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Determining treatment success in tubal ectopic pregnancies – the predictive value of the delta neutrophil index in single-dose methotrexate therapy

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ABSTRACT

Introduction and aim. This study assessed the significance of the Delta neutrophil index (DNI) in predicting the effectiveness of single-dose methotrexate (MTX) treatment for tubal ectopic pregnancy.

Material and methods. In this retrospective study, 214 women diagnosed with tubal ectopic pregnancies and treated with MTX between 2019 and 2022 were included. Group 1 comprised 88 MTX responders, while group 2 consisted of 86 non-responders necessitating surgery. HCG and DNI levels were monitored on day 1, 4, and 7 of MTX treatment and compared between the groups.

Results. Group 1 exhibited significantly lower HCG and DNI levels compared to group 2 on days 1, 4, and 7 ($p < 0.001$). Both HCG and DNI levels on days 1 and 4 of MTX treatment were significant predictors of treatment failure, with the highest AUC observed for DNI was on day 4. In multivariate logistic regression analysis, elevated DNI levels on day 1 of MTX treatment were associated with a 5.8-fold increased risk, and DNI levels on day 4 were associated with a 4.44-fold increased risk of MTX treatment failure.

Conclusion. DNI emerges as a valuable marker for predicting the success of single-dose methotrexate treatment in tubal ectopic pregnancies, demonstrating superior predictive power compared to HCG.

Keywords. clinical prediction, ectopic pregnancy outcomes, methotrexate efficacy, neutrophil response, prognostic indication

Introduction

An ectopic pregnancy is defined as a pregnancy implanted outside the uterine cavity, with the majority of such cases occurring in the fallopian tube.¹ Ectopic pregnancy poses a significant threat to the life of the mother and is a leading cause of maternal morbidity among women of reproductive age.² Confirming the presence of an ectopic pregnancy typically involves pe-

riodic measurements of human chorionic gonadotropin (HCG) levels and transvaginal ultrasound examinations.^{3,4} The routine use of ultrasonography has greatly improved the diagnosis of ectopic pregnancies, allowing for the selection of medical treatment in a majority of cases. The liberal use of methotrexate (MTX) in medical management has significantly reduced the need for surgical intervention and associated complications

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in the treatment of ectopic pregnancies.⁵⁻⁶ In this context, single-dose MTX has emerged as a safe and effective method, with a crucial early indicator of treatment success being a substantial reduction in HCG levels between days 4 and 7 of MTX therapy.⁶

Numerous studies in the literature have sought to identify markers that predict the success of MTX treatment, but none have achieved a satisfactory level of accuracy. Therefore, the quest for more effective and sensitive markers of medical treatment success remains an ongoing challenge. Some inflammatory cytokines known to play roles in angiogenesis, inflammation, and immunity have been detected at both the implantation site and in the systemic circulation of tubal ectopic pregnancies.⁷⁻¹¹

The delta neutrophil index (DNI) is a novel marker of inflammation that can be readily assessed through routine complete blood count (CBC) testing.¹⁰⁻¹¹ DNI measures the percentage of immature granulocytes in the bloodstream. In cases of infection or inflammation, increased production of myeloid cell lines leads to an elevated presence of immature granulocytes.¹²⁻¹⁵ Recent research has explored the potential of DNI to predict various inflammatory conditions.¹²⁻¹⁸

The detection of certain inflammatory cytokines at the implantation site and in systemic circulation in ectopic pregnancy has prompted our interest in exploring the association between DNI and this condition.⁷⁻¹¹ Investigating DNI as a marker of inflammatory processes in ectopic pregnancies may provide valuable insights for determining treatment success and refining management strategies. Therefore, this study aimed to examine DNI in ectopic pregnancies, marking the first instance in the literature. This represents a crucial step in enhancing our understanding of treatment success and improving management strategies for this condition.

Aim

The primary objective of this study is to investigate the role of DNI in determining the success of single-dose MTX treatment in cases of tubal ectopic pregnancy.

Material and methods

This retrospective cohort study analyzed the medical records of pregnant women diagnosed with tubal ectopic pregnancy at a tertiary care center between 2019 and 2022. The study protocol received approval from the Institutional Review Board (IRB) on June 23, 2022 (IRB approval number: 11-23.06/09/2022). All participating women provided informed consent upon hospital admission, granting permission for their medical records to be used in future studies. The study adhered to the principles outlined in the Declaration of Helsinki.

Ectopic pregnancy was diagnosed when ultrasound imaging revealed a gestational sac located in the fallopian tubes, in conjunction with the absence of a gestational sac in the uterus and HCG levels exceeding 1500 mIU/mL, as per diagnostic criteria.

Patients admitted to the hospital with a diagnosis of tubal ectopic pregnancy undergo routine physical examinations, pelvic and transvaginal ultrasonographies, complete blood counts, and liver and kidney function tests. Patients' medical history and detailed medical background are questioned. Vital signs are closely monitored during hospitalization. Following comprehensive evaluations, decisions regarding follow-up, medical treatment, or surgical options are made in consultation with the patient based on their suitability. Women with tubal ectopic pregnancy who were clinically stable and exhibited no signs of tubal rupture or bleeding were selected for MTX treatment. However, MTX treatment was contraindicated for women with hypersensitivity reactions to this medication, pregnant or breastfeeding women, individuals with pre-existing blood disorders such as bone marrow hypoplasia, leukopenia, thrombocytopenia, or significant anemia, as well as those with rheumatoid arthritis or psoriasis, chronic liver disease, liver cirrhosis, alcoholic hepatitis, or chronic alcoholism. On the day of admission, women who were not clinically stable, exhibited signs of tubal rupture or bleeding, and did not meet the criteria for MTX treatment were selected for surgical intervention.

All women in the study cohort received a single intramuscular dose of MTX at a dosage of 50 mg/m², following established guidelines.¹⁹ No other medical treatment interventions were applied beyond MTX. Patients were monitored until the ectopic pregnancy was resolved.

In the 2018 ACOG (American College of Obstetricians and Gynecologists) guidelines, successful MTX treatment was defined as a decrease in HCG levels of 15% or more when comparing days 4 and 7, with HCG levels dropping to <0.5 mIU/mL during follow-up, and no need for additional MTX or surgery.¹⁹ Exclusion criteria encompassed women with non-tubal ectopic pregnancies, those exhibiting ectopic fetal heart activity, individuals with HCG levels exceeding 10,000 mIU/mL, and those with a tubal mass greater than 4 cm. Additionally, individuals requiring a repeat dose of MTX, surgery for pain or hemodynamic instability before day 4 of MTX treatment, those with heterotopic pregnancies, or individuals with chronic inflammatory or autoimmune diseases were also excluded from the study.

Figure 1 illustrates the study's flowchart. The study comprised a total of 174 women, selected from a pool of 331 women diagnosed with tubal ectopic pregnancies.

These women were divided into two groups based on their response to MTX treatment. Group 1 included 88 women who responded to MTX treatment, while group 2 comprised 86 women who necessitated surgical intervention after day 4 of MTX treatment. HCG levels, CBC, renal function tests, and liver function tests, were conducted on days 1, 4, 7, 14, and 21 of MTX treatment. Day 1 was considered the initial day of MTX administration. All blood tests were processed at the same laboratory. DNI was determined using the ADVIA 2120 automated hematology analyzer. Data regarding demographic characteristics, obstetric history, physical examination findings, and laboratory results were extracted from patient records and the hospital's database. The choice of surgical procedure involved either laparoscopy or laparotomy.

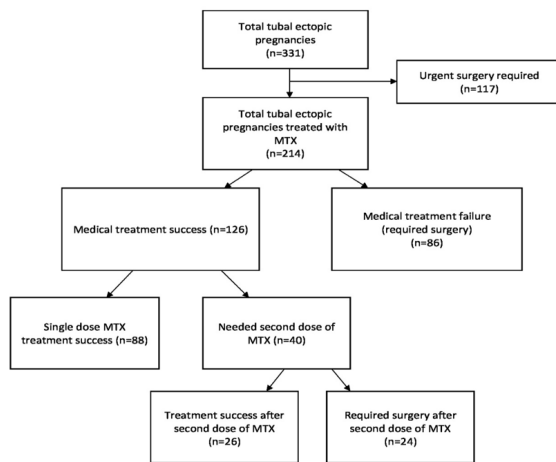


Fig. 1. Flow chart of the study

Statistical analysis

The IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Descriptive statistics were used to summarize the data. The comparison of nominal data was conducted using the independent samples t-test and presented as mean \pm standard deviation. For ordinal variables, we employed the χ^2 test, and Fisher's exact test was utilized for variables with limited sample sizes. The receiver operating characteristic (ROC) curve was used to determine cut-off values, sensitivity, specificity, and the area under the curve (AUC). Significance was considered at a 95% confidence interval (CI) with a p-value of <0.05 . To identify independent risk factors for medical treatment failure, both univariate and multivariate logistic regression analyses were performed.

Results

A total of 174 women were diagnosed with tubal ectopic pregnancies, with 88 women categorized in Group 1, indicating a response to MTX treatment, and 86 women assigned to Group 2, who did not respond to MTX treatment and required surgical intervention. The success rate of single-dose MTX treatment was 50.5%.

Table 1 presents a comparison of age, BMI, laboratory parameters, and sonographic measurements between the two groups. No statistically significant differences were observed in terms of age, body mass index (BMI), CBC results, renal and liver function tests. There was a significant difference in HCG levels (1219.22 ± 1118.82 vs. 3493.66 ± 4488.96 IU/L, 1541.7 ± 1728.29 vs. 4709.79 ± 6504.37 IU/L, 1363.58 ± 1363.51 vs. 2945.75 ± 2241 IU/L, $p < 0.001$) and DNI levels ($0.99 \pm 0.61\%$ vs. $2.4 \pm 1.51\%$, $0.90 \pm 0.57\%$ vs. $4.92 \pm 2.98\%$, $1.07 \pm 0.58\%$ vs. $2.98 \pm 1.89\%$, $p < 0.001$) (Table 1). When comparing the differences in HCG and DNI levels between the 1st and 4th days of MTX treatment within the groups, a significant difference was observed in both HCG and DNI levels between these time points ($p < 0.001$).

Table 2 presents a comparison of the medical, obstetric, and gynecologic histories of the two groups, revealing no significant differences between them.

Table 1. Comparison of demographic, laboratory and ultrasonographic findings of groups*

	MTX success group (n=88)	MTX failure group (n=86)	p
Age (y)	30.12 \pm 4.23	30.51 \pm 5.1	0.587
BMI (kg/m ²)	25.75 \pm 4.44	25.46 \pm 4.22	0.656
Endometrial thickness (mm)	9.38 \pm 4.35	10.7 \pm 4.71	0.056
Adnexal mass size (cm)	1.55 \pm 0.95	1.5 \pm 1.13	0.721
Hb (mg/dL)	12.37 \pm 1.22	12.4 \pm 1.18	0.882
WBC	7901 \pm 2275	8024 \pm 2091	0.711
PLT	263 \pm 58	262 \pm 56	0.920
AST	16.2 \pm 6.29	16.26 \pm 6.11	0.947
ALT	16.98 \pm 8.63	16.75 \pm 8.54	0.858
LDH	224.94 \pm 101.26	201.3 \pm 78.22	0.087
BUN (mg/dL)	11.75 \pm 5.97	11.45 \pm 4.65	0.716
Creatinine (mg/dL)	0.6 \pm 0.08	0.61 \pm 0.09	0.255
1 st day HCG (mIU/mL)	1219.22 \pm 1118.82	3493.66 \pm 4488.96	<0.001
4 th day HCG (mIU/mL)	1541.7 \pm 1728.29	4709.79 \pm 6504.37	<0.001
7 th day HCG (mIU/mL)	1363.58 \pm 1363.51	2945.75 \pm 2241	<0.001
14 th day HCG (mIU/mL)	710.67 \pm 606.32	780.87 \pm 899.51	0.654
1 st day DNI (%)	0.99 \pm 0.61	2.4 \pm 1.51	<0.001
4 th day DNI (%)	0.90 \pm 0.57	4.92 \pm 2.98	<0.001
7 th day DNI (%)	1.07 \pm 0.58	2.98 \pm 1.89	<0.001
14 th day DNI (%)	1 \pm 0.96	1.17 \pm 0.95	0.232

* BUN – blood urea nitrogen, DNI – delta neutrophil index, HCG – human chorionic gonadotropin

ROC analyses of HCG and DNI levels on the 1st and 4th days of single-dose MTX treatment are presented in Figure 2 and Figure 3. Notably, both HCG and DNI levels on the 1st and 4th days of MTX treatment were found to be significant in predicting MTX treatment failure. For detailed values, Table 3 provides the optimal cut-off points, AUC, sensitivity, and specificity values for HCG and DNI levels on the 1st and 4th day of MTX treatment.

