Gated Sodium ChannelNaV1.1. *ACSChemNeurosci*. 2022;13(6):834-845.doi: 10.1021/acschemneuro.1c00846
10. Hawash M, Jaradat N, Abualhasan M, et al. Evaluation of cytotoxic, COX inhibitory, and antimicrobial activities of novel isoxazole-carboxamide derivatives. *Lett Drug Des Discov*. 2023;20(12):1994-2002. doi: 10.2174/1570180819666220819151002
11. Pir M, Agirbas H, Budak F, Sahin O. Synthesis, characterization, antimicrobial activity, and QSAR studies of some new 6-substituted phenyl 3-(4-chlorophenyl)-3a,4,8,8a-tetrahydro-[1,3,2]dioxaborepino [5,6-d]isoxazoles. *Heteroat Chem*. 2017;28(2):1-12. doi: 10.1002/hc.21363
12. Saravanan G, Alagarsamy V, Dineshkumar P. Synthesis, analgesic, anti-inflammatory and in vitro antimicrobial activities of some novel isoxazole coupled quinazolin-4 (3 H)-one derivatives. *Arch Pharm Res*. 2021;44:1-11. doi: 10.1007/s12272-013-0262-8
13. Shahinshavali S, Sreenivasulu R, Guttikonda VR, Kolli D, Rao MVB. Synthesis and Anticancer Activity of Amide Derivatives of 1,2-Isoxazole Combined 1,2,4-Thiadiazole. *Russ J Gen Chem*. 2019;89(2):324-329. doi: 10.1134/S1070363219020257
14. Burra S, Voora V, Rao CP, Vijay Kumar P, Kancha RK, David Krupadanam GL. Synthesis of novel forskolin isoxazole derivatives with potent anti-cancer activity against breast cancer cell lines. *Bioorganic Med Chem Lett*. 2017;27(18):4314-4318. doi: 10.1016/j.bmcl.2017.08.033
15. Hawash M. Recent advances of tubulin inhibitors targeting the colchicine binding site for cancer therapy. *Biomolecules*. 2022;12(12):1843. doi: 10.3390/biom12121843
16. Hawash M, Jaradat N, Eid AM, et al. Synthesis of novel isoxazole–carboxamide derivatives as promising agents for melanoma and targeted nano-emulgel conjugate for improved cellular permeability. *BMC Chem*. 2022;16(1):1-12. doi: 10.1186/s13065-022-00839-5
17. Shaik A, Bhandare RR, Palleapati K, Nissankararao S, Kancharlapalli V, Shaik S. Antimicrobial, antioxidant, and anticancer activities of some novel isoxazole ring containing chalcone and dihydropyrazole derivatives. *Molecules*. 2020;25(5):1047. doi: 10.3390/molecules25051047
18. Chithra VS, Reji TF, Brindha J. Synthesis and Structure--Activity Relationship Study of Novel Isoxazole derivatives as Promising Antioxidants. *Asian J Res Chem*. 2018;11(1):65-68. doi: 10.5958/0974-4150.2018.00014.7
19. Ketan V, Pooja S, Purti M, Anshul B. Antituberculosis activity of pyrazoles. *Res J Chem Environ*. 2022;26(10):184-198. doi: 10.25303/2610rjce1840198
20. Abdul Manan MAF, Cordes DB, Slawin AMZ, et al. The Synthesis and Evaluation of Fluoro-, Trifluoromethyl-, and Iodomuscimols as GABA Agonists. *Chem - A Eur J*. 2017;23(45):10848-10852. doi: 10.1002/chem.201701443.
21. Rahman MU, Rathore A, Siddiqui AA, Parveen G, Shahar Yar M. Synthesis and antihypertensive screening of new derivatives of quinazolines linked with isoxazole. *Biomed Res Int*. 2014;2014. doi: 10.1155/2014/739056.
22. Agrawal N, Mishra P. Synthesis, monoamine oxidase inhibitory activity and computational study of novel isoxazole derivatives as potential antiparkinson agents. *Comput Biol Chem*. 2019;79:63-72. doi: 10.1016/j.compbiolchem.2019.01.012.
23. Pedada SR, Yarla NS, Tambade PJ, et al. Synthesis of new secretory phospholipase A2-inhibitory indole containing isoxazole derivatives as anti-inflammatory and anticancer agents. *Eur J Med Chem*. 2016;112:289-297. doi: 10.1016/j.ejmech.2016.02.025.
24. Aggarwal R, Bansal A, Mittal A. Synthesis and antimicrobial activity of 3-(2-thienyl)-4-arylazo--5-hydroxy- 5-trifluoromethyl-Δ2-isoxazolines and 3-(2-thienyl)-4-arylazo- 5-trifluoromethylisoxazoles. *J Fluor Chem*. 2013;145(2010):95-101. doi: 10.1016/j.jfluchem.2012.10.005.
25. Kryštof V, Cankař P, Fryšová I, et al. 4-Arylazo-3 , 5-diamino-1 H -pyrazole CDK Inhibitors : SAR Study , Crystal Structure in Complex. *J Med Chem*. 2006;49(22):6500-6509. doi: 10.1021/jm0605740.
26. Perez C. Antibiotic assay by agar-well diffusion method. *Acta Biol Med Exp*. 1990;15:113-115.
27. Garcia EJ, Oldoni TLC, Alencar SM de, Reis A, Loguercio AD, Grande RHM. Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. *Braz Dent J*. 2012;23:22-27. doi: 10.1590/S0103-64402012000100004.

28. Arya GC, Kaur K, Jaitak V. Isoxazole derivatives as anticancer agent: A review on synthetic strategies, mechanism of action and SAR studies. *Eur J Med Chem*. 2021;221:113511. doi: 10.1016/j.ejmech.2021.113511.
29. Hawash M, Jaradat N, Bawwab N, Salem K, Arafat H. Design, synthesis, and biological evaluation of phenyl - isoxazole - carboxamide derivatives as anticancer agents. *Heterocyclic Communications*. 2021:133-141. doi: 10.1515/hc-2020-0134
30. Hawash M, Kahraman DC, Ergun SG, Cetin-Atalay R, Baytas SN. Synthesis of novel indole-isoxazole hybrids and evaluation of their cytotoxic activities on hepatocellular carcinoma cell lines. *BMC Chem*. 2021;15(1):1-14. doi: 10.1186/s13065-021-00793-8

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ORIGINAL PAPER

Knowledge of esophageal cancer and preventive behaviors among nursing students – a cross-sectional study

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ABSTRACT

Introduction and aim. There is a gap in nursing student understanding, knowledge, and preventive behaviors concerning esophageal cancer. This study aimed to investigate knowledge, risk factors, and preventive behaviors among Turkish nursing students.

Material and methods. A descriptive cross-sectional survey was performed. The questionnaire was conducted online over a four-month period. A convenience sample of 688 undergraduate nursing students was recruited from health science faculty at three universities in Türkiye. The survey was performed using a self-administered questionnaire. The reliability coefficients of the knowledge test were calculated, yielding Cronbach's alpha (0.952), KR-20 (0.952), and KR-21 (0.945), respectively. A significance level $p<0.05$ was accepted.

Results. The mean age of the study group was 20±1.86 (min: 17, max: 32). The study group consists of 487 women (70.8%) and 201 men (29.2%). Most of the study population report never using alcohol and smoking (88.4%, 73.1%, respectively), and reported paying attention to oral hygiene (88.5%). Nursing students had a low family history of EC (0.4%), obesity (10.2%), and vitamin deficiency (19.2%) which are risk factors. Knowledge of esophageal cancer risks was low ($\bar{x}$=14.34±9.53; Min=0; Max=31). Total knowledge scores have higher for students with complaints of EC, vitamin deficiency, and diagnosis of Human Papilloma Virus disease ($p<0.05$).

Conclusion. This study showed that there are significant gaps in the knowledge of nursing students and these need to be addressed through an improved nursing curriculum. In this context, the study can be used as important evidence and a resource in the issues that should be given priority to in the training and research needed to increase the knowledge and awareness of future nurses about esophageal cancer.

Keywords. esophageal cancer, esophageal cancer knowledge, esophageal cancer preventive behaviors, nursing students

Introduction

Esophageal cancer (EC) is the eighth most commonly diagnosed type of cancer. It is responsible for 5.5% of cancer-related mortality, and the five-year survival rate is less than 20%.[1-3] There has been a marked and steady increase in the incidence of EC in the Western world.[1] Esophageal cancer makes up about 1% of all cancers diagnosed in the United States, but it is much more common in some other parts of the world, such as Iran, northern China, India, and southern Africa.[4] According to the cancer data from the Ministry of Health, EC is among the top 10 cancers in the Turkish community.

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Disease morbidity and mortality rate (3.8%, and 2.1% respectively) in Turkish society have been determined to be much lower than expected, which is attributed to the underreporting of cancer statistics in Türkiye.[5]

Evidence suggests some potential risk factors for EC, although the principal risk factors and etiology are not fully understood.[3] However, some risks such as male sex, family history, advanced age, low socioeconomic status in addition to *Helicobacter pylori* (HP), diseases such as gastroesophageal reflux, Barrett's esophagus (BE), head and neck cancer, scleroderma and hypertension and lifestyle habits such as smoking, alcohol use, unhealthy habits, and nutrition-related risks factors are known.[2,6-8] Current studies suggest that smoking and drinking, use of hot beverages and low intake of fruits and vegetables may contribute to a high incidence of EC.[7-10] Additionally, long-term use of proton pump inhibitors (7-10 times) and aspirin use (13%) increase the risk of EC.[9,11]

Knowing the epidemiology, risks, screening methods, signs, and symptoms of EC is the cornerstone for developing a prevention strategy. Dysphagia, weight loss, unexplained abdominal discomfort, and stool changes are common symptoms of EC.[2] Chemotherapy, radiotherapy, and surgery are the methods used for treatment. Furthermore, only 20-30% of patients are eligible for curative surgery at diagnosis.[12] Many treatments, including targeted therapy and immunotherapy do not provide satisfactory survival advantages as in other cancer populations.[2] Additionally, the recurrence rate after all treatments is quite high. Therefore, preventive initiatives are vital.[13] In the prevention of EC, avoiding risk factors and adopting a healthy lifestyle is the most important point.[14]

Esophageal cancer is a type of cancer in which the least information is known about risk factors, symptoms, diagnosis, and treatment among cancer types by the public.[12,13] Nurses play a vital role in educating and giving care needed to cope with the late and long-term consequences of cancer diseases.[15] In this framework, nurses have a great responsibility to raise public awareness about EC and creating action groups.[16] Due to their profession, nurses take responsibility for early diagnosis, prevention, and screening programs for cancer. Student nurses can inform patients about risk factors, prevention methods, and prevention programs to healthy individuals, and direct risky individuals to screening programs. In screening programs, they can undertake behaviors such as participating in screening tests with nurses and communicating the results to individuals. They can also provide education and counseling on lifestyle changes and regular health checks. In individuals with cancer, they can play a role as an assistant in diagnosis, treatment, and care processes. Therefore, it is important that nursing students, who can play a key role in these programs in the future, have the right knowledge and positive behaviors regarding cancer. Additionally, although there are some studies on the risk factors of EC, as far as we know, no study has evaluated nursing student knowledge about EC risk factors, diagnosis, symptoms, and treatment.[3,6]

Aim

This study aimed to determine preventive behaviors, risks, and knowledge of EC among nursing students.

Material and methods

Ethics approval

The institutional permits and approval from the University Ethics Committee (Decision Number: 2021-SBB-0320) were obtained before the study. All participants gave written consent. The participants were told that they were not obligated to participate in the study and had the right to withdraw from the study. Questionnaires were anonymous, and data remained confidential throughout the study.

Study participants

This descriptive, cross-sectional study was conducted with nursing students from three health schools in the West Black Sea area in Türkiye. There were 1517 students in three schools. The margin of error (d) was determined as 5%, confidence level 95%, and response rate 50%, and the sample number was 307 with the Raosoft Sample Calculator.[17] The inclusion and exclusion criteria are as follows: Inclusion Criteria: being 18 years of age and over, and registered in the 2022 academic year. Exclusion Criteria were being under 18 years of age. All 1517 students who met the inclusion criteria were enrolled, and the study was completed with 688 students who agreed to participate in the study.

Data collection

This study was conducted between February and May 2022. The questionnaire was prepared by researchers based on the literature. A pilot test was conducted with 10 nursing students from each school to eliminate bias, validate the questionnaire, and assess and administer the survey. Minor revisions were made after the pilot testing by the researchers. Completion of the questionnaire took an average of 15 minutes. The questionnaires were filled out, via the Google Forms link. The researchers stayed with students while they completed the questionnaire in the classroom. There were no missing data in the completed questionnaire.

Study tools and scoring systems

The questionnaire consisted of three parts with a total of fifty-three items. Part 1 consisted of 8 items on socio-demographic data (e.g., age, gender, class, income

status, education about EC, and educational resources). Part 2 consisted of 14 items to evaluate nursing student knowledge about preventive behaviors (harmful behavior in nutrition, unhealthy habits such as alcohol use, smoking, and lack of exercise) and risks (family history of EC, Barrett's esophagus, high body mass index, head/neck surgery cancer, HP, HPV virus, vitamin deficiencies such as A, B, C, E, Folic acid) for EC. The questions were multiple-choice and closed-ended questions. Part 3 consisted of 31 items (socio-demographic risk factors (4 items) and diseases associated with EC (6 items), risks associated with health habits (7 items), risks related to nutrition (7 items), and symptoms, diagnosis, and treatment (7 items)) used to determine nursing student' knowledge about EC.

Scores from the knowledge test range from 0 to 31, "1" was given to the true answer, "0" to the false answer and "0" to the "I have no idea" answer. The number of correct answers was divided by the number of questions, their percentages were calculated and a knowledge index was obtained. In addition, the total number of items of each dimension of the Esophageal Cancer Risk Knowledge Test was averaged and used in statistical analyses. Since the answers to the questions were "1" true and "0" false, internal consistency was calculated by Cronbach alpha and the Kuder-Richardson methods (KR-20, and 21). The Cronbach α-values were found to be 0.738, 0.814, 0.883, 0.873, and 0.858, respectively with total reliability Cronbach alpha (0.952). Additionally, psychometric and validity studies were not conducted. Reliability coefficients for the knowledge test were KR-20, and 21 (0.952, 0.945; respectively), and found to be high.[18,19]

Statistical analysis

Data were analyzed using SPSS V.25.0 (IBM, Armonk, New York, USA). Categorical variables were presented as frequencies, percentages, means and standard deviations. Reliability coefficients for the knowledge test were Cronbach's alpha and KR-20, 21. Data distribution was evaluated using the Kurtosis, Skewness, and Shapiro-Wilks Test. A t-test was used to examine differences in knowledge score levels according to the descriptive characteristics of the students. Statistical significance was set at $p<0.05$.

Results

The mean age of the study group was 20±1.86 (min: 17, max: 32). The study group consists of 487 women (70.8%) and 201 men (29.2%). Table 1 shows nursing student preventive behaviors against EC in the study of 688 participants. About half of students (44.3%) reported eating a diet rich in fresh fruit/vegetables at least 3 days a week, rarely consuming meat (70.9%), never using alcohol (88.4%), and never smoking (73.1%), and paying attention to oral hygiene (88.5%). More than half of the students consumed very hot beverages (58.4%) and cold beverages (71.5%), did not use protective drugs (70.6%), and never used aspirin (82.8%).

Table 1. Preventive behaviors of nursing students

	Yes n (%)	Rare n (%)	None n (%)
Nutrition rich in fresh fruits/vegetables at least three days a week	305 (44.3)	343 (49.9)	40 (5.8)
Frequent consumption of highly salted and spicy foods	204 (29.7)	361 (52.5)	123 (17.9)
Consuming meat too frequent and in enormous amounts	78 (11.3)	488 (70.9)	122 (17.7)
Drinking more than four glasses of alcohol a day	9 (1.3)	71 (10.3)	608 (88.4)
Smoking	110 (16)	75 (10.9)	503 (73.1)
Regular exercise (30 minutes, three days a week at least)	132 (19.2)	394 (57.3)	162 (23.5)
Taking care of oral hygiene	609 (88.5)	72 (10.5)	7 (1)
Frequent use of stomach protective medicines	34 (4.9)	168 (24.4)	486 (70.6)
Frequent use of aspirin	6 (0.9)	112 (16.3)	570 (82.8)
Frequent weight tracking	167 (24.3)	276 (40.1)	245 (35.6)
Extremely hot beverage consumption	199 (28.9)	402 (58.4)	87 (12.6)
Very cold beverage consumption	116 (0.9)	492 (71.5)	80 (11.6)
Using drugs (such as opium)	6 (0.9)	17 (2.5)	665 (96.7)
Nutrition with foods rich in vitamins A, B, C, E, and folic acid	244 (35.5)	409 (59.4)	35 (5.1)

Table 2 reports nursing students' risky conditions related to EC. Obesity (10.2%) and vitamin deficiency (A, C, E, and folate) (19.2%) were found to be the riskiest conditions for nursing students.

Table 2. Risky conditions related to EC of nursing students*

Risky conditions	In the past (n%)	Current (n%)	Never happened (n%)
Gastroesophageal reflux disease	54 (7.8)	63 (9.2)	571 (83)
Esophageal complaints	12 (1.7)	11 (1.6)	665 (96.7)
Obesity	65 (9.4)	70 (10.2)	553 (80.4)
Family history of EC	10 (1.5)	3 (0.4)	675 (98.1)
Family history of having head neck cancer	6 (0.9)	5 (0.7)	677 (98.4)
Diagnosis of HP in the stomach	42 (6.1)	10 (1.5)	636 (92.4)
HPV infection (such as herpes)	312 (45.3)	19 (2.8)	357 (51.9)
Vitamin deficiency (such as A, C, E, and folate)	242 (35.2)	132 (19.2)	314 (45.6)

* EC – esophageal cancer, HP – *H. pylori*, HPV – human papilloma virus

Table 3 shows the nursing students' correct answers to the statements on EC. Nursing students mostly reported a low socioeconomic status (52.5%), long-term reflux complaints (66.7%), alcohol use (69.6%), and consumption of very cold and hot beverages (58.9%) as causing EC. Additionally, difficulty swallowing (56.7%) was given mostly as a symptom of EC.

Table 4 shows the scores on the EC knowledge questionnaire and its sub-dimensions. Nursing students in the study had a low level of knowledge, scoring an average of 14.34±9.53 (range 0–31) on the questionnaire.

Table 3. Descriptive analysis of knowledge about EC of nursing students

	True n (%)
Non-modifiable risk factors	
Sociodemographic risks	
EC is more common at advanced ages	272 (39.5)
EC is more common in men	207 (30.1)
Low socioeconomic status increases the risk of EC	361 (52.5)
People with a family history of EC may develop EC	357 (51.9)
Modifiable Risk Factors	
Diseases associated with EC	
Diseases that cause enlargement of the esophagus, such as achalasia, predisposed to cancer	372 (54.1)
Long-standing reflux can damage the esophagus and increase the risk of cancer	459 (66.7)
The risk of developing EC is higher in scleroderma (hardening of the skin) disease	179 (26)
Hypertension increases the risk of EC	163 (23.7)
EC is more common in people who had head and neck cancer in the past	242 (35.2)
H. pylori microbe increases the risk of EC	347 (50.4)
Harmful Habits	
Long-term alcohol use may increase the risk of EC	447 (65)
Long-term and excessive smoking increases the risk of developing EC	479 (69.6)
Regular exercise may help reduce the risk of EC	413 (60)
Poor oral hygiene paves the way for the development of EC	461 (67)
The use of some medications like opium increases the risk of EC	402 (58.4)
Frequent use of some stomach protective drugs (proton pump inhibitors) may increase the risk of EC	317 (46.1)
Long-term and frequent use of aspirin may increase the risk of EC	304 (44.2)
Nutrition	
Consuming hot and cold beverages increases the risk of EC	405 (58.9)
A diet poor in fresh fruits and vegetables increases the risk of EC	294 (42.7)
Less meat consumption reduces the risk of EC	154 (22.4)
Eating too much red meat and processed foods such as sausages, hamburgers, and ham can increase the risk of EC	336 (48.8)
Consuming high salty and spicy foods may increase the risk of EC	394 (57.3)
Obesity increases the risk of EC	383 (55.7)
A, B, C, E, and folic acid's poor diet increases the risk of EC	340 (49.4)
Symptoms, diagnosis, and treatment	
Weight loss is one of the common symptoms of EC	249 (36.2)
Black or tar-colored stool is one of the symptoms seen in EC	233 (33.9)
Difficulty in swallowing and pain during feeding are common early symptoms of EC	390 (56.7)
Complaints such as unexplained discomfort and bloating in the abdomen are among the symptoms seen in EC	274 (39.8)
Changes in defecation habits are common in EC	243 (35.3)
Surgery is the most used treatment method in the initial period of EC	165 (24)
Chemotherapy is the most used treatment method in the late period	225 (32.7)

* EC – esophageal cancer

Table 4. Esophageal cancer risk knowledge scores

	Mean±SD	Min–Max
EC risk knowledge total score	14.34±9.53	0–31
Sociodemographic risks	1.74±1.45	0–4
Diseases associated with EC risks	2.56±2.02	0–6
Harmful habits risks	4.10±2.59	0–7
Nutritional risks	3.35±2.56	0–7
Symptoms, diagnosis, and treatment score	**2.58±2.43**	**0–7**

Table 5 presents the differentiation of EC Risk knowledge scores. Nursing students without esophageal complaints had higher demographic information (p=0.042), related diseases (p=0.017), and harmful habits (p=0.046) knowledge scores than those with esophageal complaints. Those with EC in the past or now in their family and who were diagnosed with HP had more information about the symptoms, diagnosis, and treatment of the disease than those who do not (p=0.035 and p=0.01, respectively). The knowledge of esophageal cancer was higher in those with HPV diagnosis in the past or present than in those who have never had it (p=0.001). The EC knowledge scores of those who have had or have a vitamin deficiency problem in the past or now were higher than those who did not (p=0.028).

Discussion

This study assessed nursing student preventive behaviors, risks, and knowledge of EC. Additionally, the differentiation between risk and knowledge scores was evaluated. We found that most nursing students have preventive behaviors, very few had risks, and their level of knowledge about EC was low.

Esophageal cancer is a crucial health issue with high mortality due to the advanced character of the disease; thus, detection at an early stage of diagnosis improves treatment success and preventive behaviors.[20] The literature reported that EC risk factors in Eastern and Western societies, such as age, gender, race, alcohol consumption, smoking, poor oral hygiene, and gastroesophageal reflux disease.[21] Additionally, Barrett's esophagus, low fruit/vegetable consumption, high meat intake, family history, head /neck cancer, diseases causing motor disorders of the esophagus (scleroderma, achalasia), and high-temperature beverage intake EC have been reported as most essential predominant risk factors.[22,23] Overall, most of the nursing students in our study had inadequate preventive behaviors against EC. Nursing students had not performed fully desirable behaviors such as consuming meat, never consuming alcohol, never smoking, paying attention to oral hygiene, not using stomach protective drugs, not taking aspirin, and consuming fresh fruit/vegetables (at least 3 days a week). In addition, they had undesirable behaviors like consuming very hot and cold beverages, and don't exercise regularly.

Recurrent long-term reflux complaints are the most important risk factors for the development of Barrett's and EC. In addition, some studies have indicated that obesity reflux complaints pose a risk for EC in individuals over the age of 20 years. On the other hand, deficient nutrition in terms of A, C, E, and folate also increases the risk of EC.[15,24] In this study, a few students also had risky conditions related to EC including gastroesophageal reflux complaints, obesity, and vitamin deficiency (vitamin C, E, and folate). It is important to correct

Table 5. Differentiation of EC knowledge score by risky conditions*

Characteristics	n	EC risk knowledge total score	Demographic risks knowledge score	Diseases-owned knowledge score	Harmful habits knowledge score	Nutrition-associated risk factors knowledge score	Symptoms, diagnosis, and treatment knowledge score
Gastroesophageal reflux disease		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Present or past	117	15.103±9.652	1.880±1.549	2.598±1.992	4.103±2.601	3.727±2.589	2.795±2.561
Never happened	571	14.186±9.514	1.711±1.440	2.553±2.039	4.103±2.598	3.275±2.555	2.543±2.41
t		0.947	1.144	0.218	-0.003	1.738	1.019
p		0.344	0.253	0.828	0.998	0.083	0.309
Esophageal complaints							
Present or past	23	10.609±9.423	1.130±1.392	1.565±1.727	3.044±2.755	2.435±2.727	2.435±2.465
Never happened	665	14.471±9.522	1.761±1.458	2.596±2.031	4.140±2.585	3.384±2.555	2.591±2.437
t		-1.913	-2.042	-2.402	-1.995	-1.747	-0.302
p		0.056	0.042	0.017	0.046	0.081	0.763
Obesity							
Present or past	135	14.126±9.534	1.756±1.438	2.482±1.988	4.059±2.515	3.311±2.616	2.519±2.428
Never happened	553	14.394±9.545	1.736±1.465	2.581±2.041	4.114±2.618	3.362±2.554	2.602±2.441
t		-0.293	0.140	-0.508	-0.219	-0.205	-0.357
p		0.770	0.889	0.612	0.827	0.837	0.721
Family history of EC							
Present or past	13	17.923±7.533	2.308±1.377	2.923±1.706	4.539±2.106	4.154±2.410	4.000±2.236
Never happened	675	14.273±9.563	1.729±1.459	2.554±2.036	4.095±2.606	3.336±2.566	2.559±2.434
t		1.368	1.418	0.649	0.610	1.139	2.118
p		0.172	0.157	0.516	0.542	0.255	0.035
Family history of head neck cancer							
Present or past	11	11.364±7.788	1.455±1.440	2.000±1.789	2.818±2.483	2.546±2.464	2.546±2.162
Never happened	677	14.390±9.560	1.745±1.460	2.570±2.033	4.124±2.595	3.365±2.565	2.586±2.442
t		-1.044	-0.653	-0.924	-1.657	-1.051	-0.055
p		0.297	0.514	0.356	0.098	0.293	0.956
Diagnosis of HP in stomach		Ort ± SS	Ort ± SS	Ort ± SS	Ort ± SS	Ort ± SS	Ort ± SS
Present or past	52	15.577±9.477	1.827±1.451	2.519±1.925	3.942±2.570	3.865±2.536	3.423±2.652
Never happened	636	14.241±9.542	1.733±1.461	2.565±2.039	4.116±2.600	3.310±2.564	2.517±2.408
t		0.971	0.447	-0.154	-0.464	1.504	2.588
p		0.332	0.655	0.877	0.642	0.133	0.01
HPV infection (such as herpes)							
Present or past	331	15.625±9.173	1.837±1.445	2.776±1.978	4.423±2.495	3.701±2.474	2.888±2.445
Never happened	357	13.151±9.725	1.650±1.468	2.361±2.058	3.807±2.656	3.028±2.607	2.305±2.398
t		3.426	1.682	2.693	3.130	3.467	3.155
p		0.001	0.093	0.007	0.002	0.001	0.002
Vitamin deficiency (Such as A, C, E, and folate)							
Present or past	374	15.075±9.277	1.818±1.399	2.754±1.996	4.297±2.522	3.524±2.505	2.682±2.431
Never happened	314	13.468±9.781	1.647±1.525	2.331±2.049	3.873±2.669	3.147±2.622	2.471±2.442
t		2.207	1.539	2.735	2.140	1.928	1.129
p		0.028	0.127	0.006	0.034	0.054	0.259

* t – independent groups t-test, EC – esophageal cancer, HP – H. pylori, HPV – human papilloma virus

these unwanted health habits of students. Nursing students should be increases of aware unhealthy behaviors that cause EC cancer.

Although nursing students demonstrated a profound understanding of the adverse effects of deleterious behaviors on EC, their familiarity with EC-related diseases, symptoms, diagnoses, and treatments was notably deficient. The scholarly literature underscores tobacco smoking and excessive alcohol consumption as the primary risk factors contributing to the development of EC.[22,23] Since, smoking, alcohol consumption, and lack of exercise are reasons for all types of cancer, approximately two-thirds of the students also know the relationship between these factors and EC. The literature reports that lower socioeconomic status is associated with a lower sanitation standard, dietary habits, a poorer lifestyle, and a higher degree of carcinogen exposure.[24,25] Additionally, it was shown that Barrett's esophagus increases the risk of development of EC (OR 3.0 and 6.4 respectively).[25] Furthermore, poor oral hygiene, consumption of extremely hot beverages, and high salty and spicy food are reported to increase the risk of Bar-

rett's esophagus, thus increasing the risk of EC.[22,25] Esophegeal cancer has frequently been observed in some parts of Türkiye due to traditional hot tea consumption culturally. However, reliable incidence data for EC are lacking due to insufficient cancer reporting in Türkiye.[25] In this study, about half of the students had not responded correctly when asked whether low socioeconomic status, excessive alcohol use, long-term reflux complaints, and consumption of cold and hot beverages may increase EC risk. Some nursing students are also aware of the most well-known risk factors for EC. Additionally, students have insufficient knowledge about the diseases and drugs that EC may be associated with. It has been reported that diseases such as reflux, HP, achalasia, Barrett's esophagus, scleroderma, head and neck cancers, and hypertension cause EC.[7,21,25] Not surprisingly, reflux, HP, achalasia, and Barrett's esophagus are the most well-known causes and diseases among nursing students. Similarly, in a study conducted with nurses, it was found that nurses were most familiar with reflux and HP.[26] Although there are different views in the literature regarding the risk of EC development with long-term use of opium, proton pump inhibitors, and aspirin, some studies have reported that aspirin and proton pump inhibitors increase the risk of EC in recent years.[3,9,10,27] Therefore, as expected, the knowledge level of our working group on this topic is low.

In the literature, complaints such as dysphagia, GI bleeding, persistent vomiting, and weight loss are defined as alarm symptoms in the recognition of a significant number of gastrointestinal cancers.[28,29] In this study, one-third of the nursing student knew the alarm symptoms required for the diagnosis for EC. This suggests that awareness of common risk factors in Türkiye are not well understood by nursing students. Awareness and adequate knowledge of risk factors are substantial issues in the early detection and successful treatment of EC. Nursing schools play a key role, and health education is an indispensable part of a comprehensive program. Moreover, nursing students might be responsible for the care multiple patients with diverse needs, interact with many healthcare team members, and connecting with the extended network of family and close associates of their patients.[30] Additionally, nurses roles have evolved over time to encompass health promotion, disease management, and disease prevention.[31] For this reason, Turkish nursing students must be qualified to provide give accurate information to their patients or healthy people after graduation.

The current study found that nursing students who had complaints related to esophageal diseases had significantly higher knowledge scores regarding demographic risks, related diseases, and harmful habits than other students. Additionally, students with a family history of EC had more knowledge of symptoms, diagnosis, and treatment. Similarly, in a case-control study conducted in China, young people with a family history of EC had better knowledge. Having a family history of EC might be related to higher knowledge gained during their involvement in the treatment process and their interaction with healthcare providers, and might promote awareness about EC. In other words, exposure to high-risk factors increases knowledge.[32]

Students with HP in the stomach had higher symptom, diagnosis, and treatment knowledge scores. Additionally, students with HPV had high knowledge of nutrition-associated risk factors and symptoms, diagnosis, and treatment. It is usual for students who have had reflux or HPV complaints in the past and received treatment to know symptoms, diagnosis, and treatment. Students can also use internet resources to obtain detailed information about their diseases such as HPV or reflux. They may have read that they are at risk for EC, which may have prompted them to learn more about the disease. Only one study reported that Turkish nurses have a moderate level of knowledge about diagnosis and screening of esophageal cancer, and a low level of knowledge about treatment.[33] On the other hand, no studies have evaluated the esophageal cancer knowledge of nursing students in Türkiye and other countries. These results highlight the importance of increasing awareness through nursing education, particularly regarding the prevention and health promotion of EC.

Furthermore, the literature reported that nursing student preventive health behaviors, knowledge, and optimistic attitudes are effective for national cancer prevention practices.[16] Nurses, one of the largest groups of health professionals, have responsibilities to identify risk groups, promote healthy lifestyles, prevent complications, and improve positive outcomes in health care through education and counseling in community health.[21] Additionally, previous studies have shown that supportive care, and consulting interviews could be effective in improving patient quality of life with EC.[34,35] In this context, developing preventive behaviors and knowledge for nursing students may provide several benefits to both them and the community. Nursing students may give provide training, plan comprehensive care, and contribute to health policies regarding EC. In line with this, in the future, nurses may contribute to the national prevention of EC. Furthermore, if the relationship between healthy behaviors and the prevention of EC in nursing is better understood, nurse educators and researchers could study ways to create awareness of the prevention of EC and improve nursing student knowledge. This result reflects the need to develop intervention guidelines to enhance student EC knowledge.

Study limitations

This study has potential limitations and strengths. Firstly, data are subjective, as they are based on student

self-reporting. Second, data were obtained from participants in three regions of Türkiye, therefore it cannot be generalized to other areas of the country. Third, the lack of information in the literature on the knowledge levels of nursing students on EC may have limited the discussion of the study findings in a broad way. There is also a lack of standardized, and validated questionnaires to compare results from different populations. On the other hand, the current study is the first comprehensive study to evaluate nursing student knowledge and preventive behaviors toward EC in literature. This is also a strength of the study as it brings new outputs to the literature. In this context, this study fills a gap in literature. Secondly, the strength of this study is that it was conducted in schools in three different cities of Türkiye, it has an appropriate sampling method and large sample size. Third, the reliability (Cronbach's alpha) of the knowledge test questionnaire prepared by the researchers was found to be high. The study was conducted with a measurement tool with high reliability.

Conclusion

This study indicated that nursing students had had low levels of knowledge about EC. In this context, the study can be used as important evidence and resource in determining the issues that should be given priority in the studies planned to determine the knowledge and awareness of EC that can be improved in the future. Thus, our study has a high potential to pave the way for new studies on education and action plans to increase the risk and protective behaviors of nursing students for esophageal cancer. It is essential to increase the knowledge and awareness of nurses who will educate the society about EC during their university education. In this context, it is recommended to conducting action-oriented training to increase student EC knowledge and awareness in nursing education, to include EC in their curriculum extensively, and to plan experimental studies for future studies.

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Author contributions

Conceptualization, A.B.Ç., I.I.A. and H.Y.; Methodology, A.B.Ç. and I.I.A.; Investigation, A.B.Ç., I.I.A. and H.Y.; Data Curation, A.B.Ç., I.I.A. and H.Y.; Data Analysis, A.B.Ç., I.I.A., and H.Y.; Writing – Original Draft Preparation, A.B.Ç., I.I.A. and H.Y.; Writing – Review & Editing, A.B.Ç. and I.I.A.; Supervision, A.B.Ç., I.I.A. and H.Y.

Conflicts of interest

The authors have no competing interest to declare.

Data availability

Data are available upon request from the correspondence author.

Ethics approval

The institutional permits and approval from the University Ethics Committee (Decision Number: 2021-SBB-0320) were obtained before the study.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*. 2021;71(3):209-249. doi: 10.3322/caac.21660
2. Yang J, Liu X, Cao S, et al. Understanding Esophageal Cancer: The Challenges and Opportunities for the Next Decade. *Front Oncol*. 2020; 10:1727. doi: 10.3389/fonc.2020.01727
3. Li J, Xu J, Zheng Y, et al. Esophageal cancer: Epidemiology, risk factors and screening. *Chin J Cancer Res*. 2021;33(5):535-547. doi: 10.21147/j.issn.1000-9604.2021.05.01
4. American Cancer Society. Key Statistics for Esophageal Cancer. https://www.cancer.org/cancer/esophagus-cancer/about/key-statistics.html#:~:text=The%20American%20Cancer%20Society's%20estimates,men%20and%203%2C160%20in%20women Accessed November 11, 2023.
5. TR Ministry of Health, 2021. Türkiye cancer statistics 2017 https://hsgm.saglik.gov.tr/tr/kanser-istatistikleri/yillar/2017-turkiye-kanser-i-statistikleri.html. Accessed September 11, 2023.
6. American Cancer Society, 2022. Esophageal cancer risk factors. https://www.cancer.org/cancer/esophagus-cancer/causes-risks-prevention/risk-factors.html. Accessed September 11, 2023
7. Ahuja NK, Clarke JO. Scleroderma and the esophagus. *Gastroenterol Clin North Am*. 2021;50(4):905-918.
8. Ai H, Wang Y, Gu, H. Effect of cluster nursing mode combined with blood pressure regulation on surgical tolerance of patients with esophageal cancer and hypertension. *Oncologie* 2021;23(2):185-193.
9. Brusselaers N, Lagergren J, Engstrand, L. Duration of use of proton pump inhibitors and the risk of gastric and oesophageal cancer. *Cancer Epidemiol*. 2019;62:101585. doi: 10.1016/j.canep.2019.101585
10. Huang J, Koulaouzidis A, Marlicz W, et al. Global burden, risk factors, and trends of esophageal cancer: An Analysis of Cancer Registries from 48 Countries. *Cancers*. 2021;13(1):141. doi: 10.3390/cancers13010141
11. Lin JL, Lin JX, Zheng CH, et al. Relationship between aspirin use of esophageal, gastric and colorectal cancer patient

survival: a meta-analysis. *BMC Cancer*. 2020;20(1):638. doi: 10.1186/s12885-020-07117-4
12. Ekheden I, Ebrahim F, Ólafsdóttir, et al. Survival of esophageal and gastric cancer patients with adjuvant and palliative chemotherapy-a retrospective analysis of a register-based patient cohort. *Eur J Clin Pharmacol,* 2020;76(7):1029-1041. doi: 10.1007/s00228-020-02883-3
13. Lindenmann J, Fediuk M, Fink-Neuboeck N, et al. Hazard curves for tumor recurrence and tumor-related death following esophagectomy for esophageal cancer. *Cancers.* 2020;12(8):2066. doi: 10.3390/cancers12082066
14. Palladino-Davis AG, Mendez BM, Fisichella PM, et al. Dietary habits and esophageal cancer. *Dis Esophagus.* 2015;28(1):59-67. doi: 10.1111/dote.12097
15. Altre R, Chou FY. Nursing students' knowledge and attitude toward cancer survivorship. *J Cancer Educ.* 2022;37(5):1312-1321. doi: 10.1007/s13187-020-01953-5
16. Baek SY. A study on factors affecting national cancer prevention practices health behavior of nursing students. *Journal of Digital Convergence*, 2020;18(9): 29-37.
17. Raosoft Sample Calculator. http://www.raosoft.com/samplesize.html Accessed February 1, 2022.
18. Taber KS. The Use of Cronbach's Alpha When Developing and Reporting Research Instruments in Science Education. *Research in Science Education*. 2018;48:1273-1296.
19. Anselmi P, Colledani D, Robusto E. A Comparison of Classical and Modern Measures of Internal Consistency. *Front Psychol.* 2019;10:2714. doi: 10.3389/fpsyg.2019.02714
20. Lao-Sirieix P, Fitzgerald RC. Screening for oesophageal cancer. Nature reviews. *J Clin Oncol.* 2012;9(5):278-287. doi: 10.1038/nrdp.2017.48
21. Chung CS, Lee YC, Wu MS. Prevention strategies for esophageal cancer: Perspectives of the East vs. West. Best practice & research. *J Clin Gastroenterol*. 2015;29(6):869-883. doi: 10.1016/j.bpg.2015.09.010
22. Thrift AP, Nagle CM, Fahey PP. The influence of prediagnostic demographic and lifestyle factors on esophageal squamous cell carcinoma survival. *Int J Cancer*. 2012;131(5):759-768. doi:10.1002/ijc.27420
23. Zhao W, Liu L, Xu S. Intakes of citrus fruit and risk of esophageal cancer: A meta-analysis. *Medicine,* 2018;97(13):e0018. doi: 10.1097/MD.0000000000010018
24. Aleyamma M, Preethi SG, Kunnambath R, et al. Booth sociodemographic factors and stage of cancer at diagnosis: A population-based study in South India. *J Glob Oncol.* 2019;5:1-10. doi: 10.1200/JGO.18.00160
25. Eroğlu A, Aydın Y, Altuntaş B, et al. The increasing incidence of esophageal squamous cell carcinoma in women in Türkiye. *Turk J Med Sci.* 2016;46(5):1443-1448.
26. Schneider JL, Zhao WK, Corley DA. Aspirin and nonsteroidal anti-inflammatory drug use and the risk of Barrett's esophagus. *Dig Dis Sci.* 2015;60(2):436-443.
27. Cheung KS, Leung WK. Long-term use of proton-pump inhibitors and risk of gastric cancer: a review of the current evidence. *Therap Adv Gastroenterol*. 2019;12:1756284819834511. doi: 10.1177/1756284819834511
28. Pool H, Radmard AR, Malekzadeh F, et al. Diagnostic accuracy of age and alarm symptoms for upper GI malignancy in patients with dyspepsia in a GI clinic: a 7-year cross-sectional study. *PloS One*. 2012;7(6):39173. doi: 10.1371/journal.pone.0039173
29. Walsh L, Callaghan H, Keaver, L. Physical activity knowledge, attitudes and behaviours among Irish nursing students. *Int J Health Promot Educ*. 2020;59:145-155.
30. Benner P. Educating nurses: a call for radical transformation-how far have we come?. *J Nurs Educ.* 2012;51(4): 183-184.
31. Wu M, Zhang ZF, Kampman E, et al. Does family history of cancer modify the effects of lifestyle risk factors on esophageal cancer? A population-based case-control study in China. *Intl J of Cancer.* 2011;128(9):2147-2157. doi: 10.1002/ijc.25532
32. Chang YL, Tsai YF, Hsu CL, et al. The effectiveness of a nurse-led exercise and health education informatics program on exercise capacity and quality of life among cancer survivors after esophagectomy: A randomized controlled trial. *Int J Nurs Stud.* 2020;101:103418. doi: 10.1016/j.ijnurstu.2019.103418
33. Pool MK, Nadrian H, Pasha, N. Effects of a self-care education program on quality of life after surgery in patients with esophageal cancer. *Gastroenterol Nurs.* 2012;35(5):332-340. doi: 10.1097/SGA.0b013e3182605f86
34. Yu Y, Li M, Kang R, et al. The effectiveness of telephone and internet-based supportive care for patients with esophageal cancer on enhanced recovery after surgery in China: A randomized controlled trial. *Asia Pac J Oncol Nurs.* 2022;9(4):217-228. doi: 10.1016/j.apjon.2022.02.007
35. Malmström M, Ivarsson B, Klefsgård R, et al. The effect of a nurse led telephone supportive care programme on patients' quality of life, received information and health care contacts after oesophageal cancer surgery-A six month RCT-follow-up study. *Int J Nurs Stud*. 2016;64:86-95.

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ORIGINAL PAPER

Brazilians living with diabetes do not meet basic physical activity guidelines for health – a cross-sectional study

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ABSTRACT

Introduction and aim. The purpose of this study was to describe the profile of physical activity (PA) of Brazilian adults living with diabetes mellitus living in large Brazilian urban centers, as well as to determine whether the practice aligns with the physical activity guidelines recommended for people with diabetes.

Material and methods. Cross-sectional data were acquired from the 2020 Surveillance System for Risk and Protective Factors for Chronic Non-communicable Diseases, in which about 54,000 persons aged 18 and older in all Brazilian state capitals were contacted in a telephone survey. Participants reported on their engagement in recreational physical activity and active commuting to school and/or work in the three months preceding the interview, as well as the weekly frequency and duration of these activities. They also stated whether they were living with diabetes. A descriptive analysis was performed, and statistical significance was determined using Pearson's chi-squared test.

Results. In 2020, 7.9% of the population identified themselves living with diabetes. There was a greater frequency among older women and those with less education. Walking, water aerobics, and general gymnastics were the most common kinds of physical activity reported by people with diabetes. Moreover, over half of them (54.5%) were inactive, and 15% matched the physical activity criteria. The majority (90%) practiced PA for 30 minutes or more per day, while 87% of those who were active and exercised 1 to 2 times per week did not meet the requirements of the Ministry of Health.

Conclusion. In 2020, 7.9% of the population identified themselves as having diabetes. There was a higher frequency among older women and those with less schooling. In the sample as a whole, approximately 70% of people living with diabetes were inactive (54.5%) or did not meet the minimum BP recommendations for people with diabetes. The duration of each session seemed to be in line with the recommendations, however, the lack of regularity caused by the low weekly frequency meant that the minimum recommended target could not be achieved. Efforts involving the continued monitoring of people living with diabetes and counseling in Primary Health Care to opt for a more physically active life, seem to be promising acts for a healthier life, pending a definitive resolution to the disease.

Keywords. diabetes mellitus, exercise, health promotion, preventive medicine, public health

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Introduction

Diabetes mellitus (DM) is a chronic illness with numerous etiologies that is defined by a rise in blood glucose concentrations that, over time, can harm the body, notably the heart, brain, eyes, kidneys, nerves, and blood vessels.[1] Diabetes is caused by a combination of genetic and environmental factors, and the risk increases with age, obesity, and physical inactivity.[2]

According to the Pan American Health Organization (PAHO) and the World Health Organization (WHO), diabetes will be responsible for around 333 million deaths globally by 2025, with 284 million of those fatalities occurring in poorer nations.[3] By 2035, 20 million additional cases are projected in Latin American countries, primarily among the lower-income population.[4] Brazil now has the fourth-largest number of people with diabetes in the world (9.4% of the population) that is responsible for roughly 54,000 fatalities every year.[5]

The term physical activity (PA) is a non-drug intervention for the control of diabetes and associated complications.[3] Physical activity is understood in this study as any physical movement produced by the skeletal muscles that result in an increase in energy expenditure beyond rest.[6] However, at some points in the text, the term "physical exercise" may appear, as this was also originally used in the Vigitel questionnaire to facilitate communication with the respondent.

The immediate and chronic alterations in insulin action on glucose metabolism are the most apparent effect of PA for people with diabetes.[7-9] Colosimo et al. found a higher decrease in glycated hemoglobin in the active (intervention) groups than in the control (inactive) groups in their systematic review and meta-analysis.[10] The American Diabetes Association and the American College of Sports Medicine both encourage PA to promote insulin cellular action and glucose management.[11] There is considerable evidence that greater levels of PA are related to a decreased risk of cardiovascular disease, improves self-esteem and independence, and lowers the probability of early all-cause mortality.[12-14]

The effects on insulin action in response to a single session of moderate PA are maximal up to 24 hours after the PA session and gradually decrease until it approaches baseline values 72 hours later.[15] The increase in glucose absorption by the muscles in non-diabetics is followed by an increase in glucose production by the liver, keeping blood glucose steady. Under the same conditions of exertion, the use of blood glucose by active muscles in people with diabetes is often greater than the hepatic synthesis of glucose, resulting in a reduction in blood glucose.[15] As a result of the temporary nature of the alterations generated by PA in insulin activity, numerous organizations throughout the world advocate the PA practice for adults living with diabetes.[16-18]

To improve insulin cellular action, the American Diabetes Association and the American College of Sports Medicine recommend 30 minutes of moderate- to vigorous-intensity aerobic PA five days a week, for a total of 150 minutes each week. This should be carried out across at least three days per week, with no more than two consecutive days. Resistance exercises (weight training) of moderate to intense intensity should be conducted at least twice a week on non-consecutive days, in addition to aerobic activities.[19-21] The WHO recommendations for adults (18–64 years), include strong recommendations based on overall moderate-certainty evidence on weekly volumes of aerobic and muscle-strengthening physical activity. Many of the benefits of physical activity are observed within average weekly volumes of 150–300 min of moderate intensity or 75–150 min of vigorous intensity, or an equivalent combination.[22] The Brazilian Ministry of Health adheres to worldwide WHO guidelines.[5]

This study aimed to describe the pattern of leisure-time PA practice of adults with diabetic (18 years of age) living in the 26 Brazilian state capitals and the Federal District, served by at least one fixed telephone line, from the perspective that PA is a non-drug therapeutic component for people with diabetes and should be carried out systematically to maximize results. Second, to ensure that the PA, organized in accordance with the guidelines of the Brazilian Ministry of Health.

Aim

The purpose of this study was to describe the profile of PA of Brazilian adults with diabetes mellitus living in large Brazilian urban centers, as well as to determine whether the practice aligns with the PA guidelines recommended for people with diabetes.

Material and methods

The hypothesis pursued

This study was based on the hypothesis that Brazilian adults living with diabetes do not meet the minimum recommendations for physical activity recommended by the World Health Organization in order to achieve substantial health effects.

Study design

A descriptive study was conducted using secondary data collected by the Surveillance of Risk and Protective Factors for Chronic Non-communicable Diseases by Telephone Survey (VIGITEL) system, referring to a cross-sectional survey conducted in the capitals of Brazilian states and the Federal District, in 2020. A prior paper has the Vigitel methodology.[23]

In 2020, Vigitel set a minimum sample size of 1,000 persons for each city to estimate the frequency of the key risk factors for chronic non-communicable ill-

nesses in the adult population (≥18 years) with a 95% confidence coefficient and a maximum error of two percentage points. Sex-specific estimates using sample weights that proportionality the difference between the sexes are projected to have maximum errors of three percentage points.[24]

A telephone interviewing organization conducted the interviews between February and December 2016. The interview crew consisted of 40 interviewers, two supervisors, and a coordinator; they had prior training and were overseen during the system's operation by the Center for Epidemiological Research in Nutrition and Health at the University of São Paulo. An electronic questionnaire used to collect information about the people's demographic, socioeconomic, and behavioral factors, as well as questions about the organization of leisure-time PA. Sex (female and male) and domicile by area (North, Northeast, Southeast, South, and Midwest) were the factors evaluated in the sample's sociodemographic makeup.

Sampling

Vigitel's sampling methodologies seek to acquire probabilistic samples of people (≥18 years old) residing in homes with at least one fixed telephone line in each capital of the 26 Brazilian states and the Federal District. Because of the limitations presented by the Covid-19 epidemic on data collecting in 2020, each city was required to have a minimum sample size of 1,000 people. With this sample, we can estimate the frequency of any risk or protective factor in the adult population with a 95% confidence level and a maximum error of three percentage points. Maximum errors of four percentage points are estimated for sex-specific estimates, assuming equal proportions of men and women in the sample.[24]

The initial stage of Vigitel's sample involves drawing at least 5,000 telephone lines in each city. This draw, which is systematic and stratified by postal address code, is made from the telephone providers' electronic registry of fixed home lines. The lines drawn in each city are then reshuffled and divided into 200 replicas, each with the same proportion of lines per ZIP code as the original register. In 2020, 183,600 phone lines were first drawn, with an average of 6,800 per city, divided into 34 replicates of 200 lines each. To meet the minimal number of about 1,000 interviews in each capital, an average of 32 replicas were deployed each city, ranging from 16 to 92 replicas.[24]

In the second step of Vigitel sampling, one adult (≥18 years old) from the home is drawn. This stage is completed after determining whether of the lines drawn are eligible for the system. Lines that relate to firms, no longer exist or are out of service, or do not reply to six phone attempts made on various days and hours, including Saturdays, Sundays, and nighttime periods, and are most likely associated with closed residences, are ineligible for the system. Vigitel called 183,600 phone lines dispersed in 876 replicates throughout all 26 state capitals and the Federal District in 2020, identifying 47,031 eligible lines.[24]

The following questions used to identify leisure-time PA practitioners: "In the last three months, have you practiced any type of physical exercise or sport?" With an affirmative response, the respondent moved on to the following question: "What is the main type of physical exercise or sport you have practiced?" The interviewer had to choose the first activity indicated by the responder from a list of 16 alternatives.

Weekly frequency of physical activity

The number of days per week that the respondent practiced PA determined the weekly frequency of PA. The data was gathered by asking, "How many days a week do you usually practice physical exercise or sport?" The responses divided into four categories: every day, 5 to 6 days, 3 to 4 days, and 1 to 2 days each week. The duration of each session's effort determined by the question: "On the day you exercise or do sport, how long does this activity last?" The responses divided into seven categories: less than 10, 10 to 19, 20 to 29, 30 to 39, 40 to 49, 50 to 59, and 60 minutes or more. Activities that take fewer than 10 minutes, weren't taken into account in the computation.[20]

The same criterion employed by VIGITEL in the 2020 edition, utilized to determine the intensity of the endeavor[25]. Walking, treadmill walking, aqua aerobics, general gymnastics, swimming, cycling, and volleyball, therefore classed as moderate-intensity physical activity routines. Vigorous-intensity PA included jogging, treadmill running, weight training, aerobics, martial arts/fighting, soccer, basketball, and tennis.

Classification of physical activity level

The participant PA levels were classified as sufficient or insufficient based on the target recommended by the Brazilian Ministry of Health for people with diabetes. Sufficient was defined as at least 150 minutes per week of aerobic or resistance exercise spread over three days per week and no more than two consecutive days.[5] Inactive during leisure time, allocated to the participant who did not engage in any leisure-time PA at least once per week for the three months preceding the survey. The notion active during leisure time was allocated to the participant who did some PA at least once a week for the three months preceding the survey.

We utilized the question "Has a doctor ever told you that you have diabetes?" to identify individuals having a previous medical diagnosis of DM.

Inference of estimates for the total adult population of each city

To extend the results to the population without a landline, post-stratification weights were used. Based on census data from the corresponding year, the "Rake"

technique, used to estimate the total population of each capital. This method uses interactive procedures that take into account successive comparisons between estimates of the distribution of each sociodemographic variable in the Vigitel sample and in the city's total population. These comparisons result in the finding of weights which, when applied to the Vigitel sample, equate its sociodemographic distribution to the distribution estimated for the city's total population. The distribution of each sociodemographic variable estimated for each city in 2020 was obtained from projections that took into account the distribution of the variable in the 2000 and 2010 Demographic Censuses and its average annual variation (geometric rate) in the intercensal period.[24]

Physical activity and its domains

Physical activity is understood as any movement produced by the skeletal muscles that requires energy expenditure greater than rest, covering various domains, such as leisure time activities, work and home activities and active commuting. Unlike physical exercise, PA does not necessarily require systematic execution.[25,26]

Leisure-time physical activity is recreational activity. The Vigitel survey offers a few options to choose from: walking, treadmill walking, weight training, aqua aerobics, gymnastics in general, swimming, martial arts and wrestling, cycling, volleyball/football, dancing, running, treadmill running, aerobics, football/futsal, basketball and tennis.

Physically active is equivalent to completing at least 150 minutes of moderate-intensity PA per week, or 75 minutes of vigorous-intensity physical activity per week, or any equivalent between them. PA lasting less than 10 minutes was not taken into account when calculating the weekly sum.

Physically inactive means not practicing any physical activity in their free time, not making any significant physical effort at work, not actively commuting (more than 10 minutes per commute or 20 minutes per day) and not taking part in heavy household chores.

Ethical aspects

Informed consent was obtained orally when the interviewees were contacted by telephone. The Vigitel project was approved by the Ministry of Health's National Research Ethics Committee for Human Beings (CAAE: 65610017.1.0000.0008). The literature review study that gave rise to this article is exempt from ethical analysis, as determined by Resolution 510/2016 – CNS, as it deals with publicly accessible secondary data, under the terms of Law No. 12,527, of November 18, 2017.[27]

Statistical analysis

The prevalence of PA among patients with diabetes, as well as their corresponding 95% confidence intervals, given by individual level of PA, region, and kind of PA. Pearson's chi-squared test, used to investigate potential changes in PA prevalence based on the presence or absence of DM. We employed a statistically significant difference, defined as a p-value less than 0.05. For data processing and statistical analysis, Stata® 11.0.

Results

One hundred twenty seven thousand telephones lines were initially allocated for the 2020 Vigitel study. Following pre-established criteria, 77,671 suitable lines were calculated, of which 53,210 responded to the interview, comprising 20,258 men and 32,952 women, showing a system success rate of 68.5% and an average interview time of around 11 minutes, ranging from 4 to 59 minutes. The proportion of people with a previous medical diagnosis of diabetes ranged from 5.3% in Boa Vista to 10.4% in Rio de Janeiro. Diabetes was more common in Rio de Janeiro (12%), São Paulo (11.1%), and Belo Horizonte (11%) among women, and less common in Palmas and Manaus (5.8%) and Teresina (6.5%) (data not presented in tables or figures).[24]

The study removed 1,535 participants (2.9%) who, although reporting practicing PA, did not provide information on the weekly frequency or duration of the activity. As a result, the calculation basis included 51,675 research participants.

Table 1. Proportional distribution of people with diabetes, aged ≥ 18 years, according to PA level, by sex – Vigitel, Brazilian state capitals and Federal District, 2020*

	PA level of people with diabetes					
	Total		men		women	
	%	95%CI	%	95%CI	%	95%CI
Inactive + Ins. active	69.5	77.2–58.2	60.9	71.9–59.2	72.8	82.1–51.5
Active	45.5	43.3–51.6	54.1	49.2–58.3	42.2	39.4–45.1

* Percentage weighted to adjust the sociodemographic distribution of the Vigitel sample to the distribution of the adult population of each city projected for 2020

Table 2. Percentage of adults living with diabetes (≥ 18 years of age) who achieved the level of PA recommended by the WHO, by sex – Vigitel, Brazilian state capitals and the Federal District, 2020*

		PA sufficient	
Regions	Prev	men	women
		% 95%CI	% 95%CI
North	6.1%	14.5 (12.1–16.7)	12.3 (10.1–14.2)
Northeast	8.2%	14.2 (12–15.4)	12.1 (10.3–15.6)
Center-West	8%	18.2 (14.4–20.8)	15.5 (13–18.1)
Southeast	10.1%	16.3 (14.2–18.4)	13.8 (11.6–16.9)
South	8.3%	15.3 (13.5–17.9)	13.0 (10.9–15)

* Weighted percentage to adjust the sociodemographic distribution of the Vigitel sample to the distribution of the adult population of each city projected for 2020, 95% CI – 95% confidence interval, Prev – prevalence of diabetes

Table 3. Percentage of people with diabetes, aged ≥ 18 years, according to the main type and level of PA reported, by sex – Vigitel, Brazilian state capitals and Federal District, 2020*

People living with diabetes	PA Sufficient						PA Insufficient					
Modalities	**Total %**	**(95%CI)**	**M %**	**(95%CI)**	**W %**	**(95%CI)**	**Total %**	**(95%CI)**	**M %**	**(95%CI)**	**W%**	**(95%CI)**
Walking	74.2	68.4–80.5	71.6	62.2–81	76.8	69.5–83.9	54.6	45.0–66.1	49.0	31.9–68.5	60	43.9–72.7
Treadmill walking	3.2	11.8–4.6	3.7	0.2–6.6	2.7	0.8–4.5	0.8	0.005–1.4	1.3	0.2–3	0.3	0.1–0.7
Jogging	1.3	0.4–2.6	1	0.1–2	1.6	0.05–40	2.5	0.4–5.8	2.4	0.8–5.3	2.8	0.2–7.7
Treadmill jogging	0.2	0.002–0.5	0.2	01–0.5	0.3	0.07–0.8	1.2	1.0–3.2	2.7	2.4–7.1	–	–
Bodybuilding	3.1	0.8–4.2	4.2	0.08–7.6	1.9	0.1–2.4	1.3	0.1–2.5	2.3	0.8–5.3	0.5	0.2–1.1
Aerobic gymnastics	1.4	0.01–2.5	2.1	0.6–4.8	0.7	0.004–1.2	0.9	0.4–1.8	1.3	1.3–3.8	0.2	0.06–0.5
Hydrogymnastics	5.1	1.9–8.4	2.2	0.9–5.1	7.9	2.3–12.4	9.1	1.7–16.3	3.5	0.1–6.5	13.1	15.8–25.9
General gymnastics	2.5	1.6–4.7	1.3	1.7–3.1	3.8	1.8–7.3	4.4	0.3–8.8	1.1	0.3–2.7	7.3	0.09–14.1
Swimming	0.7	0.05–1.8	0.9	0.5–2.1	0.6	0.4–1.2	0.9	0.004–1.6	1.2	0.6–2.8	0.5	0.05–1.3
Fights	0.1	0.01–0.1	0.5	0.2–1.3	–	–	0.4	0.3–1	0.8	0.8–2.4	–	–
Cycling	3.2	1.1–4.2	5.6	1.7–8.4	0.8	0.07–1.1	0.7	1.1–13.1	1.1	0.2–2.3	0.4	0.1–0.8
Football	2.7	0.08–3.1	4.8	1.6–6.9	–	–	9.1	4.9–13.2	21	10.7–31.2	–	–
Basketball	–	–	–	–	–	–	–	–	–	–	–	–
Volleyball	0.1	0.01–0.2	0.04	0.01–0.1	0.1	0.008–0.2	0.07	0.04–0.2	0.2	0.1–0.5	–	–
Tennis	–	–	–	–	–	–	0.01	0.08–0.3	0.2	0.2–0.7	–	–
Others	2.1	1.6–5.6	1.9	0.6–5.2	2.8	1.2–6.9	14	5.3–21.2	11	1.9–20.6	14.8	4.3–23.7

*Weighted percentage to adjust the sociodemographic distribution of the Vigitel sample to the distribution of the adult population of each city projected for 2020, 95%CI – 95% confidence interval

People with DM were distributed unequally according to schooling, with 74.2% (95%CI: 71.6–76.8) having up to 8 years of schooling, 16.9% (95%CI: 14.5–18.7) having 9 to 11 years, and 8.9% (95%CI: 7.9–12.6) having more than 12 years (p=0.001). Diabetes was more frequent in older adults in both sexes, reaching roughly 1% of individuals aged 18 to 24 and 20% of participants aged 65 or older.

The categorization of the degree of PA of patients having a previous DM, is shown in Table 1. More over half of the patients (55%) were inactive in their spare time, and only 15% met the Ministry of Health's recommended PA threshold. Table 2 displays the percentage of participants who met the PA objective by area of residence. Women's PA levels were lower across the board, with the Northeast area outperforming the rest of the country. Reaching the goal was greater in the Midwest (18.2%; 95%CI: 14.4–20.8) and lower in the Northeast (14.2%; 95%CI: 12–15.4) among males. The Midwest area had the greatest PA goal accomplishment among women (15.5%; 95%CI: 13–18.1).

The primary PA reported by people with diabetes are shown in Table 3. Three out of every four people who completed the goal reported walking (74.2%; 95%CI: 68.4–80.5). Walking was reported less often by people with diabetes and lower levels of PA (54.6%; 95%CI: 45–66.1). Males were more likely to practice soccer than females.

Table 4, depicts the weekly frequency and length of PA sessions. PA frequency of 3 to 4 days per week, reported by 44.5% (95%CI: 42.8–46). Only 1.5% of the most active indicated a weekly frequency of one to two days. On active days, more than half of people with dia-

Table 4. Percentage distribution of people with diabetes (≥ 18 years of age), according to weekly frequency and daily duration of PA and level of PA (sufficient, insufficient) – Vigitel, Brazilian state capitals and Federal District, 2020*

		General population	People with diabetes
% (95%CI)		% (95%CI)	
PA SUFFICIENT			
Frequency (weekly)	Every day	22.8 (21.3–24.3)	25.7 (17.9–5.0)
	5–6	26.9 (25.4–28.3)	35.5 (26.4–44.5)#
	3–4	44.5 (42.8–46)	37.4 (28.2–46.5)#
	1–2	5.8 (0.5–6.6)	1.4 (0.7–3.7)#
Duration (minutes)	<10	–––	–––
	10–19	0.4 (0.1–0.6)	0.2 (0.1–0.5)
	20–29	0.6 (0.1–0.9)	1.7 (0.8–2.6)#
	30–39	5.1 (4.4–6.6)	6.3 (5.1–7.8)
	40–49	10.9 (9.8–12.8)	13.6 (8,2–18.9)
	50–59	13.5 (11.0–16.3)	17.5 (15.0–22.1)#
	≥60	69.2 (68.5–71.4)	60.7 (52.7–68.3)#
PA INSUFFICIENT			
Frequency (weekly)	Every day	2.4 (0.1–3.6)	3.9 (0.9–8.9)
	5–6	1.7 (1.0–2.4)	4.4 (0.5–8.8)#
	3–4	2.9 (2.2–3.6)	12 (4.8–19.2)#
	1–2	93.0 (91.3–94.3)	79.7 (70.2–88.6)#
Duration (minutes)	<10	0.6 (0.3–0.8)	0.6 (0.1–1.1)
	10–19	3.4 (4.9–8)	4.6 (5.8–23.3)
	20–29	4.2 (3.4–5)	5.6 (3.7–9.5)
	30–39	4,1 (3.3–5.2)	9.9 (1.2–18.5)#
	40–49	8.3 (6.9–9.7)	14 (12.4–16.3)#
	50–59	11.8 (10.9–14.4)	14.5 (8.6–20.4)
	≥60	67.6 (64.9–70.3)	50.8 (38.8–62.6)#

* Weighted percentage to adjust the sociodemographic distribution of the Vigitel sample to the distribution of the adult population of each city projected for 2020, 95%CI – confidence interval,

– statistically significant difference (Pearson chi-square test)

betes exercised for 60 minutes or more, and three-quarters exercised for 30 minutes or more every session. They exercised 1 to 2 days per week for 76.8% (95%CI: 61.6–91.9) of men and 81.6% (95%CI: 70–93.2) of women with DM who did not meet the objective.

Discussion

The purpose of this study was to describe the structure of leisure-time PA for people with diabetes and to see if it met the guidelines of the Brazilian Ministry of Health. Walking, water aerobics, and gymnastics were the most common forms of PA reported, with women outnumbering males in all places questioned. The ultimate weekly amount of exercises resulted in 15% of people with diabetes meeting their PA goal.

The rise in the prevalence of DM complications has an impact on health-care management, raising the expense of disease prevention and treatment. Health surveillance strategies based on the development of improved living behaviors are critical to disease management. In this regard, and in accordance with WHO worldwide recommendations, the Brazilian Ministry of Health revised its strategy for diabetic self-care in 2013.

The most recent ones support the 2006 fundamental principles for practicing PA. Previously, the recommendations emphasized aerobic activity with a steady increase in length until attaining the aim of 30 to 60 minutes per day, 5 to 7 days per week. At least 150 minutes per week is now advised, with aerobic activities spaced out over three days per week and no more than two consecutive days, and resistance exercises (weight training) encompassing as many muscle groups as feasible. The suggestions do not mention whether the time spent on resistance exercises contributes to the aim.[5]

It is critical to discuss the weekly frequency and length of activities when establishing the weekly volume of PA. Although the daily length of activities is consistent with the requirements for 87% of active people with diabetes, the lower weekly frequency is a significant impediment to meeting the goal. In this regard, the major goal should be to increase the frequency of PA without affecting the duration of activities, which is recommended for most people with diabetes.

When you consider that PA-mediated insulin sensitization is peak 12 to 48 hours after the exercise session and gradually recovers to pre-activity levels, it is normally no more than 72 hours.[28] Training's effect on insulin sensitivity may remain a little longer, maybe because part of its benefits are mediated by muscle mass gain.[29] According to Thomas et al., the insufficient amount of effort may explain why many people with diabetes with insufficient PA have not improved their blood glucose control to the same level as adequately active people with diabetes.[30]

The most dangerous circumstance for people with diabetes' health was the complete lack of PA for the majority of people with diabetes (55%). Although different guidelines differ on the optimal way to obtain the minimum amount of PA to achieve significant health effects, all guidelines agree that the magnitude of the positive effect of PA on people with diabetes is greater when inactive people incorporate some degree of PA than when moderately active people increase their activity. In this regard, beginning to practice some PA appears to be the most crucial step for the majority of people with diabetes.

Walking was chosen as the primary form of physical activity by nearly 74.2% of people with diabetes. This rate was greater than the general population (43%). Walking is a moderate-intensity aerobic activity, and it was the exercise that contributed the most to meeting the PA goal.[25] This is in consistent with the Ministry of Health's recommendations, which favor volume over high intensity exercises.[5]

PA-mediated insulin sensitization occurs as a result of both aerobic and resistance exercise (weight training). The method by which various forms of exercise act appears to be distinct.[30] With this in mind, the Ministry of Health suggests mixing the two modes in a complimentary rather than alternative manner.[5] Resistance exercise (weight training) has been found to be safe for persons in their forties and fifties, including those at high risk of cardiac events.[22]

According to the findings of this survey, just 3% of the participants listed weight training as their primary activity. According to the opening remark in the preceding paragraph, not practicing weight training is contrary to MOH guidelines. However, as noted under the method's limitations, this information should be evaluated with caution due to the Vigitel survey's restriction of not defining types of activities carried out concurrently with the main activity. As a result, bodybuilding may be underrepresented in this study.

The emphasis on general care, focusing on individual criteria, stands out as a guiding concept. Knowledge of a prior DM diagnosis and the beneficial effects of PA on the illness might support the concept that people with diabetes should be or remain more physically active, hence protecting against disease progression and the formation of comorbidities. The decision to live a more active life is also influenced by the availability, opportunity, and safety of access to and permanency in public locations conducive to PA practice. As a result, it makes sense to integrate intersectional efforts to increase public health by promoting PA.

People with diabetes might benefit from better intersectional public planning. Measures to beautify the physical environment near residential areas, such as the creation of safe environments on sidewalks and at street crossings with reduced traffic speeds, the concentration of public access points for practicing PA away

from places with heavy traffic and preventive policing, extending the opening hours of facilities for practicing PA and publicizing the existence of appropriate points for PA, may prove beneficial.

The study's strengths include data on leisure-time physical activity of a large sample of Brazilian adults living with diabetes residing in the capitals of the Brazilian states and the Federal District, which has been tracked on a regular basis by the Vigitel system since 2006. This study provides useful information on the organization of PA in order to strengthen activities aimed at encouraging people with diabetes to be more active, minimizing access disparities between sex and age groups, and guaranteeing appropriate and secure public venues for PA. The relevance of Primary Health Care services as an ideal location to provide advice on the benefits of PA, considering its closeness and penetration among patients with DM, cannot be overstated.

Study limitations

When evaluating the findings, some limitations of this study should be acknowledged. The Vigitel data was collected only through conversations with landline phone owners. The use of post-stratified weights attempted to reduce any discrepancies between the entire population and the study population. However, residual selection bias may exist. The Vigitel survey's question on the kind of PA is not multiple choice, which makes it easy to underestimate some types of PA. Furthermore, the real amount of work cannot be proven. Direct measurements of PA might fulfill this constraint, but they are inappropriate for large-sample surveys like this one. Positive cases of illness knowledge were not validated on the spot. It should be recognized that some individuals interviewed may have been diabetic and were ignorant of their status. The utilization of a representative sample of the Brazilian population (around 30%) with solid validity and repeatability data is the study's strength.[29]

Conclusion

In 2020, 7.9% of the population identified themselves as having diabetes. There was a higher frequency among older women and those with less schooling. In the sample as a whole, approximately 70% of people living with diabetes were inactive (54.5%) or did not meet the minimum BP recommendations for people with diabetes. The duration of each session seemed to be in line with the recommendations, however, the lack of regularity caused by the low weekly frequency meant that the minimum recommended target could not be achieved. Efforts involving the continued monitoring of people living with diabetes and counseling in Primary Health Care to opt for a more physically active life, seem to be promising acts for a healthier life, pending a definitive resolution to the disease.

Declarations

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Author contributions

Conceptualization, D.F.L., A.A.S, D.C.S, D.M. and L.A.L.; Methodology, D.F.L., A.A.S, D.C.S, D.M. and L.A.L.; Software, D.F.L. and L.A.L; Validation, D.F.L., A.A.S, D.C.S, D.M. and L.A.L.; Formal Analysis, D.F.L. and L.A.L.; Investigation, D.F.L., A.A.S, D.C.S, D.M. and L.A.L.; Resources, D.F.L. and L.A.L; Data Curation, D.F.L., A.A.S, D.C.S, D.M. and L.A.L.; Writing – Original Draft Preparation, D.F.L.; Writing – Review & Editing, D.F.L., A.A.S, D.C.S, D.M. and L.A.L.; Visualization, D.F.L. and L.A.L.; Supervision, D.F.L., A.A.S, D.C.S, D.M. and L.A.L; Project Administration, D.F.L. and L.A.L.; Funding Acquisition, D.F.L. and L.A.L.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data available on request from the authors.

Ethics approval

The Vigitel project was approved by the Ministry of Health's National Research Ethics Committee for Human Beings (CAAE: 65610017.1.0000.0008).

References

1. World Health Organization (WHO). 2006. *Technical report: Definition and diagnosis of people with diabetes mellitus and impaired glycaemic regulation*. Geneva; 2006.
2. American Diabetes Association (ADA). Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2010;33:62-69. doi: 10.2337/dc10-S062
3. Khan MAB, Hashim MJ, King JK, et al. Epidemiology of Type 2 Diabetes - Global Burden of Disease and Forecasted Trends. *J Epidemiol Glob Health*. 2020;10(1):107-111. doi: 10.2991/jegh.k.191028.001
4. Sun H, Saeedi P, Karuranga S, et al. People with diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract*. 2022;183:109119. doi: 10.1016/j.diabres.2021.109119
5. Muzy J, Campos MR, Emmerick I, Silva RS, Schramm JMA. Prevalence of diabetes mellitus and its complications and characterization of healthcare gaps based on triangulation of studies. *Cad. Saúde Pública*. 2021;37(5):e00076120. doi: 10.1590/0102-311x00076120
6. Kanaley JA, Colberg SR, Corcoran MH, et al. Exercise/Physical Activity in Individuals with Type 2 Diabetes:

A Consensus Statement from the American College of Sports Medicine. *Med Sci Sports Exerc*. 2022;1;54(2):353-368. doi: 10.1249/MSS.0000000000002800

7. Stamatakis E, Straker L, Hamer M, Gebel K. The 2018 Physical Activity Guidelines for Americans: What's New? Implications for Clinicians and the Public. *J Orthop Sports Phys Ther*. 2019;49(7):487-490. doi: 10.2519/jospt.2019.0609
8. Jiang Y, Tan S, Wang Z, Guo Z, Li Q, Wang J. Aerobic exercise training at maximal fat oxidation intensity improves body composition, glycemic control, and physical capacity in older people with type 2 diabetes. *J Exerc Sci Fit*. 2020;18(1):7-13. doi: 10.1016/j.jesf.2019.08.003
9. Daryabor G, Atashzar MR, Kabelitz D, Meri S, Kalantar K. The Effects of Type 2 People Diabetes Mellitus on Organ Metabolism and the Immune System. *Front Immunol.* 2020;22(11):1582-96. doi: 10.3389/fimmu.2020.01582
10. Colosimo S, Tan GD, Petroni ML, Marchesini G, Tomlinson JW. Improved glycaemic control in patients with type 2 diabetes has a beneficial impact on NAFLD, independent of change in BMI or glucose lowering agent. *Nutr Metab Cardiovasc Dis*. 2023;33(3):640-648. doi: 10.1016/j.numecd.2022.12.010
11. Sugimoto K, Ikegami H, Takata Y, et al. Glycemic Control and Insulin Improve Muscle Mass and Gait Speed in Type 2 diabetes: The MUSCLES-DM Study. *J Am Med Dir Assoc*. 2021;22(4):834-838.e1. doi: 10.1016/j.jamda.2020.11
12. Sacre JW, Harding JL, Shaw JE, Magliano DJ. Declining mortality in older people with type 2 diabetes masks rising excess risks at younger ages: a population-based study of all-cause and cause-specific mortality over 13 years. *Int J Epidemiol*. 2021;30;50(4):1362-1372. doi: 10.1093/ije/dyaa270
13. Zhang PY. Cardiovascular disease in diabetes. *Eur Rev Med Pharmacol Sci*. 2014;18(15):2205-2214 .
14. King CJ, Moreno J, Coleman SV, Williams JF. Diabetes mortality rates among African Americans: A descriptive analysis pre and post Medicaid expansion. *Prev Med Rep*. 2018;4(12):20-24. doi: 10.1016/j.pmedr.2018.08.001
15. Flores-Opazo M, McGee SL, Hargreaves M. Exercise and GLUT4. *Exerc Sport Sci Rev*. 2020;48(3):110-118. doi: 10.1249/JES.0000000000000224
16. Rezaeeshirazi R. Aerobic Versus Resistance Training: Leptin and Metabolic Parameters Improvement in Type 2 Diabetes Obese Men. *Res Q Exerc Sport*. 2022;93(3):537-547. doi: 10.1080/02701367.2021.1875111
17. Kanaley JA, Colberg SR, Corcoran MH, et al. Exercise/Physical Activity in Individuals with Type 2 Diabetes: A Consensus Statement from the American College of Sports Medicine. *Med Sci Sports Exerc*. 2022;54(2):353-368. doi: 10.1249/MSS.0000000000002800
18. Martin JC, Awoke MA, Misso ML, Moran LJ, Harrison CL. Preventing weight gain in adults: A systematic review and meta-analysis of randomized controlled trials. *Obes Rev*. 2021;22(10):e13280. doi: 10.1111/obr.13280
19. Collins KA, Ross LM, Slentz CA, Huffman KM, Kraus WE. Differential Effects of Amount, Intensity, and Mode of Exercise Training on Insulin Sensitivity and Glucose Homeostasis: A Narrative Review. *Sports Med Open*. 2022;14;8(1):90. doi: 10.1186/s40798-022-00480-5
20. World Health Organization (WHO). 2010. *WHO guidelines on physical activity and sedentary behaviour*. World Health Organization; Geneva, 2010.
21. Lima DF, Lima LA, Sampaio AA. Recreational physical activity in Brazilian older adults: secondary analysis of the 2018 Vigitel survey. *Geriatr Gerontol Aging.* 2022;16:e0220015. doi: 10.53886/gga.e0220015
22. Bull FC, Al-Ansari SS, Biddle S, Borodulin K, Buman MP, Cardon G, et al. Br *J Sports Med*. 2020;54:1451-1462. doi:10.1136/bjsports-2020-102955
23. Balko AB, Palavissini CFC, Souza DC, Lima LA, Lima DF. Nutritional ergogenic resources in sport: lights and shadows on their use. *Research, Society and Development*. 2022;11:1-8. doi: 10.33448/rsd-v11i1.25056
24. Lima DF, Lima LA, Sampaio AA. Recreational physical activity in Brazilian older adults: secondary analysis of the 2018 Vigitel survey. *Geriatr Gerontol Aging.* 2022;16:e0220015. doi: 10.53886/gga.e0220015
25. Ainsworth BE, Haskell MC, Whitt ML, et al. Compendium of physical activities: um update of activity codes and MET intensities. *Med Sci Sports Exerc*. 2000;32:498-516. doi: 10.1097/00005768-200009001-00009
26. Stachelski RA, Torrilhas B, Camboin FF, et al. Therapeutic Exercise in Plantar Fasciitis: A Systematic Review with Meta-Analysis. *Muscles, Ligaments and Tendons Journal*. 2024;14(1):29-45. doi: 10.32098/mltj.03.2024.04
27. Lima DF, Ferreto LED, Buzanello MR. Consent for processing medical records data. *Rev bioét*. 2023;31:e3589EN. doi: 10.1590/1983-803420233589EN
28. Assumpção D, Ruiz AMP, Borim FSA, et al. Eating Behavior of Older Adults with and Without Diabetes: The Vigitel Survey, Brazil, 2016. *Arq Bras Cardiol*. 2022;118(2):388-397. doi: 10.36660/abc.20201204
29. U.S. Department of Health and Human Services- USDHHS. 1997; *Guidelines for school and community programs to promote lifestyle physical activity among young people*. Centers for Disease Control and Prevention (CDC) Atlanta, Georgia, 1997;46:1-36.
30. Lima DF, Piovani VGS, Lima LA. Recreational soccer practice among adults, in Brazilian capitals, 2011-2015. *Epidemiol Serv Saude*. 2018;27(2):e2017284. doi: 10.5123/S1679-49742018000200013

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ORIGINAL PAPER

Effect of lipopolysaccharide on the development of oxidative-nitrosative stress in salivary glands and soft periodontal tissues of rats under conditions of water avoidance stress

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ABSTRACT

Introduction and aim. Violation in the salivary glands will inevitably cause changes in periodontium, and periodontitis can disrupt the functioning of the salivary glands. The purpose of the work is to evaluate changes in NO-synthase and arginase activities, pro- and antioxidant balance in rat salivary glands and soft periodontal tissues during administration of bacterial lipopolysaccharide (LPS) and water avoidance stress (WAS) modeling.

Material and methods. The experiment was performed on 24 rats. The animals were divided into 4 groups: control, WAS, animals injected intraperitoneally with 0.4 μg/kg of bacterial LPS of *Salmonella typhi*, WAS+LPS.

Results. Water avoidance stress led to decrease of inducible NO-synthase (iNOS) activity in salivary glands by 1.63 times, but decreased arginase activity by 1.15 times, superoxide production increased by 1.53 times, catalase activity decreased by 1.2 times, and malonic dialdehyde (MDA) increased by 1.19 times compared to the control. Lipopolysaccharide led to increase of constitutive NO-synthases (cNOS) activity in salivary glands by 1.48 times, but decreased arginase activity by 6.15 times, catalase activity increased by 2.6 times and superoxide dismutase activity decreased by 2.74 times, and MDA increased by 6.84 times compared to the control. Water avoidance stress + LPS in salivary glands led to decrease of cNOS and arginase activity by 1.09 and 1.19 times, respectively, superoxide production increased by 1.88 times, catalase and superoxide dismutase activity decreased by 1.06 times and 1.34 times, respectively, and MDA increased by 2.44 times compared to the control.

Water avoidance stress led to increase of iNOS activity in periodontium by 1.44 times and arginase activity decreased by 1.37 times, superoxide production increased 1.32 times, catalase activity and superoxide dismutase activities decreased by 1.27 times and by 1.53 times, respectively, and MDA increased by 1.31 times compared to the control. Lipopolysaccharide led to increase of iNOS activity in the periodontium by 3.88 times, arginase activity decreased by 2.69 times, superoxide production increased 1.64 times, catalase activity increased by 4.32 times, and MDA increased by 4.51 times compared to the control. Water avoidance stress + LPS in periodontium led to increase of iNOS and cNOS activities by 1.95 times and 1.53, respectively, arginase activity decreased by 1.39 times, superoxide production increased 1.66 times, catalase activity increased by 1.11 times, and MDA increased by 1.53 times compared to the control.

Conclusion. The combination of LPS and WAS leads to changes in NO production and oxidative stress in salivary glands and the periodontium.

Keywords. bacterial lipopolysaccharide, nitric oxide, salivary glands, soft periodontal tissues, water avoidance stress

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Introduction

Salivary glands and the periodontium are part of a single functional system that forms homeostasis in the oral cavity. Patients with generalized chronic periodontitis have an increased risk of developing primary Sjogren's syndrome.[1] Violation of saliva secretion caused by Sjogren's syndrome leads to increased inflammatory processes in periodontal tissues.[2,3] The functional state of salivary glands and the periodontium are interconnected through common metabolic patterns. For instance, excessive amount of palmitic acid in blood can lead both to Sjogren's syndrome development and inflammation in the periodontium.[2] Terrizzi et al. in their study showed that during adaptation to hypoxia in salivary glands, secretion drops, while levels of prostaglandin E_2 increase.[3] Increased levels of prostaglandin E_2 in turn lead to excessive alveolar bone loss, thus mimicking the bone loss observed during periodontitis.[3] Lin et al. showed in their research that changes in oral microbiota caused by xerostomia form a vicious circle and exacerbate xerostomia further.[4]

Thus, a violation of the physiological functioning of the salivary glands will inevitably cause changes in the functional state of the periodontal tissues, and periodontitis can disrupt the functioning of the salivary glands.

Lipopolysaccharide (LPS) is an important factor in the virulence of gram-negative bacteria and a powerful activator of innate and adaptive immune responses, as well as being capable of triggering intracellular signaling of tissue destruction. It plays an important role in the pathogenesis of periodontitis, where a large number of gram-negative species is a typical determinant of the periodontal microbiota. Translocation of LPS into the circulation causes endotoxemia, which is involved in the pathogenesis of many inflammatory conditions, including atherosclerotic cardiovascular disease, obesity, liver disease, diabetes, metabolic syndrome, and oral inflammatory diseases, and is therefore considered a risk factor. It is known that patients with periodontitis have an increased concentration of circulating LPS and metabolic disturbances, which can be the cause or consequence of endotoxemia.[5] Bacterial endotoxin causes inflammation in the salivary glands, which leads to impaired secretion of saliva.[6,7]

Systemic inflammatory response can change composition of saliva drastically, leading to absence of proteins necessary for oral homeostasis.[8] Recent studies have revealed the role of small extracellular vesicles in saliva as powerful diagnostic tools for periodontitis development, hinting of presence of a special interconnection between metabolism of the periodontium and salivary glands.[9] The presence of oxidative stress markers in saliva corresponds to development of oxidative stress in the periodontium, however, whether salivary glands are involved in increase of oxidative stress biomarkers in saliva is still under question.[10]

The Scientific School of Professor Tarasenko substantiated biochemical mechanisms of stress-induced damage to salivary glands and periodontal tissues.[11] Stress causes drastic changes in the composition of saliva, characterized by changes in the electrolyte composition, protein secretion, and an increase in inflammatory markers.[12,13] Psychological stress leads to an imbalance in the immune homeostasis of periodontal tissues, which can lead to the development of chronic periodontitis and/or an increase in the destruction of biopolymers of periodontal tissues.[14]

The combined effect of chronic stress and bacterial LPS on periodontal soft tissues and salivary glands is poorly understood.

Aim

To evaluate changes in the activity of NO-synthase and arginase, pro- and antioxidant balance in salivary glands and soft periodontal tissues of rats under the conditions of administration of bacterial lipopolysaccharide against the background of water avoidance stress modeling.

Material and methods

Ethical approval

Research was conducted in accordance with the standards of the Council of Europe Convention on Bioethics "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (1997), general ethical principles of experiments on animals approved by the First National Congress on Bioethics of Ukraine (September 2001) and other international agreements and national legislation in this area. The rats were kept in a vivarium accredited in accordance with the "Standard rules of order, equipment and maintenance of experimental biological clinics (vivarium)." All experimental procedures were approved by Bioethical Committee of Poltava State Medical University (Record № 212 from 27.01.2023).

Sample and experimental groups

The experiment was performed on 24 sexually mature male Wistar albino rats weighing 190–240 g. The animals were divided into 4 groups of 6 rats.

I – control group, intact animals, which were intraperitoneally injected with 0.2 mL of a 0.9% aqueous solution of sodium chloride.

II – water avoidance stress group (WAS group), simulated stress according to the water avoidance stress modelling protocol.

III – a group of rats injected with lipopolysaccharide (LPS group) according to pyrogenal injection protocol.

IV – group of rats intraperitoneally injected with pyrogenal (WAS+LPS group) that were subjected to the

water avoidance stress modelling protocol and pyrogenal injection protocols.

The conditions for keeping animals in the vivarium were standard. Animals were removed from the experiment on the 30th day and blood sampling was performed from the right ventricle of the heart under thiopental anesthesia.

Water avoidance stress modelling protocol

The rats were placed on a platform (8×6 cm) in the middle of a plastic container with a diameter of 90 cm and height of 50 cm filled with water of 25°C to 1 cm below the level of the platform. Rats avoided water by staying on the platform for 1 hour during 30 days.[15]

Pyrogenal injection protocol

Bacterial LPS of *Salmonella typhi* (pyrogenal) was injected intraperitoneally with 0.4 μg/kg of in the first week 3 times and then once a week throughout the duration of the experiment.[16,17]

Biochemical analysis

For biochemical analysis, we used 10% periodontal soft tissues and salivary gland homogenate and blood serum. Periodontal soft tissues and salivary gland homogenate was obtained after homogenization of 1 g of rat periodontal soft tissues or 1 g of salivary gland with 9 mL of 0.2 M Tris-buffer solution (Trisaminomethane-hydrochloric acid buffer, pH=7.4). Then it was centrifuged at 3000 g for 10 minutes. The upper layer (supernantant) was used for further biochemical analysis. Blood plasma was obtained after addition of 0.109 M sodium citrate at ratio 9:1 and subsequent centrifugation at 3000 g for 10 minutes.

Cortisol concentration was determined in the aliquot of 0.1 mL of blood plasma of rats.

To determine the concentration of cortisol, 2 mL of ammonium tetramethylhydroxide pentahydrate solution (100 mg of ammonium tetramethylhydroxide pentahydrate was dissolved in 5 mL of distilled water, then 5 mL of the resulting solution was mixed with 45 mL of methyl alcohol) and 2 ml of nitroblue tetrazolium chloride solution (100 mg of nitroblue tetrazolium chloride in 50 mL of methyl alcohol). As a result, a red-colored dye was formed with a maximum light absorption at a wavelength of 510 nm.[18]

Total NO-synthase (gNOS) activity was evaluated by the increase of nitrites after incubation of 10% liver homogenate (0.2 mL) for 30 min in the incubation solution (2.5 mL of 0.1 M trisbuffer, 0.3 mL of 320 mM aqueous solution of L-arginine and 0.1 ml of 1 mM $NADPH^+H^+$ solution). To determine the activity of constitutive NOS (cNOS) 1% solution of aminoguanidine hydrochloride was used and the incubation time was extended to 60 min.[19,20] The activity of inducible NOS (iNOS) was calculated by the formula: iNOS=gNOS-cNOS.

Adrenaline auto-oxidation reaction in an alkaline environment with the generation of superoxide was used to determine SOD activity. SOD activity was calculated in conventional units (c.u., 1 unit indicates a 50% inhibition of the reaction rate) by comparison of speed of adrenaline auto-oxidation in presence of tissue homogenate and without it.[21]

The method of catalase activity estimation was based on the determination of colored products formed by the reaction of hydrogen peroxide with ammonium molybdate. The amount of hydrogen peroxide decomposed in the presence of a sample containing catalase can help us to make a conclusion about the activity of catalase.[21]

Free malonic dialdehyde (MDA) specifically reacts with 1-methyl-2-phenyl-indole in a mixture of methanol and acetonitrile to form chromogen (carbocyanine dye) with a maximum light absorption at a wavelength of 586 nm.[22]

Peroxynitrite concentration was measured by using its reaction with potassium iodide under pH 7.0 in 0.2 M phosphate buffer with the same pH, which yields I_3 with maximum absorbance at 355 nm wavelength.[19]

The method for the determination of nitrosothiols was based on the determination of the difference in the concentration of nitrites (NO_2^-) using Griess reagent (modified by Ilosvay) before and after oxidation of nitrosothiol complexes (SNO) to nitrites with a solution of mercuric chloride ($HgCl_2$).[23]

Sulfides specifically react with N-N-dimethyl-para-phenylenediamine in the presence of Fe^{3+} ions and excess of hydrochloric acid to form a red-pink chromogen with a maximum light absorption at a wavelength of 667 nm.[24]

The method for estimation of superoxide anion radical production was based on nitroblue tetrazolium (NBT) reduction by superoxide with the formation of diformazan, a dark blue insoluble precipitate.[24]

OMP were measured by definition of additional carbonyl groups in side chains of amino acids by reaction of 2,4-dinitrophenylhydrazine with carbonyl groups.[25]

Statistical analysis

Statistical processing of the results of biochemical studies was carried out using a pairwise comparison using the non-parametric Mann-Whitney method. All statistical calculations were performed in the Microsoft office Excel program and its extension Real Statistics 2019 developed by Charles Zaiontz. The difference was considered statistically significant at $p<0.05$.

Results

The level of cortisol in the blood of experimental animals is shown in Fig. 1.

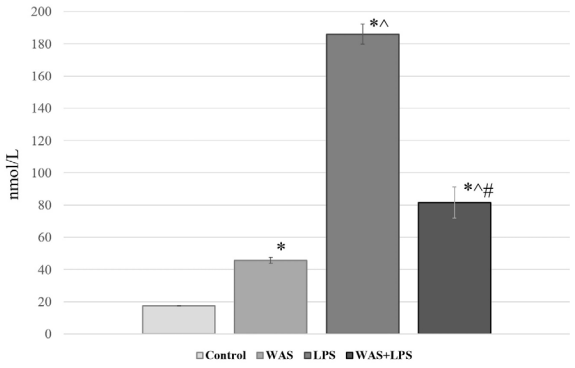

Fig. 1. Cortisol concentration in the blood of rats under the conditions of administration of bacterial lipopolysaccharide against the background of water avoidance stress modeling, M±m, * – indicates that data is statistically significantly different form control group ($p<0.05$), ^ – indicates that data is statistically significantly different form WAS group ($p<0.05$), # – indicates that data is statistically significantly different form LPS group ($p<0.05$), WAS – water avoidance stress, LPS – lipopolysaccharide

Biochemical changes in the salivary glands of rats under the conditions of administration of bacterial lipopolysaccharide against the background of water avoidance stress modeling

The activity of iNOS in the salivary glands of rats under the conditions of modeling water avoidance stress decreased by 1.63 times compared to the control group of animals. The activity of iNOS in the salivary glands of rats under the conditions of LPS administration increased 2 times compared to the group of rats with stress syndrome. The activity of iNOS in salivary glands of rats under conditions of combined exposure to water avoidance stress and administration of LPS increased by 1.35 times compared to the group of rats with stress syndrome and decreased by 1.48 times compared to the group of rats that were administered LPS (Table 1).

The activity of cNOS in the salivary glands of rats under the conditions of modeling water avoidance stress decreased by 0.1 times compared to the control group of animals. cNOS activity in the salivary glands of rats under the conditions of LPS administration increased 1.48 times compared to the control group of animals and 1.54 times compared to the group of rats with stress syndrome. cNOS activity under conditions of combined exposure to WAS and LPS administration decreased by 1.09 times compared to the control group of animals, by 1.05 times compared to the group of rats with stress syndrome, and by 1.61 times compared to the group of rats that were administered LPS.

The activity of arginase in the salivary glands of rats under the conditions of modeling water avoidance stress increased 1.15 times compared to the control group of animals. The activity of arginase in the salivary glands of rats under the conditions of LPS administration decreased by 6.15 times compared to the control group of animals and by 7.07 times compared to the group of rats with stress syndrome. The activity of arginase in the salivary glands of rats under conditions of combined exposure to water avoidance stress and LPS administration decreased by 1.19 times compared to the control group and by 1.37 times compared to the group with stress syndrome and increased by 5.15 times compared to the group of rats, who were administered LPS.

Table 1. Biochemical changes in the salivary glands of rats under the conditions of administration of bacterial lipopolysaccharide against the background of water avoidance stress modeling (M±m)[a]

Parameters	Control group	WAS group	LPS group	WAS+LPS group
Inducible NO-synthase, μmol/min per g of protein	0.8±0.08	0.49±0.02*	0.98±0.07^	0.66±0.03^#
Constitutive NO-synthase, μmol/min per g of protein	0.0614 ±0.0002	0.0591 ±0.0006*	0.0908 ±0.0004*^	0.0564 ±0.0005*^#
Arginase activity, μmol/min per g of protein	1.66±0.014	1.91±0.03*	0.27±0.04*^	1.39 ±0.003*^#
S-NO, μmol/g	0.29 ±0.0008	0.095±0.003*	0.84±0.08*^	0.13 ±0.0042*^#
$ONOO^-$, μmol/g	2.17±0.04	3.8±0.11*	6.08±0.17*^	4.31±0.13*^#
NO_2^- concentration, nmol/g	7.9±0.11	6.28±0.17*	36.38±0.23*^	6.59±0.28*#
Superoxide anion radical production, nmol/s per g	0.64±0.004	0.98±0.004*	0.78±0.08^	1.2±0.004*^#
Catalase activity, μkat/g	0.55±0.004	0.46±0.004*	1.43±0.01*^	0.52±0.007*^#
Superoxide dismutase activity, c.u.	2.11±0.07	0.77±0.03*	2.77±0.35^	1.58±0.19*^#
Malondialdehyde concentration, μmol/g	6.26±0.04	13.73±0.1*	42.83±4.37*^	15.28±0.06*^#
OMP, c.u.	0.034 ±0.0015	0.091 ±0.0009*	0.0802 ±0.002*^	0.105 ±0.0012*^#
Sulfide anion concentration, μmol/g	8.17±0.103	21.16±0.42*	28.4±0.1*^	17.92±0.08*^#

[a] * – indicates that data is statistically significantly different form control group ($p<0.05$), ^ – indicates that data is statistically significantly different form WAS group ($p<0.05$), # – indicates that data is statistically significantly different form LPS group ($p<0.05$), WAS – water avoidance stress, LPS – lipopolysaccharide

The concentration of nitrosothiols in the salivary glands of rats under the conditions of modeling water avoidance stress decreased by 3.05 times compared to the control group of animals. The concentration of nitrosothiols in the salivary glands of rats under the conditions of LPS administration increased 2.9 times compared to the control group of animals and 8.84 times compared to the group of rats with stress syndrome. The concentration of nitrosothiols in the salivary glands of rats under conditions of combined exposure to water avoidance stress and administration of LPS decreased

by 2.23 times compared to the control group and by 6.46 times compared to the group of animals administered LPS and increased by 1.37 times compared to the group with stress syndrome.

The concentration of $ONOO^-$ in the salivary glands of rats under the conditions of modeling water avoidance stress increased 1.75 times compared to the control group of animals. The concentration of $ONOO^-$ in the salivary glands of rats under the conditions of LPS administration increased 2.8 times compared to the control group of animals and 1.6 times compared to the group of rats with stress syndrome. The concentration of $ONOO^-$ in the salivary glands of rats under conditions of combined exposure to water avoidance stress and administration of LPS increased by 1.99 times compared to the control group and by 1.13 times compared to the group with stress syndrome and decreased by 1.41 times compared to the group animals that were injected with LPS.

The concentration of NO_2 in the salivary glands of rats under the conditions of modeling water avoidance stress decreased by 1.26 times compared to the control group of animals. The concentration of NO_2 in the salivary glands of rats under the conditions of LPS administration increased 4.61 times compared to the control group of animals and 5.79 times compared to the group of rats with stress syndrome. The concentration of NO_2 in the salivary glands of rats under conditions of combined exposure to water avoidance stress and administration of LPS decreased by 1.2 times compared to the control group and by 5.52 times compared to the group of animals that were administered LPS.