Table 2. Comparison of medical and obstetric history of groups*

	MTX success group	MTX failure group	p
	(n=88)	(n=86)	
	n (%)	n (%)	
History of ectopic pregnancy	15 (17)	10 (11.6)	0.308
Obstetric history			
Gravidity	3 (1–6)	3 (1–6)	0.287
Parity	1 (0–3)	1 (0–4)	0.144
Abortion	0 (0–3)	0 (0–3)	0.346
Smoking	24 (27.3)	34 (39.5)	0.086
History of PID	1 (1.1)	5 (5.8)	0.092
Contraceptive method	6 (6.8)	6 (7)	0.967
Current IUD user	4 (4.5)	6 (7)	0.491
Additional disease	7 (8)	6 (7)	0.806
Operation history	29 (33)	38 (44.2)	0.128
C-section	17	22	0.798
Salpingectomy	10	11	0.685
Appendectomy	2	1	0.545
Cholecystectomy	–	3	0.912
Inguinal hernia operation	–	1	0.934
Adnexal mass	83 (94.3)	83 (96.5)	0.490
Free intraabdominal fluid	18 (20.5)	18 (20.9)	0.938

* PID – pelvic inflammatory disease, IUD – intrauterine device

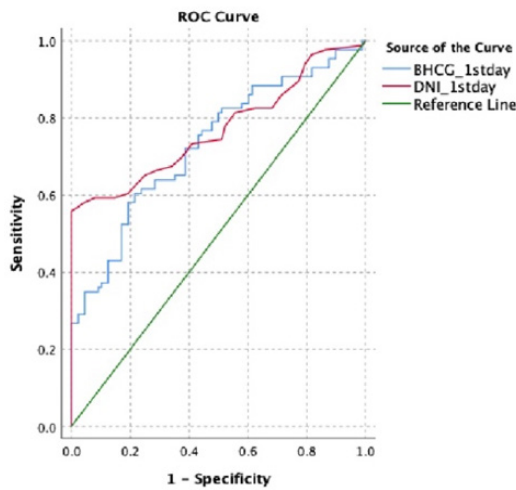


Fig. 2. ROC analysis of HCG and DNI levels on the 1st day of treatment

Table 3. AUC values and cuff off points of DNI and HCG for MTX failure*

	Cut value	AUC	p	Sensitivity	Specificity	95% Confidence interval	
						Lower Bound	Upper Bound
1st day DNI (%)	1.35%	0.771	<0.001	67.4%	65.9%	0.686	0.844
4th day DNI (%)	1.35%	0.862	<0.001	79.2%	75%	0.797	0.926
1st day HCG (mIU/mL)	1687	0.731	<0.001	65.1%	64.8%	0.654	0.812
4th day HCG (mIU/mL)	1838	0.724	<0.001	72.2%	70.5%	0.642	0.807

* DNI – delta neutrophil index

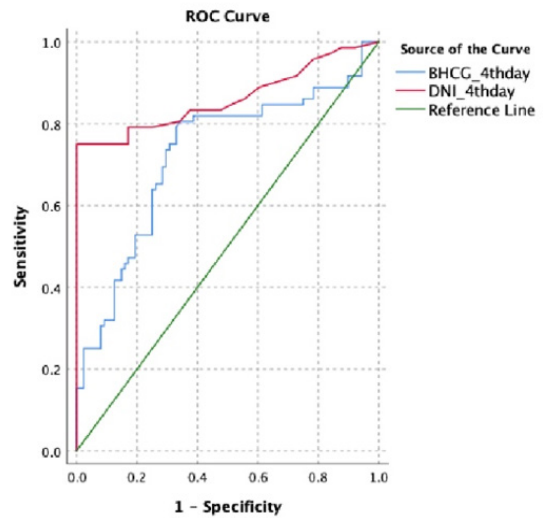


Fig. 3. ROC analysis of HCG and DNI levels on the 4th day of treatment

The correlation analysis revealed a weak, positive correlation between HCG and DNI levels. In univariate analysis, elevated HCG and DNI levels on the 1st and 4th day of MTX treatment emerged as independent risk factors for MTX treatment failure. Moving forward, multivariate logistic regression analysis demonstrated that elevated DNI levels on the 1st day of MTX treatment carried a 5.8-fold increased risk, while DNI levels on the 4th day of MTX treatment were associated with a 4.447-fold higher risk of MTX treatment failure (Table 4).

Table 4. Univariate and multivariate analysis and DNI and HCG*

	Univariate	p	Multivariate	p
	Odds Ratio (95% CI)		Odds Ratio (95% CI)	
1 st day DNI (%)	3.120 (2.114–4.602)	<0.001	5.8 (2.325–14.468)	<0.001
4 th day DNI (%)	2.927 (1.990–4.304)	<0.001	4.447 (2.307–8.575)	<0.001
1 st day HCG (mIU/mL)	1.001 (1–1.001)	<0.001	1.001 (1–1.001)	0.294
4 th day HCG (mIU/mL)	1 (1–1.001)	<0.001	1.001 (1–1.001)	0.440
Initial HCG (mIU/mL)	1.634 (0.945–2.893)	0.456		
Smoking status	1.744 (0.921–3.300)	0.088	–	
History of ectopic pregnancy	0.640 (0.270–1.517)	0.311	–	
History of PID	5.37 (0.614–46.953)	0.129	–	
History of IUD	1.575 (0.429–5.789)	0.494	–	
Contraception usage	1.025 (0.317–3.312)	0.967	–	
Adnexal mass	1.667 (0.386–7.201)	0.494	–	

* DNI – delta neutrophil index, IUD – intrauterine device, PID – pelvic inflammatory disease

Discussion

Maternal morbidity and mortality remain pressing healthcare concerns, particularly in many underdeveloped and some developing countries. Notable progress has been made through early diagnosis and medical in-

tervention using methotrexate in cases of ectopic pregnancy. Predicting the success of MTX treatment not only helps prevent complications like tubal rupture, optimizes patient follow-up, but also alleviates the anxiety experienced by affected women.

Our study delved into the association between DNI and tubal ectopic pregnancy, prompted by the detection of certain inflammatory cytokines at the implantation site and in systemic circulation.⁷⁻¹¹ Investigating DNI as a marker of inflammatory processes in ectopic pregnancies may provide valuable insights for determining treatment success and refining management strategies. This represents a significant advancement in the literature, as it marks the first instance of examining DNI in this context. By identifying high DNI levels on both day 1 and day 4 as potential indicators of MTX treatment success, our findings offer a promising avenue for improving patient outcomes.

Prior research has suggested that higher pretreatment HCG levels are associated with increased rates of treatment failure.^{7,22-23} However, in our study, no significant correlation was observed between pretreatment HCG levels and treatment outcomes. This discrepancy could be attributed to our selection criteria, which included only women eligible for MTX treatment with HCG concentrations less than 10,000 mIU/ml. However, our study contributes to the ongoing discussion on optimal tubal treatment criteria, highlighting the need for further research to establish universally applicable guidelines.

Helmy et al. noted that there is no clear-cut endpoint for the initial serum HCG level that can be universally employed as a cutoff value for determining optimal tubal treatment. Nevertheless, they found that women with an initial HCG level exceeding 2,121 mIU/mL were more likely to experience MTX treatment failure than their counterparts.¹¹ In a study conducted by Levin et al., a decrease in HCG concentration from day 1 to day 4, coupled with a slight increase in HCG concentration one day before MTX treatment, was identified as a predictor of medical treatment success for ectopic pregnancy.⁷ It was also demonstrated that an elevation in HCG levels before MTX administration is associated with treatment failure.²⁰⁻²² Levin et al. reported low success rates for treatment with a single dose of MTX in two separate studies.^{7,22} Our results similarly reflect a low success rate of MTX treatment (50.5%), likely influenced by our stringent inclusion and exclusion criteria. Predictors of single-dose MTX treatment were evaluated, with the requirement for repeated MTX dosing considered indicative of treatment failure.

Previous studies have consistently highlighted the presence of inflammation and alterations in the tubal microenvironment in cases of tubal ectopic pregnancies. This inflammatory response triggers the activation of numerous growth factors.²³ Cekmez et al. conducted

research investigating the role of systemic inflammatory markers in predicting the success of single-dose MTX treatment. They observed that mean platelet volume (MPV) and neutrophil-lymphocyte ratio (NLR) levels were lower in the treatment failure group.¹⁰ Another studies found that HCG, NLR, and platelet-lymphocyte ratio (PLR) levels were found to be associated with tubal rupture.^{24,25} Our study adds to this body of evidence by identifying DNI as a potential predictor of MTX treatment failure. However, it's important to acknowledge the limitations of our retrospective design and reliance on medical records data.

DNI's potential to distinguish between complicated and noncomplicated appendicitis has also been explored in previous studies. Shin et al. discovered that DNI stands out as the sole marker significantly predicting perforation, and its predictive capability for perforation tends to increase with age.²⁶ Meanwhile, Kim et al. examined the predictive value of DNI for acute appendicitis in children. They noted that the median levels of DNI and CRP were higher in the complicated group, although initial CRP values emerged as the sole predictor of complications.²⁷ The CRP value has not been assessed in our study as it is not a routinely examined parameter in cases of ectopic pregnancy.

In our study, we identified that DNI levels exceeding 1.35% serve as a reliable predictor of MTX treatment failure. Notably, DNI levels were found to be higher in the MTX treatment failure group. This could potentially be attributed to the increased presence of immature granulocytes in circulation, resulting from inflammation in the fallopian tube. In our multivariate regression analyses, both DNI levels on day 1 and day 4 independently emerged as risk factors for predicting MTX treatment failure. The observed increase in DNI between days 1 and 4 may signify a heightened biological response in cases of ectopic pregnancy, possibly associated with the lower success rates of MTX treatment.

The main limitations of our study are attributed to its retrospective design and the reliance on data extracted from medical records. To provide a more robust understanding of the predictive role of DNI in women with ectopic pregnancy, future studies with a prospective nature and a larger sample size are warranted.

Conclusion

Nonetheless, our findings suggest that DNI emerges as a valuable marker for predicting the success of single-dose methotrexate treatment in tubal ectopic pregnancies, demonstrating superior predictive power compared to HCG. It could prove invaluable in determining which women are likely to benefit from MTX treatment. Early prediction has the potential to optimize treatment timing, reduce patient burden, and enhance overall patient care.

Declarations

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Author contributions

Conceptualization, B.K. and C.K.; Methodology, B.K. and G.N.K.; Software, B.K. and C.K.; Validation, B.K., C.K. and G.N.K.; Formal Analysis, B.K.; Investigation, C.K.; Resources, S.C.; Data Curation, B.K. and C.K.; Writing – Original Draft Preparation, B.K.; Writing – Review & Editing, Y.E.U. and S.C.; Visualization, S.C.; Supervision, Y.E.U.; Project Administration, B.K.

Conflicts of interest

The authors declare that they have no conflict of interest.

Data availability

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

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. The study protocol received approval from the Institutional Review Board (IRB) on June 23, 2022 (IRB approval number: 11-23.06/09/2022).


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Effects of vitamin D3 supplementation on lipid profile and renal indices in rat model of drug induced renal injury

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ABSTRACT

Introduction and aim. Renal injury is associated with decreased renal function, hypovitaminosis D, deranged calcium-phosphate metabolism and dyslipidemia, thus increasing risk for chronic kidney disease and cardiovascular diseases. However, reports on the effects of vitamin D on drug induced renal injury are few. The aim was to investigate the possible role of vitamin D supplementation in reversing deranged lipid profile and renal function post drug induced renal injury.

Material and methods. Wister male rats (36) were randomly divided into group 1, 2 and 3 (n=12). Single dose of Adriamycin was given to all except group 1 (control) to induce renal injury. Group 2 left untreated, group 3 given vitamin D₃ for 28 days. Serum urea, creatinine, total protein, total cholesterol (TC), triglycerides (TG), LDL-C, HDL, apolipoprotein (Apo) A and B were measured. C-reactive protein (CRP) and nitric oxide were assessed in kidney homogenate.

Results. Vitamin D₃ significantly brought down levels of serum creatinine, TC, LDL CRP, nitric oxide and increased the levels of Apo A, albumin, HDL. Serum urea, TG and Apo B in group 3 were not significantly different after vitamin D₃ administration. Histological examination revealed improvement in glomerular mesangialisation.

Conclusion. Vitamin D₃ may improve renal health, through its positive impact on dyslipidemia, inflammation, and oxidative stress in drug induced renal injury.

Keywords. adriamycin, chronic kidney disease, dyslipidemia, oxidative stress, renal injury, vitamin D

Introduction

Chronic kidney disease (CKD) is a term that includes all degree of decrease in the kidney functions resulting from damage, and can be defined as a persistent abnormality in kidney structure or function (e.g. glomerular filtration rate (GFR) <60 mL/min/1.73 m² or albuminuria ≥30 mg per 24 hours) for more than 3 months.¹ Kidney disease is an under-recognized public health crisis.² It is a global problem affecting 8–16% of the world population and more common in women (14%) than men (12%).³ Renal disease affects an estimated 15% of

the adult population in the United States of America and its prevalence ranged from 10.7–13.9% in sub-Saharan Africa and approximately 11.4% when adjusted for age and gender in Nigeria.⁴

Renal disease occurs as a result of abnormalities in the structure or function of the kidney and it is linked to several complications including, cardiovascular disease, hypertension, anemia, mineral bone disorders, volume overload, electrolytes and acid-base derangements.⁵ Renal injury is most commonly attributed to diabetes and hypertension, other causes include glomerulonephri-

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tis, inherited diseases, such as polycystic kidney disease; immune diseases; kidney cancer, kidney stones, frequent untreated and/or long-lasting urinary tract infections, drugs/medications, toxic metal poisoning, renal artery stenosis etc.⁶ Regardless of the cause of renal injury, late diagnosis and poor management often results in progression to kidney failure, complications in other organs and death.⁷

Adriamycin (doxorubicin hydrochloride, ADR) an anti-tumor antibiotic important in the treatment of malignancies, such as prostate cancer, breast cancers and leukemia.⁸ ADR causes focal segmental glomerulosclerosis in rodents, which is characterized by proteinuria, progressive glomerulosclerosis, and tubulointerstitial injury.⁹ As a result, pharmacological research on human chronic renal disorders frequently uses the rodent form of ADR nephropathy as an experimental paradigm as employed in this present study.

Individuals with CKD exhibit an elevated cardiovascular risk manifesting as coronary artery disease, heart failure, arrhythmias, and sudden cardiac death.⁵ It is also associated with dyslipidemia and a systemic, chronic proinflammatory state contributing to vascular and myocardial remodeling processes resulting in atherosclerotic lesions, vascular calcification, and vascular senescence as well as myocardial fibrosis and calcification of cardiac valves.¹⁰ The general mechanisms underlying the dyslipidemia of renal disease may be summarized as altered metabolism of postprandial lipoproteins, altered metabolism of other triglyceride-rich lipoproteins, changes in the route of reverse cholesterol transport, structural changes of lipoproteins, postribosomal modifications of lipoproteins, insulin resistance, proteinuria, and increased lipoprotein(a).¹¹ Regardless of the cause of disease, adverse outcomes include progression to kidney failure, complications in other organs and tissues, and death.

CKD is also commonly associated with disorders of mineral and bone metabolism, manifested by either one or a combination of the following; abnormalities of calcium, phosphorus, parathyroid hormone, fibroblast growth factor 23 (FGF23), and vitamin D metabolism; abnormalities in bone turnover, mineralization, volume linear growth, or strength; extraskeletal calcification.¹² Vitamin D (25(OH)D) deficiency has also been shown by previous studies to be associated with the complications of renal injury.¹³ Low serum level of vitamin D is often associated with hypocalcemia, increased blood pressure, hyperparathyroidism, reduced bone density and cardiovascular disease.¹⁴ Vitamin D might be important in the regulation of lipid metabolism and may have implications for preventing the progression of CKD and aid in the management of CKD and ultimately CVD. Also, lower baseline vitamin D levels have been found to correlate with atherogenic blood lipid profiles, while 25(OH)D supplementation has been

found to influence the levels of serum lipids in that it lowers the levels of total cholesterol, triglycerides, and LDL cholesterol and increases the levels of HDL cholesterol in pregnancy complications like gestational diabetes mellitus and preeclampsia.^{15,16} Thus, dietary reversal of vitamin D-deficiency and treatment with vitamin D analogs could be therapeutic options for renal and cardiovascular related diseases. Recent studies on the role of Vitamin D in nephropathies reported renoprotective effects of Vitamin D through anti-inflammatory and antifibrotic mechanisms, however little is known on its role on cardiometabolic markers in kidney related pathologies.¹⁷

Aim

Hence, this study explored the efficacy of vitamin D supplementation in the amelioration of the complications of renal injury via its effects on cardiometabolic and renal markers implicated in the pathology of CKD. Overall, the study's significance lies in the potential of vitamin D supplementation to improve health outcomes in renal injury and prevent development of associated cardiovascular complications.

Materials and Methods

Experimental rats

This is an experimental study carried out at the Lead City Animal House between March, 2023 to June, 2023. Handling of experimental animals was as approved by the institution's ethical committee. Thirty-Six (36) male Wistar albino rats (weight between 190 and 250g) were purchased from Rainbow farms in Ojo, Ibadan Nigeria. Rat pellets (Vital Feed®, Nigeria) and unlimited water were provided to the animals, which were housed in well-ventilated plastic cages and allowed to get use to a 12-hour light/dark cycle for a week before the treatments started.

Body weight determination

Following their acclimatization, the body weight of rats was measured using weighing balance and initial weight recorded. This was used to calculate the dose of ADR (injection) administered. The body weight was subsequently determined weekly to adjust the doses of the treatment. After treatment in all groups, the final weight was measured.

Animal grouping and administration of ADR, vitamin D3 (cholecalciferol)

The male Wistar rats were divided into three groups and twelve (12) Wistar rats were randomly selected into each group. Group 1 served as the untreated control and were not given any treatment throughout the duration of the experiment.

After one week of acclimatization, ADR was administered to group 2 and 3 once intravenously at a single dose of 7.5 mg/kg of body weight at the first day to in-

duce nephrotoxicity in the experimental rats. The vehicle for ADR administration was 0.9% NaCl. The group 3 was the ADR treated group and were given oral cholecalciferol (200 IU/day). Vitamin D was administered at the dosage of 200 IU/kg body weight of Cholecalciferol (Vitabiotics®) and given orally using a gavage once daily for 28 days between 8:00–10:00 hrs.

Termination of treatment, and collection of organ

On the last day of treatment (28th day), all experimental animals were made to fast overnight with access to water only, and their final body weights taken. All experimental animals were euthanized using an approved method under the institutional guidelines and blood was collected through their orbital plexus into plain bottles and serum separated within 30 minutes of blood collection by centrifuging at 4000rpm for 15 minutes and kept at -20°C prior biochemical analysis. The kidney was surgically harvested for histopathological examination, and then homogenized at a PH of 7.4 with homogenizing buffer (1.15% potassium chloride solution and 50 mM Tris-HCl). For biochemical analysis, the homogenate was centrifuged for 10 minutes at 4°C and 12,500 rpm and kept at -20°C prior analysis.

Biochemical analysis

Serum albumin was determined by dye binding method known as bromocresol green (BCG) method as described by Duomas et al. using Randox Laboratories Limited diagnostic kits, England.¹⁸ Total protein was evaluated by biuret method as described by Tietz using Sigma Diagnostic Kit, USA.¹⁹ Creatinine was determined by alkaline picrate method as described by Murray using Randox Laboratories Limited Diagnostic kits, England.²⁰ Urea was quantified by enzymatic colorimetric UV method as described by Fawcett et al. using Sigma Diagnostic Kit, USA.²¹ C-reactive protein (CRP) was quantified by turbidimetric immunoassay as described by Tietz on an automated chemistry analyzer (LW C 100plus).²² Nitric oxide was determined by spectrophotometric method as described by Risa A Ridnour.²³ Plasma total cholesterol was analyzed by the cholesterol CHOD-PAP method, an enzymatic end point method as described by Allain et al.²⁴ HDL-C was quantified by precipitation through the procedure adopted by Lopez-Virella.²⁵ Serum triglycerides (TG) by GPO-PAP method as described by Trinder.²⁶ All lipid profile tests were done on an automated chemistry analyzer (LW C 100plus). LDL-C was calculated using Friedwald's formula as shown:

$$\text{LDL-cholesterol (mg/dl)} = \text{Total Cholesterol} - \text{HDL-cholesterol-TAG}/5.^{27}$$

Apolipoprotein A and B were estimated by immunoturbidimetric method as described by Labeur et al., on an automated chemistry analyzer (LW C 100plus).²⁸

Histopathological study

Histology of the kidney was carried out by a method described by Avwioro at the Anatomic Pathology Department, University College Hospital, Ibadan Nigeria.²⁹

Statistical analysis

Prism Graphpad, version 6.4, was used for data analysis. The data was subjected to descriptive statistics. One-way analysis of variance (ANOVA) was used to determine the statistical difference in mean between the three groups and level of significance was set at $p < 0.05$.

Results

There was a significant decrease in the body weight after treatment in the ADR only and ADR+vitamin D group as shown in Figure 1.

The serum levels of albumin was significantly lower ($p < 0.05$) in the ADR group when compared with control, a significant increase occurred after treatment with vitamin D as shown in Figure 2.

The serum levels of creatinine was significantly higher ($p < 0.05$) in the ADR group when compared with control while a significant decrease ($p < 0.05$) occurred after treatment with vitamin D in the ADR+vitamin D group as shown in Figure 4.

The serum levels of urea was significantly higher ($p < 0.05$) in the ADR group when compared with control but there was no significant difference in urea levels after treatment with vitamin D in the ADR+vitamin D group as shown in Figure 5.

Serum TC and TG levels were significantly higher ($p < 0.05$) in the Adriamycin group, while a significant decrease in TC occurred after vitamin D treatment in ADR+vitamin D group and there was no difference in TG after vitamin D treatment in ADR+vitamin D group as shown in Figures 6 and 9 respectively.

The serum levels of LDL was significantly higher ($p < 0.05$) in the ADR group when compared with control but there was no significant difference in urea levels after treatment with vitamin D in the ADR+vitamin D group as shown in Figure 8.

The serum levels of HDL, Apo A1 were significantly lower ($p < 0.05$) in the ADR group, while a significant increase occurred after vitamin D treatment in ADR+vitamin D group when compared with the ADR as shown in Figures 7 and 10 respectively.

There was no significant change in the levels of TP and Apo B in all groups after treatment as shown in Figures 3 and 11 respectively.

The CRP level in kidney homogenates was significantly higher ($p < 0.05$) in the ADR group when compared with control while a significant decrease occurred after vitamin D administration in ADR+vitamin D group when compared with the ADR group as shown in Figure 12.

Plate show severe tubular necrosis (black arrow) and glomerular messangialisation (green arrow) in group 2. There was only tubular necrosis (black arrow) seen in Group 3 as shown in Figure 14.

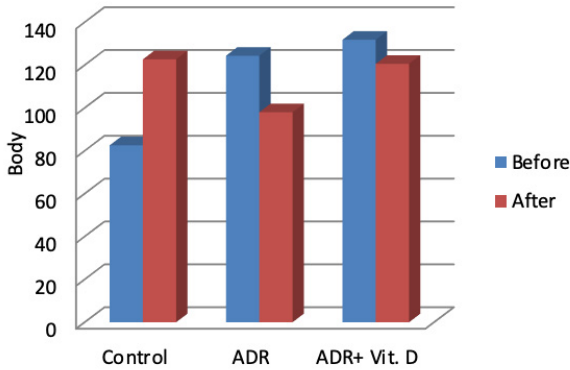


Fig. 1. Effects of vitamin D₃ on bodyweight in ADR-induced nephrotoxic rats

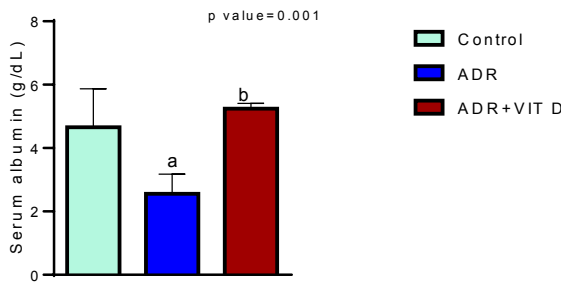


Fig. 2. Effects of vitamin D₃ on serum albumin in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

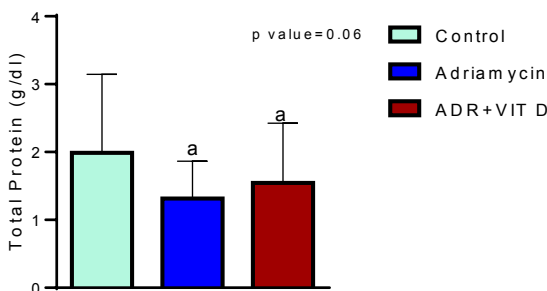


Fig. 3. Effects of vitamin D₃ on serum total protein in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

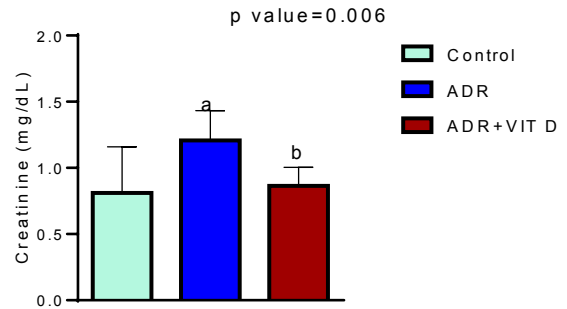


Fig. 4. Effects of vitamin D₃ on serum creatinine in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

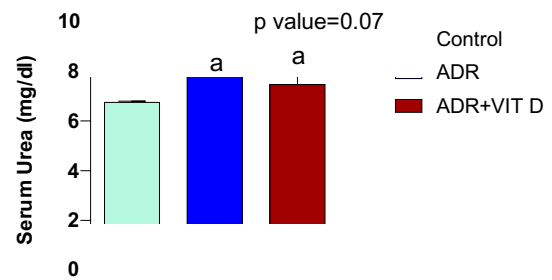


Fig. 5. Effects of vitamin D₃ on serum urea in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

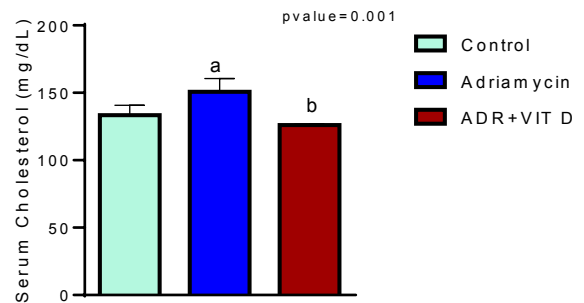


Fig. 6. Effects of vitamin D₃ on serum total cholesterol in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

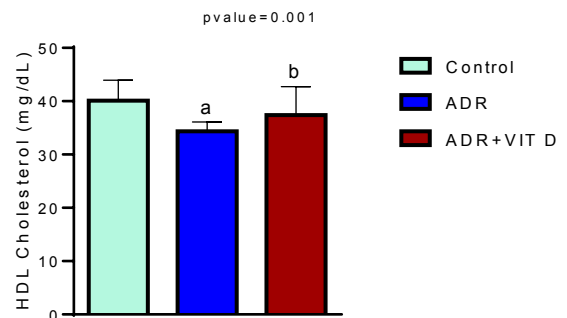


Fig. 7. Effects of vitamin D₃ on serum HDL-C in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

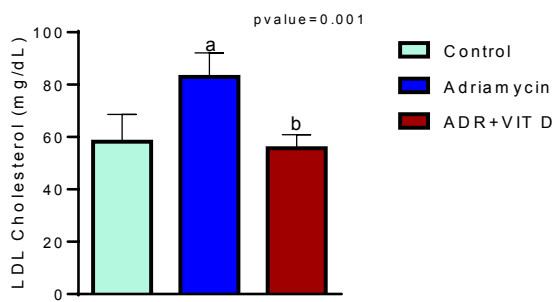


Fig. 8. Effects of vitamin D₃ on serum LDL-C in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

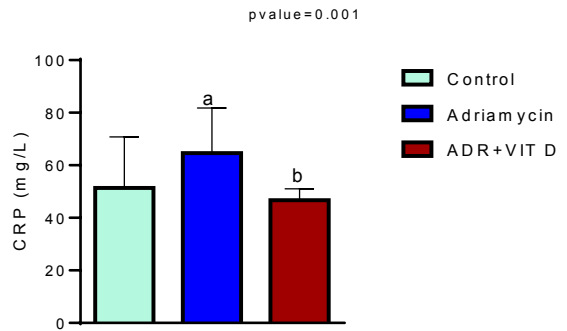


Fig. 12. Effects of vitamin D₃ on CRP levels in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

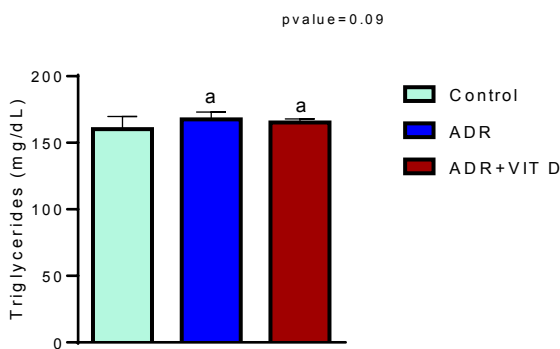


Fig. 9. Effects of vitamin D₃ on serum TG in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

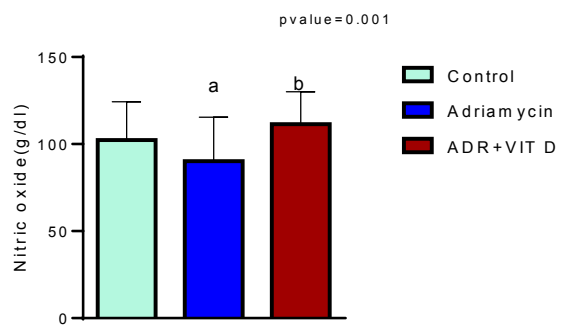


Fig. 13. Effects of vitamin D₃ on nitric oxide levels in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

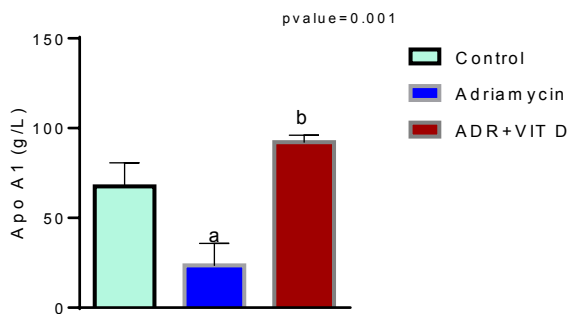


Fig. 10. Effects of vitamin D₃ on serum Apo A in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

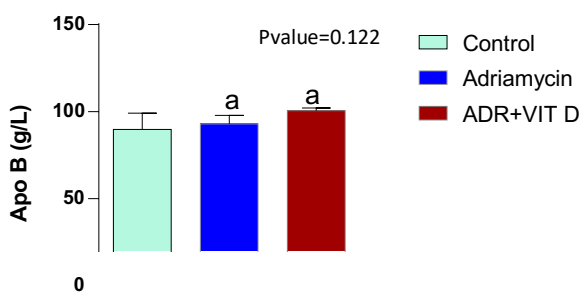


Fig. 11. Effects of vitamin D₃ on serum Apo B in ADR-induced nephrotoxic rats (a – significant when compared with control, b – significant when compared with ADR group)