Analyzing antiradical protection and production of reactive oxygen species (ROS) under the conditions of combined exposure to water avoidance stress and LPS administration, we found that SAR production in the salivary glands of rats under the conditions of water avoidance stress simulation increased 1.53 times compared to the control group of animals. SAR production in the salivary glands of rats under the conditions of LPS administration decreased by 1.26 times compared to the group of rats with stress syndrome. SAR production in the salivary glands of rats under conditions of combined exposure to water avoidance stress and LPS administration increased 1.88 times compared to the control group of rats, 1.22 times compared to the group with stress syndrome, and 1.54 times compared to the group of rats, who were administered LPS.

Catalase activity in the salivary glands of rats under the conditions of modeling water avoidance stress decreased by 1.2 times compared to the control group of animals. Catalase activity in the salivary glands of rats under the conditions of LPS administration increased 2.6 times compared to the control group of animals and 3.11 times compared to the group of rats with stress syndrome. Catalase activity in the salivary glands of rats under conditions of combined exposure to water avoidance stress and administration of LPS decreased by 1.06 times compared to the control group of rats and by 2.75 times compared to the group of animals administered LPS and increased by 1.13 times compared to group with stress syndrome.

The activity of SOD in the salivary glands of rats under the conditions of LPS simulation decreased by 2.74 times compared to the control group of animals. The activity of SOD in the salivary glands of rats under the conditions of LPS administration increased 3.6 times compared to the group of rats with stress syndrome. The activity of SOD in the salivary glands of rats under conditions of combined exposure to water avoidance stress and LPS administration decreased by 1.34 times compared to the control group of rats and by 1.75 times compared to the group of animals administered LPS and increased by 2.05 times compared to group with stress syndrome.

The concentration of MDA in the salivary glands of rats under the conditions of modeling water avoidance stress increased by 1.19 times compared to the control group of animals. The concentration of MDA in the salivary glands of rats under the conditions of LPS administration increased 6.84 times compared to the control group of animals and 3.12 times compared to the group of rats with stress syndrome. The concentration of MDA in the salivary glands of rats under conditions of combined exposure to water avoidance stress and LPS administration increased 2.44 times compared to the control group and 1.11 times compared to the group with stress syndrome and decreased 2.8 times compared to the group of animals, who were administered LPS.

The content of OMP in the salivary glands of rats under the conditions of modeling water avoidance stress increased 2.68 times compared to the control group of animals. The content of OMP in the salivary glands of rats under the conditions of LPS administration increased by 2.36 times compared to the control group of animals and decreased by 1.13 times compared to the group of rats with stress syndrome. The content of OMP in the salivary glands of rats under conditions of combined exposure to water avoidance stress and LPS administration increased 3.09 times compared to the control group of animals, 1.15 times compared to the group with stress syndrome and 1.31 times compared to the group of animals, who were administered LPS.

The concentration of sulfide anion in the salivary glands of rats under the conditions of modeling water avoidance stress increased by 2.59 times compared to the control group of animals. The concentration of sulfide anion in the salivary glands of rats under the conditions of LPS administration increased by 3.48 times compared to the control group of animals and by 1.34

times compared to the group of rats with stress syndrome. The concentration of sulfide anion in the salivary glands of rats under conditions of combined exposure to water avoidance stress and LPS administration increased 2.19 times compared to the control group and decreased 1.18 times compared to the group with stress syndrome and 1.58 times compared to a group of animals that were injected with LPS.

Biochemical changes in the soft periodontal tissues of rats under the conditions of administration of bacterial lipopolysaccharide against the background of water avoidance stress modeling

The activity of iNOS in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress increased by 1.44 times compared to the control group of animals. The activity of iNOS in the periodontal soft tissues of rats under the conditions of LPS administration increased by 3.88 times compared to the control group of animals and by 2.69 times compared to the group of rats with stress syndrome. The activity of iNOS in the periodontal soft tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 1.95 times compared to the control group of animals and by 1.35 times compared to the group of rats with stress syndrome and decreased by 1.99 times compared to the group of rats that were injected with LPS (Table 2).

The activity of cNOS in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress decreased by 1.04 times compared to the control group of animals. cNOS activity in the periodontal soft tissues of rats under the conditions of LPS administration increased by 3.03 times compared to the control group of animals and by 3.15 times compared to the group of rats with stress syndrome. cNOS activity in the periodontal soft tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 1.53 times compared to the control group of animals, by 1.59 times compared to the group of rats with stress syndrome, and decreased by 1.99 times compared to the group of rats that were injected with LPS.

The activity of arginase in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress decreased by 1.37 times compared to the control group of animals. The activity of arginase in the soft periodontal tissues of rats under the conditions of LPS administration decreased by 2.69 times compared to the control group of animals and by 1.97 times compared to the group of rats with stress syndrome. The activity of arginase in the soft periodontal tissues of rats under conditions of combined exposure to water avoidance stress and administration of LPS decreased by 1.39 times compared to the control group and increased by 1.94 times compared to the group of rats that were administered LPS.

The concentration of nitrosothiols in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress decreased by 6.03 times compared to the control group of animals. The concentration of nitrosothiols in the periodontal soft tissues of rats under the conditions of LPS administration decreased by 2.73 times compared to the control group of animals and increased by 2.21 times compared to the group of rats with stress syndrome. The concentration of nitrosothiols in the soft periodontal tissues of rats under conditions of combined exposure to water avoidance stress and administration of LPS decreased by 3.15 times compared to the control group and by 1.15 times compared to the group of animals injected with LPS and increased by 1.91 times compared to the group with stress syndrome.

Table 2. Biochemical changes in the soft periodontal tissues of rats under the conditions of administration of bacterial lipopolysaccharide against the background of water avoidance stress modeling (M±m)[a]

Parameters	Control group	WAS group	LPS group	WAS+LPS group
Inducible NO-synthase, μmol/min per g of protein	0.43±0.03	0.62±0.02*	1.67±0.14*^	0.84±0.03*^#
Constitutive NO-synthase, μmol/min per g of protein	0.0474 ±0.0002	0.0456 ±0.0002*	0.1438 ±0.0115*^	0.0724 ±0.0007*^#
Arginase activity, μmol/min per g of protein	1.75±0.003	1.28±0.02*	0.65±0.01*^	1.26±0.01*#
S-NO, μmol/g	0.41±0.004	0.068 ±0.005*	0.15±0.003*^	0.13 ±0.0005*^#
$ONOO^-$, μmol/g	1.67±0.03	2.44±0.01*	1.87±0.04*^	2.32 ±0.007*^#
NO_2^- concentration, nmol/g	3.14±0.06	4.26±0.11*	22.04±0.23*^	3.65±0.11*^#
Superoxide anion radical production, nmol/s per g	0.82±0.03	1.08±0.008*	0.5±0.01*^	1.36±0.01*^#
Catalase activity, μkat/g	0.38±0.001	0.3±0.001*	1.64±0.008*^	0.42 ±0.001*^#
Superoxide dismutase activity, c.u.	6.36±0.47	4.17±0.18*	9.86±1.29^	6±0.32^
Malondialdehyde concentration, μmol/g	9.4±0.1	12.32±0.13*	42.39±0.22*^	14.41±0.1*^#
OMP, c.u.	0.027 ±0.0004	0.077 ±0.0004*	0.048 ±0.0004*^	0.097 ±0.0006*^#
Sulfide anion concentration, μmol/g	1.81±0.06	4.19±0.05*	4.11±0.04*	2.57±0.04*^#

[a] * – indicates that data is statistically significantly different form control group ($p<0.05$), ^ – indicates that data is statistically significantly different form WAS group ($p<0.05$), # – indicates that data is statistically significantly different form LPS group ($p<0.05$), WAS – water avoidance stress, LPS – lipopolysaccharide

The concentration of $ONOO^-$ in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress increased by 1.46 times compared

to the control group of animals. The concentration of $ONOO^-$ in the periodontal soft tissues of rats under the conditions of LPS administration increased by 1.12 times compared to the control group of animals and decreased by 1.3 times compared to the group of rats with stress syndrome. The concentration of $ONOO^-$ in the soft periodontal tissues of rats under conditions of combined exposure to water avoidance stress and administration of LPS increased by 1.39 times compared to the control group and by 1.24 times compared to the group of animals injected with LPS and decreased by 1.05 times compared to the group with stress syndrome.

The concentration of NO_2 in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress decreased by 1.36 times compared to the control group of animals. The concentration of NO_2 in the periodontal soft tissues of rats under the conditions of LPS administration increased by 7.02 times compared to the control group of animals and by 5.17 times compared to the group of rats with stress syndrome. The concentration of NO_2 in the soft periodontal tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 1.16 times compared to the control group and decreased by 1.17 times compared to the group with stress syndrome and by 6.04 times compared to with a group of animals that were injected with LPS.

SAR production in the soft periodontal tissues of rats under water avoidance stress simulation conditions increased by 1.32 times compared to the control group of animals. SAR production in the periodontal soft tissues of rats under the conditions of LPS administration decreased by 1.64 times compared to the control group of animals and by 2.16 times compared to the group of rats with stress syndrome. SAR production in the periodontal soft tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 1.66 times compared to the control group of rats, by 1.26 times compared to the group with stress syndrome and by 2.72 times compared to with a group of rats that were injected with LPS.

Catalase activity in the soft periodontal tissues of rats under water avoidance stress simulation conditions decreased by 1.27 times compared to the control group of animals. Catalase activity in the periodontal soft tissues of rats under the conditions of LPS administration increased by 4.32 times compared to the control group of animals and by 5.47 times compared to the group of rats with stress syndrome. Catalase activity in the soft periodontal tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 1.11 times compared to the control group of rats and by 1.4 times compared to the group with stress syndrome and decreased by 3.9 times compared to the group of animals that were injected with LPS.

The activity of SOD in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress decreased by 1.53 times compared to the control group of animals. The activity of SOD in the soft periodontal tissues of rats under the conditions of LPS administration increased by 2.36 times compared to the group of rats with stress syndrome. The activity of SOD in the soft periodontal tissues of rats under conditions of combined exposure to water avoidance stress and administration of LPS decreased by 1.64 times compared to the group of animals with stress syndrome.

The concentration of MDA in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress increased by 1.31 times compared to the control group of animals. The concentration of MDA in the periodontal soft tissues of rats under the conditions of LPS administration increased by 4.51 times compared to the control group of animals and by 3.44 times compared to the group of rats with stress syndrome. The concentration of MDA in the periodontal soft tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 1.53 times compared to the control group and by 1.17 times compared to the group with stress syndrome and decreased by 2.94 times compared to with a group of animals that were injected with LPS.

The content of OMP in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress increased by 2.85 times compared to the control group of animals. The content of OMP in the periodontal soft tissues of rats under the conditions of LPS administration increased by 1.78 times compared to the control group of animals and decreased by 1.6 times compared to the group of rats with stress syndrome. The content of OMP in the periodontal soft tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 3.59 times compared to the control group of animals, by 1.26 times compared to the group with stress syndrome and by 2.02 times compared to with a group of animals that were injected with LPS.

The concentration of sulfide anion in the soft periodontal tissues of rats under the conditions of modeling water avoidance stress increased by 2.31 times compared to the control group of animals. The concentration of sulfide anion in the soft periodontal tissues of rats under the conditions of LPS administration increased by 2.27 times compared to the control group of animals. The concentration of sulfide anion in the periodontal soft tissues of rats under conditions of combined exposure to water avoidance stress and LPS administration increased by 1.42 times compared to the control group of rats and decreased by 1.63 times compared to the group with stress syndrome and by 1.6 times compared to the group of animals that were injected with LPS.

Discussion

Role of nitric oxide and competitive L-arginine metabolism in physiology and pathology of oral cavity

Nitric oxide has an antibacterial properties regarding periodontal pathogens.[26] These properties are connected to ability of nitric oxide to transform to nitrite (NO_2^-) and peroxynitrite ($ONOO^-$) depending on the state of oxygen involved in the reaction. Simple oxygen (O_2) reacts with NO resulting in NO_2^- formation, while superoxide anion radical, as an active form of oxygen ($O_2^{\cdot-}$), reacts with NO with $ONOO^-$ formation. Both these reactive forms of nitrogen can cause nitration of bacterial proteins.[27] In periodontium the main producer of NO is iNOS derived from tissue macrophages polarized by pro-inflammatory (M1) phenotype.[28] Arginases metabolize L-arginine to L-ornithine, which is in turn transformed by ornithine decarboxylase to putrescine and other polyamines. Polyamines are potent stimulants of cell division and are necessary for high speed regeneration of tissues of oral cavity. However, increased activity of arginase during inflammation may have adverse effects, because it signifies the shift in macrophage polarization by anti-inflammatory (M2) phenotype, which will impede host defense mechanisms related to nitric oxide production.[29] Therefore iNOS/Arginase ration can be used as a marker of predominance of macrophage polarization in specific tissues, because both enzymes are markers of different states of macrophages.[30] Increased expression of iNOS on the background of decreased expression of arginase evidences about predominant M1 polarization of tissue macrophages, an active stage of host immune response and increased exudation.[31] Vice versa, increased expression of arginase on the background of decreased expression of iNOS evidences about predominant M2 polarization of tissue macrophages, a regeneratory stage of inflammation and decreased immune response.[32]

Mechanisms of influence of water avoidance stress on biochemical parameters in salivary glands and soft periodontal tissues

Simulation of water avoidance stress leads to a decrease in nitric oxide production by iNOS in the salivary glands, which may be related to the inhibitory effect of the increased cortisol concentration observed in this group on the activation of the transcription factor NF-κB.[33] It is worth noting that our results differ from those of other scientists regarding the effect of water avoidance stress on iNOS activity.[34,35] The decrease in iNOS activity observed in our studies may be related to a longer (30 days) duration of water avoidance stress simulation, which will lead to the development of the "exhaustion" stage of the general adaptation syndrome, in contrast to the 10-day simulation, where mechanisms may prevail, characteristic of the "maximum adaptation" stage of the general adaptation syndrome. It is also worth noting that in the soft tissues of the periodontium, we observed an increase in the activity of iNOS. This may indicate a higher rate of exhaustion of adaptive capabilities in the salivary glands, when compared with the soft tissues of the periodontium. Another mechanism underlying the described changes in iNOS activity in salivary glands under conditions of water avoidance stress may be the characteristics of resident salivary gland macrophages, which have a greater tendency to polarize according to the anti-inflammatory (M2) phenotype, in contrast to resident soft tissue macrophages periodontal tissues.[36]

Changes in the activity of arginases and constitutive NO-synthase isoforms observed in salivary glands and periodontal soft tissues are probably associated with increased competition for the reaction substrate in the iNOS-arginase-cNOS triangle. Among these enzymes, iNOS has the greatest affinity for the substrate, therefore, an increase in iNOS activity leads to a decrease in the activity of arginases in the soft tissues of the periodontium, while in the salivary glands we observe the opposite changes. Constitutive isoforms of NO-synthase have the lowest affinity for the substrate, therefore, with an increase in the activity of either arginase or iNOS, they remain in conditions of substrate deficiency, which causes a decrease in their activity both in the salivary glands and in the soft tissues of the periodontium. This can lead to vasoconstriction of the vessels of the microcirculatory channel of the soft tissues of the periodontium and salivary glands and play a leading role in the development of endothelial dysfunction under these conditions.

In the salivary glands, against the background of a decrease in the concentration of nitric oxide deposited in nitrosothiols and the concentration of nitrites, an increase in the concentration of peroxynitrites is observed during water avoidance stress modeling. Therefore, the nitric oxide required for the formation of peroxynitrite probably does not originate from the L-arginine-dependent pathway of its formation. In the literature, there is no data on the powerful ability of the salivary glands to reduce nitric oxide from nitrates and nitrites that come with food.[37] In the soft tissues of the periodontium, on the contrary, there is a violation of the utilization of nitric oxide, which is produced in excess as a result of increased iNOS activity, which is evidenced by a decrease in the concentration of nitrosothiols and an increase in the concentration of nitrites and peroxynitrites.

Water avoidance stress is accompanied by increased superoxide anion radical production, depletion of antioxidant systems, and increased oxidative damage to lipids and proteins in salivary glands and soft periodontal tissues. The increased production of SAR may be a consequence of the dissociation of the endothelial isoform of NO-synthase from its substrate, which is confirmed by

the reduced activity of cNOS in this group both in the soft periodontal tissues and in the salivary glands.[38] Research shows that the stress hormone corticosterone directly modulates the function of mitochondria, leads to the development of oxidative stress and an increase in the level of homocysteine (toxic to mitochondria). Glucocorticoid receptor signaling also affects mitochondrial function during chronic stress. High levels of ROS (superoxide, hydrogen peroxide and hydroxyl radical) disrupt the functioning of mitochondria.[39] An increase in the concentration of sulfides both in the soft periodontal tissues and in the salivary glands under the conditions of simulation of water avoidance stress may be a reaction of the resident microflora of these tissues aimed at overcoming the consequences of oxidative stress.[40]

Mechanisms of influence of bacterial lipopolysaccharide on biochemical parameters in salivary glands and soft periodontal tissues

The introduction of bacterial LPS leads to an increase in the activity of iNOS against the background of a sharp decrease in the activity of arginases, which releases the substrate for cNOS and explains their increased activity in the salivary glands and soft tissues of the periodontium. This effect of bacterial LPS on L-arginine-dependent enzymes is explained by its ability to promote the polarization of macrophages according to the pro-inflammatory (M1) phenotype and activate the transcription factor NF-κB.[21,41,42] At the same time, an increase in both nitrosothiols, nitrites, and peroxynitrites is observed in the salivary glands under the conditions of increased production of nitric oxide from iNOS upon the introduction of bacterial LPS. In the soft tissues of the periodontium, only the concentration of peroxynitrites and nitrites is increased. An increased concentration of nitrites in the salivary glands and soft tissues of the periodontium may indicate a decrease in the activity of the nitrate-nitrite reductase pathway for the formation of nitric oxide. Although bacterial LPS can induce xanthine oxidase activity and increase nitrite reduction via the dehydrogenase domain of the xanthine oxidoreductase complex, the increased cortisol concentration in this group may negate this effect, since corticosteroids have an inhibitory effect on xanthine oxidase activity.[43,44]

The absence of an increase in the production of SAR in the salivary glands and a decrease in its production in the soft periodontal tissues of rats in the group of animals that were injected with LPS is associated with the accumulation of cortisol in this group of animals, the concentration of which even exceeds that in the WAS group. The accumulation of cortisol is associated with the inhibitory effect of LPS on the activity of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), an enzyme that converts cortisol into an inactive form.[45] For activity, 11β-HSD1 requires a cofactor in the form of NAD^+ as an electron acceptor. A decrease in the activity of this enzyme under the influence of LPS will lead to a decrease in the $NADH^+H^+/NAD^+$ ratio, which will reduce the production of SAR from the mitochondrial electron transport chain.[46]

Cortisol is also able to increase the activity of antioxidant enzymes, which explains the changes in the activity of SOD and catalase in salivary glands and soft periodontal tissues under the influence of LPS.[47] It is worth noting that this effect of LPS on SAR production, SOD and catalase activity may depend on the type of bacterial LPS, the organ, and duration of exposure.

The increase in the intensity of LPO and oxidative protein damage observed in the salivary glands and soft tissues of the periodontium in the group of animals injected with LPS indicates the possible initiation of LPO processes by other ROS (hydroxyl radical, hydrogen peroxide, carbonyl radical, etc.) and/or active forms of nitrogen (nitrite radical, peroxynitrite), against the background of insufficient compensation from the enzymatic link of the antioxidant system (SOD and catalase) and an increase in the concentration of sulfide anion (free radical acceptor).

Features of the combined effect of bacterial lipopolysaccharide and water avoidance stress on biochemical indicators in salivary glands and soft periodontal tissues

The combined effect of bacterial LPS and WAS on salivary glands and periodontal soft tissues has an antagonistic effect on iNOS activity, and increases iNOS activity relative to the control group due to LPS exposure. The combined effect of bacterial LPS and WAS also has an antagonistic effect on cNOS and arginase activity and leads to a decrease in cNOS activity and an increase in arginase activity, which corresponds to a WAS-dependent trend. WAS under the conditions of LPS administration to rats provides a decrease in the concentration of nitrosothiols and nitrites and prevents LPS-induced increase in their concentrations.

The combined effect of bacterial LPS and WAS on salivary glands and soft periodontal tissues increases the production of SAR compared to all studied groups, which indicates the predominance of the stimulating effect of WAS on its production. The effect on antioxidant enzymes depends to a greater extent on WAS than from the action of LPS. WAS limits LPO processes when combined with LPS action, but increases damage to protein structures in salivary glands and soft periodontal tissues.

The combined effect of LPS and WAS on the salivary glands and periodontal soft tissues leads to a decrease in the concentration of sulfide anion in comparison with the isolated WAS and LPS exposure groups, which may indicate an increased use of sulfide anion in this group to compensate for the increased production of ROS.

Our study revealed simultaneous changes in salivary glands and soft periodontal tissues during induction of

general adaptation syndrome by WAS modelling. Several studies revealed that during general adaptation syndrome there are functional changes in salivary glands and increased chewing, which is mediated by brain-derived neurotrophic factor (BDNF).[48,49] Elevated chewing may overload tissues of periodontium. At the same time psychological stress can induce changes in bacterial composition of oral microbiota.[50] BDNF during periodontitis is hypermethylated and downregulated, thus its increased concentration during general adaptation syndrome may be a pathogenetic link connecting development of oxidative stress in salivary glands and soft periodontal tissues observed in our study.[51]

Low grade systemic inflammation caused by intraperitoneal administration of bacterial LPS can also cause compensatory increase of BDNF in organs and systems most affected by inflammatory injury, like was shown by Shi J. et al. in their study on example of acute lung injury.[52] However, some scientists consider elevation of BDNF concentration in response to systemic inflammatory states to be an adaptive response aimed at removal of adverse effects caused by inflammation.[53] On the other hand, there are evidences that BDNF can exacerbate inflammatory processes and aggravate damage to organ and tissue.[54] There is information in scientific literature, that prolonged stress can also lead to depletion of BDNF secretion, which subsequently leads to depression and metabolic disorders such as diabetes mellitus type 2, etc.[55]

We did not evaluate the changes in BDNF concentrations in salivary glands and soft periodontal tissues in our study, which makes it a lucrative field for further investigations of mechanisms underlying the changes in production of nitric oxide, activity of antioxidant enzymes and lipid peroxidation observed in our study. Perspectives of further research are not limited to a single factor like BDNF concentration. The role of redox-sensitive transcriptional factors like NF-κB, STAT3, AP-1, Nrf2, p38-MAPK must also be considered and extensively studied, since, as mentioned above, they all or some of them may be either effectors or inductors of changes observed in our study.

Study limitations

The limitations of our study are: small number of animals in study groups, absence of functional tests to evaluate function of salivary glands, absence of assessment of clinical state of periodontium. Another limitation of our study is that we did not assessed the state of oral microbiota, which could have played a major role in observed changes.

Conclusion

Water avoidance stress leads to a decrease in the production of nitric oxide in the salivary glands and to an increase in the production of nitric oxide in the soft periodontal tissues of rats in an L-arginine-dependent manner, increases the formation of reactive forms of oxygen and nitrogen, increases the processes of lipid peroxidation and damage to protein structures.

Bacterial lipopolysaccharide leads to an increase in the production of nitric oxide in an L-arginine-dependent way both in the salivary glands and in the soft tissues of the periodontium, promotes the formation of both deposited and reactive metabolites of nitric oxide, intensifies the processes of lipid peroxidation and damage to protein structures on against the background of a compensatory increase in the activity of antioxidant enzymes.

The combined effect of bacterial lipopolysaccharide and water avoidance stress leads to a decrease in the production of nitric oxide in the salivary glands and to an increase in its production in the soft tissues of the periodontium of rats in an L-arginine-dependent way, increases the formation of reactive forms of oxygen and nitrogen, enhances the processes of lipid peroxidation and damage to protein structures against the background of exhaustion of the activity of antioxidant enzymes.

Declarations

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The authors declare no financial support.

Author contributions

Conceptualization, O.A. and A.M.; Methodology, A.M.; Software, O.A.; Validation, V.P., O.T., O.A. and A.M.; Formal Analysis, V.P., O.T., O.A. and A.M.; Investigation, V.P., O.T., O.A. and A.M.; Resources, A.M.; Data Curation, A.M. and O.A.; Writing – Original Draft Preparation, V.P. and O.T.; Writing – Review & Editing, O.A. and A.M.; Visualization, V.P.; Supervision, A.M.; Project Administration, O.A. and A.M.; Funding Acquisition, O.A. and A.M.

Conflicts of interest

Authors declare that there is no known conflict of interest regarding this paper.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

All experimental procedures were approved by Bioethical Committee of Poltava State Medical University (Record № 212 from 27.01.2023).

References

1. Lin TC, Tseng CF, Wang YH, Yu HC, Chang YC. Patients with chronic periodontitis present increased risk for primary Sjögren syndrome: a nationwide population-based

cohort study. *Peer J.* 2018;6:e5109. doi: 10.7717/peerj.5109
2. Shikama Y, Kudo Y, Ishimaru N, Funaki M. Potential Role of Free Fatty Acids in the Pathogenesis of Periodontitis and Primary Sjögren's Syndrome. *Int J Mol Sci*. 2017;18(4):836. doi: 10.3390/ijms18040836
3. Terrizzi AR, Conti MI, Martínez MP, Fernández-Solari J. The Process of Acclimation to Chronic Hypoxia Leads to Submandibular Gland and Periodontal Alterations: An Insight on the Role of Inflammatory Mediators. *Mediators Inflamm*. 2018;2018:6794508. doi: 10.1155/2018/6794508
4. Lin B, Zhao F, Liu Y, et al. Alterations in Oral Microbiota of Differentiated Thyroid Carcinoma Patients With Xerostomia After Radioiodine Therapy. *Front Endocrinol (Lausanne).* 2022;13:895970. doi: 10.3389/fendo.2022.895970
5. Pussinen PJ, Kopra E, Pietiäinen M, et al. Periodontitis and cardiometabolic disorders: The role of lipopolysaccharide and endotoxemia. *Periodontol 2000.* 2022;89(1):19-40. doi: 10.1111/prd.12433
6. Shinomiya T, Kawaguchi M, Okubo M, Kosuge Y, Yoshikawa M. mRNA expression and localization of LPS-induced β-defensin isoforms in rat salivary glands. *Bull Tokyo Dent Coll.* 2014;55(3):139-147. doi: 10.2209/tdcpublication.55.139
7. Seil M, El Ouaaliti M, Abdou Foumekoye S, Pochet S, Dehaye JP. Distinct regulation by lipopolysaccharides of the expression of interleukin-1β by murine macrophages and salivary glands. *Innate Immun.* 2012;18(1):14-24. doi: 10.1177/1753425910377101
8. Proctor GB, Shaalan AM. Disease-Induced Changes in Salivary Gland Function and the Composition of Saliva. *J Dent Res.* 2021;100(11):1201-1209. doi: 10.1177/00220345211004842
9. Han P, Bartold PM, Ivanovski S. The emerging role of small extracellular vesicles in saliva and gingival crevicular fluid as diagnostics for periodontitis. *J Periodontal Res.* 2022;57(1):219-231. doi: 10.1111/jre.12950
10. Chen M, Cai W, Zhao S, et al. Oxidative stress-related biomarkers in saliva and gingival crevicular fluid associated with chronic periodontitis: A systematic review and meta-analysis. *J Clin Periodontol.* 2019;46(6):608-622. doi: 10.1111/jcpe.13112
11. Tarasenko LM, Petrushanko TA. *Stress and periodontium.* Poltava; 1999.
12. Keremi B, Beck A, Fabian TK, et al. Stress and Salivary Glands. *Curr Pharm Des.* 2017;23(27):4057-4065. doi: 10.2174/1381612823666170215110648
13. Matsytska YK, Akimov OY, Mykytenko AO. Influence of corvitin and metformin on biochemical changes in lacrimal glands of rats during water avoidance stress modeling. *Oftalmologicheskii Zhurnal.* 2022;97(3):39-44. doi: 10.31288/oftalmolzh202233944
14. Spector AM, Postolache TT, Akram F, Scott AJ, Wadhawan A, Reynolds MA. Psychological Stress: A Predisposing and Exacerbating Factor in Periodontitis. *Curr Oral Health Rep.* 2020;7:208-215. doi: 10.1007/s40496-020-00282-2
15. Yamamoto K, Takao T, Nakayama J, et al. Water avoidance stress induces frequency through cyclooxygenase-2 expression: a bladder rat model. *Int J Urol.* 2012;19(2):155-162. doi: 10.1111/j.1442-2042.2011.02905.x
16. Mykytenko AO, Akimov OY, Neporada KS. Influence of lipopolysaccharide on the development of oxidative-nitrosative stress in the liver of rats under conditions of chronic alcohol intoxication. *Fiziol Zh.* 2022;68(2):29-35. doi: 10.15407/fz68.02.029
17. Yelinska AM, Kostenko VO. Combined effects of quercetin and modulators of redox sensitive factors on the indicators of systemic inflammatory response, carbohydrate and lipid metabolism in rats exposed to intraperitoneal and intragingival administration of salmonella typhi lipopolysaccharide. *Actual Problems of the Modern Medicine: Bulletin of Ukrainian Medical Stomatological Academy.* 2020;20(1):13-18. doi: 10.31718/2077-1096.20.1.13
18. Lane MB, Flatland B, Olin SJ, Fecteau KA, Rick M, Giori L. Analytic performance evaluation of a veterinary-specific ELISA for measurement of serum cortisol concentrations of dogs. *J Am Vet Med Assoc.* 2018;253(12):1580-1588. doi: 10.2460/javma.253.12.1580
19. Akimov OYe, Kostenko VO. Functioning of nitric oxide cycle in gastric mucosa of rats under excessive combined intake of sodium nitrate and fluoride. *Ukr Biochem J.* 2016;88(6):70-75. doi: 10.15407/ubj88.06.070
20. Yelins'ka AM, Akimov OYe, Kostenko VO. Role of AP-1 transcriptional factor in development of oxidative and nitrosative stress in periodontal tissues during systemic inflammatory response. *Ukr Biochem J.* 2019;91(1):80-85.
21. Mykytenko AO, Akimov OYe, Yeroshenko GA, Neporada KS. Influence of NF -κB on the development of oxidative-nitrosative stress in the liver of rats under conditions of chronic alcohol intoxication. *Ukr Biochem J.* 2022;94(6):57-66. doi: 10.15407/ubj94.06.057
22. Gérard-Monnier D, Erdelmeier I, Régnard K, Moze-Henry N, Yadan JC, Chaudière J. Reactions of 1-Methyl-2-phenylindole with Malondialdehyde and 4-Hydroxyalkenals. Analytical Applications to a Colorimetric Assay of Lipid Peroxidation. *Chem Res Toxicol.* 1998;11(10):1176-1183.
23. Gaston B, Reilly J, Drazen JM, et al. Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways. *Proc Natl Acad Sci USA.* 1993;90:10957-10961.
24. Mykytenko A, Akimov O, Shevchenko O, Neporada K. Role of sulfide anion in the development of chronic alcoholic hepatitis under the conditions of modulation of adenosine monophosphate kinase – a correlational study. *Eur J Clin Exp Med.* 2023;21(3):567-575. doi: 10.15584/ejcem.2023.3.24
25. Beregova TV, Neporada KS, Skrypnyk M, et al. Efficacy of nanoceria for periodontal tissues alteration in glutamate-induced obese rats-multidisciplinary considerations for personalized dentistry and prevention. *EPMA J.* 2017;8(1):43-49. doi: 10.1007/s13167-017-0085-7
26. Feura ES, Yang L, Schoenfisch MH. Antibacterial ac-

tivity of nitric oxide-releasing carboxymethylcellulose against periodontal pathogens. *J Biomed Mater Res A.* 2021;109(5):713-721. doi: 10.1002/jbm.a.37056

27. Borisov VB, Forte E. Bioenergetics and Reactive Nitrogen Species in Bacteria. *Int J Mol Sci.* 2022;23(13):7321. doi: 10.3390/ijms23137321
28. Galler KM, Weber M, Korkmaz Y, Widbiller M, Feuerer M. Inflammatory Response Mechanisms of the Dentine-Pulp Complex and the Periapical Tissues. *Int J Mol Sci.* 2021;22(3):1480. doi: 10.3390/ijms22031480
29. Yilmaz M, Demir E, Gürsoy M, Firatli E, Loimaranta V, Gürsoy UK. Salivary levels of BAFF, TWEAK, and soluble CD163 and salivary arginase activity before and after periodontal treatment. *J Periodontal Res.* 2023;58(3):646-654. doi: 10.1111/jre.13124
30. Silva MLF, Alves PM, Souza DM, et al. Analysis of Macrophage Activation Markers in an Experimental Model of Cutaneous Leishmaniasis Treated with Photodynamic Therapy Mediated by 5-Aminolevulinic Acid. *Photobiomodul Photomed Laser Surg.* 2019;37(5):298-304. doi: 10.1089/photob.2018.4574
31. Oliveira-Brito PKM, Rezende CP, Almeida F, Roque-Barreira MC, da Silva TA. iNOS/Arginase-1 expression in the pulmonary tissue over time during Cryptococcus gattii infection. *Innate Immun.* 2020;26(2):117-129. doi: 10.1177/1753425919869436
32. Gao JM, Yi SQ, Wu MS, et al. Investigation of infectivity of neonates and adults from different rat strains to Toxoplasma gondii Prugniaud shows both variation which correlates with iNOS and Arginase-1 activity and increased susceptibility of neonates to infection. *Exp Parasitol.* 2015;149:47-53. doi: 10.1016/j.exppara.2014.12.008
33. Dong J, Qu Y, Li J, et al. Cortisol inhibits NF-κB and MAPK pathways in LPS activated bovine endometrial epithelial cells. *Int Immunopharmacol.* 2018;56:71-77. doi: 10.1016/j.intimp.2018.01.021
34. Gurel SG, Sogut I, Hurdag C, Gurel A, Tutar A, Cikler-Dulger E. Effect of fulvic acid on gastric mucosa damage caused by chronic water avoidance stress. *Biotech Histochem.* 2022;97(3):199-206. doi: 10.1080/10520295.2021.1926541
35. Traini C, Idrizaj E, Garella R, et al. Otilonium Bromide treatment prevents nitrergic functional and morphological changes caused by chronic stress in the distal colon of a rat IBS model. *J Cell Mol Med.* 2021;25(14):6988-7000. doi: 10.1111/jcmm.16710
36. McKendrick JG, Emmerson E. The role of salivary gland macrophages in infection, disease and repair. *Int Rev Cell Mol Biol.* 2022;368:1-34. doi: 10.1016/bs.ircmb.2022.02.001
37. Oliveira-Paula GH, Pinheiro LC, Tanus-Santos JE. Mechanisms impairing blood pressure responses to nitrite and nitrate. *Nitric Oxide.* 2019;85:35-43. doi: 10.1016/j.niox.2019.01.015
38. Wu Y, Ding Y, Ramprasath T, Zou MH. Oxidative Stress, GTPCH1, and Endothelial Nitric Oxide Synthase Uncoupling in Hypertension. *Antioxid Redox Signal.* 2021;34(9):750-764. doi: 10.1089/ars.2020.8112
39. Du J, Zhu M, Bao H, et al. The Role of Nutrients in Protecting Mitochondrial Function and Neurotransmitter Signaling: Implications for the Treatment of Depression, PTSD, and Suicidal Behaviors. *Crit Rev Food Sci Nutr.* 2016;56(15):2560-2578. doi: 10.1080/10408398.2013.876960
40. Uchiyama J, Akiyama M, Hase K, Kumagai Y, Kim YG. Gut microbiota reinforce host antioxidant capacity via the generation of reactive sulfur species. *Cell Rep.* 2022;38(10):110479. doi: 10.1016/j.celrep.2022.110479
41. Orecchioni M, Ghosheh Y, Pramod AB, Ley K. Macrophage Polarization: Different Gene Signatures in M1(LPS+) vs. Classically and M2(LPS-) vs. Alternatively Activated Macrophages. *Front Immunol.* 2019;10:1084. doi: 10.3389/fimmu.2019.01084
42. Liu L, Guo H, Song A, et al. Progranulin inhibits LPS-induced macrophage M1 polarization via NF-κB and MAPK pathways. *BMC Immunol.* 2020;21(1):32. doi: 10.1186/s12865-020-00355-y
43. Ives A, Nomura J, Martinon F, et al. Xanthine oxidoreductase regulates macrophage IL1β secretion upon NLRP3 inflammasome activation. *Nat Commun.* 2015;6:6555. doi: 10.1038/ncomms7555
44. Wu L, Zhou C, Wu J, Chen S, Tian Z, Du Q. Corticosterone Inhibits LPS-Induced NLRP3 Inflammasome Priming in Macrophages by Suppressing Xanthine Oxidase. *Mediators Inflamm.* 2020;2020:6959741. doi: 10.1155/2020/6959741
45. Kim EN, Jeong GS. Inhibitory Effect of Periodontitis through C/EBP and 11β-Hydroxysteroid Dehydrogenase Type 1 Regulation of Betulin Isolated from the Bark of Betula platyphylla. *Pharmaceutics.* 2022;14(9):1868. doi: 10.3390/pharmaceutics14091868
46. Plecitá-Hlavatá L, Engstová H, Holendová B, et al. Mitochondrial Superoxide Production Decreases on Glucose-Stimulated Insulin Secretion in Pancreatic β Cells Due to Decreasing Mitochondrial Matrix NADH/NAD+ Ratio. *Antioxid Redox Signal.* 2020;33(12):789-815. doi: 10.1089/ars.2019.7800
47. Wang Y, Liu Z, Liu C, Liu R, Yang C, Wang L, Song L. Cortisol modulates glucose metabolism and oxidative response after acute high temperature stress in Pacific oyster Crassostrea gigas. *Fish Shellfish Immunol.* 2022;126:141-149. doi: 10.1016/j.fsi.2022.05.010
48. Saruta J, To M, Sakaguchi W, Kondo Y, Tsukinoki K. Brain-derived neurotrophic factor is related to stress and chewing in saliva and salivary glands. *Jpn Dent Sci Rev.* 2020;56(1):43-49. doi: 10.1016/j.jdsr.2019.11.001
49. Almohaimeed HM, Albadawi EA, Mohammedsaleh ZM, et al. Brain-derived Neurotropic factor (BDNF) mediates the protective effect of Cucurbita pepo L. on salivary glands of rats exposed to chronic stress evident by structural, biochemical and molecular study. *J Appl Oral Sci.* 2021;29:e20201080. doi: 10.1590/1678-7757-2020-1080
50. Paudel D, Kuramitsu Y, Uehara O, et al. Proteomic and

microbiota analyses of the oral cavity during psychological stress. *PLoS One.* 2022;17(5):e0268155. doi: 10.1371/journal.pone.0268155

51. Chen H, Feng X, Yang Q, Yang K, Man S. Expression Pattern and Value of Brain-Derived Neurotrophic Factor in Periodontitis. *Int Dent J.* 2023;73(4):542-549. doi: 10.1016/j.identj.2023.03.002
52. Shi J, Song S, Wu K, Liang G, Wang A, Xu X. Role of brain-derived neurotrophic factor in endotoxaemia-induced acute lung injury. *Exp Physiol.* 2023;108(12):1456-1465. doi: 10.1113/EP091228
53. Huang Q, Wu M, Wu X, Zhang Y, Xia Y. Muscle-to-tumor crosstalk: The effect of exercise-induced myokine on cancer progression. *Biochim Biophys Acta Rev Cancer.* 2022;1877(5):188761. doi: 10.1016/j.bbcan.2022.188761
54. Montone RA, Camilli M, Del Buono MG, et al. Brain-derived neurotrophic factor in patients with acute coronary syndrome. *Transl Res.* 2021;231:39-54. doi: 10.1016/j.trsl.2020.11.006
55. Claro AE, Palanza C, Mazza M, et al. Why do we not reverse the path? Stress can cause depression, reduction of brain-derived neurotrophic factor and increased inflammation. *World J Psychiatry.* 2022;12(9):1264-1267. doi: 10.5498/wjp.v12.i9.1264

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REVIEW PAPER

Genetic factors contributing to the development of inguinal hernias – a narrative review

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ABSTRACT

Introduction and aim. Inguinal hernias are one of the major disorders in the field of general and visceral surgery and can be viewed as multifactorial diseases. Although the molecular mechanism that led to predistortion to inguinal herniation still remain unclear, is well known that defects leading to improper closure of the inguinal canal during fetal development and mechanisms contributing to weaker muscles of the abdominal wall can greatly increase the risk of developing the latter disease.

Material and methods. A literature search was performed in all major electronic databases using keywords and Boolean operators to retrieve all available literature related to the topic. Due to the narrative nature of the review, there were no specific inclusion and exclusion criteria.

Analysis of the literature. Genetic factors, undoubtedly, can interfere with these mechanisms and therefore play major role in developing hernias. To this end, the present narrative review provides an overview of genes with altered expression and genetic polymorphisms associated with inguinal herniation. Moreover, the results of genome-wide association studies (GWAS) exploring susceptible genetic loci associated with the disease have been reported.

Conclusion. Nevertheless, more case-control studies and GWAS need to be conducted in different ethnic populations so as to provide better insights into the topic.

Keywords. genes, genetics, genome-wide association, inguinal hernias, polymorphisms, studies

Introduction

The inguinal canal is an oblique tubular passage that runs in the lower abdominal wall, exactly above the groin region, containing the spermatic chord in males and the round ligament of the uterus in females. An inguinal hernia is formed when abdominal viscera protrude through the inguinal canal, and is usually presented as bulges in the groin. Inguinal hernias are indeed one of the most prevalent clinical conditions, with a prevalence of approximately 9.61% in men and 1.31% in women globally.[1] They are classified into direct hernias, which are those that are characterized by protrusion through the wall of the inguinal canal, and indirect hernias, which are formed by protrusion through the inguinal ring.[2,3] Indirect inguinal hernias are more common in children and usually occur due to birth defects of the inguinal canal opening that allow the protrusion of abdominal viscera through the canal.[4,5] Direct inguinal hernias, on the other hand, are more common in middle-aged and older patients and are often caused by the weaking of the abdominal musculature.[4]

Today, the only existing method of treating inguinal hernias, is through open or laparoscopic surgery.[6]

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However, there is still a chance that the hernia reoccurs even after a successful surgical operation, making the disease a heavier burden for the patient.[7] Different factors have been identified that increase the risk of developing direct and indirect inguinal hernias, as well as the risk of post-surgery recurrence.[8,9] Recently, studies have also shown that different genetic factors may also increase the risk of developing both direct and indirect inguinal hernias, suggesting that they can be studied as multifactorial diseases.[4] Indeed, several studies have been able to identify strong inheritance patterns of predisposition to inguinal hernias amongst different families.[10] This would allow clinicians and researchers to gain better insights into the prevention of inguinal hernias and provide patients with more information on the risk of post-surgery recurrence through means of molecular testing.

Aim

The present narrative review, aims to explore the role of genetics in the development of inguinal hernias has been analyzed in detail, with the hope of providing an overview of the current available evidence.

Material and methods

A systematic search was performed in the electronic databases of PubMed, Scopus, EMBASE and Google Scholar to retrieve all available literature on the role of genetic factors in inguinal herniation. A combination of the keywords "Genetics", "Genes", "GWAS", "Inguinal", "Hernias" and "Herniation" were used in combination with the operators "AND" and "OR". Due to the narrative nature of the review, there were no specific inclusion and exclusion criteria. Overall, all articles, including original research and reviews, written in the English language and with relevant information were included in the synthesis of the review.

Analysis of the literature

Molecular mechanisms of inguinal hernia development

There are various molecular mechanisms that can increase the predisposition of developing inguinal hernias. A broader understanding of these mechanisms is required in order to comprehend the genetic backgrounds of inguinal herniation.

The role of the extracellular matrix in inguinal hernias

The extracellular matrix (ECM), is one of the important factors contributing to the pathogenesis process of inguinal hernias.[4] Indeed, the composition of the ECM can determine the strength of the lower abdominal wall muscles and therefore contribute to direct herniation.[11] Moreover, research has shown that the ECM plays a crucial role in fetal morphogenesis and thus, defects in the ECM can cause inadequate closure of the inguinal ring, contributing to the formation of indirect hernias, especially in children.[12,13] Two of the main components of the extracellular matrix are collagen and elastin fibers, which have been related to many genetic disorders and predisposition to various diseases.[14] Indeed, regarding inguinal hernias, researchers have discovered that the total quantity of collagen, especially that of type I collagen is significantly less in the fascia transversalis and peritoneal samples of most patients with direct or indirect inguinal hernias.[15] On the other hand, the quantity of collagen type III has been found to be increased in the fascia transversalis of patients with inguinal hernias.[16] It is known that collagen type III is characterized by less mechanical resistance and is associated with fragility and decreased collagen alignment in the ECM.[17] Thereby, a substitution of type I collagen by type III collagen may result in higher predisposition to inguinal hernias. In fact, studies have found that the collagen type I/III ratio is higher in the abdominal wall and peritoneum of patients with inguinal hernias, but no statistically significant difference has been found between patients with direct and indirect hernias.[18] Simultaneously, elastin fiber levels have been found to be decreased in patients with both direct and indirect inguinal hernias and contribute to weaker muscles of the abdominal wall.[18,19] It is also worth mentioning that other proteins and glycoproteins such as fibulins, fibronectin and tenascins are of high significance in the structure and mechanical resistance of the ECM.[20] Indeed, it has been discovered that in patients with direct and indirect inguinal hernias, the expression of fibulin-3 is downregulated.[21] Furthermore, patients with Ehlers-Danlos Syndrome who present tenascin-X deficiency are at a higher risk of developing inguinal hernias.[22] However, regarding fibronectins, no correlation has been yet found with inguinal herniation in human samples.[23]

The role of growth and differentiation factors in inguinal hernias

The formation of the inguinal canal begins around 8 to 10 weeks after gestation and is the route for testicular decent in male fetuses.[24] The formation and closure of the canal, nonetheless, continue until the final stages of gestation up to the third trimester and any defects in the process may result in higher predisposition to indirect inguinal herniation.[24,25] Studies have found that factors contributing to smooth muscle cell differentiation in the fetus may result in defects of the inguinal canal in children.[26] Moreover, it has been suggested that the production of androgens and epithelial transformation factors contribute to the formation of the inguinal canal, and therefore defects in these mechanisms may result in higher risks of developing indirect inguinal hernias.[27]

Figure 1 presents a summary of the molecular mechanisms contributing to predisposition to inguinal herniation.

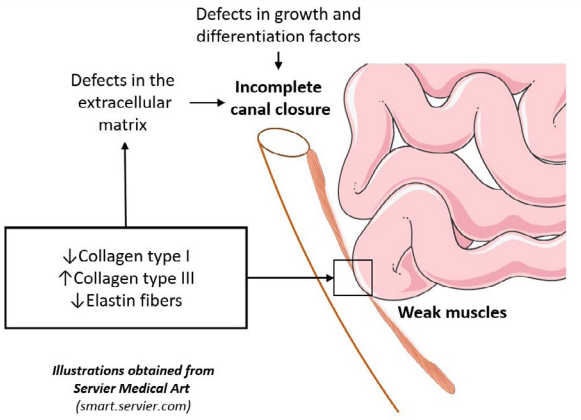

Fig. 1. Molecular mechanisms contributing to predisposition to inguinal herniation

Genes and genetic polymorphisms related to inguinal hernias

In the recent years, many genes have been found to be related to inguinal hernias and some of their polymorphisms have been statistically associated with higher risk of herniation.[4] Hence, these genes have been overviewed in this review article.

Collagen genes

The collagen genes, especially those coding for type I collagen have been found to be associated with both direct and indirect inguinal hernias. Indeed, Sezer et.al found that the +1245G/T polymorphisms in the collagen type I alpha 1 (COL1A1) gene are associated with inguinal herniation, finding approximately fourfold odds of the polymorphisms in patients with direct and indirect inguinal hernias, compared to controls.[28] This polymorphism has also been associated with other conditions such as osteoporosis and predisposition to cruciate ligament injuries and is not specific to the abdominal wall.[29,30] Moreover, a cohort study in 2022 concluded that the hazard ratio of inguinal herniation was approximately twofold in a population with collagenopathies compared to initially healthy individuals.[31]

The elastin gene

The elastin gene (ELN) codes for the protein tropoelastin is another very significant component of the ECM.[32] A case-control study by Rodrigues et.al identified that the point missense mutation g28197A>G in the ELN gene leading to an amino acid substitution in the hydrophobic domain of tropoelastin, is significantly associated with direct inguinal herniation.[33] It is also worth mentioning that the 2012DeltaG and 2039DeltaC frameshift mutations in the ELN gene have been found to be associated with congenital cutis laxa, a disease reported to increase predisposition to inguinal herniation.[34,35]

Matrix metalloproteinase genes

The matrix metalloproteinase (MMP) genes as well as their tissue inhibitors genes (TIMPs) are known to be linked to the composition of the ECM and collagen expression within the matrix.[36,37] In the case of inguinal hernias, tissue samples obtained from the abdominal wall have shown upregulation of MMP-1, MMP-2, MMP-9 and MMP-13 and simultaneously, downregulation of the inhibitors TIMP-1, TIMP-2 and TIMP-3.[38-40] Nevertheless, a search conducted from inception until May 2023 in the PubMed and SCOPUS databases retrieved no study relating polymorphisms of these genes with inguinal herniation, suggesting that such studies have not been conducted yet.