Discussion

Our findings shows that vitamin D supplementation has no significant effects on the weight loss associated with nephrotoxicity induced by adriamycin. The weight loss after the ADR treatment might be associated with loss of appetite for food, loss of nutrients, loss of muscle mass, protein energy wasting from the kidney as a result of the glomerular injury and nephrotoxicity caused by ADR administration in all the test groups.^{30,31} Adriamycin can induce weight loss and cause muscle atrophy by influencing lipid and glucose metabolism through induction of peroxisome proliferator-activated receptor gamma (PPAR γ) and 5' adenosine monophosphate-AMP-activated protein kinase.³² The active form of vitamin D can promote and induce apoptosis in adipocytes as reported by previous study.³³ In a double-blind clinical research of overweight and obese women (aged 20–40) taking supplements containing 50,000 IU of vitamin D per week for 6 weeks, it was reported that there was a significant reduction in the BMI, weight, and waist circumference.³⁴ Studies have shown that there is an inverse relationship between vitamin D and WC and some have reported inability of vitamin D to modify body weight.³⁵

High levels of high-molecular-weight proteins are found in the urine of individuals with nephrotoxicity and other cases of renal injury. Ideally, the glomeruli limit the

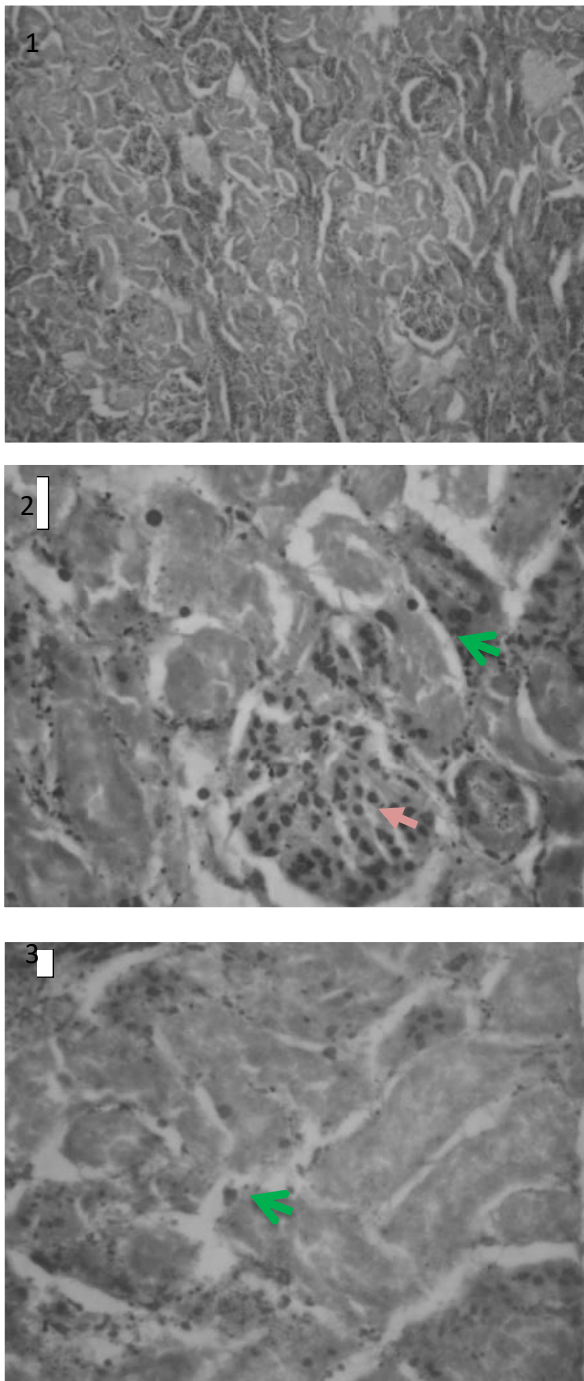


Fig. 14. Photomicrograph of the kidney, plate show severe tubular necrosis (pink arrow) and glomerular mesangialisation (green arrow)

transport of these proteins from the blood to the nephron lumen but this function is affected in the case of renal injury.³⁶ Such proteins are albumin and urea which are important in the prompt discovery of renal injury and specifically, dysfunctionality in glomerular filtration.³⁷ In our study, there was a significant reduction in serum albumin as shown in Figure 2 in ADR only group which is expected because high ADR dose induces nephrotoxicity as seen in previous studies.³⁸ Increased albuminuria, and reduced serum albumin is a marker of kidney injury and damage,

albuminuria and the presence of other macromolecules in the tubular fluid may initiate tubular damage and accelerate the progression to CKD. However, we noticed that after vitamin D therapy, serum albumin levels were significantly increased. This is similar to the work of Liyanage et al.³⁹ Vitamin D may influence albumin levels through a mechanism that involves a decline in renin expression in the kidney and angiotensin II, as well as transforming growth factor- β .⁴⁰ In individuals with chronic kidney disease, proteinuria is a surrogate predictive indicator for progression of kidney damage.⁴¹ There was an increase in serum protein level though it was not statistically significant. This might be as a result of the lingering tubular necrosis seen in the photomicrograph (Fig. 14, group 3) which implies that though vitamin D improves renal injury, renal repair is not absolute. Improvements in renal and cardiovascular outcomes have been associated with a decrease in proteinuria.⁴² Damage to the glomerular basement membrane and endothelium surface, are glomerular abnormalities that can result in proteinuria. Vitamin D has been reported to preserve the structural integrity of the slit diaphragm which help to protect the podocytes. The glomerulus expresses the VDR, particularly in podocytes and parietal epithelial cells thereby preserving the integrity of the podocyte via the coordination of the activity of heparanase promoter and urokinase receptor during genetic expression of nephrin and podocin.⁴³ This suggests that vitamin D might be effective in improving the integrity of the glomerulus in renal related diseases.

There have been controversies about the effects of vitamin D on serum creatinine, a clinical trial involving Vitamin D supplementation in 16 patients with renal diseases reported an increase in serum levels of creatinine though GFR remained unchanged, this is as a result of enhanced muscle production of creatinine.⁴⁴ Another study reported increase in creatinine levels with concomitant decrease in GFR.⁴⁵ Our study observed a contrary result; a significant decrease in creatinine after vitamin D therapy in ADR induced renal damage though GFR was not assessed. The discrepancies in creatinine levels after vitamin D administration might be as a result of the drug: ADR used in the induction of renal damage in our study. Human studies involving large sample size need to be conducted to resolve these conflicting results.

High creatinine to urea ratio in renal injury has been linked to poor clinical outcomes like chronic heart failure, myocardial infarction and ischemic stroke.⁴⁶ Fortunately, our study observed a decrease in creatinine levels after vitamin D treatment though the increased urea levels in the ADR group were not brought down by vitamin D administration as seen in Fig. 5. This is similar to the work of Vanholder et al.⁴⁷

The dyslipidaemia observed in the ADR groups was resolved after treatment with vitamin D as seen in Fig.

6-11. This is similar to the findings of Hager et al.⁴⁸ There was an increase in HDL, apolipoprotein A, and a decrease in serum levels of TG and LDL after vitamin D treatment in the ADR group. This is an interesting finding, though similar results have been reported in studies involving patients with diabetes mellitus, findings on kidney-related diseases are few.¹⁵ Dyslipidaemia in kidney injury is associated with increased risk of cardiovascular complications, development of glomerulosclerosis and progressive kidney disease.⁴⁹ Vitamin D has been suggested to increase the amount of calcium absorbed from the intestine, the intracellular calcium in the liver can stimulate microsomal triglycerides transfer protein. This increase in intracellular calcium invariably help in the formation and secretion of VLDL via stimulation of MTP. This action result in the decrease of serum triglycerides levels.³⁵ Also, the positive effects of Vitamin D on lipid profile might be due to its role in cholesterol synthesis, by increasing the activity of lipoprotein lipase in adipose tissue and through inhibition of SREBP-2 which regulates the level of lipogenic genes and controls lipid synthesis.^{50,51} In the skeletal muscle cell, calcitriol alters lipid partitioning and lipid droplet packaging in a way that increases lipid turnover.⁴⁵

CRP is an acute phase reactant and a potent indicator of systemic inflammation produced in response to tissue damage.⁵² In our study, the reduced CRP serum levels in nephrotoxic group indicate that there is an increased inflammatory process involved in nephrotoxicity caused by ADR. Previous research has demonstrated the anti-inflammatory properties of vitamin D through its inhibition of the NF- κ B pathway, a transcription factor that increases inflammation by modifying the expression of adhesion molecules and cytokines' genes.⁵³ Vitamin D also affects inflammation mediated by TLRs, (trans-membrane receptors) on mediators of innate immune response like monocytes and macrophages.⁵³ It has been shown that the infiltration of inflammatory cells in the interstitium correlates directly with proteinuria in renal injury, therefore when there is reduction in inflammation; it impacts positively on proteinuria as seen in our study.³⁷

Nitric oxide has a vital role in inhibition of tubular reabsorption of sodium, mediation of natriuresis and glomerular hemodynamics.⁵⁴ In cases of renal injury, these functions mediate the beneficial role of NO in maintaining renal health, however high concentration of serum NO might be detrimental because NO reacts readily with oxygen and nitrogen species thereby contributing to a state of oxidative stress.⁵⁴ NO levels were reduced in the ADR group as seen in previous studies involving renal diseases.⁵⁵ Interestingly, after treatment with vitamin D, the reduced levels significantly increased to levels similar to the control group, this agrees with the study of Tony wolf who gave college-aged Africa Americans vitamin D supplements for four weeks, and reported improvement in NO mediated microvascular function.⁵⁶ Vitamin D has

been reported to enhance the production of endothelium enzyme called endothelial NO synthase which helps in the generation of NO essential for prevention of arterial stiffness, regulation of blood pressure and associated cardiovascular issues.⁵⁷

As seen in our study, vitamin D was able to a certain degree resolve the glomerular damage though tubular necrosis was still present (Fig 14, group 3). The renoprotective effects of vitamin D might thus be mediated locally by directly affecting the podocyte, or theoretically other glomerular cells, like the glomerular endothelium. Activated vitamin D has been demonstrated to have a role in the treatment of glomerulonephritis through vitamin D receptor action which regulates the heparanase promoter activity and modulates the urokinase receptor, guaranteeing podocyte preservation.⁵⁸ It also controls the podocyte distribution by modulating mRNA synthesis and protein expression of nephrin and podocin.^{59,60}

Thus, this present study demonatrated that vitamin D might be beneficial in the reversal of renal injury and amelioration of cardiometabolic complications associated with chronic kidney disease, however there were some limitations associated with our study. The GFR and neutrophil gelatinase associated lipocalin were not determined in the experimental rat because urine samples were not collected, this would have further clarified the role of vitamin D on glomerular function and assessment of renal health. Also, the study did not assess the impact of vitamin D administration on parathyroid hormone, further studies are encouraged in this area.

Conclusion

The results of this study show that vitamin D might have therapeutic potential in the amelioration of the complications associated with adriamycin induced renal injury or other injuries to the kidney, thereby slowing down the progression into chronic kidney disease and development of cardiovascular diseases. The exact mechanisms involved in the reno-protective and anti-proteinuric effects of vitamin D are not well known. Therefore, human clinical trials involving large sample size is necessary to establish the findings of this study.

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Declarations

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Author contributions

Conceptualization, A.S.; Methodology, M.A. and O.S.; Software, A.S.; Validation, A.S, O.L and O.S.; Formal

Analysis, A.S.; Investigation, M.A.; Resources, O.S.; Data Curation, A.S and M.A.; Writing – Original Draft Preparation, A.S. and M.A.; Writing – Review & Editing, A.S. and O.S.; Visualization, M.A, O.S. and O.L.; Supervision, A.S.; Project Administration, A.S. and O.L.; Funding Acquisition, A.S. and M.A.

Conflicts of interest

Authors declare no conflicting interest.

Data availability

The datasets generated during and/or analyzed during the current study are available in the repository of the Department of Biochemistry, Lead City University, Ibadan, Nigeria.

Ethics approval

Ethical approval (Reference Number: LCUERB906) was obtained from Lead City University Ethical Review Board (LCU/ERB) to perform this animal research and the animals were treated in accordance with the guidelines of the committee.

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Trends of in-hospital maternal mortality during the COVID-19 pandemic in southern Morocco – analysis of a time series from 2016–2022

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ABSTRACT

Introduction and aim. The aim of this study was to compare trends of in-hospital maternal mortality in southern Morocco before and after the COVID-19 pandemic emerged in the Kingdom in March 2020.

Material and methods. We conducted a retrospective study of a time series of hospital maternal deaths from January 2016 to December 2022. Data were collected from maternal death registers and monthly reports from maternity departments of six public hospitals in Souss Massa region (one regional and five provincial hospitals).

Results. 216 maternal deaths occurred during the study period, 112 before and 104 during the pandemic. The highest annual maternal mortality ratio (MMR) was observed in 2021 (207.41 deaths/100,000 live births). Trends of monthly MMR showed a significant increase during the pandemic compared with the pre-pandemic period ($p=0.002$). Furthermore, an extremely high MMR value (977.57/100,000 live births) was noted in August 2021 (the Delta wave).

There was a significant difference in the distribution of causes of maternal death. Proportion of indirect causes were tripled during the pandemic (from 8% to 24%), with 17/104 (16.3%) deaths related to SARS-CoV-2 pneumonia.

Conclusion. In-hospital MMR increased in public hospitals of the Souss Massa region during the COVID-19 pandemic, particularly in the third quarter of 2021.