The Wilms tumor protein gene

The Wilms tumor protein gene (WT1) codes for a transcription factor, responsible for the development of the urogenital system and has been associated with the development of certain malignancies including nephroblastoma and hematological cancers.[41] Interestingly, the rs3809060 polymorphism of the gene has been found to be associated with inguinal hernias, where the GT and TT genotypes increase the risk of herniation in adult males.[42]

The EGF-containing fibulin-like extracellular matrix protein-1 gene

The EGF-containing fibulin-like extracellular matrix protein-1 gene (EFEMP1) is another very significant protein regulating the composition of the extracellular matrix.[43] Peng et.al, discovered that the expression of EFEMP1 is downregulated in the fascia transversalis of patients with direct inguinal hernias compared to apparently healthy controls.[21] Furthermore, it has been discovered that the EFEMP1 rs2009262 polymorphism is associated with inguinal hernias adults, where the TC and CC genotypes in females increase the risk of herniation in adult females.[42]

The T-box transcription factor genes

The T-box transcription factors are a family of proteins vital for embryonic development, including the development of the abdominal cavity and the genitourinary system.[44] The T-box transcription factor 2 (TBX2) and T-box transcription factor 3 (TBX3) genes have been found to be related to predisposition to indirect inguinal hernias.[45,46] In fact, the g.59476307G>C DNA sequence variant (DSV) within the TBX2 promoter gene has been found to be connected with indirect herniation.[45] Similarly, the deletion variant g.4820_4821del within the TBX3 gene promoter, has been found to significantly decrease the promoter's activity and as a result lead to

herniation predisposition.[46] These polymorphisms have been found to be correlated to the development of other embryological development disorders such as ventricular septal defects and thus, are not specific only to inguinal herniation risk.[47,48]

The lysyl oxidase like-1 gene

The lysyl oxidase like-1 gene encodes for an enzyme necessary for the biosynthesis of elastin and the cross-linking of collagen molecules.[49] A study by Pascual et.al, discovered that the expression of the enzyme is significantly downregulated in the fascia transversalis of patients with direct inguinal hernias.[50] The downregulation of LOX1, leads to the formation of a mechanically weaker ECM with less elastin fibers and therefore contributes to the process of herniation.[51]

Sirtuin genes

The sirtuin (SIRT) gene family, encoding for a total of seven significant proteins, has been found to contribute to muscle formation and differentiation and act as transcription factors.[52] In the case of inguinal hernias, it has been discovered that expression of the SIRT1 gene is correlated with incomplete closure of the inguinal canal and thus, indirect inguinal herniation. In fact, two DSVs namely g.69644213G>A and g.69644268T>A, and one single nucleotide polymorphism (SNP), g.69643707A>C of the SIRT1 gene, have been found to increase the risk of developing indirect inguinal hernias in a case-control study.[53]

Table 1 summarizes all genetic variations relating to inguinal hernia predisposition.

Table 1. Genetic variations increasing the risk for inguinal herniation

Gene	Variation	Type of variation	Subtype of inguinal hernias associated	References
COL1A1	+1245G/T	Insertion polymorphism	Direct and indirect inguinal hernias	[28]
ELN	g28197A>G	SNP	Direct and indirect inguinal hernias	[33]
WT1	rs3809060	SNP	Direct and indirect inguinal hernias (Males only)	[42]
EFEMP1	rs2009262	SNP	Direct and indirect inguinal hernias (Females only)	[42]
TBX2	g.59476307G>C	DSV	Indirect inguinal hernias	[45]
TBX3	g.4820_4821del	DSV	Indirect inguinal hernias	[46]
SIRT1	g.69644213G>A	DSV	Indirect inguinal hernias	[53]
	g.69644268T>A	DSV	Indirect inguinal hernias	
	g.69643707A>C	SNP	Indirect inguinal hernias	

Genome-wide association studies on inguinal hernias

Several genome-wide association studies (GWAS) have been conducted in order to identify significant genes and susceptible genetic loci related to inguinal hernias. Until today, five GWAS have been conducted in the UK, Japan and the USA, some in multiethnic populations, and they have reported significant results.[54-58] Table 2 summarizes the characteristics of these GWAS.

Table 2. Genome-wide association studies on inguinal hernias

Study	Year	Country	Patients	Controls	Number of loci identified
Jorgenson et al.[54]	2015	USA	5295	67,510	4
Hikino et al.[55]	2021	Japan	1983	172,507	23
Ahmed et al.[56]	2022	UK	18,791	93,955	24
Choquet et al.[57]	2022	USA (multiethnic)	33,491	694,927	63
Fadista et al.[58]	2022	UK	28,707	343,103	69

Overall, numerous susceptibility loci have been identified, out of which some include the genes which had been screened in previous smaller case-control studies, such as ELN, WT1, EFEMP1 and LOX1.[54,55,57,58] Sex-specific genes in males have also been reported to be included as susceptibility loci in inguinal hernias.[57] It is worth mentioning that two studies screened genetic loci for different types of hernias and some susceptibility loci overlapped in different types of herniation.[56,58]

Discussion

Although the molecular mechanisms contributing to inguinal herniation are not yet fully comprehended, it is clear that genetic factors do indeed contribute to the formation of both direct and indirect inguinal hernias[4]. Moreover, studies have shown that certain genetic polymorphisms associated with inguinal herniation such as the WT1 polymorphism are sex-specific rs3809060 and this could explain the higher prevalence of inguinal herniation amongst males.[42] With so many genetic polymorphisms and susceptibility loci found to be associated with inguinal herniation, the condition can henceforth be viewed as a multifactorial disease. This would mean that surgeons and pathologists could also possibly include means of molecular testing in cases of inguinal hernias to provide themselves and patients with better clinical images.

Nevertheless, there are still some genes such as MMPs and TIMPs which have been shown to present altered expression levels in patients with inguinal hernias, but the relationship between their polymorphisms and inguinal herniation is still unknown.[38,39]

Conclusion

Therefore, more studies need to be conducted in this direction so as to discover whether polymorphisms of the genes are associated with the disease or even discover epigenetic mechanisms which alter their expression. Moreover, the majority of the genome-wide association studies for inguinal hernias, except one multiethnic

study in the USA, have been conducted in Caucasian and Asian population. Hence, more studies are required in other ethnic populations so as to decrease the risk of reporting biased results. It is also worth mentioning that most studies are conducted mainly on male populations and thus it is difficult to generalize the results amongst the population, indicating the need for conducting further studies involving both males and females in a normalized distribution.

Declarations

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Author contributions

Conceptualization, D.K.; Methodology, D.K.; Software, D.K.; Validation, D.K.; Formal Analysis, D.K.; Investigation, D.K.; Resources, D.K.; Data Curation, D.K.; Writing – Original Draft Preparation, D.K.; Writing – Review & Editing, D.K.; Visualization, D.K.; Supervision, D.K.; Project Administration, D.K.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data that support the findings of this study are available on request from the corresponding author.

References

1. Abebe MS, Tareke AA, Alem A, Debebe W, Beyene A. Worldwide magnitude of inguinal hernia: Systematic review and meta-analysis of population-based studies. *SAGE Open Med*. 2022;10:20503121221139150. doi: 10.1177/20503121221139150
2. Holzheimer RG. Inguinal Hernia: classification, diagnosis and treatment--classic, traumatic and Sportsman's hernia. *Eur J Med Res*. 2005;10(3):121-134.
3. Shakil A, Aparicio K, Barta E, Munez K. Inguinal Hernias: Diagnosis and Management. *Am Fam Physician*. 2020;102(8):487-492.
4. Öberg S, Andresen K, Rosenberg J. Etiology of Inguinal Hernias: A Comprehensive Review. *Front Surg*. 2017;4:52. doi: 10.3389/fsurg.2017.00052
5. Yeap E, Pacilli M, Nataraja RM. Inguinal hernias in children. *Aust J Gen Pract*. 2020;49(1-2):38-43. doi: 10.31128/ajgp-08-19-5037
6. O'Brien J, Sinha S, Turner R. Inguinal hernia repair: a global perspective. *ANZ J Surg*. 2021;91(11):2288-2295. doi: 10.1111/ans.17174
7. Campanelli G, Pettinari D, Nicolosi FM, Cavalli M, Avesani EC. Inguinal hernia recurrence: classification and approach. *Hernia*. 2006;10(2):159-161. doi: 10.1007/s10029-005-0053-3
8. Carbonell JF, Sanchez JL, Peris RT, et al. Risk factors associated with inguinal hernias: a case control study. *Eur J Surg*. 1993;159(9):481-486.
9. Burcharth J, Pommergaard HC, Bisgaard T, Rosenberg J. Patient-related risk factors for recurrence after inguinal hernia repair: a systematic review and meta-analysis of observational studies. *Surg Innov*. 2015;22(3):303-317. doi: 10.1177/1553350614552731
10. Öberg S, Sæter AH, Rosenberg J. The inheritance of groin hernias: an updated systematic review with meta-analyses. *Hernia*. 2022;27(6):1339-1350. doi: 10.1007/s10029-022-02718-3
11. Csapo R, Gumpenberger M, Wessner B. Skeletal Muscle Extracellular Matrix – What Do We Know About Its Composition, Regulation, and Physiological Roles? A Narrative Review. Review. *Front Physiol*. 2020;11:253. doi: 10.3389/fphys.2020.00253
12. Rozario T, DeSimone DW. The extracellular matrix in development and morphogenesis: a dynamic view. *Dev Biol*. 2010;341(1):126-140. doi: 10.1016/j.ydbio.2009.10.026
13. Walma DAC, Yamada KM. The extracellular matrix in development. *Development*. 2020;147(10): 175596. doi: 10.1242/dev.175596
14. Naba A, Clauser KR, Ding H, Whittaker CA, Carr SA, Hynes RO. The extracellular matrix: Tools and insights for the "omics" era. *Matrix Biology*. 2016;49:10-24. doi: 10.1016/j.matbio.2015.06.003
15. Harrison B, Sanniec K, Janis JE. Collagenopathies—Implications for Abdominal Wall Reconstruction: A Systematic Review. *Plastic and Reconstructive Surgery – Global Open*. 2016;4(10):1036. doi: 10.1097/gox.0000000000001036
16. Kral JG, Levine RG. Increases in type III collagen gene expression and protein synthesis in patients with inguinal hernias. *Ann Surg*. 1995;221(1):116-117.
17. Asgari M, Latifi N, Heris HK, Vali H, Mongeau L. In vitro fibrillogenesis of tropocollagen type III in collagen type I affects its relative fibrillar topology and mechanics. *Sci Rep*. 2017;7(1):1392. doi: 10.1038/s41598-017-01476-y
18. Meyer AL, Berger E, Monteiro O, Jr., Alonso PA, Stavale JN, Gonçalves MP. Quantitative and qualitative analysis of collagen types in the fascia transversalis of inguinal hernia patients. *Arq Gastroenterol*. 2007;44(3):230-234. doi: 10.1590/s0004-28032007000300010
19. Rodrigues Junior AJ, Rodrigues CJ, da Cunha AC, Jin Y. Quantitative analysis of collagen and elastic fibers in the transversalis fascia in direct and indirect inguinal hernia. *Rev Hosp Clin Fac Med Sao Paulo*. 2002;57(6):265-270. doi: 10.1590/s0041-87812002000600004
20. Holle AW, Young JL, Van Vliet KJ, et al. Cell–Extracellular Matrix Mechanobiology: Forceful Tools and Emerging Needs for Basic and Translational Research. *Nano Letters*. 2018;18(1):1-8. doi: 10.1021/acs.nanolett.7b04982
21. Peng X, Guo Z, Zhang Y, Sun B, Zhang Q. EFEMP1 in Direct Inguinal Hernia: correlation with TIMP3 and Regulation Toward Elastin Homoeostasis as Well as Fi-

broblast Mobility. *J Invest Surg*. 2022;35(1):203-211. doi: 10.1080/08941939.2020.1811812
22. Rymen D, Ritelli M, Zoppi N, et al. Clinical and Molecular Characterization of Classical-Like Ehlers-Danlos Syndrome Due to a Novel TNXB Variant. *Genes (Basel)*. 2019;10(11):843. doi: 10.3390/genes10110843
23. Klinge U, Zheng H, Si ZY, Schumpelick V, Bhardwaj R, Klosterhalfen B. Synthesis of type I and III collagen, expression of fibronectin and matrix metalloproteinases-1 and -13 in hernial sac of patients with inguinal hernia. *Int J Surg Investig*. 1999;1(3):219-227.
24. Taghavi K, Geneta vP, Mirjalili SA. The pediatric inguinal canal: Systematic review of the embryology and surface anatomy. *Clinical Anatomy*. 2016;29(2):204-210. doi: 10.1002/ca.22633
25. Skandalakis JE, Colborn GL, Androulakis JA, Skandalakis LJ, Pemberton LB. Embryologic And Anatomic Basis Of Inguinal Herniorrhaphy. *Surgical Clinics of North America*. 1993;73(4):799-836. doi: 10.1016/S0039-6109(16)46086-X
26. Mouravas VK, Koletsa T, Sfougaris DK, et al. Smooth muscle cell differentiation in the processus vaginalis of children with hernia or hydrocele. *Hernia*. 2010;14(2):187-191. doi: 10.1007/s10029-009-0588-9
27. Hutson JM, Albano FR, Paxton G, et al. In vitro fusion of human inguinal hernia with associated epithelial transformation. *Cells Tissues Organs*. 2000;166(3):249-258. doi: 10.1159/000016738
28. Sezer S, Şimşek N, Celik HT, et al. Association of collagen type I alpha 1 gene polymorphism with inguinal hernia. *Hernia*. 2014;18(4):507-512. doi: 10.1007/s10029-013-1147-y
29. Stępien-Słodkowska M, Ficek K, Eider J, et al. The +1245g/t polymorphisms in the collagen type I alpha 1 (col1a1) gene in polish skiers with anterior cruciate ligament injury. *Biol Sport*. 2013;30(1):57-60. doi: 10.5604/20831862.1029823
30. Wu J, Yu M, Zhou Y. Association of collagen type I alpha 1 +1245G/T polymorphism and osteoporosis risk in post-menopausal women: a meta-analysis. *International Journal of Rheumatic Diseases*. 2017;20(7):903-910. doi: 10.1111/1756-185X.13052
31. Chang HH, Juan YS, Li CC, Lee HY, Chen JH. Congenital collagenopathies increased the risk of inguinal hernia developing and repair: analysis from a nationwide population-based cohort study. *Sci Rep*. 2022;12(1):2360. doi: 10.1038/s41598-022-06367-5
32. Duque Lasio ML, Kozel BA. Elastin-driven genetic diseases. *Matrix Biol*. 2018;71-72:144-160. doi: 10.1016/j.matbio.2018.02.021
33. Rodrigues C, Yoo J, Junior A. Elastin (ELN) gene point mutation in patients with inguinal hernia. *Genet Mol Biol*. 2006;29:45-46. doi: 10.1590/S1415-47572006000100009
34. Zhang MC, He L, Giro M, Yong SL, Tiller GE, Davidson JM. Cutis laxa arising from frameshift mutations in exon 30 of the elastin gene (ELN). *J Biol Chem*. 1999;274(2):981-986. doi: 10.1074/jbc.274.2.981
35. Kun Y, Mengdong S, Cong F, Ran H. Congenital Cutis Laxa: A Case Report and Literature Review. Case Report. *Front Surg*. 2022;9:814897. doi: 10.3389/fsurg.2022.814897
36. Van Doren SR. Matrix metalloproteinase interactions with collagen and elastin. *Matrix Biology*. 2015;44-46:224-231. doi: 10.1016/j.matbio.2015.01.005
37. Arpino V, Brock M, Gill SE. The role of TIMPs in regulation of extracellular matrix proteolysis. *Matrix Biology*. 2015;44-46:247-254. doi: 10.1016/j.matbio.2015.03.005
38. Antoniou GA, Tentes IK, Antoniou SA, Simopoulos C, Lazarides MK. Matrix Metalloproteinase Imbalance in Inguinal Hernia Formation. *Journal of Investigative Surgery*. 2011;24(4):145-150. doi: 10.3109/08941939.2011.558610
39. Isik A, Gursul C, Peker K, Aydın M, Fırat D, Yılmaz İ. Metalloproteinases and Their Inhibitors in Patients with Inguinal Hernia. *World J Surg*. 2017;41(5):1259-1266. doi: 10.1007/s00268-016-3858-6
40. Serra R, Buffone G, Costanzo G, et al. Altered metalloproteinase-9 expression as least common denominator between varicocele, inguinal hernia, and chronic venous disorders. *Ann Vasc Surg*. 2014;28(3):705-709. doi: 10.1016/j.avsg.2013.07.026
41. Sugiyama H. WT1 (Wilms' tumor gene 1): biology and cancer immunotherapy. *Jpn J Clin Oncol*. 2010;40(5):377-387. doi: 10.1093/jjco/hyp194
42. Yen HC, Chen IC, Lin GC, et al. Sex-specific genetic variants associated with adult-onset inguinal hernia in a Taiwanese population. *Int J Med Sci*. 2023;20(5):607-615. doi: 10.7150/ijms.82331
43. Livingstone I, Uversky VN, Furniss D, Wiberg A. The Pathophysiological Significance of Fibulin-3. *Biomolecules*. 2020;10(9):1294. doi: 10.3390/biom10091294
44. Papaioannou VE. The T-box gene family: emerging roles in development, stem cells and cancer. *Development*. 2014;141(20):3819-3833. doi: 10.1242/dev.104471
45. Zhang Y, Han Q, Fan H, Li W, Xing Q, Yan B. Genetic analysis of the TBX2 gene promoter in indirect inguinal hernia. *Hernia*. 2014;18(4):513-517. doi: 10.1007/s10029-013-1199-z
46. Zhao Z, Tian W, Wang L, et al. Genetic and functional analysis of the TBX3 gene promoter in indirect inguinal hernia. *Gene*. 2015;554(1):101-104. doi: 10.1016/j.gene.2014.10.031
47. Chen D, Qiao Y, Meng H, et al. Genetic analysis of the TBX3 gene promoter in ventricular septal defects. *Gene*. 2013;512(2):185-188. doi: 10.1016/j.gene.2012.10.066
48. Pang S, Liu Y, Zhao Z, Huang W, Chen D, Yan B. Novel and functional sequence variants within the TBX2 gene promoter in ventricular septal defects. *Biochimie*. 2013;95(9):1807-1809. doi: 10.1016/j.biochi.2013.05.007
49. Greene AG, Eivers SB, Dervan EWJ, O'Brien CJ, Wallace DM. Lysyl Oxidase Like 1: Biological roles and regulation. *Exp Eye Res*. 2020;193:107975. doi: 10.1016/j.exer.2020.107975

50. Pascual G, Rodríguez M, Mecham RP, Sommer P, Buján J, Bellón JM. Lysyl oxidase like-1 dysregulation and its contribution to direct inguinal hernia. *Eur J Clin Invest*. 2009;39(4):328-337. doi: 10.1111/j.1365-2362.2009.02099.x
51. Vallet SD, Ricard-Blum S. Lysyl oxidases: from enzyme activity to extracellular matrix cross-links. *Essays Biochem*. 2019;63(3):349-364. doi: 10.1042/ebc20180050
52. Houtkooper RH, Pirinen E, Auwerx J. Sirtuins as regulators of metabolism and healthspan. *Nat Rev Mol Cell Biol*. 2012;13(4):225-238. doi: 10.1038/nrm3293
53. Han Q, Zhang Y, Li W, et al. Functional sequence variants within the SIRT1 gene promoter in indirect inguinal hernia. *Gene*. 2014;546(1):1-5. doi: 10.1016/j.gene.2014.05.058
54. Jorgenson E, Makki N, Shen L, et al. A genome-wide association study identifies four novel susceptibility loci underlying inguinal hernia. *Nat Commun*. 2015;6:10130. doi: 10.1038/ncomms10130
55. Hikino K, Koido M, Tomizuka K, et al. Susceptibility loci and polygenic architecture highlight population specific and common genetic features in inguinal hernias: genetics in inguinal hernias. *EBioMedicine*. 2021;70:103532. doi: 10.1016/j.ebiom.2021.103532
56. Ahmed WU-R, Patel MIA, Ng M, et al. Shared genetic architecture of hernias: A genome-wide association study with multivariable meta-analysis of multiple hernia phenotypes. *PLOS ONE*. 2022;17(12):0272261. doi: 10.1371/journal.pone.0272261
57. Choquet H, Li W, Yin J, et al. Ancestry- and sex-specific effects underlying inguinal hernia susceptibility identified in a multiethnic genome-wide association study meta-analysis. *Human Molecular Genetics*. 2022;31(13):2279-2293. doi: 10.1093/hmg/ddac003
58. Fadista J, Skotte L, Karjalainen J, et al. Comprehensive genome-wide association study of different forms of hernia identifies more than 80 associated loci. *Nat Com*. 2022;13(1):3200. doi: 10.1038/s41467-022-30921-4

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REVIEW PAPER

An overview of incidence and mechanisms promoting weight gain as an adverse effect of oral minoxidil therapy for androgenetic alopecia

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ABSTRACT

Introduction and aim. Androgenetic alopecia, with a mechanism based on the excessive response of hair follicles to androgens, affects a majority of people at some point in their lives, prompting them to seek therapy. Current treatment options for this condition include oral minoxidil, a medication associated with an adverse effect of fluid retention, potentially resulting in weight gain for certain individuals. In contemporary scientific literature, there aren't many articles focusing solely on this specific side effect. The objective of this review is to explore links between taking oral minoxidil and fluid retention leading to weight gain in patients with androgenetic alopecia by examining available studies in order to understand the mechanisms behind this phenomenon and the dose dependence of fluid retention.

Material and methods. A review of the literature was performed to find connections between oral minoxidil therapy and water retention-induced weight gain.

Analysis of the literature. Clinical trials have demonstrated that low dose oral minoxidil therapy, within the range of 0.5 to 5 mg daily, leads to an improvement in both hair count and density. The incidence of side effects such as hypertrichosis, fluid retention, headache, dizziness, and insomnia, is relatively infrequent. Fluid retention rates varied between 0.22% in the Tanaka study and 10% of patients in the Panchaprateep study. The discontinuation of treatment was necessary in some instances, with the highest rate of 2.4% cases in the Jimenez-Cauhe study. A comparative analysis of studies on oral minoxidil use for refractory hypertension, within the range of 10 to 40 mg daily, revealed that nearly all patients required adding a diuretic to control fluid retention. Some patients discontinued the treatment due to the severity of side effects. In instances of minoxidil overdose, serious complications, including generalized edema, myocardial infarction, stroke, and pleural effusion, were observed. Across these studies, all patients recovered following the discontinuation of minoxidil treatment. The underlying mechanism behind oral minoxidil induced sodium and fluid retention, contributing to weight gain, is associated with alterations in the neurohumoral system, increased plasma renin activity, changes in renal hemodynamics with relocation of the blood circulation from outer to inner cortex, and tubular effect that can be connected to minoxidil ability to act as an opener of potassium channels in the thick ascending limb of the loop of Henle causing greater reabsorption of sodium and chloride.

Conclusion. The frequency and severity of water retention promoting weight gain in individuals taking oral minoxidil are dose dependent. In most patients, minoxidil is a safe and effective treatment option for androgenetic alopecia. In some cases, due to rapid weight gain of 5 pounds or more, adding a diuretic or discontinuation may be required. Further research is necessary to better understand the mechanisms and dose dependence of minoxidil induced fluid retention, which promotes weight gain.

Keywords. androgenetic alopecia, fluid retention, minoxidil side effects, oral minoxidil

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Introduction

Androgenetic alopecia (AGA) is a condition preset by genetic factors that affects up to 50% of men and women.[1] The underlying process leading to AGA is an excessive response of hair follicles to androgens.[2] Over time, dihydrotestosterone (DHT) causes the gradual miniaturization of terminal hair, leading to balding especially of the temples and the crown. Hair is an important part of an individual's self-image, thus the consequences of androgenetic alopecia are largely psychological.[1,2] According to recent studies, approximately 60% of women and 30% of men suffering from AGA experience emotional distress that can lead to anxiety and depression.[3] Nowadays, therapies for AGA include oral minoxidil, oral finasteride and dutasteride, platelet-rich plasma, low-level light therapy, and hair transplantation.[4] Minoxidil, being a direct acting peripheral vasodilator, was first introduced to general medicine in the 1970s as a treatment for refractory hypertension. The initial trials of this drug displayed an adverse effect of hypertrichosis in 80% of patients, which led in 1981 to the development of a topical minoxidil solution and in 1987 to creating low dose oral minoxidil (LDOM) therapy for AGA.[5] However, despite causing improvements in both hair diameter and density, the medicine seems to have also an array of possible adverse effects, such as weight gain, fluid retention, pericardial effusion, hypertrichosis, hirsutism, GI intolerance, hypotension, tachycardia, headache, periorbital oedema, and insomnia.[6-8]

Aim

In this review, we aim to explore the prevalence and mechanisms of oral minoxidil induced water retention leading to weight gain in patients with AGA.

Material and methods

For this review, PubMed, Medline, and Google Scholar were searched to find articles concerning the impact of LDOM therapy for AGA and the prevalence of adverse effects, with an emphasis on fluid retention leading to possible weight gain. As a comparison, we also analyzed minoxidil treatment for refractory hypertension and oral minoxidil overdose. We performed a search using the keywords "androgenetic alopecia", "oral minoxidil", "low dose oral minoxidil", "minoxidil adverse effects", "minoxidil fluid retention", "minoxidil weight gain."

Articles underwent a careful analysis and were included based on their relevance to our review. The inclusion criteria encompassed abstracts and full text-format published articles, including review articles, randomized control trials, and clinical trials, written in English. We excluded duplicates and articles irrelevant to our topic. The initial search, employing the keywords and criteria present in this study, resulted in identification of 237 publications consisting of abstracts and full texts. Following this, duplicate and irrelevant scientific papers were excluded. Figure 1 illustrates the inclusion and exclusion criteria applied during this process. Subsequently, a total of 115 manuscripts were identified for detailed evaluation, which led to the selection of 42 articles and reports that are incorporated in this literature review.

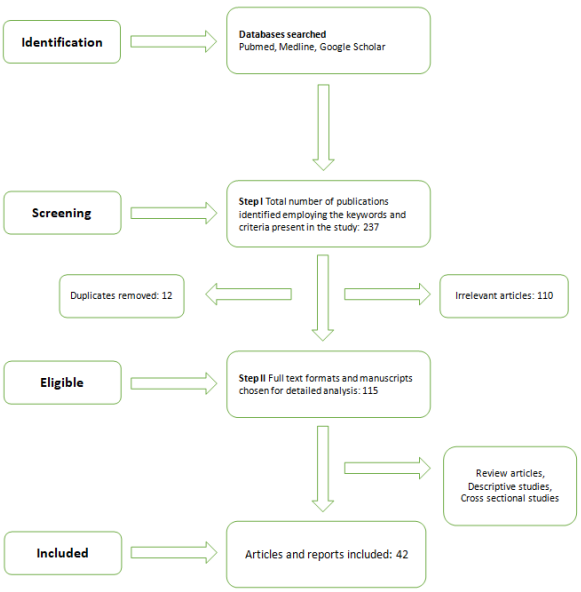

Fig. 1. A flow chart figure displaying the sequential steps from the initiation of the literature research to the finalization of chosen publications

Analysis of the literature

Hair anatomy and hair growth cycle

Hair is a structure that consists of terminally differentiated keratinized dead cells. In its composition, we can distinguish the follicle and the shaft (Fig. 2).[9] The division of the hair follicle separates the upper part, including the infundibulum and the isthmus, from the lower part, consisting of the bulb and the suprabulbar region. The bulb includes the dermal papilla that is a group of specialized mesenchymal fibroblasts, capillaries along with nerve endings, and the hair matrix built by quickly proliferating keratinocytes. Hair follicles remain anchored in the subcutis layer and undergo the phases of the hair growth cycle. The hair shaft, built by trichocytes, is visible above the skin.[9-11] The normal hair growth cycle (Fig. 3) is divided into 4 phases: growth (anagen), involution (catagen), resting (telogen), and shedding (exogen).[9,12] The anagen phase lasts 2-7 years, which makes it the longest part of the cycle. In this phase, cells at the lower part of the hair divide rapidly, simultaneously cells of the matrix migrate outward. The catagen phase lasts around 3 weeks and is a period of short transition. In this phase, the hair shafts lose their connections to the papillae and contract. The telogen phase lasts around 3 months. In this phase, the matrix regresses and the papilla retracts to a location near

the bulge. No significant proliferation or apoptosis happens in this phase. In the exogen phase, active hair shed and new hair continue to grow.[9]

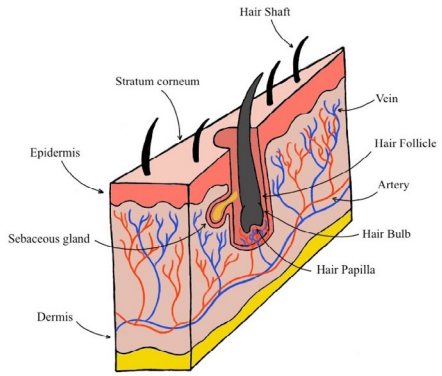

Fig 2. Anatomy of the hair[9]

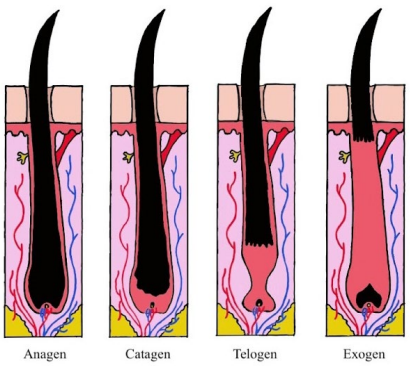

Fig 3. Hair growth cycle[9]

At any given moment, around 85–90% of the hair remains in the anagen phase, 2% of the follicles are in the catagen phase, and 10–15% in the telogen phase.[12,13] However, according to the newest data, this percentage of telogen hair can be overestimated, and in reality, only 3.6% remain in this phase.[14]

Androgenetic alopecia

Androgenetic alopecia is the most prevalent type of hair loss worldwide and affects approximately 30% of men and 20% of women in their 30s, increasing to 50% of men and 40% of women in their 50s. This further increases to 80% of men and 50% of women by the time they reach their 70s. The disease starts after puberty, progresses with age, and is the most common among Caucasians.[1,2,12,15-17] Hair loss usually starts after puberty on the frontal hairline with bitemporal recession, subsequently it's followed by diffuse thinning of the hair and eventually balding on the vertex. As AGA progresses, the bald spot at the crown connects with the frontal receding hairline, leaving only an island of hair at the frontal scalp. With the further progression of AGA, the island disappears. At this point, hair is left only in the occipital and parietal zones. The Hamilton Norwood scale is typically used to analyze the degree of AGA in men, while the Ludwig scale is used to assess the extent of AGA in women. AGA might be a factor contributing to a higher risk of type 2 diabetes, cardiovascular disease, and benign prostatic hyperplasia.[18-23] The mechanism of AGA has both a genetic and hormonal background, with excessive response of the hair follicles to androgens being the main factor. Over time androgens promote gradual miniaturization of terminal hair into intermediate and vellus hair. This process clinically manifests itself as continuous hair shortening and thinning, leading to hair loss among patients with AGA.[12,24] Moreover, elevated blood levels of dihydrotestosterone and 5-alpha reductase type 2 are frequent in patients with AGA. DHT has 10 times higher affinity to androgen receptors and is a strong hormone promoting the development of male characteristics, whereas 5-alpha reductase type 2 is an enzyme converting testosterone into DHT in the hair follicles. Even though blood levels of testosterone may not vary between patients with and not affected by AGA, the blood concentrations of unbound testosterone are elevated in individuals with AGA.[1,12,25] AGA is usually perceived as a minor dermatologic condition. However, it has been observed that about 60% of female patients and 30% of male patients with AGA frequently suffer from emotional distress, leading to anxiety as well as depression.[2,3]

Nowadays, there are continuously more new treatment options available for AGA. These include topical and oral minoxidil, topical and oral finasteride, oral spironolactone, topical pyrilutamide, intradermal botox injections, platelet-rich plasma, low-level light therapy, microneedling, and finally hair transplant. In this review, we're going to focus solely on the increasingly popular off- label use of oral minoxidil therapy for AGA.[12,15]

Hypertension

In this literature review, along with exploring the impact of LDOM treatment on patients with androgenetic alopecia, we're also reporting the adverse effects associated with the use of higher doses of oral minoxidil therapy among individuals with refractory hypertension. High blood pressure, being one of the most frequently occurring chronic illnesses leading to premature deaths in the world population, is defined as average systolic blood pressure (SBP) ≥ 140 mmHg, average diastolic blood pressure (DBP) ≥ 90 mmHg, or usage of antihypertensive agents.[26] Resistant hypertension, being the most severe form of hypertension, can be diagnosed when more than 3 antihypertensive drugs (diuretic included) belonging to different classes at the highest doses recommended are not able to reduce the blood pressure.[26-28]

Minoxidil

Oral minoxidil is a direct acting arteriolar vasodilator. The drug was first introduced in general medicine in

the 1970s as a treatment option for refractory hypertension, dosed at 10 to 40 mg daily with a maximal recommended dose not exceeding 100 mg.[5,6,29,30] Even though minoxidil has a plasma half-life of approximately 3 to 4 hours, its blood pressure lowering effect may persist for up to 72 hours, which implies the accumulation of the drug in the body.

As a result of reaction with sulfotransferase, minoxidil transforms into minoxidil sulfate. Being the active metabolite, minoxidil sulfate opens ATP-sensitive K^+ channels located in arterial smooth muscle cells, leading to hyperpolarization of the sarcolemma, which inhibits Ca^{2+} entry and causes vasodilation. This mechanism promotes hypotensive effect. Minoxidil induced arteriolar vasodilation can activate the sympathetic nervous system through aortic and carotid baroreceptors impulses, causing tachycardia, as well as an increased plasma renin activity that leads to aldosterone synthesis. That's why, when used among patients with resistant hypertension, minoxidil should be prescribed along with beta blocker to prevent tachycardia and a diuretic to avoid sodium and fluid retention.[5,8,30-32] The adverse effects of oral minoxidil are dose dependent, and besides an increase in heart rate as well as possible water retention, they include hypertrichosis affecting about 80% of individuals, hirsutism, postural hypotension, fatigue, dizziness, nausea, pericardial effusion, periorbital edema, GI intolerance, and insomnia.[6-8,31-35] The reported high prevalence of hypertrichosis among patients using minoxidil led in 1981 to the development of 5% topical minoxidil solution applied on the scalp, and subsequently in 1987, low dose oral minoxidil treatment. Off-label LDOM therapy dosed at 0.5-1 mg daily for women and 2.5–5 mg daily for men appears to be very effective in individuals suffering from AGA with a relatively good safety profile. Recently, this treatment is gaining popularity among patients because oral administration is simply easier than applying the topical solution. In 1988 minoxidil solution in concentration of 2% was approved by FDA, and in 1998 minoxidil 5% solution gained acceptance for topical use. Oral minoxidil to this day is an off-label treatment for AGA. The Polish Dermatological Society since 2018 recommends in men 5% minoxidil solution applied once or two times a day on the scalp with a provided applicator and in women 2% minoxidil solution used topically on the hair two times a day or 5% minoxidil solution used topically on the hair once a day as a treatment of first choice. Once applied, minoxidil solution should remain on the scalp for at least 2 hours to absorb. Both oral and topical minoxidil are not recommended for children and during pregnancy.[5,6,24,36,37] The exact mechanism of minoxidil hair growth promotion is not entirely known. By opening ATP-sensitive K^+ channels in arterial smooth myocytes of the scalp, leading to vasodilation, minoxidil increases the blood flow and allows a better delivery of oxygen and nutrients to the hair. Through inducing the Wnt/β-Catenin pathway, it elevates VEGF concentration in the cells of dermal papilla, and promotes vascularization as well as prolongs the growth phase of hair cycle. It is also reported that minoxidil inhibits interleukin 1α and prostacyclin synthesis at the same time promoting leukotriene B4 and prostaglandin E2 production, therefore it presents anti-inflammatory properties. Minoxidil might also act as an antiandrogen by inhibiting the expression of 5α-reductase, which converts testosterone to DHT, and inducing 17β-dehydrogenase in the hair follicles to quicker transform testosterone to its less biologically active forms. Due to lack of full understanding how exactly minoxidil causes hypertrichosis, further studies are required.[5,8]

Mechanism of minoxidil induced fluid retention leading to possible weight gain

The metabolism of oral minoxidil mainly happens in the liver, where it conjugates with glucuronic acid. After metabolization, minoxidil gets eliminated from the body through the kidneys. Even though it takes approximately 3 to 4 hours from administration to excrete the drug, the vasodilating effect remains for roughly 72 hours. Oral minoxidil has been linked to retention of sodium and fluid leading to weight gain, pericardial effusion with unknown mechanism, pulmonary hypertension caused by high pressure in the pulmonary artery along with increased cardiac output, and in extreme cases, coronary heart disease resulting potentially from the increased oxygen demand due to higher heart rate as well as cardiac output. Sodium and fluid retention causing weight gain is dose dependent and occurs due to alterations in neurohumoral system, increased plasma renin activity, changes in renal hemodynamic including relocation of the blood circulation from the outer to the inner kidney cortex, along with directly tubular effects that can be connected to minoxidil's ability to act as an opener of potassium channels. Through activating the potassium channels located in the thick ascending limb of the loop of Henle, minoxidil boosts the activity of Na^+ $2Cl^-$ K^+ cotransporters and leads to greater reabsorption of sodium and chloride. Minoxidil can promote rapid 5 pounds or more weight gain. Early identified fluid retention is easier to manage with diuretics. Therefore, a patient's weight should be monitored daily to pinpoint individuals experiencing fluid retention. In some cases, discontinuation of minoxidil treatment may be required due to extreme fluid retention.[31,32,35]

Clinical findings of oral minoxidil weight gain

In this segment of the review, we will display a short summary regarding side effects of LDOM therapy for AGA, oral minoxidil dosed at 10–40 mg daily as a treat-

ment for refractory hypertension, and oral minoxidil overdose, with emphasis on fluid retention. Through this process, we found the link between dosage of oral minoxidil and prevalence of sodium and fluid retention, leading to weight gain, which in some cases caused discontinuation of treatment.

Across the 5 studies regarding the use of LDOM treatment for AGA, an improvement of hair count and density was observed. The incidence of side effects was low. The most common adverse effects included: hypertrichosis, fluid retention along with lower limbs edema, headache, dizziness, and insomnia.[6,24,37-39] Fluid retention led to discontinuation of treatment in several individuals.[6,37,39]

Across the 4 studies, when minoxidil was used as an antihypertensive agent, in 1 review the safety profile was similar to LDOM therapy for AGA because low doses of minoxidil were used, whereas in 3 trials the incidence of side effects was higher.[30,33,40,41] Many patients required adding a diuretic to control sodium and fluid retention, leading to weight gain.[30,33,41] Some patients had to discontinue the use of minoxidil because of the severity of side effects.[33,40,41]

In 2 studies displaying minoxidil overdose, the most severe side effects were observed. These included generalized edema, myocardial infarction, stroke, passing out, prolonged tachycardia, fluid retention and pleural effusion.[36,42] Intubation and use of a diuretic was needed.[42] Patients recovered after discontinuation of the treatment.[36,42]

LDOM therapy for AGA clinical trials

In the multicenter study conducted in 2021 by Sergio Vano-Galvan et al. 1404 patients mostly with AGA 82.4% (1157 patients) who received LDOM for not less than 3 months were analyzed in order to describe the safety profile of this treatment. The dosing was titrated in 1065 patients and fixed in 339, so in fact 2469 cases could be analyzed. The doses ranged from 0.03 mg to 15 mg. Adverse effects affected 20.6% (509 patients). The displayed side effects were hypertrichosis 15.1% (374 patients), dizziness 1.7% (43 patients), fluid retention and pedal edema 1.3% (32 patients), tachycardia 0.9% (21 patients), headache 0.4% (9 patients), periorbital edema 0.3% (7 patients) and insomnia 0.2% (6 patients). The treatment was discontinued in 1.7% (43 patients). Fluid retention led to discontinuation in 8 cases. The incidence of side effects was similar in AGA and other alopecia types. The authors conclude that LDOM is a safe treatment option for alopecia with infrequent side effects. Based on their data, they propose to start dosing for females from 0.5 mg a day and increase by 0.25 mg every 12 weeks to a max dose 2.5mg, for males from 2.5mg a day and increase by 1.25 every 12 weeks to a max dose 5mg.[6] In a prospective open labeled and single arm study conducted in 2020 by Panchaprateep and Lueangarun, 30 males with AGA were given 5mg of oral minoxidil daily. The study duration was 24 weeks. After this time both efficacy as well as safety of the drug were assessed. All of the patients displayed improvement in hair count and density. Most common side effect was hypertrichosis 93% (28 males). Pedal edema affected 10% of the participants (3 males). In this study minoxidil was found both safe and effective. However, the authors of this trial emphasized that oral minoxidil should be used with caution among patients with high blood pressure and elevated risk of congestive heart failure.[38] In the interventional trial conducted between January 2017 to May 2020 performed by Sanabria et al. 435 participants taking LDOM (≤5 mg/d) at 3 trichology clinics in Brazil were interviewed regarding possible adverse effects of this drug in AGA treatment. The most common side effects were hypertrichosis reported by 55.4% (117 females and 124 males), headache 9% (22 females and 17 males) and insomnia 7% (14 females and 15 males). Generalized edema was reported by 1.1% of patients (3 females on 1mg oral minoxidil and 2 males on 2.5mg and 5mg). Lower limbs edema affected 6% of participants (19 females and 6 males). Facial edema affected 1% of participants (2 females and 3 males). All in all 6 patients discontinued using low dose oral minoxidil treatment for androgenetic alopecia due to fluid retention in the body. The results indicated that LDOM is a relatively-safe option for AGA, but patients should be warned about possible adverse effects including hypertrichosis, headaches, insomnia and edema.[37] In the controlled study conducted in 2019 by Jimenez-Cauhe et al. 41 males with AGA were included. The focus of this trial was to assess the effectiveness and possible side effects of LDOM in men. The doses prescribed in this study were 2.5 mg (10 participants) and 5 mg (31 participants) for at least 6 months. Clinical images before and after therapy were assessed by 3 dermatologists. 37 patients displayed clinical improvement, 4 showed stabilization and no worsening was observed. The most common side effect was hypertrichosis 24.3% (10 males) and lower limbs edema 4.8% (2 males). All in all, 1 patient discontinued the treatment due to pedal edema. Fluid retention affected patients taking 5 mg of oral minoxidil daily. The conclusion of the study states that oral minoxidil treatment at 5 mg daily brought positive effects and its safety profile was acceptable. In order to determine optimal dosing further controlled studies need to be conducted.[39] In a study conducted by Tanaka et al. between 2011 and 2017 a total of 18918 Asian men with AGA were prescribed a combination therapy consisting of oral finasteride 1mg once a day, topical minoxidil with concentration 5% and oral 2.5mg minoxidil twice a day as well as 4 ml per procedure injectable solution containing lidocaine, minoxidil, retinyl palmitate, caffeine, amino acids, vitamins and panthenol once a month. The aim of this study was evaluation of efficacy and safety of the combination therapy for AGA in Asian men. In order to

analyze the results photographs were taken before and after. After the treatment all patients displayed significant improvement. According to this study side effects affected 4.2% of the participants (802 males). Most common were pain due to injection 3.4% (651 participants) and slight bleeding in 0.3% (56 participants). When it comes to edema, swelling was observed in 0.22% (42 patients). Other side effects were itching and erythema linked to the use of topical minoxidil as well as decreased libido linked to the use of oral finasteride. Tanaka and coauthors conclude their study with a statement that their combination therapy is both effective and safe, however further studies are necessary to assess the safety of the therapy if higher doses are used.[24]

Minoxidil therapy used to treat hypertension clinical trials

In a multicentric retrospective review in 2023 Jimenez-Cauhe et al. analyzed 254 patients that had received LDOM as a treatment for alopecia in the past and had had high blood pressure or arrhythmia at that time. Side effects appeared in 6.8% (26 patients) and these were dizziness 3.1% (8 patients), water retention 2.6% (7 patients), other adverse effects included tachycardia, headache, general feeling of weakness. Six participants discontinued LDOM of hypertension due to adverse effects. The authors concluded that treatment of hypertension and with low dose oral minoxidil displays an advantageous safety profile.[40] In a clinical trial conducted in 1981 by Hagstam et al., minoxidil was prescribed to 25 patients suffering from refractory hypertension. At the beginning of this study 18 patients were diagnosed with kidney failure, presenting high serum creatinine levels. Soon after the onset of this trial 6 patients discontinued the treatment due to adverse effects or inability to control high blood pressure and only 19 individuals remained receiving the treatment for 0.5 to 4.5 years. Minoxidil had to be combined with beta-blocker and diuretic in order to reduce its side effects. During the treatment, all of the patients developed fluid retention that was kept under control with the use of diuretics. One patient died; however, it was not caused by minoxidil, but by the high uremia linked to starting dialysis. The authors found minoxidil to be successful in treating hypertension in patients with advanced renal disease.[33] In a clinical trial conducted in 1980 by Meier et al. 11 patients with severe hypertension impossible to control with conventional therapy were given diazoxide 400mg daily and minoxidil 17.7 mg daily in a crossover approach. Both treatments managed to decrease blood pressure. Both caused hypertrichosis, more severe with minoxidil. Both caused sodium and fluid retention leading to weight gain, which could be controlled with diuretic therapy.[30] In a clinical trial conducted in 1979 by Joekes et al. 47 patients with refractory hypertension received oral minoxidil 5-40 mg daily for up to 57 months. In 45 out of 47 patients a decrease in blood pressure was achieved on this drug. When it comes to adverse effects: 3 patients had to withdraw oral minoxidil due to sodium retention causing congestive heart failure, 2 female patients discontinued treatment because of hirsutism, 1 patient because of postural hypotension. In 34 patients due to sodium retention adding a diuretic was required. The authors of the study were unable to tell if adding the diuretic is always necessary. They claimed that if no edema, weight gain or postural hypotension were observed, minoxidil could be prescribed "well alone."[41]

Oral minoxidil overdose

In a multicentric retrospective review in 2022 by Moreno-Arrones et al. the authors focused their attention on 12 women (0.7% of patients) who developed serious side effects out of 1700 patients taking LDOM for AGA from January 2018 to October 2020. The dosing varied from 0.5mg to 1mg daily. When it comes to side effects: generalized oedema affected 6 patients, passing out affected 6 patients, stroke affected 1 patient and myocardial infarction affected 1 patient. After analyzing the composition of capsules that patients were receiving it turned out that they were taking higher doses than prescribed because of a compounding error at the pharmacy (the doses really varied between 50mg and 1000mg per pill). The patient who had a stroke received a 1000 times higher dose than the prescribed one and the patient who experienced myocardial infarction took a 200 higher dose. Out of the 12 women described in this case report, generalized edema affected those that took between 50 mg and 500mg of minoxidil. While the woman who took 50 mg presented swelling of the whole body in the first week, with higher doses this side effect appeared after the first intake. All women fully recovered after discontinuation of treatment. The authors concluded that the most severe side effects happened due to a pharmacist error at the dose at least 10 times higher than used in LDOM. Furthermore, they stated that adverse effects are dose dependent and while taking regular doses the safety of this treatment is high.[36] In a case report presented by Farell and Epstein from 1999 the authors describe an overdose of minoxidil. Patient drank a bottle containing 5% minoxidil solution resulting in a total dose of 3g oral minoxidil. Adverse effects were severe hypotension, prolonged tachycardia as well as fluid retention alongside pleural effusion. Intubation, antihypotensive agents and several days of furosemide therapy were necessary. In this case fluid retention and pleural effusion caused by oral minoxidil overdose were managed with furosemide.[42]

Conclusion

Oral minoxidil is an increasingly popular off label treatment option for AGA. Some patients find it more convenient than recommended by the Polish Dermatological

Society topical solution. Clinical findings have shown that both incidence and severity of adverse effects, including sodium and fluid retention leading to weight gain among individuals taking oral minoxidil are dose dependent. Low dose oral minoxidil treatment for patients with androgenetic alopecia based on 0.5 to 2 mg daily for women and 2.5 to 5 mg daily for men is proved to be effective with a relatively good safety profile.[5,6] Besides hypertrichosis, which is highly prevalent, the occurrence of other side effects including water retention and lower limbs edema appears to be low in this group, not exceeding 10% of the patients. Only in several cases due to severity of oedema discontinuation of the treatment was necessary. Furthermore, in this review we provided a comparison of the frequency and gravity of LDOM therapy adverse effects to those occurring in the treatment involving oral minoxidil to cure refractory hypertension dosed at 10 to 40 mg daily and an oral minoxidil overdose. With higher doses the incidence of side effects was respectively higher. Edema affected the majority of the patients and adding a diuretic was needed. The most extreme adverse effects occurred during oral minoxidil overdose, which required intensive care in some individuals.