Keywords. COVID-19 pandemic, maternal mortality, southern Morocco, time series

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Introduction

The tenth revision of the International Classification of Diseases (ICD-10) defines maternal death as the death of a woman during pregnancy, childbirth or the postpartum period, from any cause related to or aggravated by the pregnancy or its management.¹ A WHO systematic analysis indicated that hemorrhages, gestational hypertensive diseases, and sepsis were the main causes of maternal death worldwide. While indirect causes accounted for more than 25% of causes.² Several factors increased the risk of maternal death, such as maternal age over 35 years (OR=2.63).³ Avoidable deaths were not completely attributable to pregnancy complications, they were also related to socio-economic factors and healthcare efficiency.⁴

The maternal mortality ratio (MMR) is an indicator of healthcare quality, whose reduction is a major global challenge.⁵ It is defined as the number of maternal deaths during a given period per 100,000 live births (LB) during the same period.¹ Over the last few decades, the MMR has decreased worldwide, although there were disparities between countries depending on their income levels and maternal health policies.⁶ Indeed, maternal death is an uncommon outcome in high-income countries, mainly linked to pre-existing chronic conditions. While it is more common in low-income countries, due to lack of access to skilled maternal healthcare.⁷ Pregnancy related mortality was also found higher in rural than in urban areas even in high-income countries such as United States.⁸

Morocco is a developing country that has adopted several policies aimed to improve maternal and perinatal health. It achieved a decrease of 69.9% in maternal mortality ratio over the period 2000-2020, falling from 244 to 72.6 deaths per 100,000 live births. An MMR lower than global estimates for the North Africa region for 2020 (103 deaths/100,000 live births).⁹ This performance meets the WHO's Agenda 2030 target (to reduce maternal deaths to less than 70 per 100,000 live births).¹⁰ Consequently, Morocco is at stage 4 of the maternal mortality transition (MMR <100), whereas it was at stage 3 (MMR 0–299) in 2000.⁴ Although several official reports have been published on maternal mortality in Morocco, few local studies have examined causes of deaths.¹¹

The Souss Massa region was merged with the Daraa region in southern Morocco before the 2015 territorial organization. According to the report of the confidential survey of maternal deaths, Souss Massa-Daraa was the leading region in terms of pregnancy-related deaths.¹² This national survey, report that 79% of maternal deaths occurred in public hospitals. The Moroccan report 'Health in figures' for the year 2022, indicated that the rate of childbirth in public facilities was around 56%, 83% of which were recorded in public hospitals.¹³

The emergence of the COVID-19 pandemic has reduced the progress achieved in public health and caused an excess of mortality rates worldwide.¹⁴ This fact prompts to examine the effect of this pandemic on maternal mortality rates and causes. Initially, as a direct consequence of pregnant women contamination by SARS-CoV-2.¹⁵ Furthermore, as an indirect result of the healthcare services perturbation.¹⁶ A systematic review reported an increase of maternal mortality rate compared with the pre-pandemic period (RR=1.37).¹⁷ The World Health Organization has stated that the COVID-19 pandemic may have contributed to the lack of progress on reduction of MMR. However, it was not possible to comprehensively assess this impact, as published global data on maternal mortality during the pandemic were limited and mainly concerned high-income countries.⁹

Morocco, in turn, was not exempt from of the COVID-19 pandemic consequences. Just one year after the first case appeared on March 2020, more than 9,000 deaths have been reported, despite the several measures implemented to reduce the impact of the COVID-19 pandemic on public health.¹⁸ Indeed, the Moroccan government has adopted a strategy based on prevention of the SARS-CoV-2 transmission.¹⁹ Regarding perinatal health, the Ministry of Health has circulated an urgent notice requesting healthcare workers to ensure the usual activity of maternal care services during the pandemic.²⁰ To this aim, a digital platform was created to promote prenatal monitoring during the lockdown with the possibility of face-to-face consultations, imposing protective masks. Priority was given to pregnant women for PCR testing, particularly during the screening and admission procedure. Furthermore, specific care channels were introduced in hospitals for pregnant women with confirmed or suspected COVID diagnosis to reduce the risk of hospital-acquired infection. The organizational diagrams for cases management, diagnosis procedures, and therapeutic protocols were detailed in a national guide, published in May 2020.²¹

Aim

In order to improve the evidence on the impact of COVID-19 on maternal mortality, the aim of this study was to assess trends of in hospital MMR in Souss Massa region in southern Morocco during the COVID-19 pandemic relative to pre-pandemic period. The secondary objective was to compare causes of death, age, and geographical origin of deceased women in both periods.

Material and methods

Type and period of the study

We conducted a comparative multicentric, retrospective study of a time series of maternal deaths occurring in

public hospitals of the Souss Massa region over seven years, extending from January 2016 to December 2022.

Study setting

The Souss-Massa region has been one of Morocco's 12 regions since the 2015 territorial reorganization. It is located at the junction of the north and south of the country. The region covers 7.6% of the national territory, including 6 provinces: Agadir Ida Outanane, Inezgane Ait Melloul, Taroudant, Tiznit, Chtouka Ait Baha, and Tata. The rural area is extensive in the region (154 rural versus 21 urban communes). According to the High Commission for Planning, urban population is estimated in 2022 at 1881521 and rural population at 1095607 (Detailed presentation of the region is available on the website www.hcp.ma). As for public healthcare provision in the region, there are 198 physicians and 304 specialists. There are 2,466 nurses and health technicians, including 517 midwives. The maternal health care programs target 446,917 childbearing women. In 2022, 16.66% of deliveries in the public sector were carried in primary maternity facilities and 83.33% in hospitals. The private sector is competitive, number of privately owned clinics in the region is 24 with 293 physicians and 460 specialists.¹³

This regional study was conducted in public hospitals in Souss Massa: the Agadir regional hospital, which receives patients from all over the region, and is also the COVID-19 reference hospital. And the five provincial hospitals proper to each of the remaining provinces. These hospitals were provided with units of isolation and management for suspected and confirmed COVID-positive pregnant women. However, critical cases were transferred to the regional hospital when required.

Study eligibility criteria

Inclusion criteria

The maternal death notification system was introduced in Morocco in 2009 notify any death of a woman aged between 15 and 49 in each province. Monthly reports were performed including main feathers of deceased women. Confidential audits then determine the main cause and whether it was a maternal death.¹² The current series included women aged between 15 and 49 years who died in hospital during pregnancy, childbirth, or post-partum, for obstetric or non-obstetric reasons. This was an exhaustive collection of all cases of maternal deaths over the period 2016–2022, in the six public hospitals of the region.

Exclusion criteria

The study excluded: 1) deaths that do not meet the definition of maternal death, 2) deaths occurring before admission to hospital.

Private clinics were excluded from our study given that pregnant women admitted there were commonly transferred to regional public hospitals in event of major complications.²²

Data sources

This study was carried out with the authorization of the Regional Health and Social Protection Directorate in the Souss-Massa region.

The data collected for this time series were based on maternal death registers from the maternity departments in the included hospitals and monthly reports of these maternity departments.

Outcomes and data collection process

The first case of SARS-CoV-2 infection was recorded in Morocco on March 2, 2020. It was followed by the Moroccan government's declaration of a state of public health emergency and restrictions in response to the pandemic. These measures were lasted for three years and periodically updated according to the epidemiological situation in the kingdom.

In this study, pre-pandemic maternal trends were described to provide a baseline for comparing changes after the emergence of COVID-19.

The pre-pandemic period was defined from January 2016 to February 2020.

The post-pandemic period was extended from March 2020 to December 2022.

Data collected in the present study were those recorded monthly in registers of hospital maternal deaths. These included serial number of maternal death, date of death, maternal age, province, area of residence (urban or rural), location of death (hospital department), and cause of death. A compilation sheet was used to extract individual data from the registers for each case included on the time series.

Maternal causes of death were defined according to the International Classification of Diseases (ICD-10), excluding causes of late maternal death:¹

Direct causes: Hemorrhage, hypertensive disorders, sepsis, obstetric embolism...

Indirect causes: SARS-CoV-2 infection, other maternal diseases complicating pregnancy, childbirth, and the postpartum period.

The authors classified the causes of death based on what was notified on the maternal death registers. It should be noted that the primary cause is the one that is listed after the audit decision.

Monthly in hospital MMR were calculated using the following formula: $Monthly\ MMR = \frac{Number\ of\ maternal\ deaths\ occurring\ in\ hospital\ settings\ during\ a\ given\ month}{Number\ of\ live\ births\ in\ the\ same\ month\ in\ the\ same\ hospital\ settings} \times 100,000$

The annual in hospital MMR was calculated for each given year.

$Annual\ MMR = \frac{\text{Number of maternal deaths occurring in hospital settings during a given year}}{\text{Number of live births in the same year in the same hospital settings}} \times 100,000$

The number of live births (LB) was extracted from the monthly statistical reports issued by each hospital's maternity department. It was then summed to estimate the monthly number of live births for all included hospitals. The annual number of live births was calculated using the sum of the monthly number of live births in all hospitals for each year.

Similarly, the number of maternal deaths was calculated monthly and annually based on cases notified on the maternal mortality registers for each hospital. It was then summed to estimate the overall number of deaths occurring in the included hospitals per month and per year.

Statistical analysis

The analysis method used was a comparative model of pre- and post-pandemic MMR on a time series. This model is also used to examine characteristics of deceased women.

The categorical variables were presented as numbers and percentages. The age group, area of residence, and site of death were compared using the chi-square test (χ^2) or Fisher's exact test, depending on the application conditions of each test.

The normality of the distribution was verified with the Shapiro-Wilk test, the MMR was a continuous variable with a non-Gaussian distribution. Therefore, the central tendency was described using the median and interquartile range for each period. The pre and post pandemic MMR medians were compared using the non-parametric Mann-Whitney test.

Combined curves were adopted to illustrate changes in MMRs, maternal deaths, and live births over time. Additionally, the geographical distribution of deaths was mapped using ArcGIS 10.8 software.

Data were entered and analyzed using the statistical software Jamovi version 2.3.28. A p-value <0.05 was considered statistically significant.

Ethical considerations

This study was conducted respecting the ethics and dignity of the participants. Ethical approval was granted by the Ethics Committee for Biomedical Research of the Faculty of Medicine and Pharmacy Mohammed V in Rabat (Reference: CERB G/23). In this study, the ethical standards of the institutional and/or national research committee were applied in all procedures performed involving human participants. Confidentiality and anonymity criteria were respected as charted by the Declaration of Helsinki and its later amendments.

Results

Trends in in-hospital MMR in the Souss Massa region before and during the COVID-19 pandemic

As showed in figure 1, the in-hospital MMR in the Souss Massa region decreased between 2016 and 2017 by 35.7%. It then increased by 36.2% and 47.8% in 2018 and 2019 respectively. During the COVID-19 pandemic, the MMR declined slightly in 2020 (131.6 deaths/100,000 live births) and then increased significantly in 2021, with 207.41 deaths/100,000 live births. And it achieved 156.06 per 100.000 live births in 2022. Number of maternal deaths was variable over the study period. The highest value was recorded in 2021 (44 deaths) and the lowest in 2017 (26 deaths). In terms of live births (LB), there was a gradual decline. there has been a gradual decline. It fell from 28062 to 25557 in 2016-2019, and from 22792 to 21145 in 2020-2022 (Fig. 1).

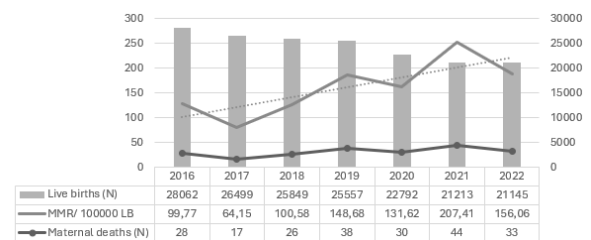


Fig. 1. Annual trends of MMRs, maternal deaths, and live births in SOUSS MASSA region hospitals from 2016 to 2022

The median monthly of MMR in the pre-pandemic period was 93.3 (81.7-125) deaths/100,000 LB. During the COVID-19 pandemic, the median was 116 (101-207) deaths/100,000 LB. The comparison of medians showed a significant increase in MMR relative to the pre-pandemic period ($p=0.002$). A peak was noted in August 2021 with an extremely high MMR value (977.57 deaths/100,000 live births). Another increase was noted in May 2020 (359.89 deaths/100,000 live births). In addition, the highest number of maternal deaths was recorded in August 2021 (17 deaths). However, no deaths occurred during in January and October 2017. The number of live births fluctuated, showing a declining trend throughout the pandemic. The minimum value was recorded in February 2021 (147 LB), and the maximum value (2593 LB) was noted in June 2017 (Fig. 2).

Figure 3 showed that the province of Taroudant had the highest frequency of maternal deaths before and after the pandemic, while Tiznit and Tata were the least affected. Maternal deaths in the province of Inezgane Ait Melloul increased by 46% during the pandemic.

Distribution of maternal deaths by age, area of residence, site, and cause of death

Table 1 showed that the indirect causes were more frequent during the pandemic. Of the 104 mater-

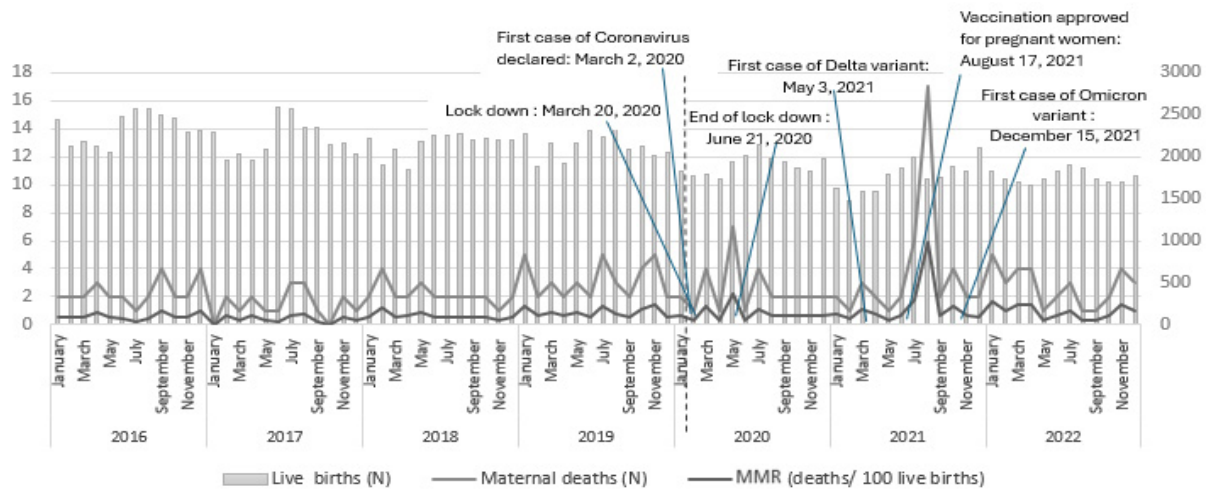


Fig. 2. Monthly trends of MMRs, maternal deaths, and live births in Souss Massa hospitals from 2016 to 2022

nal deaths recorded during the pandemic, 17 deaths (16.3%) were caused by SARS-CoV-2 infection. However, hypertensive disorders (eclampsia, pre-eclampsia, HELLP syndrome) were the leading cause of maternal death, representing respectively 37.5% and 34.8% of direct causes during the pandemic and pre-pandemic periods.