The mechanism of minoxidil induced sodium as well as fluid retention and subsequently weight gain is based on the drug's ability to affect the neurohumoral system, increase plasma renin activity and change renal hemodynamic along with promoting a direct tubular effect. These processes can cause a rapid weight gain of 5 pounds or more that if detected early can be in most of the cases easily managed with the use of a diuretic. Some patients may require discontinuation of the treatment due to severe oedema. After ceasing treatment fluid retention resolves on its own.

Even though low dose oral minoxidil therapy is a very effective and mostly safe treatment option for androgenetic alopecia, the adverse effects in some individuals are quite severe, therefore further studies are necessary to fully understand the mechanism and dose dependance of water retention leading to weight gain in patients taking LDOM therapy for AGA.

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Author contributions

Conceptualization, M.P. and Ł.C.; Methodology, Ł.C.; Software, J.F.; Validation, A.K. and M.P.; Formal Analysis, J.F.; Investigation, Ł.C., M.P., A.K. and J.F.; Resources, M.P., A.K. and J.F.; Data Curation, Ł.C.; Writing – Original Draft Preparation, Ł.C. and M.P.; Writing – Review and Editing, Ł.C., M.P., A.K. and J.F.; Visualization: M.P.; Supervision: M.P., A.K. and Ł.C., Project Administration, Ł.C., M.P., A.K. and J.F.

Conflict of interest

The authors declare no conflicts of interest.

Data availability

Not applicable.

References

1. Ho CH, Sood T, Zito PM. Androgenetic Alopecia. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2022.
2. Asfour L, Cranwell W, Sinclair R. Male Androgenetic Alopecia. In: Feingold KR, Anawalt B, Blackman MR, et al., ed. *Endotext*. South Dartmouth (MA): MDText.com, Inc.; 2023.
3. Tabolli S, Sampogna F, di Pietro C, Mannooranparampil TJ, Ribuffo M, Abeni D. Health status, coping strategies, and alexithymia in subjects with androgenetic alopecia: a questionnaire study. *Am J Clin Dermatol*. 2013;14(2):139-145. doi: 10.1007/s40257-013-0010-3
4. Ly NY, Fruechte S, Hordinsky MK, Sadick N, Arruda S, Farah RS. Medical and procedural treatment of androgenetic alopecia - Where are we? *J Am Acad Dermatol*. 2023;89(2):36-39. doi: 10.1016/j.jaad.2023.05.004
5. Graczyk A, Waśkiel-Burnat A, Rakowska A, Rudnicka L. The use of minoxidil in diseases associated with hair loss. *Dermatology Review*. 2022;109(6):437-452. doi: 10.5114/dr.2022.126603
6. Vañó-Galván S, Pirmez R, Hermosa-Gelbard A, et al. Safety of low-dose oral minoxidil for hair loss: A multicenter study of 1404 patients. *J Am Acad Dermatol*. 2021;84(6):1644-1651. doi: 10.1016/j.jaad.2021.02.054
7. Beach RA, McDonald KA, Barrett BM, Abdel-Qadir H. Side effects of low-dose oral minoxidil for treating alopecia. *J Am Acad Dermatol*. 2021;84(5):239-240. doi: 10.1016/j.jaad.2020.12.038
8. Gupta AK, Talukder M, Shemar A, Piraccini BM, Tosti A. Low-Dose Oral Minoxidil for Alopecia: A Comprehensive Review. *Skin Appendage Disord*. 2023;9(6):423-437.doi: 10.1159/000531890
9. Grymowicz M, Rudnicka E, Podfigurna A, et al. Hormonal Effects on Hair Follicles. *Int J Mol Sci*. 2020;21(15):5342. doi: 10.3390/ijms21155342
10. Schneider MR, Schmidt-Ullrich R, Paus R. The hair follicle as a dynamic miniorgan. *Curr Biol*. 2009;19(3):132-142. doi: 10.1016/j.cub.2008.12.005
11. Martel JL, Miao JH, Badri T. Anatomy, Hair Follicle. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2022.
12. Devjani S, Ezemma O, Kelley KJ, Stratton E, Senna M. Androgenetic Alopecia: Therapy Update. *Drugs*. 2023;83(8):701-715. doi: 10.1007/s40265-023-01880-x
13. Lepselter J, Elman M. Biological and clinical aspects in laser hair removal. *J Dermatolog Treat*. 2004;15(2):72-83. doi: 10.1080/09546630310023152
14. Hernandez I, Alam M, Platt C, et al. A technique for more precise distinction between catagen and telogen human hair follicles ex vivo. *J Am Acad Dermatol*. 2018;79(3):558-559. doi: 10.1016/j.jaad.2018.02.009

15. Nestor MS, Ablon G, Gade A, Han H, Fischer DL. Treatment options for androgenetic alopecia: Efficacy, side effects, compliance, financial considerations, and ethics. *J Cosmet Dermatol*. 2021;20(12):3759-3781. doi: 10.1111/jocd.14537
16. Famenini S, Slaught C, Duan L, Goh C. Demographics of women with female pattern hair loss and the effectiveness of spironolactone therapy. *J Am Acad Dermatol*. 2015;73(4):705-706. doi: 10.1016/j.jaad.2015.06.063
17. Salman KE, Altunay IK, Kucukunal NA, Cerman AA. Frequency, severity and related factors of androgenetic alopecia in dermatology outpatient clinic: hospital-based cross-sectional study in Türkiye. *An Bras Dermatol*. 2017;92(1):35-40. doi: 10.1590/abd1806-4841.20175241.
18. Martinez-Jacobo L, Villarreal-Villarreal CD, Ortiz-López R, Ocampo-Candiani J, Rojas-Martínez A. Genetic and molecular aspects of androgenetic alopecia. *Indian J Dermatol Venereol Leprol*. 2018;84(3):263-268. doi: 10.4103/ijdvl.IJDVL_262_17
19. Guarrera M, Cardo P, Arrigo P, Rebora A. Reliability of hamilton-norwood classification. *Int J Trichology*. 2009;1(2):120-122. doi: 10.4103/0974-7753.58554
20. Duskova M, Starka L, Hill M, Dolezal M, Simunkova K, Cermakova I. Is there male androgenetic alopecia the sign of male equivalent of polycystic ovary syndrome or metabolic syndrome? *Endocrine Abstracts*. 2006;11:366.
21. Cannarella R, Condorelli RA, Mongioì LM, La Vignera S, Calogero AE. Does a male polycystic ovarian syndrome equivalent exist? *J Endocrinol Invest*. 2018;41(1):49-57. doi: 10.1007/s40618-017-0728-5
22. Ramsamy K, Subramaniyan R, Patra AK. An observational Study of the Association between Androgenetic Alopecia and Size of the Prostate. *Int J Trichology*. 2016;8(2):62-66. doi: 10.4103/0974-7753.188034
23. Rodríguez-Gutiérrez R, Salcido-Montenegro A, González-González JG. Early Clinical Expressions of Insulin Resistance: The Real Enemy to Look For. *Diabetes Ther*. 2018;9(1):435-438. doi: 10.1007/s13300-017-0348-2
24. Tanaka Y, Aso T, Ono J, Hosoi R, Kaneko T. Androgenetic Alopecia Treatment in Asian Men. *J Clin Aesthet Dermatol*. 2018;11(7):32-35.
25. YunSI, Lee SK, Goh EA, et al. Weekly Treatment with SAMiRNA Targeting the Androgen Receptor Ameliorates Androgenetic Alopecia. *Sci Rep.* 2022;12:1607. doi: 10.1038/s41598-022-10024-2
26. Sumna VM, Malhotra S, Gupta S, Goswami K, Salve HR. Prevalence and Associated Factors of Hypertension Among Adolescents in a Rural Community of North India. *Cureus*. 2023;15(10):47934. doi: 10.7759/cureus.47934
27. Doroszko A, Janus A, Szahidewicz-Krupska E, Mazur G, Derkacz A. Resistant Hypertension. *Adv Clin Exp Med*. 2016;25(1):173-183. doi: 10.17219/acem/58998
28. Lamirault G, Artifoni M, Daniel M, Barber-Chamoux N, Nantes University Hospital Working Group On Hypertension. Resistant Hypertension: Novel Insights. *Curr Hypertens Rev*. 2020;16(1):61-72. doi: 10.2174/1573402115666191011111402
29. Gilmore E, Weil J, Chidsey C. Treatment of essential hypertension with a new vasodilator in combination with beta-adrenergic blockade. *N Engl J Med*. 1970;282(10):521-527. doi: 10.1056/NEJM197003052821001
30. Meier A, Weidmann P, Glück Z, et al. Comparison of oral diazoxide and minoxidil in refractory hypertension. *Klin Wochenschr*. 1980;58(13):681-687. doi: 10.1007/BF01478605
31. Sica DA. Minoxidil: an underused vasodilator for resistant or severe hypertension. *J Clin Hypertens (Greenwich)*. 2004;6(5):283-287. doi: 10.1111/j.1524-6175.2004.03585.x
32. Suchonwanit P, Thammarucha S, Leerunyakul K. Minoxidil and its use in hair disorders: a review. *Drug Des Devel Ther*. 2019;13:2777-2786. doi: 10.2147/DDDT.S214907
33. Hagstam KE, Lundgren R, Wieslander J. Clinical experience of long-term treatment with minoxidil in severe arterial hypertension. *Scand J Urol Nephrol*. 1982;16(1):57-63. doi: 10.3109/00365598209179641
34. Devine BL, Fife R, Trust PM. Minoxidil for severe hypertension after failure of other hypotensive drugs. *Br Med J*. 1977;2(6088):667-669. doi: 10.1136/bmj.2.6088.667
35. Berger K. Minoxidil side effects and how to avoid them. https://www.singlecare.com/blog/minoxidil-side-effects/. Published May 14, 2020. Accessed September 29, 2023.
36. Moreno-Arrones OM, Rodrigues-Barata R, Morales C, et al. Serious Adverse effects From Compounding Errors With Low-Dose Oral Minoxidil for Alopecia Treatment. *Actas Dermosifiliogr*. 2022;113(7):725-727. doi: 10.1016/j.ad.2021.03.003
37. Sanabria B, Vanzela TN, Miot HA, Müller Ramos P. Adverse effects of low-dose oral minoxidil for androgenetic alopecia in 435 patients. *J Am Acad Dermatol*. 2021;84(4):1175-1178. doi: 10.1016/j.jaad.2020.11.035
38. Panchaprateep R, Lueangarun S. Efficacy and Safety of Oral Minoxidil 5 mg Once Daily in the Treatment of Male Patients with Androgenetic Alopecia: An Open-Label and Global Photographic Assessment. *Dermatol Ther (Heidelb)*. 2020;10(6):1345-1357. doi: 10.1007/s13555-020-00448-x
39. Jimenez-Cauhe J, Saceda-Corralo D, Rodrigues-Barata R, et al. Effectiveness and safety of low-dose oral minoxidil in male androgenetic alopecia. *J Am Acad Dermatol*. 2019;81(2):648-649. doi: 10.1016/j.jaad.2019.04.054
40. Jimenez-Cauhe J, Pirmez R, Müller-Ramos P, et al. Safety of Low-Dose Oral Minoxidil in Patients With Hypertension and Arrhythmia: A Multicenter Study of 264 Patients. *Actas Dermosifiliogr.* 2024;115(1):28-35. doi: 10.1016/j.ad.2023.07.019
41. Joekes AM, Thompson FD, O'Regan PF. Clinical use of minoxidil (Loniten). *J R Soc Med*. 1981;74(4):278-282. doi: 10.1177/014107688107400408
42. Farrell SE, Epstein SK. Overdose of Rogaine Extra Strength for Men topical minoxidil preparation. *J Toxicol Clin Toxicol*. 1999;37(6):781-783. doi: 10.1081/clt-100102457

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REVIEW PAPER

A sight into the pathogenesis and treatment of thyroid-associated ophthalmopathy

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ABSTRACT

Introduction and aim. Thyroid-associated ophthalmopathy (TAO), often referred to as thyroid eye disease or Graves' ophthalmopathy, is a syndrome characterized by autoimmune inflammation affecting the eye muscles, connective tissue, and orbital fat. The aim of this literature review is to present TAO and integrate the available data in the literature regarding the pathogenesis and treatment methods. Based on these, the authors aim to examine whether, despite the extensive knowledge already available on TAO, there are still issues to be investigated.

Material and methods. In this literature review, books and scientific publications in both Polish and English languages have been assessed. The search criteria included keywords such as TAO, Graves' disease, thyroid-associated ophthalmopathy. The evaluation covered the following databases: PubMed, Scopus, Google Scholar.

Analysis of the literature. Typically, the eyeball is not involved, but in exceptional cases, corneal ulceration may occur, or inflammation of the optic nerve may ensue. TAO most commonly occurs in the course of hyperthyroidism in Graves' disease, involving up to 25–50% of cases. The coexistence of autoantigens shared between the thyroid and orbital tissues is considered the primary cause of TAO when it occurs concurrently with hyperthyroidism, later in its course, or even preceding the manifestation of hyperthyroidism, with or without concurrent thyroid dysfunction. TAO is generally bilateral, although dominance on one side is often observed. Common symptoms include eye pain, photophobia, diplopia, varying degrees of proptosis, and impaired vision. The cornerstone of treatment lies in managing hyperthyroidism, as TAO cannot be cured without it.

Conclusion. First-line treatment involves glucocorticoids, with radiation therapy as a supplementary option, and in cases unresponsive to pharmacological treatment, surgical intervention may be necessary.

Keywords. Graves' disease, TAO, thyroid-associated ophthalmopathy, thyroid eye disease

Introduction

Thyroid-associated ophthalmopathy (TAO) constitutes a clinical syndrome associated with hyperthyroidism in Graves' disease and represents its most common extrathyroidal manifestation.[1-9] It involves autoimmune inflammation of the soft tissues within the orbit, including the extraocular muscles, connective tissue, and orbital fat.[3,8,10-12] The estimated prevalence of this condition is 16/100,000 in women and 2.9/100,000 in men, with an overall occurrence of at least around 10/10,000.[3,13-16] Inflammation of these structures leads to their swelling, ultimately resulting in the protrusion of the eyeball from the orbital cavity. TAO occurs in 30% of individuals with Graves' and Basedow's diseases, but only 10% of them ex-

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perience a severe course necessitating immunosuppressive treatment.[9,17-19] The exact cause of TAO remains not fully understood; however, it predominantly occurs in patients with hyperthyroidism or a history of it. Nevertheless, 5–8% of TAO cases are associated with thyroid inflammation in euthyroidism or hypothyroidism.[1,9,20] The hyperthyroidism in Graves' and Basedow's diseases is induced by autoantibodies that bind to the thyroid follicular cell's thyrotropin receptor, stimulating excessive thyroid hormone production. The presence of these antibodies in almost all TAO patients suggests that immune reactivity against the thyrotropin receptor underlies both hyperthyroidism and TAO.[20] Autoimmune processes leading to fibroblast proliferation, increased adipogenesis, and extracellular matrix expansion are involved in the pathophysiology of TAO.[8] Risk factors for TAO include stressful life events, genetics, gender, age, ethnic origin, smoking, thyroid dysfunction, and antibodies against thyrotropin. Smoking is identified as the most potent modifiable risk factor for the disease.[3] Common TAO symptoms include bilateral and symmetric retraction of the eyelids, proptosis, and double vision. Additionally, the disease manifests with tearing, redness, photophobia, and eye pain, significantly impacting patients' daily activities such as driving and interpersonal interactions. Ophthalmopathy typically occurs bilaterally, but unilateral cases may arise, often associated with the dominant side of the disease process. However, in 5% of cases, true unilateral occurrence occurs, posing diagnostic challenges due to its atypical presentation.[1,8]

Aim

The aim of the article is to review the available literature on thyroid-associated ophthalmopathy and to closely examine its pathogenesis, clinical presentation, and treatment methods in order to better understand this complex disease process and develop more effective treatment approaches.

Material and methods

In this literature review, books and scientific publications in both Polish and English languages have been assessed. The search criteria included keywords such as TAO, Graves' disease, thyroid-associated ophthalmopathy. The evaluation covered the following databases: PubMed, Scopus, Google Scholar.

Analysis of the literature

Pathogenesis

Thyroid-associated ophthalmopathy (TAO) is most commonly associated with hyperthyroidism in Graves' disease, although in 5-8% of cases, it can coexist with autoimmune thyroid inflammation in euthyroidism or hypothyroidism.[1,9,20] The occurrence of TAO is linked to an autoimmune process. The enlargement of extraocular muscles, connective tissue, and orbital fat results from interactions among orbital fibroblasts, cytokines, autoantibodies, immune cells, environmental factors, and genetic factors.[7]

Due to the frequent coexistence of ophthalmopathy in Graves' disease, there is a presence of similar antigens in both conditions. In Graves' disease, the loss of tolerance to the thyrotropin receptor (TSHR) occurs, making TAO inducible by autoimmune reactions against TSHR.[9,12,21] Clinical and experimental evidence supports this, with immunohistochemical studies showing TSHR overexpression in orbital tissues of TAO patients, with the highest receptor expression in individuals with clinically active disease. The expansion of orbital fat tissue results from early TSHR activation, enhancing the differentiation of orbital preadipocytes into adipocytes. Therefore, a strong association exists between circulating TSHR antibodies and TAO occurrence.[9,11,20-25] Moreover, tests have demonstrated that TSHR levels are correlated with the activity and severity of ophthalmopathy.[12] Recent studies have indicated significantly elevated expression levels of insulin-like growth factor-1 (IGF-1) and the insulin-like growth factor-1 Receptor (IGF-1R), suggesting their potential involvement in TAO pathogenesis. It has also been shown that IGF-1R and TSHR can form functional complexes, synergistically promoting the accumulation of hyaluronic acid with cytokines, thus inducing inflammation and the expansion of orbital muscle and fat tissue.[3,9,26,27]

Another component of the etiopathogenesis of TAO is considered to be orbital fibroblasts. As a result of autoimmune reactions, these fibroblasts produce glycosaminoglycans, mainly hyaluronic acid. These hydrophilic molecules attract water, causing swelling of the orbital tissue and extraocular muscles. The increase in the volume of soft tissues within the orbit explains the accompanying symptoms of TAO, including proptosis, dysfunction of extraocular muscles, and compression of the optic nerve.[9]

The initial triggering factor for the immunologic reaction in TAO is the activation of auto-reactive T lymphocytes by the thyroid-stimulating hormone receptor (TSHR) on the surface of orbital fibroblasts. This process leads to the release of pro-inflammatory cytokines, including interleukins (IL). Interleukins are proteins that regulate the immune response in the body. In the pathogenesis of TAO, IL-1, IL-6, IL-10, IL-17, and IL-18 play a significant role in the inflammatory cascade.[3,4,6,10,12,17,22] IL-1 promotes the inflammatory process and cell adhesion, while IL-6 is involved in immune responses. It promotes the development of TH17 in naïve T lymphocytes under the influence of IL-23 and TGF-β. IL-17 is responsible for the recruitment of immune cells and contributes to tissue inflammation.[3,6] IL-18 is a pro-inflammatory cytokine that modulates the innate and

adaptive immune systems, stimulates the production of cytokines and chemokines. It activates the chemotaxis of neutrophils and lymphocytes and the production of interferon-γ (IFN-γ) by natural killer (NK) cells. Studies conducted by Myśliwiec et al. have shown that the levels of IL-18 in the serum of TAO patients were significantly higher than in control groups, and after glucocorticoid treatment, their levels decreased. There is limited research on the role of IL-18 in TAO, so further studies are needed to better understand its role.[3]

IL-6 is identified as a critical mediator in the etiopathogenesis of TAO. It activates fibroblasts to produce hyaluronan, leading to increased inflammation and tissue growth. The activation of fibroblasts simultaneously results in the overexpression of TSHR, thereby perpetuating the autoimmune response.[12] Studies have shown that serum IL-6 levels are elevated in patients with Graves' disease, particularly in those with TAO, and its levels correlate with disease activity.[3]

IL-17 recruits neutrophils to the orbital tissues, releasing reactive oxygen species and other inflammatory mediators. This exacerbates tissue damage and contributes to the clinical symptoms of TAO.[3,21] IL-10 is an anti-inflammatory cytokine and also plays a role in regulating the immune response.[6]

Initially, IL-38 was thought to have anti-inflammatory effects, but it seems to have such a role only at high concentrations. Therefore, the function of IL-38 is controversial. It is believed to limit inflammation in orbital fibroblasts in TAO patients.[3] IL-38 may have potential significance in the therapeutic process, but further research is needed to conclusively determine its action.

In TAO, the balance between pro-inflammatory and anti-inflammatory cytokines is disrupted, significantly contributing to increased and chronic inflammation and tissue remodeling in the orbital region. The precise identification of interleukins and understanding their roles in the inflammatory process in TAO could significantly contribute to the development of diagnostics and treatment for this complex disease.

In the pathogenesis of Graves' disease, 20–60% of patients have a positive family history of thyroid diseases. Patients with this disease also show an increased expression of certain human leukocyte antigen (HLA) genes more frequently than healthy individuals. The HLA-B8, DR3, DQA10501 haplotype may increase susceptibility to the disease. On the other hand, in patients with TAO, researchers have examined the frequency of major histocompatibility complex class II (MHC II) alleles in 81 Brazilian TAO patients and 161 healthy individuals in the control group. It was found that in TAO patients with greater involvement of extraocular muscles, the HLA-DRB116 allele is more common, while in patients with minimal involvement of extraocular muscles, the HLA-DRB1*03 allele is more frequent.[7,28] The role of genetic factors has also been demonstrated in studies conducted on twins and families.[9] In Danish population studies of monozygotic twins, the concordance coefficient was 35%, suggesting low penetrance of the implicated genes.[7] Several genes have been identified that predispose patients with Graves' disease to the development of TAO, but the diversity of the genetic profile of patients remains uncertain.[9] Conducted studies suggest an association between genetic predisposition and the manifestation of TAO. This provides further scope for development and the conduct of additional research to more precisely determine the genes involved in the disease process.

Additional interesting studies have been initiated on differences in the composition of gut microbiota in patients with Graves' disease, with and without TAO, as well as research on animals suggesting a crucial role of gut microbiota in TSHR-induced disease.[9] This opens up another field of research and may contribute to a better understanding and identification of factors involved in the occurrence of TAO.

Clinical presentation

The clinical picture of thyroid-associated ophthalmopathy (TAO) is typically characteristic in the majority of cases. Symptoms are mostly bilateral, with the disease process dominating on one side in 9-34% of patients; however, it remains bilateral in those cases [8]. Truly unilateral TAO occurs in only about 5% of cases.[1,8] The most common symptom of TAO is eyelid retraction, present in 90-98% of patients, often changing with gaze – a phenomenon known as Kocher's sign, where the upper border of the sclera is exposed when looking upward. Another nearly pathognomonic sign for TAO is lateral widening of the retracted eyelid contour. Several factors contribute to eyelid retraction, including increased sympathetic stimulation of the Müller muscle, contraction of the levator muscle, and scarring between the fascia of the lacrimal gland and the levator. Another frequent symptom in TAO patients is incomplete eyelid closure, leading to dryness, foreign body sensation, roughness, tearing, photophobia, and, consequently, corneal ulceration. Soft tissue swelling in the orbit causes visible proptosis and redness. This results in a disturbance in the relaxation of the extraocular muscles, leading to double vision. In severe cases with pronounced edema, compression of the optic nerve may occur, causing visual impairment up to blindness, observed in only 5% of patients.[1,2,7,29-32] TAO is the most common cause of adult-onset strabismus.[14]

Treatment

Given its strong association with Graves' disease, TAO treatment should begin with managing hyperthyroidism. Effective TAO treatment is not possible without controlling hyperthyroidism. In the treatment process,

the priority is visual disturbances, which, depending on the presence of inflammation, can be treated with corticosteroids, radiotherapy, or orbital decompression surgery. Another priority is controlling inflammation, which can be treated with corticosteroids, steroid-sparing immunosuppressive drugs, or radiotherapy. Strabismus and changes in appearance are often treated conservatively, waiting for inflammation to resolve before considering surgical intervention.[1,2,7,9,10,14,25] It is crucial for patients to quit smoking as there is a strong association between TAO and cigarette smoking.[8,33,34]

Systemic treatment relies significantly on glucocorticoids (GCS), with an estimated effectiveness of 40-60%. However, relapses at the end of therapy or during treatment are common. The use of pulsatile GCS administration has been developed to harness the immunosuppressive effects of GCS while simultaneously reducing side effects. Methylprednisolone, given in pulses over 12 weeks at a cumulative dose of 4.5 to 7.5g, has proven effective, with a higher dose effectively reducing the overall severity of the disease.[1,3,11,19,25,35-37] Protocols utilizing cumulative methylprednisolone doses exceeding 8g have been associated with severe, even fatal, liver toxicity and should not be routinely applied.[11,19,25,35] Studies have shown a significant advantage of intravenous methylprednisolone over orally administered prednisone, with the former demonstrating better clinical response and fewer adverse effects.[1,11,25,38]

Immunosuppressive treatment options include mycophenolate mofetil, cyclosporine A, and azathioprine, usually employed as adjunctive therapy for inadequate response to GCS.[7,39-41] A randomized study compared the effectiveness of monotherapy with GCS (methylprednisolone) to combined treatment with GCS and mycophenolate sodium for 24 weeks, followed by a 12-week observation. While patients receiving both drugs showed greater improvement, the benefits of combining GCS with mycophenolate were not significantly higher. Monotherapy with azathioprine did not significantly alter the course of the disease in moderate and severe TAO over a 2-year period, though later studies lacked sufficient statistical power for clear conclusions.[11,42,43] Studies involving cyclosporine A demonstrated that its use as monotherapy was less effective than oral prednisone. However, when used in combination, both drugs proved more effective than prednisone alone, potentially useful for patients unresponsive to GCS therapy.[25]

The pathogenesis of TAO is likely attributed to immune cells and cytokines; hence, monoclonal antibodies disrupting cytokine signaling may have therapeutic applications. Although only a few drugs have been tested in randomized studies, the results are promising. In one study, 152 TAO patients treated with rituximab (RTX) showed a significant reduction in symptoms and recurrence frequency, although resolution of proptosis or improvement in double vision was not described compared to baseline values. RTX effectively inactivates TAO and limits disease recurrence, proving effective even in patients resistant to GCS therapy.[11,19,38,44-46] The use of Tocilizumab (TCZ) in a randomized clinical trial resulted in disease inactivation in 93% of TAO patients compared to 59% receiving a placebo after 16 weeks. While TCZ reduced proptosis, improvement in double vision was observed in only 5-6% of patients. TCZ appears effective in patients resistant to conventional GCS therapy.[19,38,47] The further development of therapy using monoclonal antibodies appears to be crucial in refining conservative treatment methods, given the strong association between autoimmune processes and the occurrence of TAO.

Radiation therapy is another method of treating TAO. Assessing its effectiveness is challenging due to the lack of controlled studies and frequent concomitant use of GCS, significantly complicating retrospective data analysis. Orbital radiotherapy is effective only in patients with active disease and recent progression. Patients with inactive TAO do not respond to radiotherapy. Typically, a dose of 20 Gy per side is administered in 10 fractions over 2 weeks. During orbital radiotherapy, there may be a transient worsening of inflammation, which can be easily prevented by simultaneously using GCS. This method is often used as a supplementary treatment in systemic therapy. Although the effectiveness of radiotherapy in TAO has been questioned, it remains in constant use. It is a relatively safe method, with a low risk of optic neuropathy or retinopathy.[7,19,25,48,49]

Indications for surgical treatment in TAO include optic neuropathy, double vision, and corneal exposure. The goal of surgical intervention is orbital decompression, strabismus correction, and correction of eyelid abnormalities. The most appropriate sequence in surgical treatment is typically decompression of the orbit first, followed by strabismus surgery, and finally, eyelid procedures. The majority of surgeries are performed in a state of inactive TAO when the condition has been stable for at least 6-8 months. However, decompression may be necessary urgently in cases of compressive optic neuropathy or severe proptosis with corneal ulceration.[7,25,50] Orbital decompression involves removing parts of the bone building the orbit and removing fat from the orbit. Currently, the most common surgical approach includes medial wall, orbital floor, and/or lateral wall decompression. Simultaneous decompression of bones combined with fat removal is gaining importance due to its good clinical outcomes. Surgery on the extraocular muscles aims to alleviate double vision in the primary position and downward gaze. Eyelid surgery involves gradually weakening the Müller and levator palpebrae superioris muscles to better cover the cornea with the upper eyelid. In cases of significant retraction of the lower eyelid, a spacer graft may be necessary.[7,51]

Effective treatment of TAO is possible with various therapeutic methods. The choice of treatment depends on the severity of the disease, its activity, and the individual needs and preferences of the patient. Treating TAO poses a therapeutic challenge, but continuously improving methods help achieve successful treatment and limit the lasting consequences of the disease.

Quality of life of patients

TAO exerts a significant impact on the quality of life of patients. Visual disturbances such as double or blurred vision affect their daily functioning, greatly limiting both physical and professional activities. Patients report difficulties in driving and challenges in computer work. In a descriptive study conducted on patients with varying degrees of TAO, the quality of life was assessed using the short-form standardized questionnaire, the medical outcomes study (MOS-36). In the examined group of German TAO patients, low scores were observed on the MOS-36 scale, with clear differences compared to the control group noted in the areas of social functioning, mental health, health perception, and bodily pain. These results highlight the impact of common visual symptoms on health and well-being measured using the MOS-36 scale.[1,52] In other studies, it has been shown that in patients with Graves' disease who developed TAO, the quality of life decreased regardless of the treatment applied. It is also significant that patients with TAO returned to physical health within a year, but the recovery of mental health took twice as long.[53] In the course of TAO, exophthalmos often occurs, leading to a decrease in self-acceptance among patients and a deterioration in interpersonal relationships.[1] Considering the above, paying attention to the psychological aspects of TAO patients appears to be crucial.

Conclusion

Despite significant progress in understanding TAO, certain elements are still lacking. It is essential to emphasize the need for further development in clinical research to discover new biomarkers involved in the onset of TAO. This will be crucial for a better understanding of the disease's pathogenesis and, consequently, for the development and more precise selection of treatment, particularly using monoclonal antibodies. Such an approach should help limit the occurrence of irreversible consequences of TAO and, in turn, enhance the effectiveness of the conducted therapy. Both review literature and works based on clinical research were utilized to provide a broader perspective on the presented topic.

Declarations

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Author contributions

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Conceptualization, A.K.C., M.P.K. and I.M.; Methodology, M.D.; Software, A.K.C. and M.P.K.; Validation, M.D. and I.M.; Formal Analysis, A.K.C.; Investigation, M.D.; Resources, M.P.K.; Data Curation, I.M.; Writing – Original Draft Preparation, A.K.C.; Writing – Review & Editing, A.K.C., M.P.K., M.D. and I.M.; Visualization, M.D.; Supervision, I.M.; Project Administration, A.K.C.

Conflicts of interest

The author(s) declare no competing interests.

Data availability

Not applicable.

References

1. Şahlı E, Gündüz K. Thyroid-associated Ophthalmopathy. *Turk J Ophthalmol.* 2017;47(2):94-105. doi: 10.4274/tjo.80688
2. Moledina M, Damato EM, Lee V. The changing landscape of thyroid eye disease: current clinical advances and future outlook. *Eye.* 2024. doi:10.1038/s41433-024-02967-9
3. Zhang P, Zhu H. Cytokines in Thyroid-Associated Ophthalmopathy. *J Immunol Res*. 2022;2022:2528046. doi: 10.1155/2022/2528046
4. Feldon SE, Weiner JM. Clinical significance of extraocular muscle volumes in Graves' ophthalmopathy: a quantitative computed tomography study. *Arch Ophthalmol.* 1982;100(8):1266-1269. doi: 10.1001/archopht.1982.01030040244006
5. Nunery WR, Nunery CW, Martin RT, Truong TV, Osborn DR. The risk of diplopia following orbital floor and medial wall decompression in subtypes of ophthalmic Graves' disease. *Ophthal Plast Reconstr Surg.* 1997;13:153-60. doi: 10.1097/00002341-199709000-00001
6. Smith TJ, Hoa N. Immunoglobulins from patients with Graves' disease induce hyaluronan synthesis in their orbital fibroblasts through the self-antigen, insulin-like growth factor-I receptor. *J Clin Endocrinol Metab*. 2004;89:5076-5080. doi: 10.1210/jc.2004-0716
7. Maheshwari R, Weis E. Thyroid associated orbitopathy. *Indian J Ophthalmol*. 2012;60(2):87-93. doi: 10.4103/0301-4738.94048
8. Panagiotou G, Perros P. Asymmetric Graves' Orbitopathy. *Front Endocrinol (Lausanne).* 2020;11:611845. doi: 10.3389/fendo.2020.611845.
9. Bartalena L, Tanda M. Current concepts regarding Graves' orbitopathy. *J Intern Med*. 2022; 292(5):692-716. doi: 10.1111/joim.13524
10. Taylor P, Zhang L, Lee R, et al. New insights into the pathogenesis and nonsurgical management of Graves orbitopathy. *Nat Rev Endocrinol.* 2020;16(2):104-116. doi: 10.1038/s41574-019-0305-4.

11. Neag E, Smith T. 2021 Update on Thyroid-Associated Ophthalmopathy. *J Endocrinol Invest*. 2022;45(2):235-259. doi: 10.1007/s40618-021-01663-9
12. Turck N, Eperon S, De Los Angeles Gracia M. Thyroid--Associated Orbitopathy and Biomarkers. Where We Are and What We Can Hope for the Future. *Dis Markers*. 2018; 2018:7010196. doi: 10.1155/2018/7010196
13. Weiler D. Thyroid eye disease: a review. *Clin Exp Optom*. 2017;100(1):20-25. doi: 10.1111/cxo.12472
14. Hennein L, Robbins S. Thyroid-Associated Orbitopathy. Management and Treatment. *J Binocul Vis Ocul Motil.* 2022;72(1):32-46.
15. Lazarus J. H. Epidemiology of Graves' orbitopathy (GO) and relationship with thyroid disease. *Best Pract Res Clin Endocrinol Metab*. 2012;26(3):273-279. doi: 10.1016/j.beem.2011.10.005
16. Perros P, Hegedüs L, Bartalena L, et al. Graves' orbitopathy as a rare disease in Europe. a European group on Graves' orbitopathy (EUGOGO) position statement. *Orphanet Journal of Rare Diseases*. 2017;12(1):72. doi: 10.1186/s13023-017-0625-1
17. Chin YH, Ng CH, Lee MH et al. Prevalence of thyroid eye disease in Graves' disease. a meta-analysis and systematic review. *Clin Endocrinol*. 2020;93:363-374. doi: 10.1111/cen.14296
18. Ippolito S, Cusini C, Lasalvia P, et al. Change in newly diagnosed Graves' disease phenotype between the twentieth and the twenty-first centuries. meta-analysis and meta-regression. *J Endocrinol Invest*. 2021;44:1707-1718. doi: 10.1007/s40618-020-01479-z
19. Barbesino G, Salvi M, Freitag S. Future Projections in Thyroid Eye Disease. *J Clin Endocrinol Metab*. 2022;107(1):47-56. doi: 10.1210/clinem/dgac252
20. Bahn R. Graves' Ophthalmopathy. *N Engl J Med*. 2010; 362(8):726-738. doi: 10.1056/NEJMra0905750
21. Burch HB, Wartofsky L. Graves' ophthalmopathy: current concepts regarding pathogenesis and management. *Endocr Rev*. 1993;14:747-793. doi: 10.1210/edrv-14-6-747
22. Boschi A, Daumerie C, Spiritus M, et al. Quantification of cells expressing the thyrotropin receptor in extraocular muscles in thyroid associated orbitopathy. *Br J Ophthalmol*. 2005;89:724-729. doi: 10.1136/bjo.2004.050807
23. Hai YP, Lee ACH, Frommer L, Diana T, Kahaly GJ. Immunohistochemical analysis of human orbital tissue in Graves' orbitopathy. *J Endocrinol Invest*. 2020;43:123-137. doi: 10.1007/s40618-019-01116-4
24. Wakelkamp IM, Bakker O, Baldeschi L, Wiersinga WM, Prummel MF. TSH-receptor expression and cytokine profile in orbital tissue of active vs. inactive Graves' ophthalmopathy patients. *Clin Endocrinol*. 2003;58:280-287. doi: 10.1046/j.1365-2265.2003.01708.x
25. Ahn E, Subramanian P. Treatment modalities of thyroid related orbitopathy. *Indian J Ophthalmol*. 2014;62(10):999-1002. doi: 10.4103/0301-4738.145994
26. Tsui S, Naik V, Hoa N, et al. Evidence for an association between thyroid-stimulating hormone and insulin-like growth factor-1 receptors. a tale of two antigens implicated in Graves' disease. *J Immunol*. 2008;181:4397-4405. doi: 10.4049/jimmunol.181.6.4397
27. Krieger CC, Sui X, Kahaly GJ, Neumann S, Gershengorn MC. Inhibition of TSH/IGF-1 receptor crosstalk by teprotumumab as a treatment modality of thyroid eye disease. *J Clin Endocrinol Metab.* 2021;107(4):1653-1660. doi: 10.1210/clinem/dgab824
28. Akaishi P, Cruz A, Silva F. The role of major histocompatibility complex alleles in the susceptibility of Brazilian patients to develop the myogenic type of Graves' orbitopathy. *Thyroid*. 2008;18(4):443-447. doi: 10.1089/thy.2007.0194
29. Wiersinga WM, Kahaly GJ. Graves orbitopathy. A multidisciplinary approach. *J Clin Endocrinol Metab.* 2001;86(2):501-503. doi: 10.1210/jcem.86.2.7338
30. Gilbard JP, Farris RL. Ocular surface drying and tear film osmolarity in thyroid eye disease. *Acta Ophthalmol (Copenh)*. 1983;61:108-116. doi: 10.1111/j.1755-3768.1983.tb01401.x
31. Dickinson AJ, Perros P. Controversies in the clinical evaluation of active thyroid-associated orbitopathy. Use of a detailed protocol with comparative photographs for objective assessment. *Clin Endocrinol (Oxf)*. 2001;55:283-303. doi: 10.1046/j.1365-2265.2001.01349.x
32. Frueh BR, Musch DC, Garber FW. Lid retraction and levator aponeurosis defects in Graves' eye disease. *Ophthalmic Surg*. 1986;17:216-220.
33. Cawood TJ, Moriarty P, O'Farrelly C, O'Shea D. Smoking and thyroid-associated ophthalmopathy. A novel explanation of the biological link. *J Clin Endocrinol Metab*. 2007;92:59-64. doi: 10.1210/jc.2006-1824
34. Krassas GE, Wiersinga W. Smoking and autoimmune thyroid disease. The plot thickens. *Eur J Endocrinol.* 2006;154:777-780. doi: 10.1530/eje.1.02157
35. Marino M, Morabito E, Brunetto R. Acute and severe liver damage associated with intravenous glucocorticoid pulse therapy in patients with Graves' ophthalmopathy. *Thyroid.* 2004;14(5):403-406. doi: 10.1089/105072504774193276
36. Nagayama Y, Izumi M, Kiriyama T, et al. Treatment of Graves' ophthalmopathy with high-dose intravenous methylprednisolone pulse therapy. *Acta Endocrinol (Copenh)*.1987;116(4):513-518. doi: 10.1530/acta.0.1160513
37. Dandona P, Havard CW, Mier A. Methylprednisolone and Graves' ophthalmopathy. *BMJ*. 1989;298(6676):830-830. doi: 10.1136/bmj.298.6676.830
38. González-García A, Sales-Sanz M: Treatment of Graves' ophthalmopathy. *Med Clin (Barc)*. 2021;156(4):180-186. doi: 10.1016/j.medcli.2020.07.031
39. Ye X, Bo X, Hu X, et al. Efficacy and safety of mycophenolate mofetil in patients with active moderate-to-severe Graves' orbitopathy. *Clin Endocrinol (Oxf)*. 2017;86(2):247-255. doi: 10.1111/cen.13170

40. Kahaly GJ, Riedl M, Konig J, et al. European Group on Graves' Orbitopathy (EUGOGO). Mycophenolate plus methylprednisolone versus methylprednisolone alone in active, moderate-to-severe Graves' orbitopathy (MINGO): a randomised, observer-masked, multicentre trial. *Lancet Diabetes Endocrinol.* 2018;6(4):287-298. doi: 10.1016/S2213-8587(18)30020-2
41. Bartalena L, Kahaly GJ, Baldeschi L, et al. The 2021 European Group on Graves' orbitopathy (EUGOGO) clinical practice guidelines for the medical management of Graves' orbitopathy. *Eur J Endocrinol.* 2021;185(4):43-67. doi: 10.1530/EJE-21-0479
42. Kahaly G, Riedl M, König J. Mycophenolate plus methylprednisolone versus methylprednisolone alone in active, moderate-to-severe Graves' orbitopathy (MINGO): a randomised, observer-masked, multicentre trial. *Lancet Diabetes Endocrinol*. 2018;6(4):287-298. doi: 10.1016/S2213-8587(18)30020-2
43. Kelley W, Rosenbloom F, Seegmiller J. The effects of azathioprine (imuran) on purine synthesis in clinical disorders of purine metabolism: *J Clin Invest*. 1967;46(9):1518-1529. doi: 10.1172/JCI105643.
44. Salvi M, Vannucchi G, Beck-Peccoz P. Potential utility of rituximab for Graves' orbitopathy. *J Clin Endocrinol Metab.* 2013;98(11):4291-4299. doi: 10.1210/jc.2013-1804
45. Silkiss RZ, Reier A, Coleman M, Lauer SA: Rituximab for thyroid eye disease. *Ophthalmic Plast Reconstr Surg*. 2010;26(5):310-314. doi: 10.1097/IOP.0b013e3181c4dfde
46. Chen J, Chen G, Sun H. Intravenous rituximab therapy for active Graves' ophthalmopathy: a meta-analysis. *Hormones (Athens).* 2021;20(2):279-286. doi: 10.1007/s42000-021-00282-6
47. Moreiras J, Gomez-Reino J, Perez-Pampis. Efficacy of Tocilizumab in Patients with Moderate-to-Severe Corticosteroid-Resistant Graves Orbitopathy. A Randomized Clinical Trial. *Am J Ophthalmol*. 2018;195:181-190. doi: 10.1016/j.ajo.2018.07.038
48. Bartley G, Gorman C. Perspective--Part I: the Mayo Orbital Radiotherapy for Graves Ophthalmopathy (ORGO) study lessons learned. *Ophthalmic Plast Reconstr Surg*. 2002;18(3):170-172. doi: 10.1097/00002341-200205000-00002
49. Grassi P, Strianese D, Piscopo R, Pacelli R, Bonavolonta G. Radiotherapy for the treatment of thyroid eye disease--a prospective comparison is orbital radiotherapy a suitable alternative to steroids. *Ir J Med Sci.* 2017;186(3):647-652. doi: 10.1007/s11845-016-1542-3
50. Finn AP, Bleier B, Cestari DM, et al. A retrospective review of orbital decompression for thyroid orbitopathy with endoscopic preservation of the inferomedial orbital bone strut. *Ophthalmic Plast Reconstr Surg.* 2017;33(5):334-339. doi: 10.1097/IOP.0000000000000782
51. Rootman DB, Golan S, Pavlovich P, Rootman J. Postoperative changes in strabismus, ductions, exophthalmometry, and eyelid retraction after orbital decompression for thyroid orbitopathy. *Ophthalmic Plast Reconstr Surg*. 2017;33(4):289-293. doi: 10.1097/IOP.0000000000000758
52. Kahaly GJ, Hardt J, Petrak F, Engle UT. Psychosocial Factors in Subjects with Thyroid-Associated Ophthalmopathy. *Thyroid*. 2002;12(3):237-239. doi: 10.1089/105072502753600205
53. Abraham-Nordling M, Wallin G, Traisk F, et al. Thyroid-associated ophthalmopathy; quality of life follow-up of patients randomized to treatment with antithyroid drugs or radioiodine. *Eur J Endocrinol.* 2010;163(4):651-657. doi: 10.1530/EJE-10-0475

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CASUISTIC PAPER

From diagnosis to recovery – a detailed case report on a nail bed glomus tumor

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ABSTRACT

Introduction and aim. Glomus tumors represent benign neoplastic proliferations of the glomus body, an integral thermoregulatory component within the cutaneous microvasculature. Although they can manifest at various anatomical sites, they are predominantly observed in the subungual region. The tumors present as painful, firm, purplish, solitary nodules of the extremities, especially in the nail bed. They may be solitary or multiple; solitary lesions are encapsulated and most commonly subungual, while multiple tumors are unencapsulated and rarely subungual.

Description of the case. Here, we present a case of multiple glomus tumors of the right hand in an 18-year-old female who presented with complaints of painful bluish discoloration of the right little finger for six years and progressive asymptomatic nodules on the right index finger for six months. A clinical examination revealed acute tenderness in both fingers. Love's pin test and Hildreth's test were positive. Excision of all lesions was done and sent for histopathology, which confirmed the diagnosis. The patient was symptom-free immediately following surgery. No nail deformities were noticed, and there was no recurrence of symptoms after one year of follow-up.

Conclusion. The transungual approach is a safe and effective minimally invasive surgical technique for the treatment of symptomatic nail bed glomus tumors. It offers high success rates, promising cosmetic outcomes, and minimal complications. However, careful patient selection and meticulous surgical technique are essential to avoid potential nail deformities.

Keywords. glomus tumor, subungual, transungual nail excision

Introduction

Glomus tumors denote benign neoplastic proliferations originating from the glomus body, a pivotal thermoregulatory entity within the cutaneous vasculature.[1] Clinically, these lesions manifest as indurated, violaceous nodules situated predominantly in the extremities, with a predilection for the nail bed. They may be solitary or multifocal. The key to diagnosing this condition is to be highly suspicious and conduct a thorough clinical examination.

Aim

To evaluate the feasibility and safety of the transungual technique for the complete excision of glomus tumors of the nail bed.

Description of the case

We report a case of an 18-year-old Indian female who presented to the outpatient department of Dermatology with complaints of painful bluish discoloration of the right little finger for six years and progressive asymptomatic nodules on the right index finger for six months. She gave a history of aggravation of pain upon

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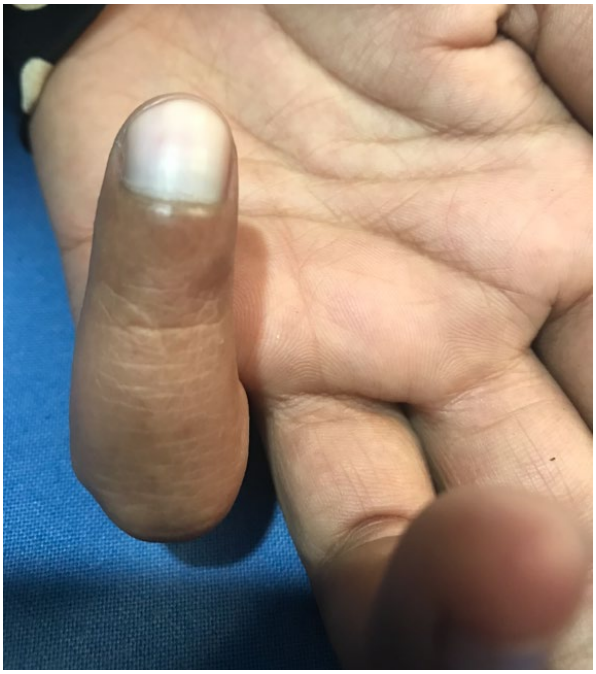

Fig. 1. A solitary 1–2 cm bluish palpable, tender, round, uniform swelling present subungually, involving the phalanx of the right little finger

exposure to cold; however, there was no characteristic triphasic color change as seen in Raynaud's phenomenon. There was no history of preceding trauma. There was no similar history in any of the immediate family members. She had been taking a homeopathic treatment in the form of topical creams and oral medications for three months prior to presentation, but had no significant relief. Examination of the hands revealed a solitary 1–2 cm bluish, palpable, painful,

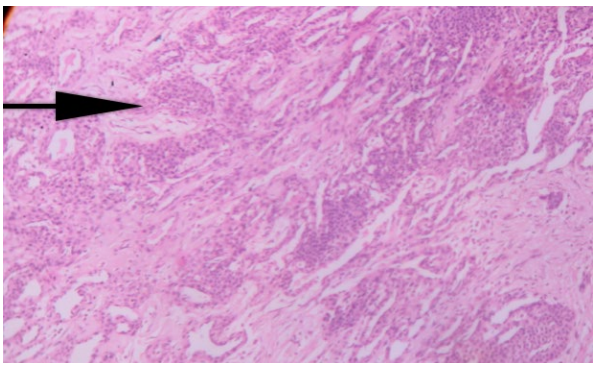

Fig. 3. Multiple dilated thin-walled blood vessels with surrounding clusters of glomus cells (H&E ×40)

round, uniform swelling present subungually, involving the phalanx of the right little finger (Fig. 1) and bluish discoloration seen over the right index finger. Obliteration of Lovibond angle with fluctuation of the nail bed and pseudo-clubbing was present. On bedside clinical examination, Love's pin test, Ice cube test, and Hildreth's test were positive. USG color Doppler revealed a well-defined hypo-echoic lesion measuring (1 × 0.4 cm) in the periungual region of the right little finger with significant internal vascularity. A clinical diagnosis of glomus tumor was made, and the patient was planned for nail avulsion surgery. Proximal nail avulsion and surgical excision of all glomus tumors were performed utilizing the transungual approach (Fig. 2), and the tissue was sent for histopathological examination, which showed numerous dilated thin-walled vascular spaces surrounded by glomus cells. These are sheets of uniform monomorphic cells with round punched-out central nuclei in a pale to eosin-

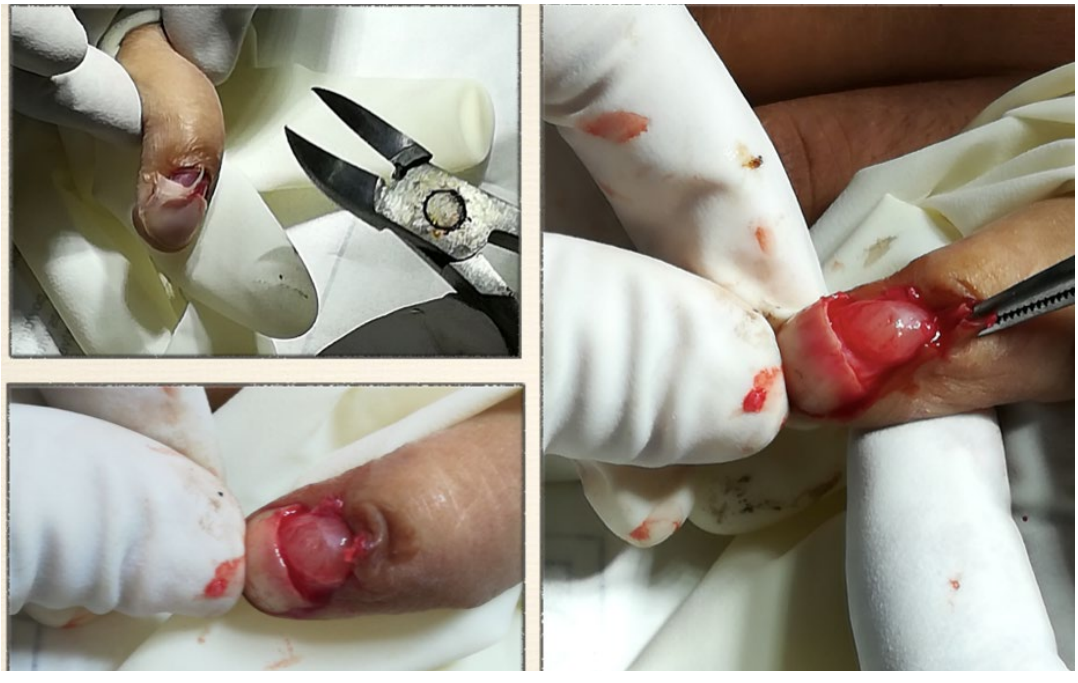

Fig. 2. Tumor resection using transungual approach with careful preservation of the nail bed

ophilic cytoplasm and well-defined margins (Fig. 3). The patient was symptom-free following surgery and developed no nail deformity or recurrence in the next year of follow-up.

Discussion

Glomus tumor is a benign vascular hamartoma that originates from the neuromyoarterial cells of endothelium-lined vascular spaces (the Sucquet-Hoyer canal).[1] It accounts for 1–5% of soft tissue tumors of the hand.[2] It typically presents with a triad of cold hypersensitivity, pain and tenderness, and occasionally nail deformities or nail discoloration.[3] The tumor usually presents as a painful, firm, purplish, solitary nodule of the extremities, especially in the nail bed. Multiple glomus tumors are described as softer, more compressible, bluish nodules and they occur with less frequency than solitary tumors. Infrequently, atypical glomus tumors have been reported to involve other sites, including muscles, joints, head and neck, stomach, penis, and rarely the mediastinum.[4,5] Surgical excision is the treatment of choice, although radiotherapy has been advocated as a primary or adjunctive modality.[6] The approach is decided according to the location of the lesion and described as periungual, paraungual and transungual. Various published reports have stated that the transungual approach is associated with subsequent nail deformity.[6,7] Jawalkar H et al., in a study of 12 glomus tumors treated with transungual excision, reported to have no recurrence or new nail deformity.[8] Subungual glomus tumors are more difficult to treat because they are small, and their total eradication requires several procedures. Local recurrences occur due to inadequate excision because of infiltrative growth and local invasion of the capsule around the glomus tumor have been reported in only 1–2% of cases. Other treatment options include sclerotherapy and laser therapy with CO_2, KTP, Nd: YAG, and pulsed dye laser, but with variable results. Prompt diagnosis and early institution of appropriate therapy help to significantly lower patient morbidity.

Conclusion

Glomus tumors of the nailbed, while seemingly small, can cause significant discomfort and disruption to daily life. A high index of suspicion and careful clinical examination is the crux of diagnosing this condition. Delayed or misdiagnosis and improper management result in undue suffering for the patient. Though bilateral glomus tumors are rare, the possibility should not be excluded in bilateral unexplained digital pain. Symptomatic glomus tumors are successfully treated with surgical excision. The transungual approach offers a minimally invasive solution with high success rates and promising cosmetic outcomes. While meticulous technique and potential for nail deformities exist, careful patient selection and experienced surgeons can ensure a positive experience.

Declarations

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Author contributions

Conceptualization, H.I.S.; Methodology, H.I.S..; Validation, H.I.S.; Formal Analysis, H.I.S.; Investigation, H.I.S.; Resources, H.I.S..; Data Curation, H.I.S.; Writing – Original Draft Preparation, H.I.S..; Writing – Review & Editing, H.I.S..; Visualization, H.I.S.

Conflicts of interest

The author discloses no conflicts of interest.

Data availability

Not applicable.

Ethics approval

The patient gave written informed consent for inclusion before participation in the study.

References

1. Chou T, Pan SC, Shieh SJ, Lee JW, Chiu HY, Ho CL. Glomus Tumor: Twenty-Year Experience and Literature Review. *Ann Plast Surg*. 2016;76(1):35-40. doi: 10.1097/SAP.0000000000000684
2. Mitchell A, Spinner RJ, Ribeiro A, Mafra M, Mouzinho MM, Scheithauer BW. Glomus tumor of digital nerve: case report. *J Hand Surg Am*. 2012;37(6):1180-1183. doi: 10.1016/j.jhsa.2012.02.035
3. Kim SW, Jung SN. Glomus tumour within digital nerve: a case report. *J Plast Reconstr Aesthet Surg*. 2011;64(7):958-960. doi: 10.1016/j.bjps.2010.11.012
4. Altorjay A, Arató G, Adame M, et al. Synchronous multiple glomus tumors of the esophagus and lung. *Hepatogastroenterology*. 2003;50(51):687-690.
5. Kiyosawa T, Umebayashi Y, Nakayama Y, Soeda S. Hereditary multiple glomus tumors involving the glans penis. A case report and review of the literature. *Dermatol Surg*. 1995;21(10):895-899. doi: 10.1111/j.1524-4725.1995.tb00719.x
6. Singhal S, Sharma S, Dixit S, De S, Chander S, Rath GK. Primary radiation therapy in the management of glomus tumours. *Indian J Cancer*. 1993;30(3):120-124.
7. Ghaly RF, Ring AM. Supraclavicular glomus tumor, 20 year history of undiagnosed shoulder pain: a case report. *Pain*. 1999;83(2):379-382. doi: 10.1016/s0304-3959(99)00094-9
8. Jawalkar H, Maryada VR, Brahmajoshyula V, Kotha GK. Subungual glomus tumors of the hand: Treated by transungual excision. *Indian J Orthop*. 2015;49(4):403-407. doi: 10.4103/0019-5413.159611

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CASUISTIC PAPER

An anomalous case report of canine impaction resultant of supernumerary fusion to mandibular incisor

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ABSTRACT

Introduction and aim. Anomalies in dental characteristics such as size, shape, number, structure, and eruption are commonly observed in clinical conditions. One such anomaly is the presence of supernumerary teeth found in various regions of the dental arch. Although frequently asymptomatic, supernumerary teeth are routinely identified during radiographic evaluations. Among the most common causes of impacted maxillary incisors is the presence of supernumerary teeth.

Description of the case. Herein, we present a rare case of lower left canine impaction subsequent to a supernumerary fusion to the mandibular left incisor in a 10-year-old Caucasian female.

Conclusion. This case contributes to the current knowledge regarding supernumerary fused teeth, emphasizing the importance of early intervention and multidisciplinary collaboration in effectively managing such developmental dental irregularities.

Keywords. fusion, impacted maxillary incisors, supernumerary tooth

Introduction

The development of occlusion and tooth eruption in pediatric dentistry often presents complex challenges characterized by deviations from the typical eruption sequence, positional irregularities, and tooth morphology abnormalities.[1,2] Various factors can influence occlusion development that disrupt tooth alignment and the harmonious relationships with neighboring teeth. Dental anomalies of numbers, particularly supernumerary teeth, have been commonly associated with delayed eruption of maxillary incisors.[1]

Supernumerary teeth, defined as teeth exceeding the standard set, have been documented since ancient times, with descriptions dating back to 23–70 A.D.[1,2] Hyperdontia, characterized by supernumerary teeth exceeding the average tooth count, is a developmental anomaly with various etiologies.[1-17] It predominantly occurs in the maxilla, with rare cases reported in the mandible.[5,6] Prevalence rates in the general population are from 0.15 to 1.9%, with a higher occurrence among males than females.[10] Occurrence rates of supernumerary teeth vary among different racial and ethnic groups.[5] In the Caucasian population, the reported prevalence ranges between 1 and 3%.[5] Among Caucasians, 90–98% of supernumerary teeth occur in the premaxillary region.[4] Asians exhibit a slightly higher frequency, exceeding 3%.[4] Black children show a prevalence of 0.42%, while children of Hispanic descent have a preponderance of 5.6%.[5] In permanent dentition, the prevalence ranges from 0.15 to 1%, with a male predominance ratio

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of 2:1.[6] Despite extensive research, the etiology of supernumerary teeth remains uncertain. However, evidence suggests a genetic basis, supported by the higher occurrence of hyperdontia among related families and its association with syndromes such as Gardner's syndrome and cleidocranial dysostosis.[12]

Supernumerary teeth can be classified according to their location, morphology and orientation.[15] According to the position of supernumerary teeth, they can be categorized as mesiodens, paramolar, and distomolar.[15] One of the most common types of supernumerary teeth are distomolars presented in the OPG below (Fig. 1).

Mesiodens are the most prevalent type of supernumerary tooth and are attributed to many complications such as maxillary central incisors impaction.[11] The mere presence of a supernumerary tooth does not solely account for delayed eruption. The shape, number, and position of supernumerary teeth play significant roles in the eruption process.[1-17] Four morphological types have been identified: conical or peg-shaped, tuberculate or invaginated, supplemental or incisiform, and odontoma-like.[16] Notably, tuberculate or invaginated supernumerary teeth have shown a higher correlation with delayed eruption of maxillary incisors.[11-16] Therefore, a thorough determination of etiology and the development of appropriate treatment plans become crucial when incisors fail to erupt within the expected timeframe.

The presence of supernumerary teeth in the anterior maxillary region often leads to various complications, including prolonged retention of deciduous teeth,

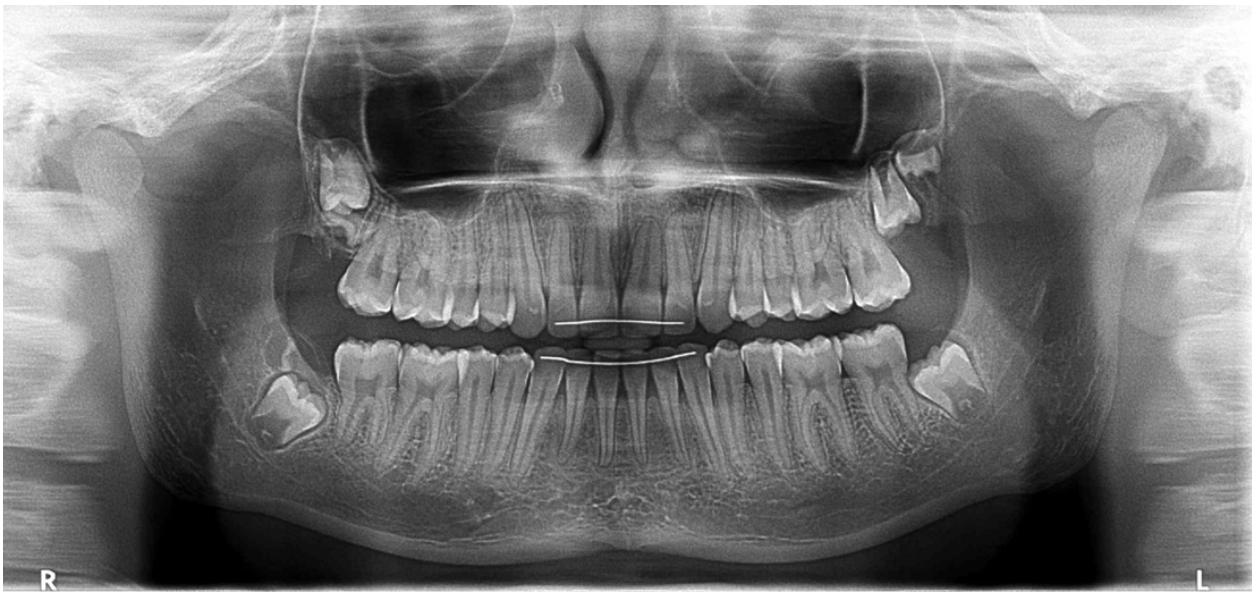

Fig. 1. Panoramic radiograph showing fourth molars in the maxilla and right distomolar in the mandible-growing horizontally

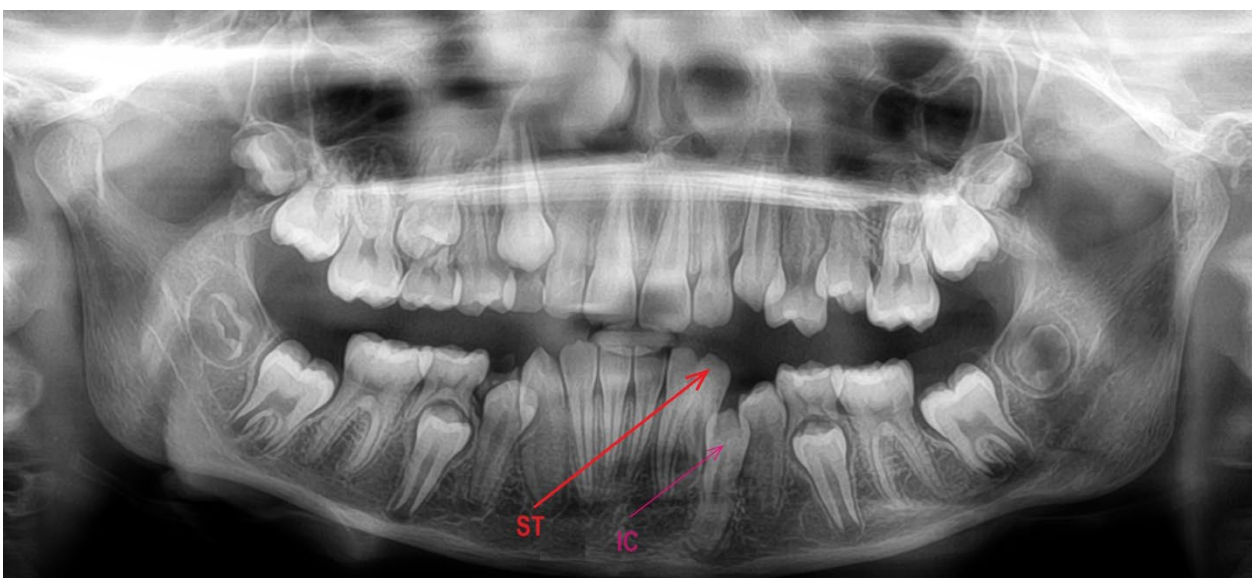

Fig. 2. Panoramic radiograph of a 10-year-old female patient showing supernumerary tooth (ST) fusion with 32 and the impaction of tooth 33 (IC): IC – impacted canine, ST – supernumerary tooth

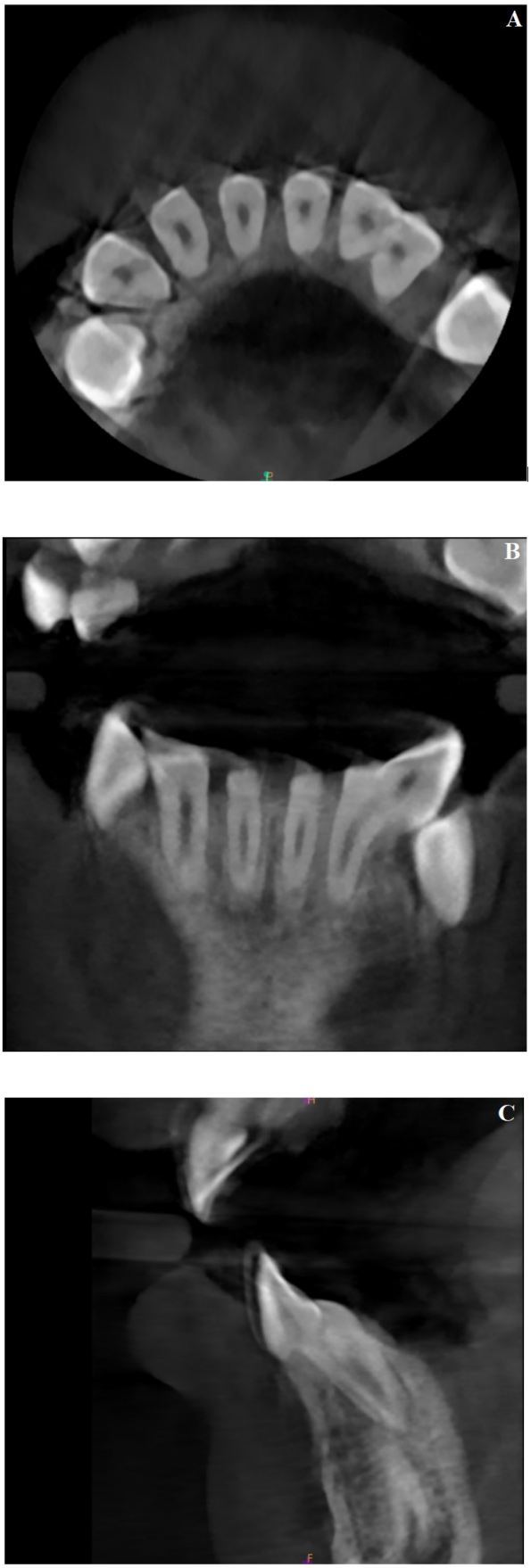

Fig. 3. A–C: CBCT scans of a 10-year-old female patient confirming the supernumerary tooth fusion to tooth number 32

delayed eruption of permanent teeth, impaction of permanent incisors, ectopic eruption, root dilaceration, and other forms of malformation.[1-18] Such complications pose unique challenges for dental professionals. Literature reports analogous cases of supernumerary tooth fusion to permanent dentition and subsequent impaction of permanent teeth. The aforementioned published literature emphasizes the possibility of complications and the need for multidisciplinary treatment modalities.[4,9,13-18] Given the significant implications of hyperdontia and tooth impaction, this research article presents a case study that offers valuable insights into the impact of supernumerary teeth on the eruption of permanent teeth.

Aim

This study aims to present a unique case report of canine impaction resultant of supernumerary fusion to mandibular incisor. We discuss a multidisciplinary approach, incorporating surgical intervention, to resolve the impaction and achieve proper alignment within the dental arch.

Description of the case

A 10-year-old Caucasian female non-syndromic patient was presented to the Department of Dental Surgery with a case of permanent lower left canine impaction. The patient has not been treated orthodontically previously. As first a general dentist revealed the disturbances and asymmetry in eruption of canines. The patient's legal guardian's consent was taken, and no medical deviations were noted. Familial history was not contributory. The presence of a canine was already detected on the right side of the mandible. The patient did not report pain on the left side of her mandible. An extraoral examination did not reveal any facial asymmetry. Clinical intraoral examination revealed the presence of an asymptomatic supernumerary tooth in the area of the left anterior mandible and a slight painless protrusion of the bone in the area of the missing, unerupted tooth 33 without symptoms of bone crepitation. A panoramic radiograph (OPG) and cone-beam computed tomography (CBCT) scan confirmed the supernumerary tooth fusion to tooth number 32, resulting in tooth number 33 impaction (Fig. 2) and (Fig. 3), respectively. A CBCT scan helped assess the degree of tooth fusion and the precise planning of the surgical procedure. It turned out to be a complete fusion of the crown of the supernumerary tooth to the crown of tooth number 32 and a fusion of their beginning root compartments. A treatment plan was proposed and initiated after thorough communication with the patient and their legal guardian. The patient was provided with all the associated risks, including follow-up treatment modalities if the impacted tooth 33 did not erupt spontaneously within a few months. Failure of eruption would require surgical exposure of the

unerupted tooth and orthodontic treatment to re-establish sufficient arch space. A surgical management approach was adopted; the supernumerary tooth fused with 32 was surgically removed. The surgical procedure was performed under infiltration anesthesia with 4% articaine with norepinephrine without detachment of the mucoperiosteal flap. The fused fragment of the crown of the supernumerary tooth was cut off using a narrow drill in the area involved by enamel, and the initial fragments of the roots of both teeth were separated. Supernumerary tooth was removed (Fig. 4). The place of cut off was thoroughly smoothed. The prognosis regarding the post-surgical pulp vitality of tooth 32 was favorable. The enamel-dentin junction after the supernumerary tooth cut off was not exposed and thus, did not require any additional protection. The socket was secured with resorbable sutures. Routine postoperative indications were given. Surgical recovery of the tooth extraction was uneventful. Within a few months after the procedure, impacted tooth 33 erupted spontaneously. The patient currently does not require orthodontic treatment. No consequential loss of dental arch space was detected. Post-surgical pulp vitality tests of the remaining tooth did not show any disturbances. A long-term clinical observation did not reveal any post-surgical complications as well as any other disturbances.

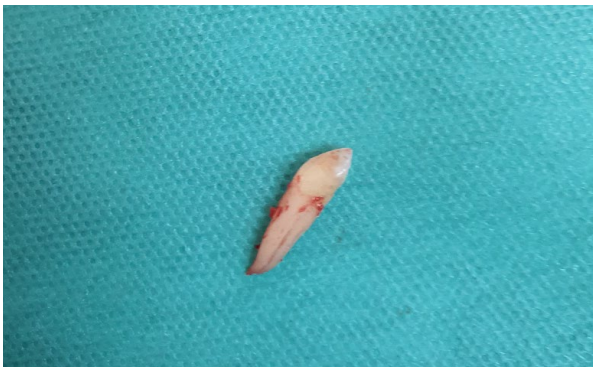

Fig. 4. Supernumerary tooth after extraction

Discussion

Supernumerary teeth are a prevalent developmental dental anomaly resulting from factors such as hyperactivity of the dental lamina, dichotomy, environmental influences, or polygenetic processes of atavism.[1-12] These additional teeth often present classical oral complications, including the impaction of adjacent teeth, dental crowding, diastema formation, tooth rotation, displacement, and occlusal interference.[14-18] Dental fusion, clinically noted as a wide tooth, is a morphological dental anomaly associated with supernumerary teeth.[13-14] Fused teeth present with a distinct unaesthetic tooth morphology and demand for differential diagnosis.[14] Literature reports that fusion's prevalence is rare in posterior and permanent teeth and more predominant in primary dentition, often involving incisors and canines.[14] The etiology of fused teeth remains speculated; many theories consider developmental, environmental factors, genetic factors, trauma or inflammation.[1-14] The presence of fused teeth is attributed to a high affinity for spacing, periodontal problems and dental caries.[14] Hence, extensive radiographic and clinical evaluation is warranted for patients diagnosed with supernumerary and fused dentition to avert the aforementioned dental complications.

Herein, we present a case of a 10-year-old female patient attributed with both dental abnormalities, 33 canine impaction, and supernumerary fusion with tooth number 32. The etiological development of her supernumerary tooth was not clear. Additionally, no associated conditions such as a cleft lip and palate or syndromes such as cleidocranial dysplasia and Gardner's syndrome were reported. A panoramic radiograph (OPG) and cone-beam computed tomography (CBCT) scan were utilized to diagnose this complex case. CBCT imaging was a preferred mode of radiological investigation, in hindsight of its maxillofacial imaging and low radiation. Moreover, CBCT imaging aided in the diagnosis of dental fusion due to its ability to display the root canal systems and teeth morphology. In this case, CBCT imaging showed the conjunction of the crowns of 32 and the supernumerary tooth and the presence of separate pulp canals. CBCT imaging differential diagnoses confirmed fusion, not gemination, associated with a single pulp canal. The supernumerary tooth was confirmed to have an incisiform morphology.

Proper diagnosis of supplementary fusion with thorough radiographic examination is a prerequisite for successful surgical management.[16-18] As observed in this case; surgical intervention was justified due to the interference of 32 and supernumerary tooth fusion in the eruption of 33. Supplementary fusion warrants a list of complications; prudent management of these teeth is necessary to maintain normal eruption and position patterns of adjacent permanent incisors.[15-18] In such cases, immediate surgical intervention is recommended unless the supernumerary tooth is a non-inverted conical type or is positioned above the apices of an adjacent permanent tooth.[16-18] Supplementary fusion imposes the challenge of mid-root connections between the root canals of fused teeth.[17] Reported case studies describe the need for endodontic treatment, with a poor prognosis of pulp vitality. Additionally, supplementary fusion incorporating the root canals increases the risk of pulpal infection.[17] In the present case, there was a low risk of iatrogenic damage as only the complete fusion of the crown and initial fusion of root compartments was detected.

The eruption of an impacted tooth post-surgical removal of a supplementary tooth calls for additional vigilance. The described case showed no significant delays in the eruption of impacted tooth 33, as it erupted

spontaneously within a few months. Literature, however, reports many instances of delayed eruption due to supernumerary involvement.[5-18] Optimal treatment modalities in such cases are offered, including surgical exposure of the unerupted tooth and orthodontic treatment to re-establish sufficient arch space.[16-18] Another treatment option is only to observe the unerupted tooth if it is symptomless and is not impacting in any way the dentition.[16-18] Delayed eruption post supernumerary removal may occur within 18 months if the tooth has arch space and is not excessively displaced.[18] This approach may, however, impose potential complications, such as crowding leading to a loss of dental arch space, midline shift, and the loss of eruptive force of adjacent teeth.[16-18] Interceptive treatment is recommended if delayed eruption patterns are detected; therefore, follow-up appointments post-supernumerary removal are crucial for clinically detecting such a complication.

Conclusion

Supplementary fusion, leading to the impaction of a tooth, is a complicated case and requires a cautious and multispecialty approach from the clinician to provide the patient with a successful outcome. Hence, early surgical and orthodontic intervention is often necessary to minimize the side effects of delayed tooth eruption due to supernumerary teeth. The diagnostic tool, CBCT imaging, is recommended to detect the degree of root canal involvement in fused supplementary dentition. This case is exemplary in enhancing our understanding of the implications of supernumerary teeth on dental development and eruption patterns. Unlike other presented cases, the described patient is atypical as she had no associated syndrome; this alludes to the need for vigilance from clinicians when screening for fused supernumerary teeth. Our case underscores the significance of early diagnosis, interdisciplinary collaboration, and appropriate treatment planning in effectively managing dental anomalies associated with impaction due to supernumerary teeth.

Declarations

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Author contributions

Conceptualization, Oles. M., A.K., Olen. M. and K.B.; Methodology, Oles. M., A.K., Olen. M. and K.B.; Formal Analysis, Oles. M., A.K., Olen. M. and K.B.; Investigation, Oles. M., A.K., Olen. M. and K.B.; Resources, Oles. M., A.K., Olen. M. and K.B.; Data Curation, K.B..; Writing – Original Draft Preparation, Oles. M., A.K. and Olen. M.; Writing – Review & Editing, Oles. M. and K.B..; Supervision, K.B.

Conflicts of interest

The authors declare no competing interests.

Data availability

No datasets were generated or analyzed during the current study.

Ethics approval

Written informed consent for publication was obtained from the patient's parent. We complied with the policy of the journal on ethical consent.

References

1. Primo LG, Wilhem RS, Bastos EPS. Frequency and characteristics of supernumerary teeth in Brazilian children: consequences and proposed treatments. *Rev Fac Odont S Paulo*. 1997;1:231-237.
2. Babu V, Kikkeri SN, Diwakar NR. A rare case of Hereditary Multiple impacted normal and supernumerary teeth. *J Clin Pediatr Dent*. 1998;23:59-62.
3. Solares R. The complications of late diagnosis of anterior supernumerary teeth: case report. *J Dent Child*. 1990;57:209-211.
4. Tay F, Pang A, Yuen S. Unerupted maxillary anterior supernumerary teeth: report of 204. *J Dent Child*. 1984;51:289-294.
5. Barton DH, Murray C. Ethnic and racial relationships and supernumerary teeth in the primary dentition. *J Indiana Dent Assoc*. 1977;56:32-33.
6. Ferreira EF, Nascimento de F, Santos RL, et al. Prevalence of dental anomalies: Supernumerary teeth in the maxillary anterior region. *Revista brasileira de odontologia*. 2012;69(1):112-115.
7. Vi de Almeida CM, Gomide MR. Prevalence of natal/neonatal teeth in cleft lip and palate infants. *Cleft Palate-Craniofac J*. 1996;33:297-299.
8. Kramer RM, Williams AC. The incidence of impacted teeth. A survey at Harlem Hospital. *Oral Surg Oral Med Oral Pathol*. 1970;29:237-241.
9. Bodenham RS. The treatment and prognosis of unerupted maxillary incisors associated with the presence of supernumerary teeth. *Br Dent J*. 1967;123:173-177.
10. Van Buggenhout G, Bailleul-Forestier I. Mesiodens. *Eur J Med Genet*. 2008;51(2):178-181.
11. Alberti G, Mondani PM, Parodi V. Eruption of supernumerary permanent teeth in a sample of urban primary school population in Genoa, Italy. *Eur J Paediatr Dent*. 2006;7(2):89-92.
12. Townsend GC, Richards L, Hughes T, et al. Epigenetic influences may explain dental differences in monozygotic twin pairs. *Aust Dent J*. 2005;50(2):95-100. doi: 10.1111/j.1834-7819.2005.tb00347.x
13. Nayak G, Shetty S, Singh I, Pitalia D. Paramolar - A supernumerary molar: A case report and an overview. *Dent Res J (Isfahan)*. 2012;9(6):797-803.

14. Zhu M, Liu C, Ren S, Lin Z, Miao L, Sun W.. Review Article Fusion of a Supernumerary Tooth to Right Mandibular Second Molar: A Case Report and Literature Review. *Int J Clin Exp Med*. 2015;8:11890-11895.
15. Ata-Ali F, Ata-Ali J, Peñarrocha-Oltra D, Peñarrocha-Diago M. Prevalence, Etiology, Diagnosis, Treatment and Complications of Supernumerary Teeth. *J Clin Exp Dent*. 2014;6(4):414-418. doi: 10.4317/jced.51499
16. Yeluri R, Hegde M, Baliga S, Munshi AK. Multiple Supernumerary Teeth Associated with an Impacted Maxillary Central Incisor: Surgical and Orthodontic Management. *Contemp Clin Dent*. 2012;3(2):219. doi: 10.4103/0976-237x.96844
17. Yagci A, Cantekin K, Buyuk SK, Pala K. The Multidisciplinary Management of Fused Maxillary Lateral Incisor with a Supernumerary Tooth in Cleft Lip Adolescence. *Case Rep Dent*. 2014;2014:459416. doi: 10.1155/2014/459416
18. Kumar A, Namdev R, Bakshi L, Dutta S. Supernumerary teeth: Report of four unusual cases. *Contemp Clin Dent*. 2012;3:71-77.

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ISSN 2544-1361 (online)
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CASUISTIC PAPER

Novaluron and indoxacarb induced methemoglobinemia – unveiling a rare poisoning

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ABSTRACT

Introduction and aim. In India, the utilization of agricultural pesticides for intentional self-poisoning is a prevalent method, and it is associated with substantially higher fatality rates compared to other self-poisoning approaches. Plethora, an agricultural insecticide containing novaluron (5.25%) and indoxacarb (4.5%), has recently been introduced and widely used in India and various other regions for its broad-spectrum lepidopteran control. While there have been documented cases of isolated self-poisoning involving indoxacarb, there is currently no literature reporting incidents of human poisoning specifically related to novaluron.

Description of the case. An 83-Year-old male was presented to the emergency department (ED) with a history of consumption of 50 mL of an insecticide suspension concentrate called 'PLETHORA' to commit suicide. He had one episode of vomiting and dizziness after the ingestion. There was associated cyanosis, and the patient was put on high-flow oxygen at 10 L/min through a face mask. The patient was diagnosed to have methemoglobinemia and was successfully treated with methylene blue and ascorbic acid. One hour post methylene blue injection showed a methemoglobin level of 1%, and the patient gradually improved. Patients presenting with novaluron and indoxacarb poisoning require supportive treatment as there is no specific antidote. There should be a high index of suspicion for methemoglobinemia in such patients, and timely management is necessary to prevent further complications. The patient was successfully managed and discharged after the 3rd day of admission.

Conclusion. The management of patients with novaluron and indoxacarb poisoning primarily involves supportive care, as there is currently no specific antidote available for these substances. Maintaining a high suspicion index for the development of methemoglobinemia and timely management of other complications is crucial for the best possible patient outcomes.

Keywords. emergency department, indoxacarb, methemoglobinemia, novaluron, poisoning

Introduction

The self-poisoning by agricultural pesticides is a common means of suicide in India.[1,2] The problem is of particular concern in rural India, where a variety of highly hazardous pesticides are easily available in the home and work settings for use in routine agricultural needs.[2] The case fatality rates are significantly higher following these hazardous pesticides compared to other means of self-poisoning, such as with sedatives and analgesics.[2] The case fatality rates following agriculture pesticide poisoning in India range from 5–70% across the reported studies.[2] There exists considerable variation

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across the suicide rates from pesticides among the Indian states, where states with good economic development and a higher proportion of populations engaged in agriculture documented a rising trend.[2] Plethora (novaluron 5.25% + indoxacarb 4.5%) is a recently introduced broad-spectrum lepidopteran insecticide for agricultural use in India and many parts of the world.[3] While incidents of isolated self-poisoning by indoxacarb have been reported, currently the documented evidence of human poisoning by novaluron is rare in the existing literature.[4,5,6] There is only one reported case of novaluron and indoxacarb combination-induced poisoning in the literature that developed acute methemoglobinemia, and there is a need to shed more light on the challenges in the management of such rare agricultural insecticide poisoning given their growing agricultural use.[6]

Aim

In this article, we present a case of self-poisoning with 'Plethora' that was presented to our emergency department (ED) with altered sensorium and cyanosis and successfully managed by a multidisciplinary team of the ED, with emphasis on the challenges encountered during treatment, culminating in the patient's discharge on the third day after presentation.

Description of the case

An 83-year-old male, presented at the ED with a history of deliberate ingestion of 50 ml of an insecticide suspension concentrate known as 'PLETHORA' (Fig. 1) in an attempt to end his life. Shortly after consuming the substance, he experienced a single episode of vomiting and dizziness. Upon inquiry, family members became aware of the incident and immediately sought medical assistance. The patient was promptly taken to a nearby local hospital, where gastric lavage was performed before being referred to our hospital.

Fig. 1. Plethora insecticide containing novaluron 5.25% and indoxacarb 4.5%

Upon arrival at our ED, the patient presented with an altered sensorium, characterized by a drowsy state and a Glasgow Coma Scale score of 13 out of 15 (E3V4M6). This altered level of consciousness is indicative of neurological impairment, affecting the patient's responsiveness and cognitive awareness. His vital signs included a pulse rate of 100 beats per minute, blood pressure of 132/82 mmHg, respiratory rate of 24 breaths per minute, and oxygen saturation of 82% on room air (Table 1).

Table 1. Vital signs of the patient during hospitalization

Sl no	Vital signs	Initial presentation	Day 1	Day 2	Day 3 (at discharge)
1	Heart rate (rate/minute)	100	72	68	75
2	Blood pressure (mm/Hg)	132/82	122/82	118/78	124/86
3	Temperature (degree Fahrenheit)	98.6	99.0	98.2	99.5
4	Respiratory rate (rate/minute)	24	18	16	16
5	Spo_2 (% in room air)	82	92	95	96

Bilateral respiratory sounds were normal. However, the patient displayed cyanosis, prompting the administration of high-flow oxygen at a rate of 10 L/min through a face mask, which effectively maintained oxygen saturation at 88%. Arterial and venous blood samples were collected for comprehensive analysis, encompassing blood gas analysis, complete blood count, renal function tests, and liver function tests. Notably, the blood sample exhibited a muddy brown coloration, which was observed during the collection process. These clinical observations collectively indicated a potential diagnosis of acute methemoglobinemia, subsequently confirmed by arterial blood gas analysis revealing a significantly elevated partial pressure of oxygen (PO_2) of 224.7 mmHg and an abnormally high methemoglobin value of 35%.

Simultaneously, blood samples were sent for testing to rule out the possibility of glucose-6-phosphate dehydrogenase (G6PD) deficiency. Meanwhile, the patient received intravenous administration of 500 mg of ascorbic acid, supplemental oxygen, and fluid therapy. Considering that the G6PD values fell within the normal range, an intravenous injection of methylene blue (50 mg, corresponding to 1 mg/kg of a 1% solution) in saline solution was administered over 5 minutes. Following the administration of methylene blue, the patient displayed the characteristic development of apple-green urine. One hour after the methylene blue injection, the methemoglobin (MetHb) level decreased to 1%, and the patient exhibited gradual improvement, with renal parameters returning to normal. Although the initial renal function tests displayed deranged results, including urea levels of 46 mg/dL, creatinine levels of 1.66 mg/dL, and a urea/creatinine ratio of 27.7, the urine output remained within the normal range (Table 2). The patient was observed in the hospital for another 48 hours along with supportive management measures and discharged on the third day of presentation. The authors attest that the manuscript adheres to the standards of

CARE guidelines for clinical case reporting. Written informed consent was obtained from the patient for publication in the journal.

Table 2. Laboratory data during hospitalization

Parameters	Initial presentation	Day 1	Day 2	Day 3 (at discharge)
pH	7.320	7.430	7.365	7.401
PaO_2 (mmHg)	224.7	110.5	96	99.1
$PaCo_2$ (mmHg)	35	30.5	36	35
Base excess (mmol/L)	- 4.3	-1.9	-1.4	-1.6
Bicarbonate (mmol/L)	18.3	22.1	24.2	22.2
Lactate (mmol/L)	3.40	1.07	1.20	1.01
Methemoglobin (%)	35	1	0.8%	0.9%
Fractional oxyhemoglobin (%)	58.4	94	94.4	96
Sodium (mEq/L)	133	140	144	145
Potassium (mEq/L)	4.41	4.01	4.05	3.68
Chloride (mEq/L)	99	–	105	110
Blood urea nitrogen (mg/dl)	21.47	–	20.73	10
Creatinine (mg/dl)	1.66	–	1.4	1.17

Discussion

Globally, pesticide mortality and morbidity associated with pesticide-related suicides pose a significant concern, particularly in developing countries like India, where agriculture plays a central role in livelihoods.[2] Each pesticide compound exhibits variations in toxicokinetics and/or dynamics, often necessitating specific medical management.[2] Novaluron, classified as a benzoylphenylurea compound, acts as a chitin synthesis inhibitor.[7] It is reported to possess lower acute toxicity towards mammals and reduced risks to non-target organisms and the environment, distinguishing it from other insecticides.[7] To date, there have been no documented cases of isolated novaluron poisoning, although animal studies have demonstrated elevated levels of methemoglobin upon exposure to the compound and one reported case of poisoning by novaluron and indoxacarb combination product.[6,8]

Indoxacarb 4.5% belongs to the oxadiazine class and functions by blocking sodium channels in the insect nervous system.[4,5] Several reports have indicated methemoglobinemia following indoxacarb ingestion.[4,5] Methemoglobinemia occurs when the ferrous (Fe^{2+}) ions of heme undergo oxidation to the ferric (Fe^{3+}) state, impairing the oxygen-carrying capacity of hemoglobin.[4,5] This process causes a leftward shift in the oxygen-dissociation curve and compromised tissue oxygenation. The metabolism of indoxacarb in the body likely involves the cleavage of the parent compound and the production of aniline metabolites and aromatic compounds capable of generating methemoglobin through metabolism.[4,9] In the present case, the presence of cyanosis and the observation of muddy brown-colored blood during sampling raised a strong suspicion of methemoglobinemia, which was subsequently confirmed by arterial blood gas analysis (ABG).

The primary approach to treating Plethora poisoning is supportive care. Depending on the patient's condition, fluid resuscitation and correction of metabolic abnormalities may be necessary.[5] The therapeutic approach to methemoglobinemia involves a systematic regimen encompassing various pharmacological interventions and supportive measures tailored to mitigate the condition's severity. Central to this strategy is the administration of methylene blue (MB), a paramount antidote utilized at a loading dose of 1–2 mg/kg over 5 minutes at intervals of 30–60 minutes, with a maximum cumulative dose not exceeding 7 mg/kg.[4,5,10] Methylene blue acts by reducing methemoglobin through the action of the nicotinamide adenine nucleotide phosphate (NADPH) reductase enzyme, significantly decreasing the half-life of methemoglobin from 15-20 hours to 40-90 minutes.[10] Concomitant administration of dextrose plays a pivotal role in this therapeutic paradigm, aiming to bolster NADPH formation, a crucial step in the reduction of methemoglobin. The addition of dextrose serves as a facilitator in the enzymatic conversion of methemoglobin back to its functional hemoglobin form. In instances where MB therapy exhibits ineffectiveness, the consideration for exchange transfusion emerges as a plausible alternative, offering a means to rapidly replace the affected blood with unaffected blood components.[11] In our case, after ruling out G6PD deficiency, the patient received 50 mg of methylene blue. Additional drugs, such as ascorbic acid, which can supplement NADH or NADPH, may be indicated to expedite methemoglobin reduction via NADPH reductase.[4,5] Ascorbic acid, a component within the minor reduction pathway of methemoglobin, assumes significance as an adjunctive therapeutic option in cases where MB therapy is contraindicated or ineffective.[10-11] The recommended dosage of ascorbic acid ranges from 1 to 3 grams administered at eight-hour intervals, contributing to the reduction of methemoglobin levels via its intrinsic properties.[10] In most instances, rapid response to methylene blue treatment is observed, although some patients may require repeated doses if symptoms persist. In our patient, a favorable response was observed following methylene blue administration, evidenced by a reduction in cyanosis and improvement in ABG values. The authors acknowledge that long-standing follow-up of the index case was not available, and that could have shed more light on the long-term effects of such rare poisoning.

Conclusion

The management of patients with novaluron and indoxacarb poisoning primarily involves supportive care, as there is currently no specific antidote available for these substances. In such cases, it is crucial to maintain a high index of suspicion for the development of methemoglobinemia. Timely and appropriate management is essential to prevent the occurrence of further complications

and ensure the best possible outcomes for these patients. There is a need to study the long-term health effects of such rare poisoning to determine the need for supportive management measures and continuous follow-up.

Declarations

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Author contributions

Conceptualization, I.M.S. and C.R.M.; Methodology, I.M.S., C.R.M. and R.V.R.; Software, I.M.S. and R.N.S.; Validation, C.R.M., R.V.R. and R.N.S.; Formal Analysis, I.M.S. and R.N.S.; Investigation, I.M.S., C.R.M. and R.N.S.; Resources, C.R.M.; Data Curation, I.M.S. and R.V.R.; Writing – Original Draft Preparation, I.M.S., C.R.M. and R.V.R.; Writing – Review & Editing, R.V.R. and R.N.S.; Visualization, R.N.S., and C.R.M.; Supervision, I.M.S. and C.R.M.; Project Administration, C.R.M.

Conflicts of interest

There are no conflicts of interest to declare from any of the authors.

Data availability

The data pertaining to the reported case is available with the Authors.

Ethics approval

Not applicable as written informed consent was taken from the participants.

References

1. Dawson AH, Eddleston M, Senarathna L, et al. Acute human lethal toxicity of agricultural pesticides: a prospective cohort study. *PLoS Med*. 2010;7(10):1000357. doi: 10.1371/journal.pmed.1000357
2. Bonvoisin T, Utyasheva L, Knipe D, Gunnell D, Eddleston M. Suicide by pesticide poisoning in India: a review of pesticide regulations and their impact on suicide trends. *BMC Public Health.* 2020;20(1):251. doi: 10.1186/s12889-020-8339-z
3. Ghosal A, Dolai AK, Chatterjee M. Plethora (Novaluron+ Indoxacarb) insecticide for the management of tomato fruit borer complex. *J Appl. Nat Sci*. 2016;8(2):919-922.
4. Prasanna L, Rao SM, Singh V, Kujur R, Gowrishankar. Indoxacarb poisoning: an unusual presentation as methemoglobinemia. *Indian J Crit Care Med.* 2008;12(4):198-200. doi: 10.4103/0972-5229.45082
5. Firoozabadi AS, Nasri-Nasrabadi Z, Marashi SM. Management of indoxacarb poisoning in a regional setting. *Indian J Crit Care Med.* 2016;20(10):627-628. doi: 10.4103/0972-5229.192067
6. Jacob J, CH KR, James J, Janardhanan N. Too Blue To Be True": Indoxacarb Induced Methemoglobinemia-A Rare Case. *Indian J Forensic Med Toxicol*. 2021;15(4):1501-1507.
7. Hwang JM, Bae JW, Jung EJ, Lee WJ, Kwon WS. Novaluron Has Detrimental Effects on Sperm Functions. *Int J Environ Res Public Health*. 2021;19(1):61. doi: 10.3390/ijerph19010061
8. National Center for Biotechnology Information. PubChem Compound Summary for CID 93541, Novaluron. https://pubchem.ncbi.nlm.nih.gov/compound/Novaluron. Accessed August 15, 2023.
9. Shih PC, Tsai TH. Methemoglobinemia following ingestion of Indoxacarb: A case report. *J Acute Med.* 2011;1(2):55-57. doi: 10.1016/j.jacme.2011.10.005
10. Iolascon A, Bianchi P, Andolfo I, et al. Recommendations for diagnosis and treatment of methemoglobinemia. *Am J Hematol.* 2021;96(12):1666-1678. doi: 10.1002/ajh.26340
11. Rehman HU. Methemoglobinemia. *West J Med*. 2001;175(3):193-196. doi: 10.1136/ewjm.175.3.193

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ISSN 2544-1361 (online)
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CASUISTIC PAPER

Evaluation of primary musculocutaneous Hodgkin's lymphoma with the aid of ^{18}F FDG PET/CT – a rare entity

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ABSTRACT

Introduction and aim. Primary musculocutaneous lymphoma is extremely rare and associated with poor prognosis. Here, we present a case of refractory primary muscular lymphoma with overlying cutaneous involvement with the aid of 18F-Fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F FDG PET/CT) for staging and treatment.

Description of the case. We present the case of a 52-year-old man presented with musculocutaneous Hodgkin's lymphoma having swelling and discolored areas over the left leg and right arm associated with itchiness. Upon examination, there was scaling, erythema over the body with Gottron's papules on knuckles and heliotrope rash. Skin and muscle biopsy were suggestive of Hodgkin's lymphoma. This patient was had primary musculocutaneous lymphoma. His diagnosis was delayed. PET/CT was performed for staging and response to treatment, which was suggestive of refractory disease.

Conclusion. PET/CT plays a vital role in diagnosis, staging, response to therapy and helps in optimized treatment for these specific patients.

Keywords. cutaneous lymphoma, muscular lymphoma, musculocutaneous Hodgkin's lymphoma, refractory

Introduction

Lymphomas are a heterogeneous group of neoplasm derived from lymphoid cell linages that vary widely in presentation, clinical features, and prognosis. Although extra nodal involvement of lymphoma is common, involvement of muscular/ cutaneous/ musculocutaneous/ musculoskeletal lymphoma is rare because of nodal disease spread into the adjacent structures i.e. secondary.[1-5] Hybrid imaging modalities like PET/CT are not routinely used for typical cutaneous lesions although if the typical cutaneous lesions have the presentation of paraneoplastic conditions as in our case, PET/CT definitely helps in early diagnosis, staging, early initiation of treatment, response evaluation, as well as changes in the treatment plan accordingly.

Aim

We present a case of primary musculocutaneous Hodgkin's lymphoma with a description of the clinical, pathological, radiological features and PET/CT findings and treatment history.

Description of the case

A 52-year-old man was presented to our hospital with complaints of swelling and discolored areas over the left leg and right arm associated with itchiness. Initially he started on treatment for varicose veins but his symptoms didn't improve. Along with that, he complained of unintentional weight loss without any history of night sweats or fever. The patient was then referred to the dermatology department for the discolored itchy lesions.