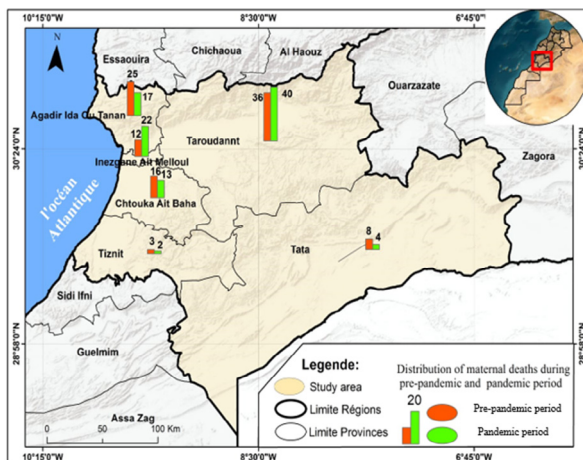


Fig. 3. Spatial distribution of hospital maternal deaths in the Souss Massa region by province of origin

The mean maternal age was 31.4 ± 6.8 years in the pre-pandemic and 32.1 ± 6.8 years during the pandemic. There was no significant association between the pandemic period and the distribution of maternal deaths, either by age group or by area of residence ($p > 0.05$). Most maternal deaths occurred among women aged over 35 and those living in rural areas for both periods (Table 1). A significant association was observed between the COVID-19 pandemic and the hospital setting of maternal death ($p = 0.005$). Intensive care units were the departments where the greatest number of deaths occurred (75.9% and 65.4% before and after

the pandemic respectively). 12 deaths occurred in a specific COVID-19-unit care (Table 1).

Table 1. Distribution of maternal deaths before and during the COVID-19 pandemic by age group, area of residence, site and causes of death*

Variables	Period (n, %)		p
	Pandemic, n=104	Prepandemic, n=112	
Age			0.395
<35 years	61 (58.7%)	72 (64.3%)	
≥ 35 years	43 (41.3%)	40 (35.7%)	
Area of residence			0.76
Urban	36 (34.6%)	41 (36.6%)	
Rural	68 (65.4%)	71 (63.4%)	
Site of death			0.005
Maternity department	21 (20.2%)	21 (18.8%)	
Intensive care units	68 (65.4%)	85 (75.9%)	
COVID-19 unit care	12 (11.5%)	0 (0%)	
Other site ^d	3 (2.9%)	6 (5.3%)	
Causes of death			
Indirect causes			
Hemorrhage ^a	19 (18.3%)	31 (27.7%)	
Hypertensive disorders ^b	39 (37.5%)	39 (34.8%)	
Sepsis	9 (8.7%)	15 (13.4%)	
Other obstetric causes ^c	12 (11.5%)	18 (16.1%)	
Direct causes			
SARS-CoV-2 infection	17 (16.3%) [†]	0 (0%)	
Other indirect causes ^d	8 (7.7%)	9 (8%)	

* ^Δ – operating room, emergency department, ^a – all types of obstetric hemorrhage, including hemorrhage due to uterine rupture, ^b – eclampsia, pre-eclampsia, HELLP syndrome, ^c – thromboembolic complications, post-surgical complications, unspecified obstetric cause, ^d – all non-obstetric causes: diabetes, meningitis, cholecystitis, pancreatitis, [#] – including one death due to pre-eclampsia complicated by SARS-CoV-2 infection

Of the 17 deaths linked to maternal infection with SARS-CoV-2, 2 deaths occurred at the start of the pan-

demic (April-May 2020), 12 deaths during the months of July-August 2021, corresponding to the Delta wave, and one death in December 2021 (Omicron wave).

Discussion

The present study indicated that there was a significant increase in hospital MMR in Souss Massa region during the COVID-19 pandemic compared to the previous four years. This increase was not pronounced at the start of the pandemic, whereas it was catastrophic at the third quarter of 2021, with a peak of maternal deaths in August. That occurred even though the Moroccan Ministry of Health has introduced preventive measures in hospitals to improve care for infected pregnant women. There was a specific circuit for women with a positive or suspected diagnosis. After admission, they were hospitalized and constantly monitored in an isolation room. A therapeutic protocol was dispensed depending on gestational age, co-morbidities, and the severity of COVID-19 disease. Laboring women were referred to a specific delivery room, if a caesarean section is indicated, they are transferred to the appropriate operating room and then returned to the isolation unit. Women with severe symptoms were transferred to the COVID-19 reference intensive care unit. In contrast, pregnant women defined as negative were oriented to the usual process. However, monitoring and reporting of any emergent symptoms were strongly recommended. After discharge, an extensive disinfection of areas was systematically performed.²¹

The increase of MMR during the pandemic could be explained by multiple factors, especially the rise of maternal deaths. Indeed, in our study, the highest number of deaths was recorded in 2021. Similarly, the fall of births and live births in public maternity units may be an important determinant. According to the official annual reports 'Health in figures' (available on the website of the Ministry of Health and Social Protection www.gov.ma), the births in public hospitals of Souss Massa fell from 2017 to 2022. There was a decline of 3.76% in 2017–2019 period and 9.8% during the pandemic (2020–2022). The lowest values were noted in 2021 and 2022, which may also explain the decrease observed in live births in current study. The fall in hospital births could be considered as an indirect effect of the pandemic on the use of maternal health services, as indicated in literature.²³

In Morocco, the Delta wave was the severest, affecting 10,000 patients daily, nationwide. The pandemic peaked in August, when intensive care units reached maximum capacity, health workers burned out, and an excessive use of oxygen and was reported.¹⁹ In our series, of 17 maternal deaths due to SARS-CoV-2 infection, 12 deaths occurred during the Delta wave (July–August 2021). A meta-analysis of studies conducted at

the start of the pandemic reported that maternal mortality rates were reassuring.²⁴ Similarly, a Turkish study indicated that in-hospital maternal mortality related to SARS-CoV-2 infection was high during the Delta wave (7%) compared to other pandemic stages (1.28% in the first wave and 2.56% in the Omicron wave).²⁵ In contrast, a high incidence of maternal deaths was observed at start of the pandemic in some countries such as Brazil and Mexico.²⁶ A Mexican study reported a 56.8% increase of MMR in 2020.²⁷

The lack of vaccination of pregnant women against COVID-19 could explain associated maternal deaths.²⁵ The Delta variant was reported to increase maternal mortality and admissions to intensive care units, particularly in unvaccinated pregnant women.²⁸ Indeed, the rise of maternal deaths incidence during the Delta wave prompted the Moroccan Ministry of Health to publish a circular on 17 August urging the vaccination of pregnant women against SARS-CoV-2.²⁹ This could explain the relative decline in MMR over the next month. Furthermore, the universal application of health insurance in Morocco, at the end of 2021–beginning of 2022, could improve equity of access to healthcare services.³⁰

The annual MMRs were high in our results compared to the latest national data published in 2018 (72.6 deaths per 100,000 LB).¹³ Nevertheless, they remained lower than the estimated global MMR in 2020 (223 deaths per 100,000 LB). This can be explained by the exclusion of primary maternity units, private clinics, and home births which account for around 40% of births in Morocco. Consequently, the results obtained were based solely on live births in public hospital maternity units.

Intra-regional disparities were noted in current study. Women living in the province of Taroudant had the highest incidence of maternal death, which could be explained by rurality and its impact on access to qualified care. This province contains only 8 urban communes, against 81 rural communes. The greatest increase in maternal deaths during the pandemic was noted in the province of Inezgane Ait Melloul, the most densely populated province in the Souss Massa region (2,168 inhabitants per km²). That might increase the risk of pregnant women exposure to SARS-CoV-2 (Detailed presentation of the Souss Massa provinces is available on the website www.hcp.ma).

Our case series showed that no significant difference in maternal age or residence area between the pre-pandemic and pandemic period. Most maternal deaths occurred among women aged over 35 and those living in rural areas for both periods. It is reported that the effect of age on maternal mortality rate is intensified by rurality.⁸ This rate is 2.5 times higher in rural areas than in urban areas in Morocco.³¹ In a large American cohort study, COVID-19-related mortality was found to be associated with maternal age over 35 years (RR=23.9).³²

Co-existing conditions and socioeconomic vulnerability increase this association.³³

The present study showed that indirect causes of maternal death was tripled during the pandemic (from 8% to 24%) due to deaths related to SARS-CoV-2 pneumonia. Hypertensive disorders remained the principal cause of maternal death in Souss Massa region with a slight growth of 2.7% during the pandemic. A Canadian cohort study showed that the incidence of pre-eclampsia did not increase significantly during the first 18 months of the pandemic.³⁴ However, it was reported that pregnant women with pre-eclampsia were vulnerable to COVID-19 complications.³⁵ In our case series, one death was due to severe pre-eclampsia associated with SARS-CoV-2 infection. In addition, intensive care units (ICU) continued to be the main site of maternal deaths even during the pandemic. This is consistent with the Moroccan confidential maternal death survey findings, in which 74% of hospital deaths occurred in ICU, followed by maternity unit.

There were considerable disparities in the maternal health impact of the pandemic between high and middle/low income countries.^{17,36} To mitigate this situation, priority must be given to equitable access to quality maternal and perinatal healthcare.¹⁷ Reducing urban/ rural disparities should be a strategic goal in maternal health policies.⁸ To accomplish this objective, it is essential to raise the rural population awareness on the importance of maternal health care. Furthermore, mapping disparities would help to identify the most vulnerable areas and to implement effective solutions.³⁷ Several recommendations can be proposed to reduce maternal mortality as enhancing the monitoring system of maternal death and perinatal care quality, and improving horizontal and vertical communication between stakeholders.³⁸ In addition, it is crucial to standardize management procedures for severe obstetric complications through validated protocols.²² In terms of human resources, Morocco needs to recruit 760 midwives each year to improve coverage to 95%, according to the International Confederation of Midwives. This should contribute to reducing the incidence of maternal deaths and stillbirths by 2030.³⁹ Moreover, resorting to public/private partnerships may optimize the human and material resources.⁴⁰

Study limitations and strengths

The current study was the first in Morocco addressing the longitudinal effect of the COVID-19 pandemic on maternal mortality in a large Moroccan region over seven years, from 2016 to 2022. This descriptive and comparative analysis provided an overview of trends in maternal deaths and live births in public hospitals in the context of the COVID-19 pandemic.

However, the study has several limitations. Collected data were limited to the maternity unit registry and

did not include the medical records of deceased women. That excluded important variables which only available on records as timing of maternal death, comorbidities, parity, prenatal care, socioeconomic status, vaccination status, and data regarding the health care quality. Exploring these variables would have allowed a more precise interpretation of the findings.

The representative of the number of maternal deaths should be treated with caution, due to the probability of under-reporting of maternal deaths, particularly outside public health care structures. Furthermore, the series only included maternal deaths occurring in public hospitals. As a result, live births in these sites were considerably lower than live births in all public and private health facilities in the region. This weakness explains the general increase in hospital MMR compared TH national statistics.

Despite the limitations, the findings can enhance understanding the effect of the COVID-19 pandemic on maternal mortality in developing countries. In addition, they can provide a useful roadmap for public health decision-makers in developing strategies to strengthen maternal health and reduce disparities in access to health care.

Conclusion

The hospital MMR increased in the Souss Massa region during the pandemic, particularly in the third quarter of 2021. Infection with SARS-CoV-2 during pregnancy appears as a new indirect cause of maternal death, this cause was more frequent during the Delta wave. Women aged over 35 and those living in rural areas were most at risk of maternal death during both the pandemic and pre-pandemic periods.

Although the increase observed in the MMR during the pandemic was significant, it is not attributable to a sole factor. Further investigations are recommended to determine all associated factors. Updating published data on national and regional maternal mortality will allow for more understanding of the effect of the pandemic, and improved management of preventable causes of maternal death.

Declarations

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Author contributions

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Conflicts of interest

The authors declared no conflicts of interest for this paper.

Data availability

Data available on request from the authors.

Ethics approval

The ethical approval was granted by the Ethics Committee for Biomedical Research of the Faculty of Medicine and Pharmacy Mohammed V in Rabat, Morocco (Reference: CERB G/23).

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Improving sleep quality among ICCU patients – evaluating the effectiveness of ear plug and eye mask interventions

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ABSTRACT

Introduction and aim. Sleep quality is crucial for patient recovery, particularly in critical care settings such as the Intensive Cardiac Care Unit (ICCU). This study aimed to investigate the effectiveness of using eye masks and earplugs in improving sleep quality among ICCU patients.

Material and methods. A quasi-experimental study was conducted using a one-group without control design. A total of 40 ICCU patients were provided with eye masks and earplugs for a minimum duration of three days. Sleep quality was assessed using the Richard Campbell Sleep Questionnaire (RSCQ) before and after the intervention. Data were analyzed using descriptive and inferential statistics.

Results. The use of eye masks and earplugs significantly improved sleep quality among ICCU patients ($p < 0.05$). There was a notable increase in sleep duration, reduction in sleep onset latency, and improvement in sleep efficiency following the intervention. These findings support the efficacy of non-pharmacological interventions in enhancing sleep quality in critical care settings.

Conclusion. The findings highlight the importance of incorporating simple interventions such as eye masks and earplugs into routine care practices in ICCUs to improve patients' sleep quality.

Keywords. earplugs, eye masks, intensive cardiac care unit, sleep quality

Introduction

Sleep is one of the fundamental needs of humans to restore health, energy, and physical well-being. The serotonin hormone and growth hormone undergo chemical changes and cellular nutrition enhancement.^{1,2} Sleep disturbances are often experienced by patients admitted to the ICCU, which can lead to increased blood pressure, heart rate, and other bodily metabolic changes.³ The quantity of sleep for patients decreases, but sleep quality, influenced by environmental factors, is most

affected.⁴ The time patients spend in continuous sleep decreases, and circadian rhythm (a person's 24-hour sleep period) and normal sleep cycles (progressing from 'light' to 'deep') are disrupted.^{5,6}

Sleep quality in critical care settings has been proven to be suboptimal, with associated factors including environmental conditions and patient-related factors.⁷ Environmental factors such as light, noise, alarm sounds from monitoring devices, care activities, diagnostic examinations, and therapeutic procedures.^{8,9} Patient-relat-

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ed factors include age, underlying diseases, pain, stress, psychological factors, circadian rhythm disturbances, and organ dysfunction.^{5,10}

Disturbed sleep quality in critically ill patients can lead to psychological and physiological disturbances, affecting patient recovery and treatment.¹¹ Other conditions that may arise due to poor sleep quality include immunodeficiency function, inspiratory muscle endurance, weaning changes, and increased pain scale in critically ill patients.^{4,12} Other studies suggest that sleep quality disturbances can result in psychological disturbances such as agitation, confusion, and delirium.^{13,14}

Interventions can be provided to improve sleep quality both pharmacologically and non-pharmacologically.¹⁵ Pharmacological interventions typically administered in ICU include sedative and analgesic drugs that help improve patient sleep quality.¹⁶ Pharmacological interventions may have side effects such as cognitive function impairment, dependency risks, decreased ventilation, and disruption of normal sleep function.^{17,18}

Therefore, non-pharmacological interventions are needed to improve sleep quality in critically ill patients. Non-pharmacological interventions help reduce side effects such as dependency when pharmacological interventions are administered.¹⁹ Non-pharmacological interventions that can be provided to improve sleep quality in the ICU include providing physical aids such as earplugs and eye masks.²⁰ Other interventions that can be provided include relaxation techniques (massage and foot soaking), music therapy, quiet time, acupuncture, and aromatherapy.^{21,22}

Various research results indicate that light and noise are causes of sleep disturbances in patients. Therefore, the use of earplugs and eye masks is chosen as one of the intervention methods to help reduce sleep disturbances, thus improving sleep quality for ICU patients.²³ The use of earplugs and eye masks is safe for coronary heart patients. In addition to being safe, earplugs and eye masks are also proven to be effective in assessing patient sleep quality, cost-effective, easy to implement in large groups, and well-tolerated by the body.^{24,25}

The use of a combination of eye masks and earplugs as a non-pharmacological intervention has not been specifically explored in intensive care settings such as the ICU. Previous studies may have included single sleep interventions, such as the use of an eye mask or earplugs alone, but this study explores the synergistic effects of using both together.²⁶ Other research results also indicate that the implementation of earplugs and eye masks post-cardiothoracic surgery is efficient and straightforward.^{27,28} Earplugs and eye masks improve sleep quality and patient satisfaction and can reduce pain intensity.²⁹ Additionally, earplugs and eye masks are proven to contribute to faster recovery, fewer morbidities, and reduced costs.

Aim

This research is conducted to the influence of providing eye masks and earplugs to improve the quality of sleep in ICU patients.

Material and methods

Study design

This study employs a quasi-experimental research method with a one-group without control approach. The study investigates the influence of providing eye masks and earplugs to improve the sleep quality of ICU patients. Patients receiving the treatment of eye masks and earplugs are examined. The sample is a portion of the total population with certain characteristics. Samples are representatives of the population being studied. Sampling is conducted using total sampling technique. The sample size for this study is 40 patients admitted to the ICU with criteria including a minimum of 3 days of hospitalization, GCS score of 14–15, bedside monitor placement, and willingness to participate as respondents (Fig. 1). The patients taken were patients with heart problems and not post-operative patients. Additionally, patients with cardiac rhythm disorders, heart failure, recent Percutaneous Coronary Intervention, severe respiratory disorders, and severe neurological or psychiatric disorders were not included as respondents in the study. Midazolam therapy was stopped before the ear plug and eye mask intervention was given, this was to determine the maximum effect of the intervention given.

The respondents were provided with earplugs, made of silicone material, and eye masks, made of cotton material, during sleep for three days. The sleep quality of the respondents was measured before they were given earplugs and eye masks. Three days after the intervention, their sleep quality was measured again. Sleep quality observations were conducted daily, but the analyzed results focused on sleep quality before and after three days of earplug and eye mask usage.

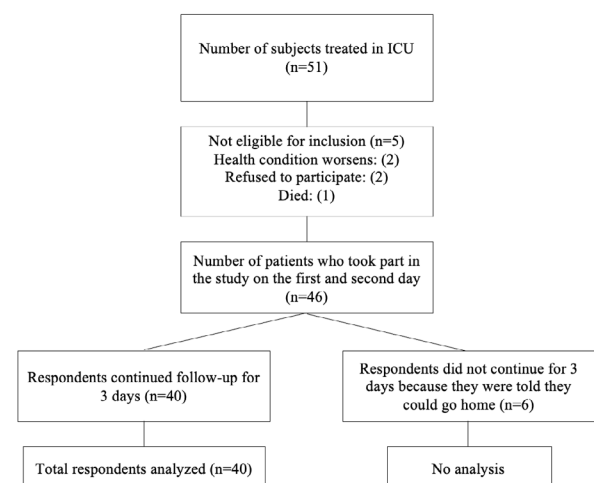


Fig. 1. Flow diagram for participant assignment in this study

Instruments

This study utilizes the Richard Campbell Sleep Questionnaire (RCSQ) to assess various aspects of an individual's sleep quality based on their experiences and perceptions. It comprises a series of questions designed to evaluate various aspects of sleep, such as sleep duration, difficulty falling asleep, sleep quality, and the impact of sleep on daily activities. The purpose of this questionnaire is to provide a comprehensive understanding of an individual's sleep quality, which can aid in the diagnosis, management, and evaluation of sleep disorders and provide useful information for intervention planning. The RCSQ consists of questions covering various aspects of sleep quality, including sleep duration, difficulty falling asleep, sleep quality, and the impact of sleep on daily well-being. Each response in the questionnaire is assigned a value based on a predefined scale. These values can then be summed or calculated in a specific manner to generate a total score reflecting overall sleep quality. Higher scores indicate better sleep quality (>50), while lower scores indicate poorer sleep quality (≤50).

Data analysis

The collected data will be analyzed using the Pearson correlation test in SPSS version 26 statistical software. This research uses the Wilcoxon test that there is an effect if the p value <0.05.

Ethical approval

The study has obtained ethical clearance with number 211.6/II.3.AU/F/KEPK/VII/2023 and received permission from the local health authorities. Adequate information for informed consent has been provided, and patient data confidentiality is maintained.

Results

Table 1, indicates that several risk factors such as gender, excess body mass index (BMI), smoking history, alcohol consumption, medication use history, and sleep problem history have a significant association with sleep quality. Eye mask and ear plug have an impact on sleep quality in patients with a minimum duration of usage of three days, like Table 2.

Discussion

Gender is a multifaceted construct that encompasses biological, psychological, and sociocultural dimensions. In the realm of sleep science, researchers have long been interested in understanding how gender influences sleep patterns, behaviors, and ultimately, sleep quality. The significance of gender in relation to sleep quality can be analyzed through both theoretical frameworks and empirical evidence.³⁰ Gender roles and expectations imposed by society can shape individuals' behaviors, in-

cluding sleep habits. Differences in body structure between men and women, such as differences in body size and composition, can also influence comfort during sleep and the tendency to experience certain sleep disorders. Traditional gender norms may prescribe different sleep patterns and expectations for men and women.³¹ For example, societal expectations regarding caregiving responsibilities and career demands may influence how individuals allocate their time for sleep.³² Additionally, gender differences in coping strategies for stress and emotional regulation may impact sleep quality. Hormones such as estrogen and progesterone in women and testosterone in men have a role in regulating sleep cycles and sleep quality. These hormonal fluctuations can influence sleep patterns and sleep experiences between both sexes.

Table 1. Characteristic of respondent (n=40)

	n	%	Median (Min–Max)	Mean±	r	p
Sex						
Male	26	65	1 (1–2)	1.35±0.48	0.39	0.013
Female	24	35				
Education						
No	17	42.5	2 (1–3)	1.72±0.71	0.92	0.571
Elementary–senior School	17	42.5				
College	6	15				
Chronic disease history						
No	28	70	1 (1–2)	1.3±0.46	0.066	0.687
Yes	12	30				
BMI						
Underweight	9	22.5	2 (1–3)	2.07±0.72	0.647	<0.001
Normal	19	47.5				
Overweight	12	30				
Smoking history						
No	17	42.5	2 (1–2)	1.57±0.5	0.239	0.038
Yes	23	57.5				
Alcohol consumption						
Never	22	55	1 (1–2)	1.45±0.51	0.414	0.008
Occasional	18	45				
History of medication use						
No	13	32.5	2 (1–2)	1.67±0.47	0.553	<0.001
Yes	27	67.5				
Sleep disorder history						
No	18	45	2 (1–2)	1.55±0.51	0.798	<0.001
Yes	22	55				

Table 2. Results of Wilcoxon test (n=40)*

	Median (Min–Max)	Mean±	p
Sleep Quality before EMEP	48 (38–72)	51.75 ± 9.98	<0.001
Sleep Quality after EMEP	60 (50–80)	62.2 ± 6.15	

* EMEP – eye mask and ear plug

Education level serves as a key determinant influencing health outcomes, including sleep quality.³³ Higher education levels are often associated with greater access to resources, health literacy, and socioeconomic status, which in turn may contribute to better sleep hygiene and overall well-being.³⁴ However, the relation-

ship between education level and sleep quality may not always be straightforward.^{35,36} Factors such as work-related stress, irregular schedules, and academic pressures may adversely affect sleep quality among individuals with higher education levels. Contrary to expectations, several studies have reported mixed or inconclusive findings regarding the relationship between education level and sleep quality.^{27,29} While some studies suggest a positive association between higher education levels and better sleep quality, others have found no significant correlation or even a negative relationship. These discrepancies may be attributed to methodological differences, sample characteristics, and confounding variables not adequately controlled for in analyses.

Chronic disease history can impact sleep quality through various pathways. The presence of chronic conditions such as diabetes, cardiovascular disease, or respiratory disorders may lead to symptoms such as pain, discomfort, or nocturnal symptoms that disrupt sleep.^{3,37} Additionally, the management of chronic diseases often involves medication regimens, which may have side effects or influence sleep patterns. Psychological factors such as stress, anxiety, and depression commonly co-occur with chronic illnesses and can further exacerbate sleep disturbances.^{11,38} Similarly, the relationship between chronic disease history and sleep quality appears to be nuanced.³⁹ While certain chronic conditions, such as chronic pain syndromes or respiratory disorders, are commonly associated with sleep disturbances, the presence of other chronic diseases may not necessarily predict poor sleep quality.⁴⁰ Additionally, factors such as disease severity, comorbidities, and treatment adherence may influence the impact of chronic diseases on sleep.

Higher BMI levels are often associated with lifestyle factors such as poor dietary habits and sedentary behaviors, which can contribute to sleep disturbances such as obstructive sleep apnea and insomnia. Moreover, excess adiposity may lead to physiological changes, including alterations in hormonal regulation and inflammation, which can further impact sleep quality.⁴¹ Numerous studies have provided empirical support for the significant relationship between BMI and sleep quality. Research consistently demonstrates that individuals with higher BMI levels are more likely to experience sleep disturbances, including sleep fragmentation, decreased sleep efficiency, and increased prevalence of sleep disorders such as obstructive sleep apnea.^{18,42} Moreover, longitudinal studies have shown bidirectional associations between BMI and sleep disturbances, suggesting a reciprocal relationship between these factors over time.

Smoking behavior, on the other hand, is linked to various adverse health outcomes, including sleep disturbances. Nicotine, a key component of cigarettes, acts as a stimulant and can disrupt sleep architecture, leading

to difficulties in falling asleep and maintaining sleep.⁹ Additionally, smoking is associated with an increased risk of respiratory conditions such as chronic obstructive pulmonary disease and sleep-related breathing disorders, which can exacerbate sleep problems.⁴³ Smoking history has been consistently linked to poor sleep quality in empirical studies. Smokers are more likely to report difficulties falling asleep, waking up during the night, and experiencing non-restorative sleep compared to non-smokers.⁴⁴ Moreover, smoking cessation interventions have been shown to improve sleep quality among former smokers, further supporting the causal relationship between smoking behavior and sleep disturbances. Although it does not directly affect sleep depth, nicotine can cause lighter sleep and easier awakening, disrupting normal sleep patterns.

Social determinants of health theory offer valuable insights into the relationship between alcohol consumption, medication use, and sleep quality. Alcohol, as a central nervous system depressant, can initially induce feelings of relaxation and drowsiness, leading individuals to believe it aids in sleep.^{45,46} However, alcohol disrupts sleep architecture by suppressing rapid eye movement sleep and increasing awakenings during the night, ultimately resulting in poorer sleep quality. Research consistently demonstrates that acute and chronic alcohol consumption are associated with sleep disturbances, including difficulties falling asleep, frequent awakenings during the night, and non-restorative sleep.⁴⁷ Moreover, longitudinal studies have shown that reducing alcohol consumption or abstaining from alcohol altogether can lead to improvements in sleep quality. The results of the analysis we carried out on the RSCQ questionnaire, in the domain we found that 22 respondents had a history of alcohol consumption. Although alcohol has an initial sedative effect that can speed up the time to sleep, consuming alcohol before bed often causes an increase in sleep latency as the alcohol begins to metabolize in the body. The stimulant effect of alcohol that lasts after a few hours can cause wakefulness in the middle of the night.

Medication use history, particularly the use of certain classes of drugs such as sedatives, antidepressants, or stimulants, can also influence sleep quality. Sedative medications, commonly prescribed for anxiety or insomnia, may lead to dependence and rebound insomnia, exacerbating sleep disturbances over time.⁴⁸ Conversely, stimulant medications used to treat conditions such as attention-deficit hyperactivity disorder can interfere with sleep onset and maintenance, leading to fragmented sleep patterns and reduced sleep quality. The result identified specific classes of medications, such as benzodiazepines, antipsychotics, and antihistamines, as having adverse effects on sleep quality.⁴⁹ Additionally, polypharmacy, or the concurrent use of multiple

medications, has been associated with increased risk of sleep-related side effects and poor sleep outcomes.

Individuals learn from their experiences and observations, and their behaviors are influenced by cognitive processes such as attention, memory, and motivation. In the context of sleep disorders, individuals with a history of sleep disturbances may develop maladaptive sleep behaviors or negative beliefs about sleep, perpetuating the cycle of poor sleep quality.^{18,31} Additionally, psychosocial factors such as stress, anxiety, and depression, which are commonly associated with sleep disorders, can further exacerbate sleep disturbances.

The Biopsychosocial Model of Health emphasizes the interaction between biological, psychological, and social factors in shaping health outcomes. Sleep disorders may arise from a combination of genetic predispositions, neurobiological abnormalities, and environmental triggers.²¹ Biological factors such as alterations in neurotransmitter systems or disruptions in circadian rhythms can contribute to the development and maintenance of sleep disturbances. Psychological factors such as maladaptive coping strategies or dysfunctional beliefs about sleep can perpetuate sleep disorders. Social factors such as work schedules, familial responsibilities, and socioeconomic status can also influence sleep quality and exacerbate sleep disturbances.^{19,22}

Individuals with a history of sleep disorders, such as insomnia, sleep apnea, or restless legs syndrome, are more likely to report poorer subjective sleep quality, increased sleep latency, and decreased sleep efficiency compared to those without sleep disorders. Objective measures, such as polysomnography, also reveal alterations in sleep architecture and sleep continuity among individuals with sleep disorders.¹⁷ Persistent sleep disturbances can lead to long-term consequences such as daytime sleepiness, impaired cognitive function, mood disturbances, and decreased quality of life. Additionally, untreated sleep disorders are associated with an increased risk of comorbidities such as cardiovascular disease, diabetes, and psychiatric disorders, highlighting the importance of early detection and intervention.^{8,30}

Optimizing the sleep environment can promote better sleep quality. External stimuli such as light and noise can disrupt sleep onset and maintenance. Eye masks provide a physical barrier to block out light, thereby creating a darker sleep environment conducive to sleep initiation and maintenance.^{50,51} Similarly, earplugs reduce the transmission of external noise, minimizing disturbances that can disrupt sleep continuity and depth. Eye masks and earplugs can serve as behavioral strategies to modify sleep-related behaviors and promote healthier sleep patterns.²⁹ By incorporating these aids into bedtime routines, individuals can signal to their bodies that it is time to sleep, facilitating the onset of sleep and enhancing sleep efficiency.