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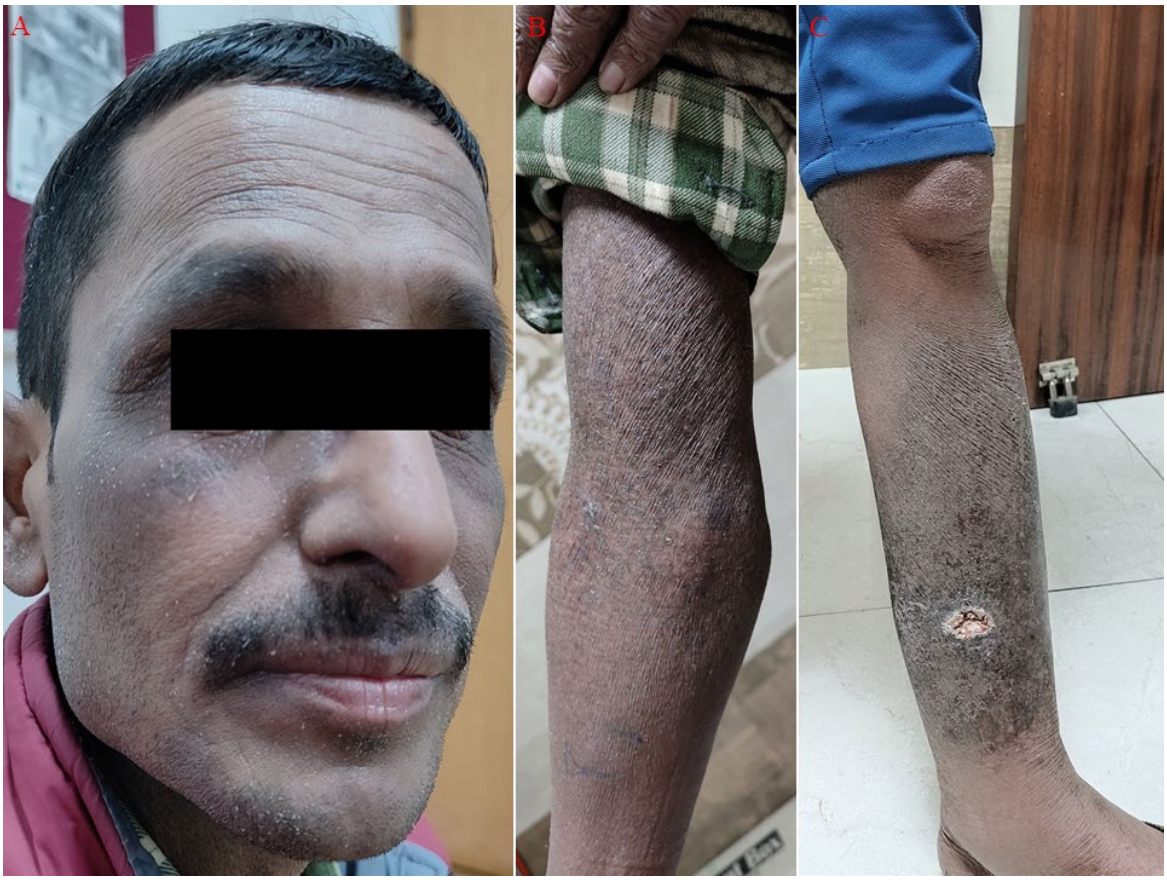

Fig. 1. A: Heliotrope rash on face, hyperpigmented scaly skin lesions of B: right arm C: and left leg

Upon examination, there was fine scaling with erythema/discoloration over the body predominantly right arm and left leg with Gottron's papules on knuckles and heliotrope rash around the both eyes (Fig. 1).

However, there was no complaint of any muscle weakness. Also, there were palpable lymph nodes in axillary and inguinal regions. For clinical evaluation contrast enhanced magnetic resonance imaging of the right arm and left leg were performed which revealed ill-defined heterogeneously enhancing soft tissue thickening with altered signals in subcutaneous tissue and muscles of flexor compartment of right arm and anterior, lateral and superficial posterior compartment of left leg. Multiple enlarged right axillary lymph nodes were also noted. The findings were suggestive of an inflammatory etiology likely dermatomyositis. The blood investigation shows raised LDH, and C reactive protein. ESR and antinuclear antibodies were positive (3+, speckled). Based on clinical suspicion, laboratory findings and radiological investigation, provisional diagnosis of erythrodermic dermatomyositis was made. The patient underwent FNAC from right axillary lymph node and skin and muscle biopsy from the left leg and right arm. The cytology findings from the lymph nodes were suggestive of Hodgkin's lymphoma and skin and muscle biopsy showed infiltration by Hodgkin's lymphoma likely mixed cellularity subtype. Immunohistochemistry was positive for CD 15 and CD 30 antigens. Then, the patient was referred to our department for a staging PET/CT, which revealed FDG avid multiple axillary, abdominal, pelvic and inguinal lymph nodes along with FDG avid mixed sclerotic lytic lesion in few dorsolumbar vertebrae and pelvis. FDG avid soft tissue thickenings were seen involving the cutaneous, subcutaneous tissue and muscle of upper arm, bilateral upper thigh & left lower leg. Patients had Deauville score 5. According to Lugano's classification, the patient was staged as IVb. The patient was then started on an ABVD regimen (adriamycin, bleomycin, vinblastine and dacarbazine).

After 3 cycles of ABVD chemotherapy, interim PET/CT was performed, which was suggestive of partial response to therapy with significant reduction of size, number and metabolic activity of lymph nodes, bony and right arm and left leg lesions (Deauville score 4). Completion chemotherapy PET/CT findings were suggestive of persistent hypermetabolism in right axillary, pelvic and inguinal lymph nodes, skeletal lesions and right arm and left leg cutaneous, subcutaneous and muscular lesions (Deauville score 4). There is appearance of similar type of cutaneous lesion in left arm. Then patient was switched to GDP regimen (gemcitabine, dexamethasone and cisplatin) and he underwent 4 cycles of chemotherapy. The PET/CT was performed after 3 months to look for response to the second line

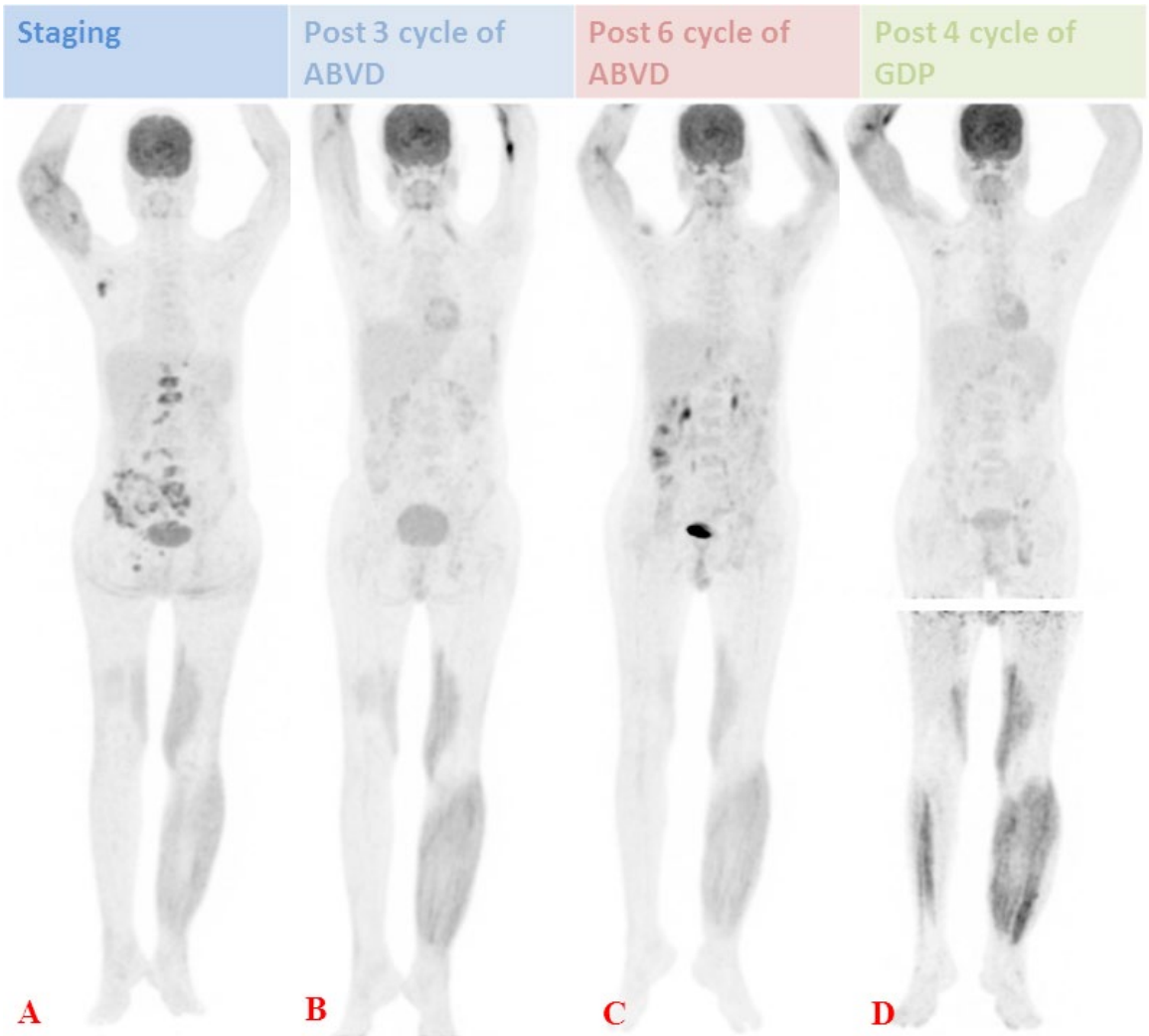

Fig. 2. Maximum intensity projection (MIP) image of ^{18}F FDG PET scan A: on staging PET demonstrate diffuse FDG uptake in right upper & bilateral lower extremities along with focal FDG uptake in right axillary, abdomino-pelvic and right inguinal region, B: interim PET demonstrate the partial response to treatment C: after 6 cycle of ABVD chemotherapy PET demonstrates appearance of new lesion in left upper extremity along with no significant change in previously seen extremity lesions after completion of chemotherapy D: post 4 cycle of GDP chemotherapy PET demonstrates decrease in metabolic activity of left upper extremity along with increased metabolic activity of previously seen extremity lesions and hypermetabolic spleen

chemotherapy which revealed that there was increase in size and metabolic activity of axillary, pelvic and inguinal lymph nodes with diffuse hypermetabolism in the spleen along with persistent cutaneous and muscular lesions (Deauville score 4). Previously metabolically active bone lesions showed sclerosis with no FDG uptake. The PET/CT findings were suggestive of refractory disease (Fig. 2 and 3).

Discussion

Hodgkin's lymphoma previously known as Hodgkin's disease is a rare monoclonal lymphoid proliferative disease with a high chance of being cured.[6] It constitutes a significant proportion of malignancy with bimodal age distribution. The etiology and pathophysiology for disease remains unclear, however a few risk factors have been identified like familial predisposition, immunocompromised state, Epstein Barr virus or Human immunodeficiency virus infection etc.[7] The WHO has divided Hodgkin's lymphoma into two major groups: classical Hodgkin's lymphoma and nodular lymphocytic predominant. The classical Hodgkin's lymphoma is more common than the nodular lymphocytic predominant and it is further divided into 4 subgroups: nodular sclerosis, mixed cellularity, lymphocyte rich and lymphocyte depleted.[8] The disease is clinically characterized by the lymph node infiltration usually in the cervical, mediastinal and axillary regions with contiguous lymph node group involvement and may have extra nodal involvement.[9]

The involvement of other organs is usually limited to spleen, liver, bone or lungs; skin involvement is rare.[10] Cutaneous lymphoma has non-specific findings

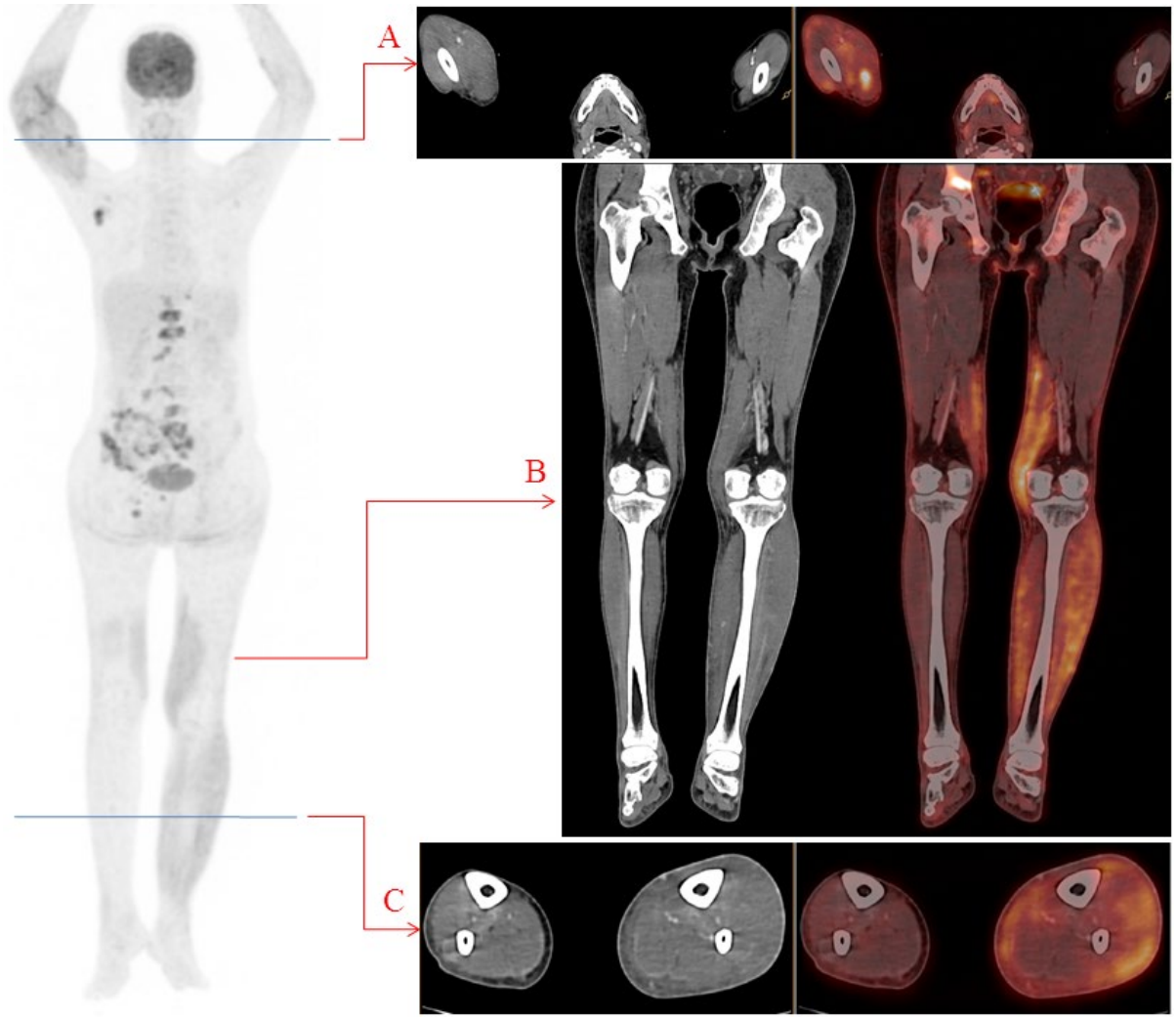

Fig. 3. Maximum intensity projection (MIP) image of staging ^{18}F FDG PET scan A: axial section at the level of mid arm of CT and corresponding PET/CT shows metabolically active musculocutaneous lesion with enlarged muscles of left extremity without CT attention changes in muscles B and C: coronal and axial sections of CT and corresponding PET/CT show metabolically active bilateral semitendinosus, left leg muscles and overlying skin lesions without CT changes in enlarged affected muscles

like pruritus, urticaria, papules, nodules, infiltrative plaques, hyperpigmentation, ichthyosis, ulcerative lesion or combined as erythroderma.[11,12] The three methods for the involvement of skin in Hodgkin's lymphoma have been proposed i.e. hematogenous dissemination, direct extension from the disease involved lymph nodes, and retrograde lymphatogenous spread from involved proximal lymph nodes. According to previously published case reports, the most common route is supposed to be retrograde lymphatogenous spread from the involved lymph nodes as in most of the cases the involved skin areas were drained by the involved lymph nodes.[13] However, in our case, there is involvement of muscle, subcutaneous and cutaneous tissue which can be either retrograde lymphatogenous spread or direct hematogenous spread. The involvement of skin and muscle classifies patient under stage IV (Ann Arbor staging system) and there is no specific therapeutic regimen for cutaneous involvement.

Lymphomatous involvement of muscles occurs in approximately 1.4% of cases, with 0.3% occurring in Hodgkin lymphoma and 1.1% in non-Hodgkin lymphoma.[3] Primary cutaneous Hodgkin's lymphoma is extremely rare entity with good prognosis.[5] Here we are dealing with the refractory musculocutaneous Hodgkin's lymphoma. Skeletal muscle lymphoma may be primary or secondary to invasion of the adjacent disease. Skeletal muscle lymphoma usually presents with painful enlargement of affected muscle representing as a diagnostic challenge to differentiate from various manifestation like deep vein thrombosis and various other benign and alignment neoplasms of muscles and bone.[3] In our patient, there were muscular lesions with cutaneous manifestations and these made definitive diagnosis

complicated and delayed. The patient was treated with six cycles of ABVD and followed by three cycles of the GDP regime. The patient showed partial remission after three cycles of chemotherapy after that refractory.

PET/CT is helpful in early diagnosis and initiation of optimal treatment in such fairly rare cases. In our case, there was a delay in diagnosis and start of the treatment. The actual disease burden is much more than clinical presentation. As on CT, skeletal muscle lymphomas are either iso/ hypodense and these lesions are metabolically active on FDG PET/CT i.e. occult disease involvement or identifying extracutaneous disease. As we know, the stage of the disease is the most important predictor of the prognosis in primary cutaneous lymphoma and PET/CT provides the actual stage of the disease which guides the clinician for optimal individualized patient centered treatment plan selection. PET/CT provides not only the actual global disease burden in primary cutaneous/ musculocutaneous lymphoma but it also provides the quantitation of overall disease burden.[14] Quantitative data helps in response evaluation, restaging, surveillance and prognostication.

Conclusion

Primary muscle/musculocutaneous lymphoma is extremely rare. Patients of musculocutaneous lymphoma usually presented with pain, muscle enlargement with or without regional lymphadenopathy along with cutaneous lesions. On CT, masses are usually isodense to muscle. FDG PET/CT play a vital role in detection of muscle/ cutaneous involvement that is occult on CT. FDG/CT is also helpful in diagnosis, staging, therapeutic response evaluation with significant impact on therapeutic decisions, restaging, surveillance and prognostication.

Declarations

Funding

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Author contributions

Conceptualization, M.M.S. and L.K.; Methodology, M.M.S.; Validation, L.K., M.M.S., S.D. and P.B.T.; Formal Analysis, S.D.; Investigation, P.B.T.; Data Curation, M.M.S.; Writing – Original Draft Preparation, L.K. and M.M.S.; Writing – Review & Editing, M.M.S.; Visualization, P.B.T.; Supervision, M.M.S. and S.D.

Conflicts of interest

There are no conflicts of interest.

Data availability

The dataset used and/or analysed during the current study available from the corresponding author on the reasonable request.

Ethics approval

Written consent to participate.

References

1. Gao S, Shu H, Yang H. Imaging features of skeletal muscle lymphoma: a case report and literature review. *BMC Med Imaging.* 2021;21(1):136. doi: 10.1186/s12880-021-00667-4
2. Balogova S, Greksak R, Mizickova M, Noskovicova L, Babal P, Lukac L. Case Report: Skeletal Muscle Lymphoma as a Result of Slow Centrifugal Migration of Untreated Primary Neurolymphomatosis? *Front Nucl Med*. 2022;2:804421. doi: 10.3389/fnume.2022.804421
3. Alamdari A, Naderi N, Peiman S, Shahi F. Non-Hodgkin lymphoma with primary involvement of skeletal muscle. *Int J Hematol Oncol Stem Cell Res.* 2014;8(3):55-57.
4. Kamura Y, Tsutsumi I, Miura Y, et al. Primary Intramuscular Classic Hodgkin Lymphoma: A Rare Case Report. *Intern Med.* 2021;60(23):3789-3793. doi:10.2169/internalmedicine.7524-21
5. Hakkou D, Belefqih I, Sabri S, et al. Primary Cutaneous Hodgkin's Lymphoma: An Extremely Rare Entity. *Cureus.* 2021;13(11):19272 doi: 10.7759/cureus.19272
6. Huang J, Pang WS, Lok V, et al. Incidence, mortality, risk factors, and trends for Hodgkin lymphoma: a global data analysis. *J Hematol Oncol*. 2022;15(1):57. doi:10.1186/s13045-022-01281-9
7. Ansell SM, Armitage JO. Management of Hodgkin Lymphoma. *Mayo Clinic Proceedings.* 2006;81(3):419-426. doi: 10.4065/81.3.419
8. Hansmann ML, Willenbrock K. WHO classification of Hodgkin's lymphoma and its molecular pathological relevance. *Pathologe.* 2002;23(3):207-218. doi: 10.1007/s00292-002-0529-1
9. Ansell SM. Hodgkin lymphoma: 2018 update on diagnosis, risk-stratification, and management. *Am J Hematol*. 2018;93(5):704-715. doi: 10.1002/ajh.25071
10. Massaro F, Ferrari A, Zendri E, Zanelli M, Merli F. Atypical cutaneous lesions in advanced-stage Hodgkin lymphoma: A case report. *World J Clin Cases*. 2019;7(17):2513-2518. doi: 10.12998/wjcc.v7.i17.2513
11. Pagliaro JA, White SI. Specific skin lesions occurring in a patient with Hodgkin's lymphoma. *Australas J Dermatol*. 1999;40(1):41-43. doi: 10.1046/j.1440-0960.1999.00315.x
12. Hsia CC, Howson-Jan K, Rizkalla KS. Hodgkin lymphoma with cutaneous involvement. *Dermatology Online Journal.* 2009;15(5):5. doi: 10.5070/d35502h87w
13. Introcaso CE, Kantor J, Porter DL, Junkins-Hopkins JM. Cutaneous Hodgkin's disease. *J Am Acad Dermatol.* 2008;58(2):295-298. doi: 10.1016/j.jaad.2005.11.1055
14. McDonald EO, Amanullah AA, Sang P, et al. The role of 18F-FDG PET/CT in primary cutaneous lymphoma: an educational review. *Ann Nucl Med*. 2023;37(6):328-348. doi: 10.1007/s12149-023-01830-3

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CASUISTIC PAPER

Combined non-surgical and surgical management of misdiagnosed cutaneous sinus tract of endodontic origin – 15 months CBCT follow-up

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ABSTRACT

Introduction and aim. An extraoral cutaneous sinus tract of endodontic origin represents a skin condition that appears due to an infection that could be primary or secondary to trauma accompanied by alveolar bony changes. It may be easily misdiagnosed and inappropriately treated due to lack of inadequate expertise on part of medical professionals followed by faulty treatment which can complicate the case further.

Description of case. This case report intends to highlight a case of a sixteen-year-old male patient referred for an extraoral cutaneous sinus tract misdiagnosed and insufficiently treated by a general physician followed by general dentists for more than a month due to lack of proficiency in the field of endodontics. The clinical and radiographic examinations confirmed the pulpal origin due to trauma related to permanent mandibular incisors. A combination of non-surgical root canal therapy followed by periapical surgery was performed leading to a significant healing of the lesion.

Conclusion. Accurate diagnosis is the key to treat draining sinus tracts of endodontic origin. Root canal treatment and endodontic surgery should be used judiciously for effectively eliminating the pathogens thus providing healing and repair mechanisms a chance to achieve the desired result.

Keywords. CBCT, cutaneous sinus tracts, diagnostic errors, healing, root canal treatment, surgical management

Introduction

Chronic periapical abscess of pulpal or endodontic origin may drain through a sinus tract intraorally or extraorally. Cutaneous sinus tract of endodontic origin is relatively rare and generally may get misdiagnosed and inappropriately treated because of the absence of any specific signs and symptoms.[1] Cutaneous sinus tract is defined as a pathologic canal leading from an enclosed area of inflammation or infection that opens to an epithelial surface of the face or the neck.[2] The causes of chronic periapical abscess may include pulpal inflammation due to caries, trauma, parafunctional habits etc. The drainage of sinus tract depends upon various factors such as the location of the involved tooth, position of the tooth root's apex in relation to muscle attachments, virulence of the microflora, decreased immunity of the host and least resistance provided by the underlying structure.[3]

There are several factors influencing the success rate of apical surgery that must be considered when considering apical surgery as a treatment alternative which

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includes: lesion size, preoperative pain, tenderness to percussion, fistula and resurgery.[4]

Aim

The main objective of this case report is to present a case of a sixteen-year-old male patient who was earlier misdiagnosed for an extraoral cutaneous sinus of endodontic origin and after identification of cause and proper diagnosis underwent a combination of non-surgical and surgical endodontic treatment with a follow up of 15 months CBCT.

Description of the case

A sixteen-year-old healthy male patient of Indian origin reported to Department of Conservative Dentistry and Endodontics with a chief complaint of occasional pus discharge and pain in lower front tooth region and chin for the past month. Medical and family history was found to be non-contributory. Dental history revealed a history of a bike accident one year prior. There was no history of unconsciousness and bleeding from the mouth or nose. The patient went to a local medical practitioner to show his chin area initially and then to a general dental practitioner where root canal treatment was initiated in 31, 32, 41 and 42. Also, a second general dental practitioner who reattempted root canal therapy in 31, 32, 41 and 42 one week back because patient's symptoms were not getting relieved. The patient also gave history of being prescribed antibiotics course and painkillers, the course of which he duly completed.

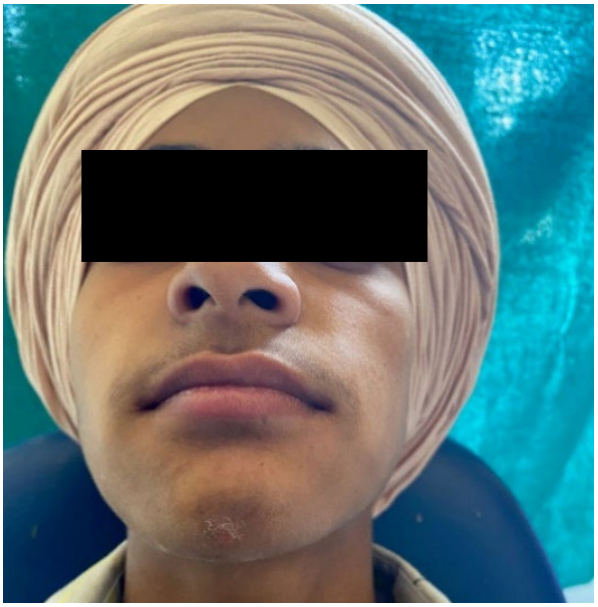
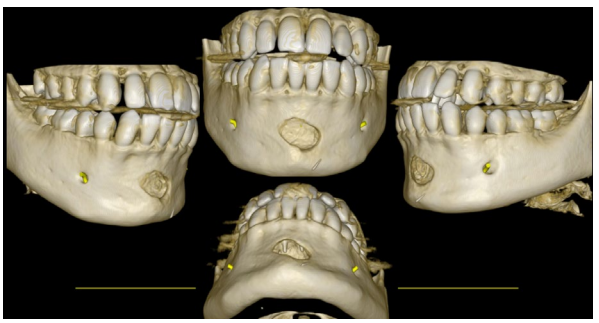


Fig. 1. Preoperative clinical photograph showing extraoral draining sinus on the chin

Clinical examination revealed an extra oral draining sinus in the chin area (Fig. 1). Intraoral examination revealed access opening done in 31, 32, 41 and 42. There was no pus discharge intraorally. Radiographic examination revealed ill-defined radiolucency in periapical area w.r.t 31, 32, 41 and 42. Radiopaque root canal filling was seen in 31 and 41 extending periapically and radiopaque material was seen in the internal structure of the lesion suggestive of a piece of Gutta Percha.

CBCT was conducted for the patient. It showed a well-defined periapical lesion with size 18 mm (Mesio-distally), 14.3 mm (Superio-inferiorly), 11.5 mm (Anterio-posterior) crossing the midline with destruction of labial cortical plate and intact lingual cortical plate in the symphyseal region and a broken piece of Gutta Percha was visualized (Fig. 2).



Fig, 2. CBCT reconstructed images showing the lesion and dislodged Gutta Percha point

Bilateral incisive canals were found to be in contact with the lesion (Fig. 3). Preoperative 3D volume was 1350 mm^3 and it was calculated using ITK-Snap software (Version 4.0.0).

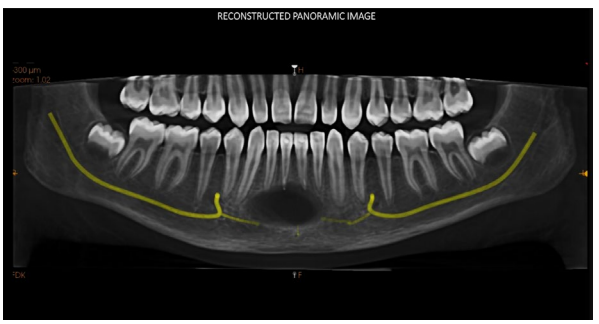


Fig. 3. CBCT reconstructed panoramic image showing the lesion and the course and the proximity of bilateral mandibular incisive nerve

A final diagnosis of previously treated 31, 32, 41 and 42 with chronic periapical abscess with extraoral cutaneous sinus tract in relation with 31 and 41 due to trauma was given. The treatment plan was formulated advising re-root canal treatment in 31, 32, 41 and 42 followed by apicoectomy in 31, 32, 41, 42 along with surgical removal of dislodged Gutta Percha.

The complete treatment plan, benefits and risks were explained to the patient and consent was taken prior to the commencement of the treatment. Also, blood hemo-

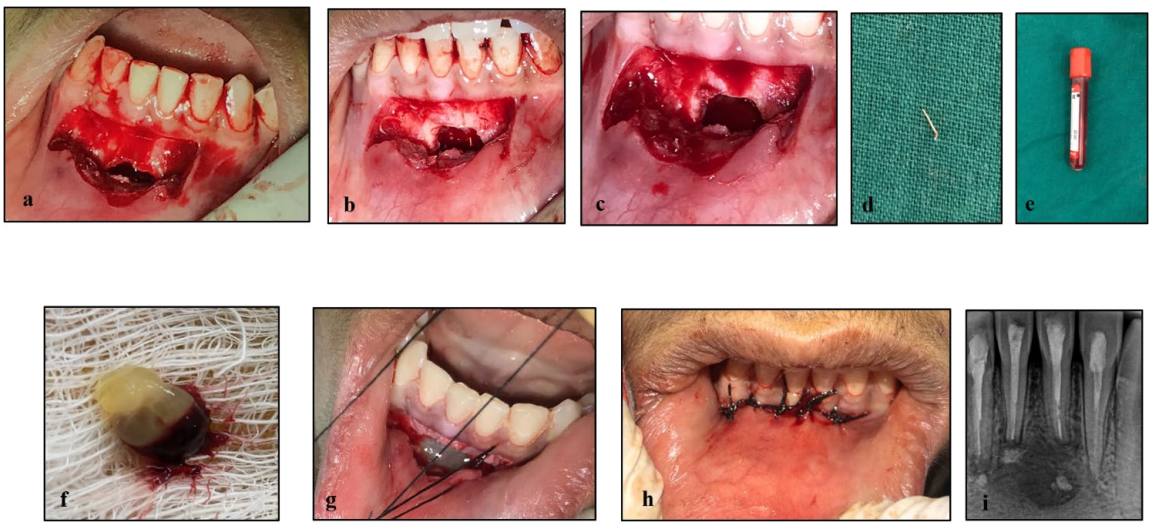


Fig. 4. Intraoperative records, A: Flap reflection, B: Visualization of GP in periapical area, C: Debrided bony cavity, D: Retrieved GP, E: Blood Sample collection, F: PRF formation, G:P RF placed, H: Flap repositioning and suturing, I: Immediate post-operative IOPA

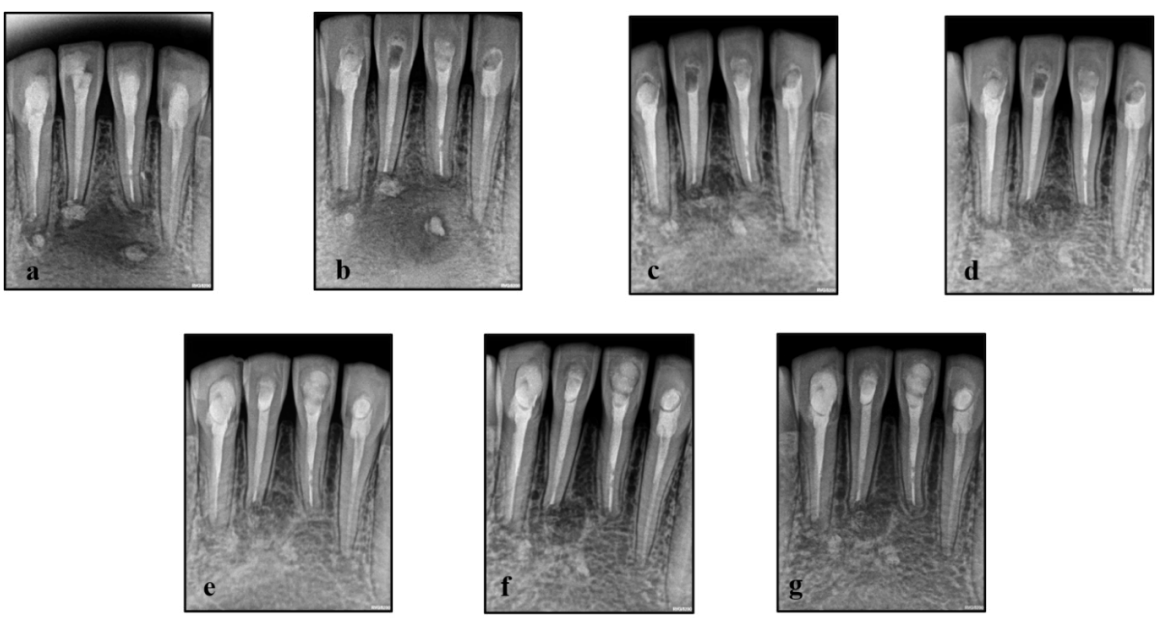


Fig. 5. Periodic radiographic follow up, A: 1 week, B: 1 month, C: 3 months, D: 6 months, E: 9 months, F: 12 months, G: 15 months

gram tests were conducted before surgery, revealing the values to be within normal physiologic limits. Indicated teeth were endodontically retreated. Access opening was modified and thorough biomechanical preparation was done followed by placement of calcium hydroxide dressing. Teeth were restored with cavit and the patient was then recalled after 2 weeks. On the next appointment, after removal of calcium hydroxide and thorough irrigation, the canals were obturated with MTA as obturating the canals with MTA offers a novel method to treat complicated endodontically involved teeth that may not have responded using traditional filling materials and sealers when extensive periapical pathosis is present.

On the day of surgery, all the steps were carried out in aseptic conditions. Preoperative mouth rinse with 0.15% chlorhexidine was done for the patient. Local anesthesia 2% lignocaine (LIGNOCAD ADR, Cadila Pharmaceuticals) containing 1:200000 epinephrine solution was administered. After the incision, a full mucoperiosteal flap was raised. Osteotomy was performed under copious water spray using a straight micromotor handpiece with a bone-cutting bur. Removal of granulation tissue was done using curettes. The dislodged Gutta Percha piece was visualized and removed from the periapical area. Bony cavity was debrided and copious irrigation with saline was done. Apical 3 mm of root were resected in 31, 32, 41 and 42. 5 ml of blood was collected from the patient and fresh PRF was formed in a centrifugation machine (10 min) followed by its placement in the bony cavity to provide growth factors and enhance

healing. Interrupted sutures were placed and post-operative instructions and medications were prescribed (Fig. 4). Suture removal was done after 1 week.

Periodic follow-ups were done at 1 week, 1 month, 3 months, 6 months, 9 months, 12 months and 15 months to track down the periapical healing (Fig. 5). 15 months follow-up CBCT was also done for the patient (Figure 6) showing remarkable healing and bone formation. Post-operative 3D volume was 52.87 mm^3 and it was calculated using ITK-Snap software (Version 4.0.0). The overall reduction in the volume of the lesion was determined to be approximately 96% at the end of 15 months.

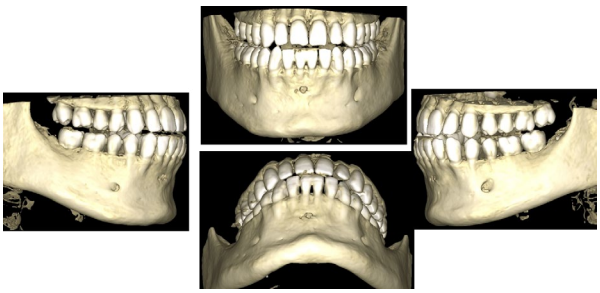


Fig. 6. 15 months CBCT follow-up

Discussion

Cutaneous sinus tract of endodontic origin are relatively uncommon lesions, so misdiagnoses and inappropriate treatment often arise.[5] Due to their presence in head and neck region, they are of special interest to medical fraternities as well especially dermatologists, ENT and general practitioners.[1] In our case also, the patient visited a general medical practitioner first followed by two general dental practitioners. However, the patient could not get appropriate treatment due the lack of their finesse in the field of endodontics. Such diagnostic and therapeutic misfortune highlights the importance of combined efforts and participation between medical and dental practitioners in the management of patients with head-and-neck lesions.[6,7]

The clinical differential diagnosis in such cases of cutaneous sinus tract includes pustule, actinomycosis, osteomyelitis, orocutaneous fistula, neoplasms, and local skin infections (carbuncle and infected epidermoid cyst). Other lesser common causes are salivary gland fistula, thyroglossal duct cyst, branchial sinus, dacryocystitis, and suppurative lymphadenitis.[8] Pustule(s) are frequently noted and generally found to have a superficial location and a short course. Actinomycosis generally shows multiple draining lesions and presence of fine yellowish granules in the purulent discharge is its characteristic finding. Osteomyelitis of jaw rarely gives rise to a cutaneous sinus and usually occurs secondary to some type of exogenic trauma, acquired infection after extraction of diseased or grossly carious teeth, impacted teeth, or retained roots. Neoplasms traditionally involves fixation to underlying structures. Carbuncle mostly shows involvement of hair follicles. Salivary gland fistula is a communication between the skin and mainly the parotid gland or the parotid duct that allows external drainage of saliva through the skin and mimics as a cutaneous sinus. Thyroglossal duct cyst and branchial sinus are developmental anomalies and they present as a midline neck mass at or below the level of the hyoid bone. The presence of history of trauma and root canal therapy in mandibular anterior teeth along with clinical and radiographic findings helped us in concluding to the final diagnosis of previously treated 31, 32, 41 and 42 with chronic periapical abscess with extraoral cutaneous sinus tract in relation with 31 and 41 due to trauma.

Mineral trioxide aggregate (MTA) was incipiently introduced to dentistry as a root-end filling and perforation repair material exhibiting excellent biocompatibility and osteoconductivity properties. MTA canal obturation can offer noticeable benefits by promoting the repair of the periodontium and the supporting structures of the tooth. The induction of cellular repair responses by a filling material that can promote cementum deposition, encourage bone formation and periodontal ligament regeneration must be considered a significant step forward when treating teeth compromised by long-standing disease and failed conventional treatments. In our case also, the canals were obturated with MTA to prevent coronal microleakage as it was a retreatment case with complicated root canal anatomy with a colossal periapical pathosis due to previous attempt for treatment.[9] Bogen G and Kuttler S (2009) presented a review and case series demonstrating the use of MTA and its significance as an obturating material in diverse clinical situations.[10]

An apicoectomy (also known as surgical endodontics, peri-apical surgery, or peri-radicular surgery) should be considered only when conventional endodontic root filling or re-treatment (root canal treatment) techniques have failed. Periapical surgery has been found to have 37% to 91% success rate depending upon the skills of the operator and the specific techniques used; and is evaluated through clinical and radiographic follow-ups.[11,12] The clinicians should provide the patient with as much information as is appropriate and pertinent and should be in terms that the patient understands. The risks and benefits related to the treatment and prognosis should be personalized for that individual patient. Also, there should be enough time for the patient to understand the information given and get a second opinion if needed. The present case also showed momentous healing in six months radiographically after the periapical surgery.

PRF has a good healing potential with various growth factors. It accelerates the new bone formation

and acts as a scaffold due to its dense fibrous nature. It releases growth factor for longer period as it gets slowly absorbed by the host. PRF in combination with bone mineral has been found to have the ability to increase the regenerative effects in intrabony defects.[13] The decision to use PRF as a grafting material was made in the present case to provide scaffold for growth factors and expedite the natural process of healing.

Cone beam computed tomography (CBCT) has become a well acknowledged imaging modality in the dentomaxillofacial region in recent times to evaluate the actual extension of the lesion and confirm the offending tooth. In comparison with periapical radiographs, CBCT examination allows more accurate measurements of the lesion limits in all three planes, the observation of its content, and also its direct relation to the root canal.[14] CBCT proved to be an adjunct in the true estimation of the size of the lesion, localization of dislodged Gutta Percha, determination of nerve proximity and finally the healing status at the end of 15 months in this present case.

Study limitations

Authors suggest that the findings or treatment plan of this particular isolated case should be cautiously applied to the general population and more cases should be reported to enrich the existing scientific literature.

Conclusion

Accurate diagnosis is the key to treat draining sinus tracts of endodontic origin. Successful management of odontogenic extraoral sinus tracts with pulpal pathology depends on both preoperative and intraoperative conditions. Surgical management can prove to be an adjunct for speedy management of such cases. Root canal treatment and endodontic surgery should be used judiciously for effectively eliminating the pathogens thus providing healing and repair mechanisms a chance to achieve the desired result.

Declarations

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Author contributions

Conceptualization, S.M. and S.R.; Methodology, S.G.; Software, S.R.; Validation, S.M., S.G. and S.N.; Formal Analysis, S.G.; Investigation, S.R.; Resources, M.M.; Data Curation, S.R.; Writing – Original Draft Preparation, S.R.; Writing – Review & Editing, S.M.; Visualization, S.G.; Supervision, S.G., S.N; Project Administration, M.M.; Funding Acquisition, M.M.

Conflicts of interest

The authors display no conflicts of interest.

Data availability

Data is available according to policy of the journal.

Ethics approval

Informed written consent was taken from the patient.

References

1. Latifa H, Dorsaf T, Amira K, Karim J, Nabiha D. Surgical management of an odontogenic cutaneous sinus tract misdiagnosed for 4 years. *Clin Case Rep*. 2022;10:05333. doi:10.1002/ccr3.5333
2. Cohenca N, Karni S, Rotstein I. Extraoral sinus tract misdiagnosed as an endodontic lesion. *J Endod*. 2003;29(12):841-843. doi: 10.1097/00004770-200312000-00015
3. Pasternak-Júnior B, Teixeira CS, Silva-Sousa YT, et al. Diagnosis and treatment of odontogenic cutaneous sinus tracts of endodontic origin: three case studies. *Int Endod J*. 2009;42:271-276. doi: 10.1111/j.1365-2591.2008.01519.x
4. Kreisler M, Gockel R, Aubell-Falkenberg S, et al. Clinical outcome in periradicular surgery: effect of patient- and tooth-related factors–a multicenter study. *Quintessence Int*. 2013;44(1):53-60. doi: 10.3290/j.qi.a28742
5. Bennani A, Jawad K. Cutaneous fistulas of dental origin. *Afr J Dent Implant*. 2018;13:8-18.
6. Kallel I, Moussaoui E, Kharret I, Saad A, Douki N. Management of cutaneous sinus tract of odontogenic origin: eighteen months follow-up. *J Conserv Dent*. 2021;24(2):223. doi: 10.4103/jcd.jcd_56_21
7. Lewandowski B, Mac-Pietrasz I. Problems with diagnosis and treatment of an odontogenic fistula of facial skin. *Pol Merkur Lekarski*. 1997;2(8):120-121.
8. Gupta M, Das D, Kapur R, Sibal N. A clinical predicament–diagnosis and differential diagnosis of cutaneous facial sinus tracts of dental origin: a series of case reports. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2011;112(6):132-136. doi: 10.1016/j.tripleo.2011.05.037
9. Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. *J Endod*. 1999;25(3):197-205. doi: 10.1016/S0099-2399(99)80142-3
10. Bogen G, Kuttler S. Mineral trioxide aggregate obturation: a review and case series. *J Endod*. 2009;35(6):777-790. doi: 10.1016/j.joen.2009.03.006
11. Torabinejad M, Corr R, Handysides R, Shabahang S. Outcomes of nonsurgical retreatment and endodontic surgery: a systematic review. *J Endod*. 2009;35(7):930-937.
12. Friedman S. The prognosis and expected outcome of apical surgery. *Endodontic Topics*. 2005;11:219-262.
13. Pavlovic V, Ciric M, Jovanovic V, Trandafilovic M, Stojanovic P. Platelet-rich fibrin: Basics of biological actions and protocol modifications. *Open Medicine*. 2021;16(1):446-454. doi: 10.1515/med-2021-0259
14. Patel S, Brown J, Pimentel T, Kelly RD, Abella F, Durack C. Cone beam computed tomography in Endodontics – a review of the literature. *Int Endod J*. 2019;52(8):1138-1152. doi: 10.1111/iej.13115

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CASUISTIC PAPER

Unmasking the nature of granulomatosis with polyangiitis – a diagnostic odyssey revealed through a compelling case report

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ABSTRACT

Introduction and aim. Granulomatosis with polyangiitis (GPA), formerly known as Wegener's granulomatosis, presents a formidable challenge in the realm of autoimmune diseases.

Granulomatosis, characterized by vasculitis and granuloma formation, exhibits diverse clinical manifestations. The rarity of GPA is evident, with an estimated incidence between 0.4 and 11.9 cases per 1 million person-years. The aim of this report is to show the complex diagnostic challenges inherent in GPA, demonstrating the diagnostic process from initial symptoms.

Description of the case. This case report unfolds the diagnostic journey of a 52-year-old Caucasian male. The presented case, initially suspected as a respiratory infection, led to a comprehensive investigation owing to persistent symptoms, abnormal blood counts, and elevated inflammatory markers. This narrative aims to depict the patient's diagnostic journey. Key diagnostic tools include ANCA testing, imaging studies, and tissue biopsy. Pulmonary nodules, lymphangitic changes, and renal involvement culminating in a GPA diagnosis confirmed by positive ANCA and anti-PR3 antibodies. The successful management of this case involved a tailored therapeutic regimen, including cyclophosphamide and methylprednisolone, addressing both vasculitic and renal components.

Conclusion. This case contributes to the understanding of atypical presentations of GPA, emphasizing the importance of a holistic and dynamic diagnostic approach.

Keywords. autoimmune diseases, granulomatosis with polyangiitis, vasculitis

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Introduction

Granulomatosis with polyangiitis (GPA), previously known as Wegener's granulomatosis, stands as a challenging autoimmune disease.[1] It is characterized by the inflammation of small- to medium-sized blood vessels (vasculitis) and the formation of granulomas in various organs.[2] In this case, we would like to present the journey of a patient's diagnostic path, which serves as a striking example of the difficulties in identifying this disorder.

Granulomatosisis a rare autoimmune disease with a relatively low incidence, estimated to range between 0.4 and 11.9 cases per 1 million people per year.[3] It affects individuals across both genders, with equal numbers of males and females.[3] Onset of this disease typically occurs between the ages of 45 and 65 years.[3] There is a higher prevalence of GPA among individuals of White ethnicity.[3] Notably, the incidence of GPA appears to have increased in recent decades, possibly due to greater awareness and improved diagnostic capabilities, particularly with the introduction of ANCA (anti-neutrophil cytoplasmic antibody) testing.[4] In a Polish study, 1491 patients (749 females and 742 males), all of whom were admitted to the hospital for the first time with a diagnosis of GPA, was conducted between 2011 and 2015. The average annual incidence of GPA in Poland was estimated at 7.7 cases per million within the population (95% CI, 4.1–11.4), and the point prevalence stood at 38.4 cases per million at the end of 2015.[5] Interestingly, it was observed that the highest rate of newly diagnosed GPA cases occurred during the month of January.[5] This temporal pattern in GPA incidence warrants further investigation to better understand the potential seasonal variations and contributing factors to the disease's occurrence.[6]

Antineutrophil cytoplasmic antibodies (ANCA) play a crucial role in the pathogenesis and diagnosis of GPA.[7] ANCA antibodies are a hallmark feature of GPA and are central point to understanding the autoimmune nature of this condition.[8] In GPA, the immune system mistakenly produces ANCA antibodies, specifically proteinase 3 (PR3-ANCA).[8] When ANCA antibodies bind to neutrophils, they activate these immune cells, leading to the release of inflammatory mediators and the initiation of an autoimmune response.[9] The activated neutrophils adhere to the blood vessel walls, contributing to inflammation and damage to the small- to medium-sized blood vessels in various organs.[9] PR3-ANCA is particularly indicative of GPA.[10] ANCA testing is often used to support the clinical diagnosis of GPA, especially when other clinical and laboratory findings are suggestive of the disease.[11]

The symptoms of GPA can vary widely from one individual to another and may affect multiple organ systems.[12] Common symptoms of GPA include constitutional signs such as fever, asthenia, recurrent low-grade fevers, and weight loss, with approximately 50% of patients experiencing these manifestations, which contributes to the complexity of its clinical presentation.[2]

Ear, nose and throat (ENT) signs are present in 70%, even up to 100% of cases at diagnosis.[2] Respiratory symptoms are prevalent, including chronic sinusitis, rhinitis, and a persistent cough with blood-tinged sputum. Shortness of breath may arise due to lung involvement, which affects 50% to 90%.[2] Renal symptoms, occurring in a significant percentage of cases, manifests with hematuria and proteinuria, often presenting as focal segmental necrotizing glomerulonephritis accompanied by extracapillary proliferation and pauci-immune crescent formation.[13]

Musculoskeletal symptoms could be present in two-thirds of patients at the onset of the disease involve joint pain and swelling.[14] Cutaneous manifestations are reported in approximately 23% of patients, including skin rashes, ulcers, and purpura resulting from blood vessel inflammation.[2,15] Ocular symptoms encompass eye inflammation, with potential complications such as redness, pain, and vision changes.[16] Neurological involvement, though less frequent, may lead to headaches, peripheral neuropathy, and severe neurological complications.[17]

To classify a patient with a diagnosis of small- or medium-vessel vasculitis as having GPA, the cumulative score had to be equal to or exceed 5 points. These criteria provide a valuable tool for accurately diagnosing GPA while distinguishing it from other vasculitis and vasculitis mimics (Table 1).[18]

Table 1. The classification criteria for GPA[18]

Criteria	Points
Bloody nasal discharge, nasal crusting, or sino-nasal congestion	3
Cartilaginous involvement	2
Conductive or sensorineural hearing loss	1
Cytoplasmic antineutrophil cytoplasmic antibody (ANCA) or anti-proteinase 3 ANCA positivity	5
Pulmonary nodules, mass, or cavitation on chest imaging	2
Granuloma or giant cells on biopsy	2
Inflammation or consolidation of the nasal/paranasal sinuses on imaging	1
Pauci-immune glomerulonephritis	1
Perinuclear ANCA or antimyeloperoxidase ANCA positivity	-1
Eosinophil count $\geq 1\times10^9$/L	-4

Researchers are exploring various strategies for the treatment of autoimmune diseases such as GPA and eosinophilic GPA (EGPA).[19] These investigations encompass evaluating the advantages of higher-dose glucocorticoid (GC) therapy over standard dosing in terms of faster inflammation control and potential reductions in relapses.[19] Prolonged use of GCs increases the risk of various adverse effects.[20] Recent trials assessing induction

therapy in GPA have encompassed both new-onset and relapsing patients.[21] Notably, in the largest trial comparing rituximab (RTX) and cyclophosphamide (CYC) for remission induction, relapsing patients exhibited higher remission rates at 6 and 12 months with RTX.[21] In contrast, patients in the CYC arm were switched to receive azathioprine (AZA) for 12–15 months.[22,23] Consequently, RTX is favored for treating relapsing patients.[21]

Researching novel immunomodulators like JAK inhibitors as alternative therapies, potentially offering fewer side effects but requiring a thorough evaluation for safety and potential unknown risks.[24] Gaining insights into the long-term effectiveness and safety of avacopan as an induction and maintenance therapy compared to standard treatments, understanding potential limitations or side effects.[25] C5a blockade can replace GCs for induction and extended use, reducing the adverse effects associated with long-term GC therapy while considering potential limitations.[25] These research objectives aim to enhance the treatment of autoimmune diseases, striving to optimize therapeutic benefits while minimizing potential harm and side effects.[19]

Aim

The aim of this report is to offer a comprehensive view of the diagnostic journey for GPA, emphasizing the efficiency of the diagnostic process. The selected case serves as an illustration of the typical diagnostic challenges associated with GPA, detailing the progression from the onset of symptoms to the eventual identification of the disease. By presenting this case, we hope to shed light on the various pitfalls, missteps, and frustrations that often accompany the diagnosis of GPA, ultimately serving as a valuable resource for medical practitioners.

Description of the case

The patient, a 52-year-old male, presented for the first time to his family doctor in early April with a persistent dry cough and weakness lasting for two weeks that was diagnosed as an upper respiratory tract infection. He had no history of chronic illnesses, but experienced occasional generalized joint pains, self-treated with NSAIDs. In his medical history, there was a 20-cigarette-per-day smoking habit and excessive alcohol consumption (1-2 beers per day). The doctor prescribed a syrup with levodropropizine and doxycycline. The patient was referred for tests (complete blood count, CRP, creatinine, lipid profile, and glucose concentration) with a follow-up appointment scheduled in 3 days).

The test results revealed leukocytosis 12.3×10^3/μL (normal range: 4.2-9.1×10^3/μL), significant thrombocytosis 527x10^3/μl (normal range: <450×10^3/μL), elevated CRP 5.42 mg/dL (normal range: 0–5 mg/dL) and increased glucose levels 6.08 mmol/L (normal range: 3.9–5.5 mmol/L). Other results, including creatinine, were within normal ranges. In mid-May, the patient returned with generalized musculoskeletal pain, tenderness, restricted mobility in shoulder and elbow joints, and bilateral hand numbness. Symptomatic treatment was initiated: etoricoxib 60 mg tablets, diclofenac in a series of 10 injections (75 mg/3 mL), and a supplement containing vitamins B12, B6, thiamine. A follow-up consultation was scheduled for a week later. During the follow-up, the patient still experienced pain and numbness, fever (up to 39°C), and loss of appetite. He reported a weight loss of approximately 10 kg in 2 months. Urgent referral to the internal medicine department was made for further diagnostic investigation.

Upon admission, the patient was generally in good condition, tachycardic (110/min), without peripheral edema but with slightly enlarged supraclavicular and axillary lymph nodes on the left side and erythematous lesions with desquamation on the upper back. Laboratory tests showed leukocytosis 15.25×10^9/L (normal range: 4.9-9.1×10^3/μL), mild normocytic anemia 10.6 g/dl (normal range: 13.7–16.5 g/dL), significant thrombocytosis 1069×10^9/L (normal range: <450×10^3/μL), elevated ESR 69 mm/h (normal range: 1–20 mm/h), CRP 213.3 mg/L (normal range: 0-5 mg/dL), hyperuricemia 566 μmol/L (normal range: 202–417 μmol/L), hyperkalemia 6.3 mmol/L (normal range: 3.5–5.1 mmol/L), D-dimer 6.99 μg/mL (normal range: 0–0.5 μg/mL), and positive rheumatoid factor (RF) at 184.4 IU/mL (normal range: <14 IU/mL). Protein and albumin levels in serum were decreased. Kidney parameters revealed an elevated creatinine concentration of 387.7 μmol/L (normal range: 62–106 μmol/L), with an eGFR of 15.14 mL/min/1.73 m^2 (normal range: >90 mL/min/1.73 m^2), and urea of 16.9 mmol/L (normal range: 2.76–8.07 mmol/L). The urinalysis revealed the absence of bacteria, leukocyturia, and significant proteinuria 0.45 g/L. Dysmorphic red blood cells were also observed. Chest X-ray revealed extensive shadowing at the apex of the right lung, an oval shadow in the lower part of the left lung, and a nodular lesion at the apex of the left lung requiring further investigation with contrast-enhanced CT.

Pulmonology consultation was initiated, and a non-contrast CT revealed a polycyclic tumor (66×65×61 mm) in the upper lobe of the right lung with pleural involvement extending to the upper pole of the right hilum, and lymphangitic carcinomatosis-like changes around the tumor (Fig. 1).

Additionally, a polycyclic tumor (26×36×43 mm) was observed at the apex of the left lung, and a 9 mm nodule with lymphangitic carcinomatosis-like changes was present in the left lung (Fig. 2).

A small amount of fluid in both pleural cavities and enlarged lymph nodes in segments 1 and 2, as well as a 30×25 mm nodule in the left segment 6, were noted. Ultrasound of lymph nodes showed altered echogenicity in several left supraclavicular lymph nodes.

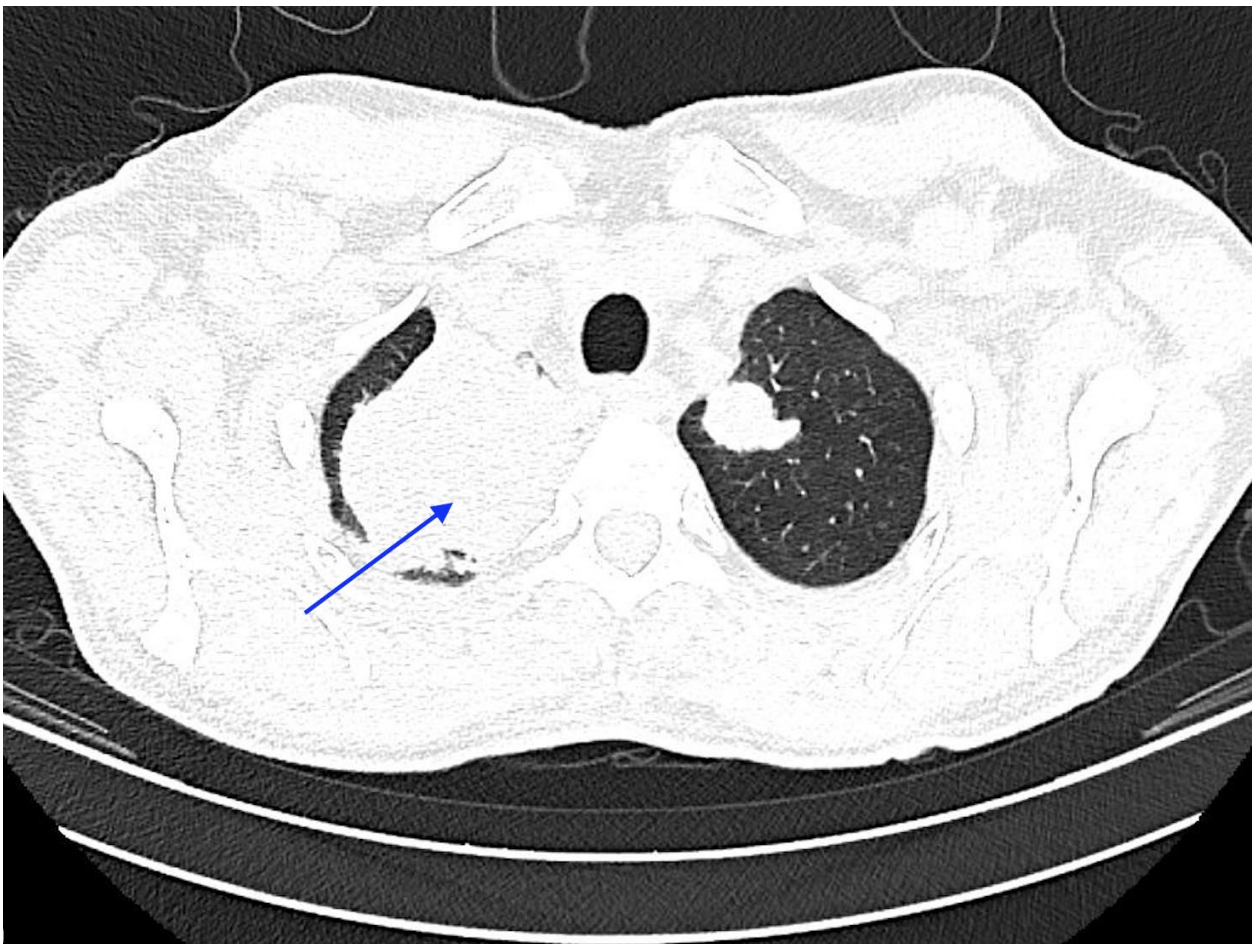

Fig. 1. CT of the chest with polycyclic tumor in the upper lobe of the right lung with pleural involvement

In-hospital treatment included intravenous hydration, broad-spectrum antibiotic therapy, allopurinol, painkillers, low-molecular-weight heparin adjusted to eGFR, and acetylsalicylic acid. Due to worsening anemia and the presence of fresh blood in the stool, acetylsalicylic acid was discontinued and 2 units of red blood cell concentrate were transfused. Furosemide was added to the therapy for lower limb edema, with a positive response. A rheumatology consultation led to the initiation of steroid therapy for suspected paraneoplastic syndrome-related bone and joint pain. A nephrology consultation was requested due to rising kidney parameters despite optimal conservative treatment and good diuresis. The patient was not deemed eligible for renal replacement therapy until metastatic spread of cancer was ruled out. A bronchoscopy was performed a week after admission, with no evidence of tumor cells in the material collected for general culture. However, a lymph node biopsy was recommended for histopathological examination, leading to an urgent referral to a highly specialized thoracic surgery department in case histopathological verification was needed.

Despite a high suspicion of cancer, a histopathological examination did not confirm the cancer hypothesis. Instead, positive results for ANCA antibodies and anti-PR-3 antibodies were obtained, leading to suspicion of granulomatosis with polyangiitis. In early July, the patient was admitted to the clinic for confirmation of the diagnosis and initiation of treatment.

Upon admission, the patient was in good general condition but with dyspnea and lower limb edema. Bronchoscopy with EBUS was performed during hospitalization to definitively rule out cancer. Immunological tests revealed positive ANCA antibodies (1:160), positive anti-PR3 antibodies (28 IU/mL), while anti-MPO and anti-GBM were negative. Kappa and lambda light chains in serum were elevated in equal proportions. Levels of immunoglobulins IgA, IgG, IgM, and complement components C3 and C4 were normal. The final diagnosis was confirmed as granulomatosis with polyangiitis.

The worsening renal function necessitated hemodialysis, and a kidney biopsy was performed due to suspected secondary membranous nephropathy. The biopsy revealed features consistent with the late stage of proliferative extracapillary glomerulonephritis with crescents. Therapy with cyclophosphamide (CTX) at a dose of 1g, along with oral methylprednisolone at a dose of 32mg and prophylactic trimethoprim/sulfamethoxazole 480 mg, was initiated. A permanent catheter was implanted in the left internal jugular vein, and 7 plas-

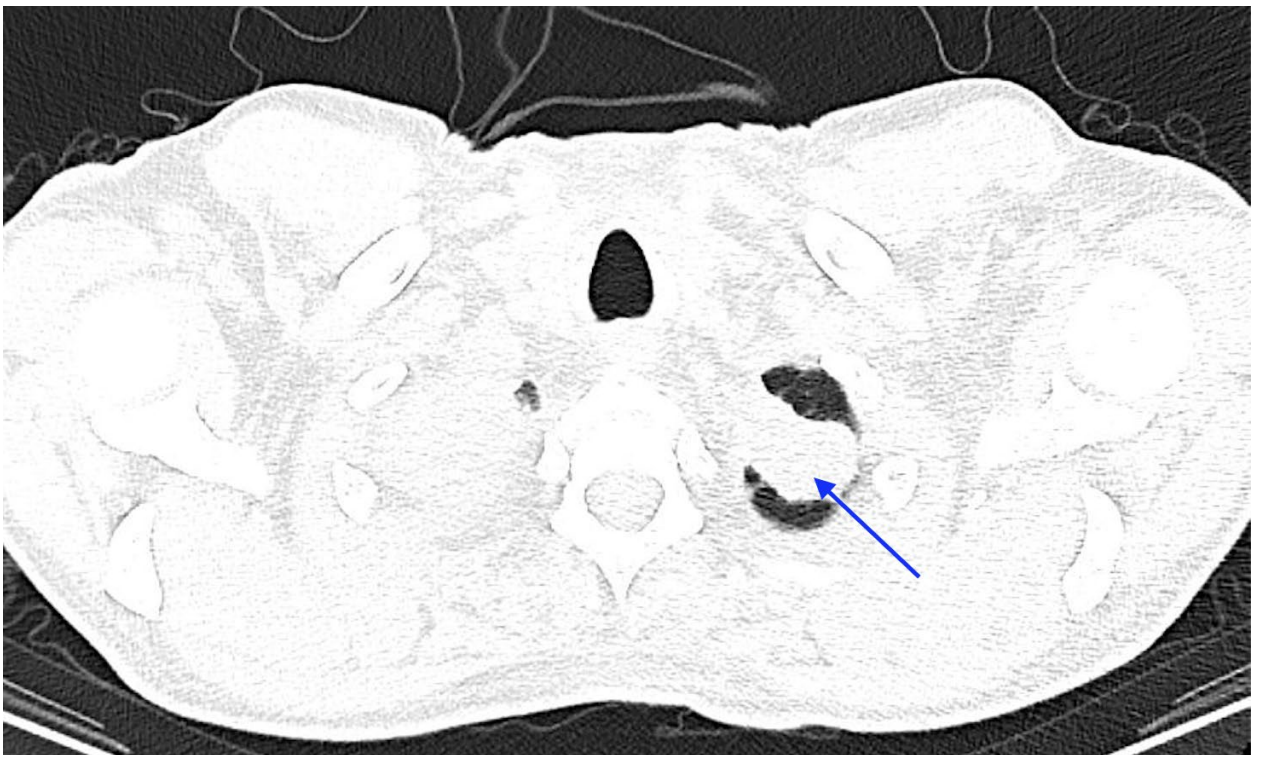

Fig. 2. CT of the chest with polycyclic tumor was observed at the apex of the left lung

mapheresis procedures were performed during hospitalization. Two weeks after the first CTX pulse, the second pulse (900 mg) was administered, and anti-PR3 antibody levels were measured at 1.4 IU/mL. The patient was discharged after three weeks of hospitalization in a stable condition, appropriate for coexisting conditions, with ongoing recommendations until the results of the histopathological examination and immunological tests (ANA, ANCA, anti-GBM, and serum protein electrophoresis) were obtained. The histopathological examination did not confirm the cancer hypothesis, but positive results for ANCA antibodies and positive anti-PR-3 antibodies were obtained. Suspecting granulomatosis with polyangiitis, the patient was admitted to the clinic in early July for confirmation of the diagnosis and initiation of treatment. In addition to methylprednisolone and trimethoprim/sulfamethoxazole, the patient received metoprolol 25 mg, amiodarone 200 mg (both medications administered due to paroxysmal atrial fibrillation episodes observed in the ward), calcitriol 0.25 μg, calcium carbonate 1000 mg, and proton pump inhibitor 20 mg. Two weeks later, the patient was readmitted to the clinic for the administration of three pulses of cyclophosphamide (CTX) at a dose of 900 mg each and assessment of disease activity. Currently, the individual has completed five pulses of CTX (fourth and fifth doses also at 900 mg each) without complications, and the methylprednisolone dose is being gradually reduced.

Discussion

The presented case outlines the complex diagnostic journey of a 52-year-old male who initially sought medical attention for a persistent dry cough and weakness, attributed to an upper respiratory tract infection. The patient's subsequent clinical course unfolded with atypical musculoskeletal symptoms, constitutional features, and unexplained laboratory abnormalities, prompting a multidisciplinary investigation. The initial therapeutic approach, combining levodropropizine and doxycycline, was chosen based on the presumptive infectious etiology. However, the persistence of symptoms and the emergence of systemic manifestations led to a comprehensive workup, revealing abnormal blood counts, elevated inflammatory markers, and deranged glucose metabolism.

Notably, the patient's history of smoking and excessive alcohol consumption raised concerns about potential contributing factors. The onset of the disease, starting with nonspecific symptoms of the upper respiratory tract, is common, yet it is not a specific symptom and may lull the doctor's vigilance.[2]

Subsequent evaluations, including imaging studies, unveiled pulmonary nodules and lymphangitic carcinomatosis-like changes. Weight loss, fever, and changes in lung imaging in a patient could suggest a developing neoplastic process. Various cases of initial suspicion of neoplastic disease are described before the diagnosis of GPA is established.[26] Despite initial suspicions of malignancy, the absence of tumor cells in bronchoscopic material and

the subsequent identification of specific autoantibodies underscore the challenges in distinguishing autoimmune conditions from neoplastic processes. The clinical presentation, coupled with positive anti-PR-3 antibodies, shifted the diagnostic focus towards GPA. The significance of renal involvement became apparent, necessitating hemodialysis and a kidney biopsy, ultimately confirming the presence of proliferative extracapillary glomerulonephritis. Plasma exchange was also used in this case to reduce the risk of end-stage cardiovascular disease in patients with acute primary disease.[27] Symptoms associated with renal failure in the form of nephritic syndrome are common, and biopsy is crucial in making the diagnosis of glomerulonephritis.[28] The initiation of a therapeutic regimen comprising cyclophosphamide, methylprednisolone, and supportive measures aimed to address both the vasculitic and renal components of GPA. The treatment strategy used is also described in other cases.[27] Prophylactic use of trimethoprim with sulfamethoxazole is important in the case of the applied therapy.[29]

The successful management of this patient, including the reduction of immunosuppressive therapy and stabilization of renal function, highlights the potential for favorable outcomes in GPA with prompt and targeted interventions. Continued follow-up, along with further investigations, including immunological markers and histopathological examinations, will be crucial for assessing treatment response and guiding ongoing care. This case contributes to the growing body of literature on atypical presentations of GPA, emphasizing the need for a holistic and dynamic approach in the evaluation and management of complex medical cases.

Conclusion

In conclusion, the diagnostic odyssey of the 52-year-old male presented here illuminates the intricate challenges associated with identifying GPA, a formidable entity within the spectrum of autoimmune diseases. The patient's journey, from the initial presentation of seemingly benign symptoms to the ultimate confirmation of GPA, underscores the complexities involved in discerning this rare autoimmune disorder.

This case report serves as a poignant reminder of the multifaceted nature of GPA, characterized by protean clinical manifestations that can mimic other conditions, leading to diagnostic ambiguity. The hurdles encountered in the diagnostic process, exemplified by the patient's atypical musculoskeletal symptoms, constitutional features, and perplexing laboratory abnormalities, underscore the importance of a meticulous and collaborative approach in unraveling the intricacies of such autoimmune disorders.

Moreover, the diagnostic challenges highlighted in this report shed light on the broader landscape of GPA, emphasizing the need for increased awareness and improved diagnostic capabilities. The evolving incidence patterns observed in recent decades, attributed partly to heightened awareness and advancements in diagnostic tools like ANCA testing, underscore the dynamic nature of our understanding of GPA.

This case not only contributes valuable insights into the diagnostic intricacies of GPA but also emphasizes the pivotal role of interdisciplinary collaboration in navigating such complex clinical scenarios. The successful management of the patient, with a tailored therapeutic approach targeting both vasculitic and renal components, serves as a beacon of hope, showcasing the potential for positive outcomes with timely and targeted interventions.

Declarations

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Author contributions

Conceptualization, P.S.; Methodology, Ł.S. and A.K.; Software, S.Z.; Resources, M.S. and A.B; Data Curation, P.S. and M.T.; Writing – Original Draft Preparation, P.S. and A.K.; Writing – Review & Editing, K.Ż. and K.K. and M.D.; Visualization, J.T.; Supervision, P.S.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Data availability

The datasets used and/or analyzed during the current study are open from the corresponding author on reasonable request.

Ethics approval

Written informed consent for publication was obtained from the patient. We complied with the policy of the journal on ethical consent.

References

1. Manti F, Battaglia C, Pelaia C, Petullà M, Bertucci B, Laganà D. Migrant cavitation as primary involvement in a particular case of granulomatosis with polyangiitis. *Radiol Case Rep*. 2022;17(10):3535-3538. doi: 10.1016/j.radcr.2022.06.074
2. Comarmond C, Cacoub P. Granulomatosis with polyangiitis (Wegener): clinical aspects and treatment. *Autoimmun Rev*. 2014;13(11):1121-1125. doi: 10.1016/j.autrev.2014.08.017
3. Panupattanapong S, Stwalley DL, White AJ, Olsen MA, French AR, Hartman ME. Epidemiology and Outcomes of Granulomatosis With Polyangiitis in Pediatric and Working-Age Adult Populations In the United States: Analysis of a Large National Claims Database. *Arthritis Rheumatol*. 2018;70(12):2067-2076. doi: 10.1002/art.40577

4. Austin K, Janagan S, Wells M, Crawshaw H, McAdoo S, Robson JC. ANCA Associated Vasculitis Subtypes: Recent Insights and Future Perspectives. *J Inflamm Res*. 2022;15:2567-2582. doi: 10.2147/JIR.S284768
5. Kanecki K, Nitsch-Osuch A, Gorynski P, Tarka P, Bogdan M, Tyszko P. Epidemiology of Granulomatosis with Polyangiitis in Poland, 2011-2015. *Adv Exp Med Biol*. 2018;1116:131-138. doi: 10.1007/5584_2018_239
6. Kain R, Exner M, Brandes R, et al. Molecular mimicry in pauci-immune focal necrotizing glomerulonephritis. *Nat Med*. 2008;14(10):1088-1096. doi: 10.1038/nm.1874
7. Kitching AR, Anders HJ, Basu N, et al. ANCA-associated vasculitis. *Nat Rev Dis Primers*. 2020;6(1):71. doi: 10.1038/s41572-020-0204-y
8. Kronbichler A, Lee KH, Denicolò S, et al. Immunopathogenesis of ANCA-Associated Vasculitis. *Int J Mol Sci*. 2020;21(19):7319. doi: 10.3390/ijms21197319
9. Frangou E, Vassilopoulos D, Boletis J, Boumpas DT. An emerging role of neutrophils and NETosis in chronic inflammation and fibrosis in systemic lupus erythematosus (SLE) and ANCA-associated vasculitides (AAV): Implications for the pathogenesis and treatment. *Autoimmun Rev*. 2019;18(8):751-760. doi: 10.1016/j.autrev.2019.06.011
10. Trivioli G, Marquez A, Martorana D, et al. Genetics of ANCA-associated vasculitis: role in pathogenesis, classification and management. *Nat Rev Rheumatol*. 2022;18(10):559-574. doi: 10.1038/s41584-022-00819-y
11. Guchelaar NAD, Waling MM, Adhin AA, van Daele PLA, Schreurs MWJ, Rombach SM. The value of anti-neutrophil cytoplasmic antibodies (ANCA) testing for the diagnosis of ANCA-associated vasculitis, a systematic review and meta-analysis. *Autoimmun Rev*. 2021;20(1):102716. doi: 10.1016/j.autrev.2020.102716
12. Cabral DA, Uribe AG, Benseler S, et al. Classification, presentation, and initial treatment of Wegener's granulomatosis in childhood. *Arthritis Rheum*. 2009;60(11):3413-3424. doi: 10.1002/art.24876
13. Morales-Angulo C, García-Zornoza R, Obeso-Agüera S, Calvo-Alén J, González-Gay MA. Manifestaciones otorrinolaringológicas en pacientes con granulomatosis de Wegener (granulomatosis con poliangeitis) [Ear, nose and throat manifestations of Wegener's granulomatosis (granulomatosis with polyangiitis)]. *Acta Otorrinolaringol Esp*. 2012;63(3):206-211. doi: 10.1016/j.otorri.2011.12.002
14. Jeleniewicz R, Suszek D, Majdan M. Musculoskeletal symptoms in a group Of granulomatosis with polyangiitis patients. *Wiad Lek*. 2018;71(1 pt 1):17-20.
15. Gomes GL, Halpern AS, Souza FH, Shinjo SK. Association between saddle nose deformity and retro-orbital mass in Wegener's granulomatosis. *Acta Reumatol Port*. 2010;35(3):340-345.
16. Byszewska A, Skrzypiec I, Rymarz A, Niemczyk S, Rękas M. Ocular Involvement of Granulomatosis with Polyangiitis. *J Clin Med*. 2023;12(13):4448. doi: 10.3390/jcm12134448
17. Zhang W, Zhou G, Shi Q, Zhang X, Zeng XF, Zhang FC. Clinical analysis of nervous system involvement in ANCA-associated systemic vasculitides. *Clin Exp Rheumatol*. 2009;27(1 Suppl 52):65-69.
18. Robson JC, Grayson PC, Ponte C, et al. 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for granulomatosis with polyangiitis. *Ann Rheum Dis*. 2022;81(3):315-320. doi: 10.1136/annrheumdis-2021-221795
19. Hellmich B, Sanchez-Alamo B, Schirmer JH, et al. EULAR recommendations for the management of ANCA-associated vasculitis: 2022 update. *Ann Rheum Dis*. 2024;83(1):30-47. doi: 10.1136/ard-2022-223764
20. Robson JC, Dawson J, Cronholm PF, et al. Patient perceptions of glucocorticoids in anti-neutrophil cytoplasmic antibody-associated vasculitis. *Rheumatol Int*. 2018;38(4):675-682. doi: 10.1007/s00296-017-3855-6
21. Stone JH, Merkel PA, Spiera R, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med*. 2010;363(3):221-232. doi: 10.1056/NEJMoa0909905
22. Specks U, Merkel PA, Seo P, et al. Efficacy of remission-induction regimens for ANCA-associated vasculitis. *N Engl J Med*. 2013;369(5):417-427. doi: 10.1056/NEJMoa1213277
23. de Joode AAE, Sanders JSF, Puéchal X, et al. Long term azathioprine maintenance therapy in ANCA-associated vasculitis: combined results of long-term follow-up data. *Rheumatology (Oxford)*. 2017;56(11):1894-1901. doi: 10.1093/rheumatology/kex281
24. Alesaeidi S, Darvishi M, Dabiri S, GhasemiAdl M, Jafarieh A, Tavakolpour S. Current Understanding and Unknown Aspects of the Treatment of Granulomatosis with Polyangiitis (Wegener's Granulomatosis): Opportunities for Future Studies. *Curr Rheumatol Rev*. 2020;16(4):257-266. doi: 10.2174/1573397116666200331130919
25. Jayne DRW, Merkel PA, Schall TJ, Bekker P; ADVOCATE Study Group. Avacopan for the Treatment of ANCA-Associated Vasculitis. *N Engl J Med*. 2021;384(7):599-609. doi: 10.1056/NEJMoa2023386
26. Gendelman O, Kuntzman Y, Shovman O, et al. Tumor-like Lesions in Patients with Granulomatosis with Polyangiitis: A Case Series. *Isr Med Assoc J*. 2021;23(6):350-352.
27. Walters GD, Willis NS, Cooper TE, Craig JC. Interventions for renal vasculitis in adults. *Cochrane Database Syst Rev*. 2020;1(1):CD003232. doi: 10.1002/14651858.CD003232.pub4
28. Luna M, Bocanegra V, Vallés PG. Granulomatosis with polyangiitis: rapidly progressive necrotizing glomerulonephritis in a pediatric patient. *Int J Nephrol Renovasc Dis*. 2014;7:153-156. doi: 10.2147/IJNRD.S57109
29. Kronbichler A, Kerschbaum J, Gopaluni S, et al. Trimethoprim-sulfamethoxazole prophylaxis prevents severe/life-threatening infections following rituximab in anti-neutrophil cytoplasm antibody-associated vasculitis. *Ann Rheum Dis*. 2018;77(10):1440-1447. doi: 10.1136/annrheumdis-2017-212861

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Competing interests statement format guidelines

The statement included in the article file must be explicit and unambiguous, describing any potential competing interest (or lack thereof) for EACH contributing author.

Examples of declarations are:

— Competing interests: The author(s) declare no competing interests.
— Competing interests: Dr X's work has been funded by A. He has received compensation as a member of the scientific advisory board of B and owns stock in the company. He also has consulted for C and received compensation. Dr Y and Dr Z declare no potential conflict of interest.
— Competing interests: "This work was supported by the [Funding Agency] under Grant [number]."

Peer-reviewers

The Eur J Clin Exp Med invites peer-reviewers to exclude themselves in cases where there is a significant conflict of interest, financial or otherwise. However, just as financial interests need not invalidate the conclusions of an article, nor do they automatically disqualify an individual from evaluating it. We ask peer-reviewers to inform the editors of any related interests, including financial interests as defined above that might be perceived as relevant. Editors will consider these statements when weighing peer-reviewers' recommendations.

Availability of materials and data

In order to maintain the integrity, transparency and reproducibility of research records, authors are encouraged to make their experimental and research data openly available either by depositing into data repositories or by publishing the data and files as supplementary information in this journal.

Data may be deposited with specialized service providers or institutional/subject repositories, preferably those that use the DataCite mechanism. Large data sets and files greater than 60 MB must be deposited in this way. For a list of other repositories specialized in scientific and experimental data, please consult databib.org or re3data.org. The data repository name, link to the data set (URL) and accession number, doi or handle number of the data set must be provided in the paper. The journal Data also accepts submissions of data set papers.

Data availability statement format guidelines

The statement should be provided as a separate section (titled 'Data Availability') at the end of the main text, before the 'References' section. Data availability statements should include, where applicable, accession codes, other unique identifiers and associated web links for publicly available datasets, and any conditions for access of non-publicly available datasets. Where figure source data are provided, statements confirming this should be included in data availability statements. Depending on the data described in the manuscript, data availability statements commonly take one of the following forms, or can be a composite of the statements below:

— The datasets generated during and/or analyzed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS].
— The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.
— All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).
— The datasets generated during and/or analyzed during the current study are not publicly available due to [REASON(S) WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.
— No datasets were generated or analyzed during the current study.
— The data that support the findings of this study are available from [THIRD PARTY NAME] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [THIRD PARTY NAME].

Correction and retraction policy

The Eur J Clin Exp Med operates the following policy for making corrections to its peer-reviewed content.

Publishable amendments must be represented by a formal online notice because they affect the publication record and/or the scientific accuracy of published information. Where these amendments concern peer-reviewed material, they fall

into one of four categories: Publisher Correction (formerly Erratum), Author Correction (formerly Corrigendum), Retraction or Addendum.

Publisher Correction (formerly Erratum). Notification of an important error made by the journal that affects the publication record or the scientific integrity of the paper or the reputation of the authors or the journal.

Author Correction (formerly Corrigendum). Notification of an important error made by the author(s) that affects the publication record or the scientific integrity of the paper, or the reputation of the authors or the journal.

Retraction. Notification of invalid results. All co-authors must sign a Retraction specifying the error and stating briefly how the conclusions are affected, and submit it for publication. In cases where co-authors disagree, the in-house editors may seek advice from independent referees and impose the type of amendment that seems most appropriate, noting the dissenting author(s) in the text of the published version.

Addendum. Notification of additional information. Addenda are published when the in-house editors decide that the addendum is crucial to the reader's understanding of a significant part of the published contribution.

Peer-review process

Initial checks

Once submitted, your manuscript will be assigned to a member of our Editorial Board, who will read the paper and decide whether it is appropriate for the journal. Manuscripts that are within scope and seem, on initial assessment, to be technically sound and scientifically valid, will be sent to external reviewers. Copies of any papers containing similar or related work under consideration or in press at other journals must be included with the submission.

Manuscripts that do not fit the journal's ethics policy or do not meet the standards of the journal will be rejected before peer-review. Manuscripts that are not properly prepared will be returned to the authors for revision and resubmission.

Peer review

Once a manuscript passes the initial checks, it will be assigned to at least two independent experts for peer-review. Reviewers will be able to access your manuscript securely using our online system, whilst maintaining referee anonymity. A double-blind review is applied, where authors' identities are unknown to reviewers and vice versa. Peer review comments are confidential and will only be disclosed with the express agreement of the reviewer.

Editorial decision

After considering the reviewer reports the Editorial Board Member will make one of the following decisions:

— Accept outright,
— Request a minor revision, where authors revise their manuscript to address specific concerns,
— Request a major revision, where authors revise their manuscript to address significant concerns and perhaps undertake additional work,
— Reject outright.

The final decision is made by the Editor-in-Chief.

Revisions

In cases where the referees or Editorial Board Member has requested changes to the manuscript, you will be invited to prepare a revision. The decision letter will specify a deadline for submission of a revised manuscript. Once resubmitted, the manuscript may then be sent back to the original referees or to new referees, at the Editorial Board Member's discretion.

A revised manuscript should be submitted via the revision link provided in the decision letter, and not as a new manuscript. Authors should attach a cover letter to explain, *point by point*, the details of the revisions to the manuscript and responses to the referees' comments. Cover letters should not contain information that could identify the authors. The destination of the cover letter file in the submission system is 'Supplementary File for Review'. Please ensure that all issues raised have been addressed in the first round of revision. Where the authors disagree with a reviewer, they must provide a clear response.

Final submission and acceptance

When all editorial issues are resolved, your paper will be formally accepted for publication. Once accepted, the manuscript will undergo professional copy-editing, English editing, final corrections, pagination, and, publication on the http://www.ejcem.ur.edu.pl/. The Eur J Clin Exp Med reserves the right to make the final decision about matters of style and the size of figures.

Appeals

Even in cases where the Eur J Clin Exp Med does not invite resubmission of a manuscript, some authors may ask the Editorial Board to reconsider a rejection decision. These are considered appeals, which, by policy, must take second place to the normal workload. In practice, this means that decisions on appeals often take several weeks. Only one appeal is permitted for each manuscript, and appeals can only take place after peer review. Final decisions on appeals will be made by the Editorial Board Member handling the paper.

Decisions are reversed on appeal only if the relevant Editorial Board Member is convinced that the original decision was a serious mistake. Consideration of an appeal is merited if a referee made substantial errors of fact or showed evidence of bias, but only if a reversal of that referee's opinion would have changed the original decision.

Similarly, disputes on factual issues need not be resolved unless they were critical to the outcome.

If an appeal merits further consideration, the Editorial Board Member may send the authors' response and the revised paper out for further peer review.

ORCID

The Eur J Clin Exp Med supports the use of ORCID. The Eur J Clin Exp Med mandates ORCID iDs for all submitting authors; this is published on the final article to promote discoverability and credit. Please provide the ORCID iDs of the authors in the title page.

Submission guidelines

Submission process

Manuscripts for the Eur J Clin Exp Med should be submitted online at https://mc04.manuscriptcentral.com/pmur. The submitting author, who is generally the corresponding author, is responsible for the manuscript during the submission and peer-review process. The submitting author must ensure that all eligible co-authors have been included in the author list (read the criteria to qualify for authorship) and that they have all read and approved the submitted version of the manuscript. To submit your manuscript, register and log in to the submission website. All co-authors can see the manuscript details in the submission system, if they register and log in using the e-mail address provided during manuscript submission.

Cover letter

A cover letter must be included with each manuscript submission. It should be concise and explain why the content of the paper is significant, placing the findings in the context of existing work and why it fits the scope of the journal. Confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal. The names of proposed and excluded reviewers should be provided in the submission system, not in the cover letter.

Accepted file formats

Authors must use Microsoft Word to prepare their manuscript. Please insert your tables, graphics (schemes, figures, etc.) in the main text after the paragraph of its first citation.

In most cases, we do not impose strict limits on word count or page number. However, we strongly recommend that you write concisely and stick to the following guidelines:

— We encourage not exceeding 20 pages for original and review papers, and 8 pages for case reports of standard computer text (1800 signs on a page).
— The main text should be no more than 4,500 words (not including Abstract, Methods, References and figure legends).
— The title should be no more than 20 words.
— The abstract should be no more than 250 words.
— Recommended font: Times New Roman, 12 points.
— Manuscript text should be double-spaced. Do not format text in multiple columns.

Types of Publications

Manuscripts submitted to the Eur J Clin Exp Med should neither be published previously nor be under consideration for publication in another journal. The main article types are as follows:

Original research manuscripts. The journal considers all original research manuscripts provided that the work reports scientifically sound experiments and provides a substantial amount of new information.

Reviews. These provide concise and precise updates on the latest progress made in a given area of research. Systematic reviews should follow the PRISMA guidelines.

The Eur J Clin Exp Med accepts also the following types of submissions: case reports, letters to the editor, commentaries, book reviews, and reports from scientific meetings and conferences.

Reporting guidelines

The guidelines listed below should be followed where appropriate. Please use these guidelines to structure your article. Completed applicable checklists, structured abstracts and flow diagrams should be uploaded with your submission; these will be published alongside the final version of your paper.

Please refer to existing guidelines for reporting methodology; e.g.:

— AGREE guidelines for clinical practice guidelines
— ARRIVE guidelines for *in vivo* animal studies
— CARE guidelines for clinical case reports
— CONSORT guidelines for clinical trials
— PRISMA guidelines for systematic reviews and meta-analyses
— SPIRIT for clinical trials
— STARD guidelines for studies of diagnostic accuracy
— STROBE guidelines for observational studies

Manuscript preparation

Your paper should consist of the following parts. Title page should be supplied as a **separate** file.

Research manuscripts should comprise:

— Title page: Title, Author list, Affiliations, Abstract, Keywords.
— Research manuscript sections: Introduction, Aim, Materials and Methods, Results, Discussion, Conclusions.
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions,

Conflicts of Interest, Data Availability, Ethics Approval, References.

Research manuscript sections:

— *Introduction*

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

— *Material and methods*

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

— *Results*

Results should be clear and concise. The section may be divided into subsections, each with a concise subheading. Tables and figures central to the study should be included in the main paper. Do not use the term "significant" unless p-values are provided. Show p-values to 2 or 3 decimal places. The Results section should be written in past tense.

— *Discussion*

This should explore the significance of the results of the work, not repeat them. Avoid extensive citations and discussion of published literature.

— *Conclusions*

Summarize the work's findings, state their importance, and possibly recommend further research.

Review manuscripts should comprise:

— Title page: Title, Author list, Affiliations.
— Abstract, Keywords, Literature review sections.
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions, Conflicts of Interest, Data Availability, References.

Structured reviews and meta-analyses should use the same structure as research articles and ensure they conform to the PRISMA guidelines.

Case reports should comprise:

— Title page: Title, Author list, Affiliations.
— Abstract, Keywords. Case reports should include a succinct introduction about the general medical condition or relevant symptoms that will be discussed in the case report; the case presentation including all of the relevant de-identified demographic and descriptive information about the patient(s), and a description of the symptoms, diagnosis, treatment, and outcome; a discussion providing context and any necessary explanation of specific treatment decisions; a conclusion briefly outlining the take-home message and the lessons learned.
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions, Conflicts of Interest, Data Availability, Ethics Approval, References.

Requirements for case reports submitted to Eur J Clin Exp Med:

— Patient ethnicity must be included in the Abstract under the Case Presentation section.
— Consent for publication is a mandatory journal requirement for all case reports. Written informed consent for publication must be obtained from the patient (or their parent or legal guardian in the case of children under 18, or from the next of kin if the patient has died).

Language Style

Manuscripts must be submitted in English (American or British usage is accepted, but not a mixture of these).

Title page

These sections should appear in all manuscript types:

Title: The title of your manuscript should be concise and informative. It should identify if the study reports (human or animal) trial data, or is a systematic review, meta-analysis or replication study. When gene or protein names are included, the abbreviated name rather than full name should be used.

Author list and affiliations: Authors' full first and last names must be provided. For each affiliation provide the details in the following order: department, institution, city, country. If available, the e-mail address of each author should also be provided. At least one author should be designated as *corresponding author*, and his or her email address and other details should be included at the end of the affiliation section.

Abstract: The abstract should be a total of about 250 words maximum. The abstract should be a single paragraph and should follow the style of structured abstracts: *Introduction and aim:* Place the question addressed in a broad context and highlight the purpose of the study; *Material and methods*: Describe briefly the main methods or treatments applied. Include any relevant preregistration numbers, and species and strains of any animals used. *Results:* Summarize the article's main findings; and *Conclusion*: Indicate the main conclusions or interpretations.

Keywords: Three to six pertinent keywords need to be added after the abstract in alphabetical order. We recommend that the keywords are specific to the article, yet reasonably common within the subject discipline.

Back matter

Supplementary materials: Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate the name and title of each element as follows Figure S1: title, Table S1: title, etc.

Acknowledgments: Thank all of the people who helped with the research but did not qualify for authorship. Acknowledge anyone who provided intellectual assistance, technical help, or special equipment or materials.

Funding statement: All sources of funding of the study should be disclosed.

Author contributions: Authors must supply an Author Contribution Statement as described in the *Author contributions statements* section.

Conflicts of interest: Authors must supply a competing interests statement. For more details please see *Competing interests policy*.

Data availability: Authors must include a Data Availability Statement in all submitted manuscripts; see *Availability of materials and data* section for more information.

Ethics approval: Example of an ethical statement: "All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of XXX (Project identification code)."

References: References must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as EndNote, Reference Manager or Zotero to avoid typing mistakes and duplicated references.

References style

In-text citations and references should be prepared according to the American Medical Association (AMA) style. Each item should be listed in numerical order.

In-text citations

Each reference should be cited in the text using superscript arabic numerals. These superscript numbers should be outside periods. If you are citing sequential references, these should be indicated with a hyphen. Nonsequential references should be separated with commas. There should not be a space between numbers. For example: The degree of respiratory muscles fatigue depends on the applied exercise protocol and the research group's fitness level.[1,2] The greatest load with which a patient continues breathing for at least one minute is a measure of inspiratory muscles strength.[3] Diabetes mellitus is associated with a high risk of foot ulcers.[4-6]

Sample Reference

In listed references, the names of all authors should be given unless there are more than 6, in which case the names of the first 3 authors are used, followed by "et al.". If the source does not have any authors, the citation should begin with the title.

To find the proper abbreviation of journal go to the National Library of Medicine PubMed Journals Database at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Journals.

Page number(s) should be inserted in full (for example: use 111–112, not 111–2).

The following are examples of individual citations made according to the required rules of editing and punctuation:

— **Article from a journal, number of authors from 1 to 6**

Author AA, Author BB, Author CC. Title of article. *Accepted Abbreviated Journal Title*. Year;Volume(Issue):Page-Page. doi (if available)

Lee JC, Seo HG, Lee WH, Kim HC, Han TR, Oh BM. Computer-assisted detection of swallowing difficulty. C*omput Methods Programs Biomed*. 2016;134(2):72-78. doi: 10.1016/j.cmpb.2016.07.010

Morris A. New test for diabetes insipidus. *Nat Rev Endocrinol*. 2019;15(10):564-565. doi: 10.1038/s41574-019-0247-x

— ***Article from a journal, number of authors* more *than 6***

Author AA, Author BB, Author CC, et al. Title of article. *Accepted Abbreviated Journal Title*. Year;Volume(Issue):Page-Page. doi (if available)

Gonzalez ME, Martin EE, Anwar T, et al. Mesenchymal stem cell-induced DDR2 mediates stromal-breast cancer interactions and metastasis growth. *Cell Rep*. 2017;18:1215-1228. doi: 10.1016/j.celrep.2016.12.079

Jordan J, Toplak H, Grassi G, et al. Joint statement of the European Association for the Study of Obesity and the European Society of Hypertension: obesity and heart failure. *J Hypertens*. 2016;34:1678-1688. doi: 10.1097/HJH.0000000000001013

— **Websites**

Author AA (if indicated). Webpage title. Name of Website. URL. Published or Updated date. Accessed date.

Cholera in Haiti. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/haiticholera/. Published October 22, 2010. Updated January 9, 2012. Accessed February 1, 2012.

Address double burden of malnutrition: WHO. World Health Organization site. http://www.searo.who.int/mediacentre/releases/2016/1636/en/. Accessed February 2, 2017.

— **Book**

Author AA, Author BB. *Title of Work*. Location: Publisher; Year:Page-Page

Doane GH, Varcoe C. *Family Nursing as Relational Inquiry: Developing Health– Promoting Practice*. Philadelphia, PA: Lippincott Williams & Wilkins; 2005:25-28.
London ML, Ladewig PW, Ball JW, et al. *Maternal & Child Nursing Care*. Upper Saddle River, NJ: Pearson Education; c2011:101-103.
— Chapter in a book
Chapter Author AA. Title of chapter. In: *Name of Book*. Edition Number. Editor AA, ed. Location: Name of Publisher; Year:Page-Page.
Grimsey E. An overview of the breast and breast cancer. In: *Breast Cancer Nursing Care and Management*. 2nd ed. Harmer V, ed. Chichester, UK: Wiley-Blackwell; 2011:35-42.
NOTE: The Editorial Board requires consistent and carefully made references prepared according to the above-mentioned AMA standards. Otherwise, the work will be sent back to the authors.

Preparing figures, schemes and tables

File for Figures and Schemes must be provided during submission and at a sufficiently high resolution (minimum 1000 pixels width/height, or a resolution of 300 dpi or higher). Common formats are accepted, however, TIFF, JPEG, EPS and PDF are preferred.

Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure (not on the figure itself) or above the table. All figures, schemes, and tables should be numbered following their number of appearance (Figure 1, Scheme 1, Figure 2, Scheme 2, Table 1, etc.).

Tables should present new information rather than duplicating what is in the text. Readers should be able to interpret the table without reference to the text.

All table columns should have an explanatory heading. To facilitate the copy-editing of larger tables, smaller fonts may be used, but no less than 8 pt. in size. Tables must be provided in an editable format in appropriate place in the main text. Tables provided as jpeg/tiff files will not be accepted. Do not submit your tables in separate files.

Abbreviations

The journal requires using only standard abbreviations. Abbreviations should be defined in parentheses the first time they appear in the abstract, main text and in figure or table captions and used consistently thereafter. Ensure consistency of abbreviations throughout the article. Keep abbreviations to a minimum.

SI Units

SI Units (International System of Units) should be used. Imperial, US customary and other units should be converted to SI units whenever possible.