Based on identification and analysis on the observation sheet using RSCQ. The domains contain sleep depth, sleep latency, awakening from sleep, ability to return to sleep when awakened and as an additional domain, namely noise. We analyzed that 22 respondents experienced a significant increase, except for the noise domain, considering that in the treatment room there was the sound of the tools used by the respondents.

One of the primary challenges in managing sleep quality in patients with heart disease is the sleep disturbances caused by physiological factors such as shortness of breath and difficulty finding a comfortable sleeping position. In this study, all patients received adequate oxygen therapy to ensure their oxygen needs were met during sleep. Additionally, patients in the ICCU were always carefully positioned to ensure maximum comfort and to prevent complications that might arise from uncomfortable positions. Proper sleep position adjustments, such as the semi-Fowler position, can help reduce symptoms of shortness of breath and improve patient comfort during sleep.

Research utilizing subjective measures such as self-reported sleep quality and objective measures such as polysomnography consistently show that individuals using these aids experience longer sleep duration, reduced sleep latency, and increased sleep efficiency compared to those without them.^{26,29} Individuals who regularly use these aids report improvements in overall sleep satisfaction, daytime functioning, and mood regulation. Additionally, intervention studies involving randomized controlled trials have shown that the provision of eye masks and earplugs to individuals with sleep disturbances leads to significant improvements in sleep parameters compared to control groups. The research has not discussed environmental noise levels, lighting levels, and patient fatigue and stress levels, so this can be added when conducting further research

Conclusion

This study investigates the influence of using eye masks and earplugs on improving sleep quality among patients in the ICCU. It was found that the use of eye masks and earplugs significantly improves patients' sleep quality, as evidenced by an increase in sleep duration, reduction in sleep onset latency, and improvement in sleep efficiency. These findings support the theories of sleep environment and cognitive-behavioral interventions as approaches to enhancing sleep quality. The implications of this study are that providing eye masks and earplugs can serve as simple and effective non-pharmacological interventions to enhance patients' sleep quality in the ICCU.

Further relevant research includes conducting comparative studies between the use of eye masks and earplugs and other non-pharmacological interventions, such as relaxation or music therapy, to evaluate the relative ef-

fectiveness of each intervention in improving sleep quality. Additionally, investigating the effects of using eye masks and earplugs on patients with other critical conditions outside the ICU would evaluate the generalizability of these findings to a broader population.

Declarations

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Author contributions

Conceptualization, B.W. and P.A.W.S.; Methodology, B.W. and W.U.; Validation, B.W. and E.R.; Formal Analysis, P.A.W.S. and W.U.; Investigation, F.K.; Resources, P.A.W.S. and E.R.; Data Curation, B.W.; Writing – Preparation of Original Draft, B.W. and P.A.W.S.; Writing – Review & Editing, B.W.; Visualization, P.A.W.S.; Supervision, B.W.

Conflicts of interest

All author declare have no conflict of interest.

Ethics approval

This study was approved by the local ethics committee (Health Research Ethics Committee in Universitas Muhammadiyah Gombong, date: July 25, 2023 decision number: 211.6/II.3.AU/F/KEPK/VII/2023).

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Morphometric analysis of dry acetabulum and iliac bones from anatomy museums in south eastern Nigeria with relation to clinical practice

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ABSTRACT

Introduction and aim. Good outcomes of total hip replacement and acetabular surgeries have been linked to proper understanding of the morphometry of acetabular and iliac bones. The aim of this study was to analyze the clinical morphometry of dry acetabulum and iliac bones in southeastern Nigeria.

Material and methods. The measurements were done on human hip bones in anatomy museums using a Vernier caliper. The acetabular and iliac bone parts were measured.

Results. A total of 148 dry hip bones were measured during the study. The mean acetabular depth and diameter are 32.10 ± 2.33 mm, and 53.93 ± 2.74 mm respectively. The mean thicknesses of the acetabular walls are 5.88 ± 1.38 mm, 12.64 ± 1.84 mm, 15.79 ± 2.07 mm and 19.74 ± 2.18 mm for the antero-inferior, antero-superior, postero-inferior and postero-superior walls respectively. The distances between landmarks on the iliac bone are: anterior-inferior-iliac-spine – posterior-inferior-iliac-spine = 112.91 ± 8.01 mm, posterior-superior-iliac-spine – anterior-inferior-iliac-spine = 135.29 ± 8.04 mm, anterior-inferior-iliac-spine – anterior-superior-iliac-spine = 34.98 ± 9.55 mm, posterior-superior-iliac-spine – upper-edge-of-acetabulum = 119.14 ± 7.83 mm and anterior-superior-iliac-spine – nearest-edge-of-acetabulum = 55.71 ± 5.60 mm. The regression model for prediction of diameter of the acetabulum using the depth of the acetabulum shows that for every 1 mm increase in the acetabular depth, the acetabular diameter increases by 0.312 times. The regression equation is “ $Y = bx + a$ ”; $Y =$ acetabular diameter, $x =$ acetabular depth, $b =$ margin of error = 0.312, and $a =$ constant determined to be 43.919.

Conclusion. The study established the mean values and the relationship between acetabular depth and diameter.

Keywords. acetabular depth, acetabular diameter, acetabular wall thickness, anterior superior iliac spine, posterior superior iliac spine

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Introduction

The hip joint socket, the acetabulum ('vinegar cup'), is a concave hemisphere whose axis is not strictly horizontal but is directed also downwards and slightly anteverted along the axis of the femoral neck. Its inferior margin is lacking, so the vinegar would run out of the cup. Deficiency at the inferior margin is the acetabular notch.^{1,2} The acetabulum is divided into four quadrants as described by Wasielewski et al. by bisecting them with an imaginary line from the anterior superior iliac spine and another line passing through the center of the acetabulum perpendicular to the first line.³ The thickest quadrant is the postero-superior, followed by the postero-inferior, then the antero-superior and the thinnest, the antero-inferior quadrant.³ It is very important to know this as well as the average length of the screws that can be placed safely at various quadrants of the acetabulum during surgery.³

The ilium forms the pelvic brim between the hip joint and the joint with the sacrum; above the pelvic brim it is prolonged, broad and wing-like, for the attachment of ligaments and large muscles.¹ The ilium is divisible into two parts, the body (corpus ossis ilii) and the ala (ala ossis ilii).¹ The following anatomical landmarks are at the anterior border of ala; the anterior superior iliac spine (ASIS) which can be palpated under the skin and the anterior inferior iliac spine (AIIS). Between these iliac spines there is incisure (incisura iliaca anterior). At the posterior border of ala are the posterior superior iliac spine (PSIS) and the posterior inferior iliac spine (PIIS). There is incisure (incisura iliaca posterior) between these spines.⁴

Acetabular morphometric parameters like depth of the cavity, diameter and acetabular notch width in addition to the head of the femur determine the stability of the hip joint.⁵

The average depth of the acetabulum from previous studies ranges from 20 mm to 32 mm.^{2,6-9} The acetabular depth has been considered by many investigators as an important measurement in defining acetabular dysplasia. The acetabulum with depth of less than 9 mm is regarded as acetabular dysplasia.¹⁰ In acetabular dysplasia, there is deficient coverage of the femoral head superolaterally and anteriorly, therefore there is decreased surface area for weight bearing. Excessively deep acetabulum can predispose the person to pincer femoroacetabular impingement syndrome, which will eventually lead to osteoarthritis of the hip joint.¹¹

The diameter of the acetabulum has been documented by previous authors. Ukoha et al. obtained a mean acetabular diameter of 55.8±3.7 mm on the right side and 54.6±3 mm on the left side.⁶ A study done by Chauhan et al. in a North Indian population obtained values of 47.10±2.9 mm on the right side and 47.48±3.05 mm on the left side.¹²

The morphometry of the acetabulum and iliac bones has been shown to vary amongst individuals from different geographic locations or regions, there is also variation amongst individual from different racial groups and ethnicities.^{13,14} Good outcome of total hip replacement surgeries, and pelvic surgeries had been linked to proper understanding of the morphometry of the acetabulum and iliac bones. For instance, it aids in the placement of fixation screws at the right location to avoid damage to adjacent neurovascular structures.¹⁵ Also, the size of the prosthetic cup in total hip replacement should be about the same as the dimensions of the native acetabulum. Knowledge of this morphometry is also applied in implant manufacture and selection for pelvic and hip surgeries. Good understanding of iliac bone morphometry also aids the surgeon to identify important bone landmarks (anterior superior iliac spine, posterior superior iliac spines etc.) during surgeries.¹⁶

Pelvic surgeries and total hip replacement surgeries are presently being done in our environment. These surgeries require acetabular components for the total hip replacement surgeries, special plates and screws for the acetabular reconstruction surgeries. These prosthesis and implants are manufactured with similar morphometries as the native bone. There is paucity of data about acetabular and iliac bone parameters in our environment and no previous study in our environment had established relationship between the depth and diameter of the acetabulum. This study was therefore designed to help in bridging this gap in knowledge.

Aim

This study aims to provide database of the values of acetabular morphometries of people of southeast Nigeria, also to establish a model for the prediction of acetabular diameter from the depth and vice versa. Mean values obtained will be of immense benefit to bioengineers who manufacture acetabular prosthesis and pelvic implants.

Material and methods

The study is a cross-sectional study conducted at the anatomy museums of Departments of Human Anatomy of six universities in the southeastern Nigeria.

Normal adult hip bones without pathology or features of wear and tear from storage and preparation were included in the study. Bones whose epiphyses had not fused were excluded from the study.

All the hip bones in the Anatomy museums which met the inclusion criteria were used for the study.

Ethical clearance was obtained from the Research and Ethics Committee of Enugu State University of Science and Technology. Ethical right permission number is ESUCOM/FBMS/ETR/2022/015.

Electronic Vernier caliper, thin metallic meter rule, examination gloves and markers were used in the study.

Depth of the acetabulum

Figure 1 shows the measurement of depth of the acetabulum. The maximum vertical distance from the deepest point in the acetabular cavity (center of the acetabulum) to the horizontal plane touching the margins of the acetabular cavity. A thin metallic meter rule was kept across the margins of the acetabular cavity and the depth of the acetabulum was measured on the Electronic Vernier caliper using the depth gauge from the deepest point in the acetabulum to the ruler.²



Fig. 1. Measurement of the depth of the acetabulum.

Diameter of the acetabulum

The distance from the junction where anterior iliac margin meets the periphery of the acetabulum to a point on the periphery of the Acetabulum nearest to the ischial tuberosity was measured with Electronic Vernier caliper.¹⁷ This measurement is shown in Figure 2.



Fig 2. Measurement of the acetabular diameter

Width of the acetabular notch

The distance between the ends of the lunate surface of the acetabulum was measured with an electronic Vernier caliper.¹⁷ This is shown in Figure 3.

Thickness of the acetabulum

The acetabulum was divided into 4 quadrants as was described by Wasielewski.³ This was done by drawing a straight line with meter rule and marker from the anterior superior iliac spine bisecting the acetabulum. Another line, perpendicular to the 1st, through the center

of the acetabulum was drawn. Figure 4 shows measurement of the acetabular wall thickness. These lines divide the acetabulum into 4 quadrants; the anterosuperior, anteroinferior, posterosuperior and posteroinferior quadrants.³ With the aid of an electronic Vernier caliper, the thickness of the acetabulum was measured at a point 2 cm from the rim of the acetabulum in each of the quadrants.



Fig 3. Measurement of the acetabular notch width.



Fig. 4. Measurement of the acetabular wall thickness

Distances between landmarks on the iliac bone

The distances between the following bony landmarks on the iliac bone were measured:

Distance between anterior inferior iliac spine (AIIS) and posterior inferior iliac spine (PIIS). This is shown in Figure 5.

Distance between the posterior superior iliac spine (PSIS) and the anterior inferior iliac spine (AIIS).

Distance between anterior inferior iliac spine (AIIS) and anterior superior iliac spine (ASIS).

Distance between the posterior superior iliac spine (PSIS) and the upper edge of the acetabulum (UEA).

Distance between the anterior superior iliac spine (ASIS) and the nearest edge of the acetabulum (NEA). This is shown in Figure 6.

Statistical analysis

Data was collated and analyzed using IBM SPSS version 26 (Armonk, NY, USA). Descriptive statistics which include means and standard deviations were obtained for continuous variables. Comparison of means was done

using student's t test. *P* value less than 0.05 was regarded as significant. Relationship between diameter and depth of acetabulum was assessed using Pearson correlation. A regression analysis was done to see if the depth of acetabulum can predict the diameter.



Fig. 5. Measuring the distance between the AIIS and PIIS



Fig. 6. Measuring the distance between the ASIS and nearest edge of the acetabulum



Fig. 7. Measuring the distance between the ASIS and AIIS

Results

One hundred and forty-eight (148) pelvic bones that met the inclusion criteria were used for the measurements. Right hip bones were 75 in number while left hip bones were 73 in number. Table 1 shows the values of the measured parameters. The mean acetabular depth is 32.10 ± 2.33 mm, the mean acetabular diameter is 53.93 ± 2.74 mm. Mean values of all the parameter are shown in the Table 1.

Table 1. The mean acetabular depth, diameter, notch width, wall thickness, and iliac bone measurements. Measured in millimeters (mm)

	n	Minimum	Maximum	Mean	Std. deviation
Acetabular depth	148	27.4	38.11	32.1	2.33
Acetabular diameter	148	47.7	60.1	53.95	2.74
Acetabular width	148	8.6	35.63	24.3	3.57
Antero-inferior wall thickness	148	2.2	8.48	5.88	1.38
Antero-superior wall thickness	148	8.8	18.42	12.64	1.84
Postero-inferior wall thickness	148	10.4	23.1	15.79	2.07
Postero-superior wall thickness	148	14.68	28.5	19.74	2.18
AIIS-PIIS	148	90.7	130.5	112.91	8.01
PSIS-AIIS	148	116.5	157.1	135.29	8.04
AIIS-ASIS	148	24.33	135.58	34.98	9.55
PSIS-UEA	148	96.44	138.70	119.14	7.83
ASIS-NEA	148	37.30	71.55	55.71	5.6

Table 2 shows there is no significant difference in the mean values of both sides.

Table 2. Comparison of mean parameters between the right and left sides. Measured in millimeters (mm)

	Right Mean \pm SD	Left Mean \pm SD	T	P
Acetabular depth	32.13 \pm 2.43	32.07 \pm 2.24	0.169	0.866
Acetabular diameter	53.86 \pm 2.54	54.04 \pm 2.95	0.382	0.703
Acetabular width	24.56 \pm 3.26	24.04 \pm 3.86	0.887	0.376
Antero-inferior wall thickness	5.82 \pm 1.37	5.94 \pm 1.38	0.517	0.606
Antero-superior wall thickness	12.49 \pm 1.68	12.79 \pm 1.99	0.959	0.339
Postero-inferior wall thickness	15.68 \pm 2.02	15.90 \pm 2.12	0.66	0.51
Postero-superior wall thickness	19.48 \pm 2.42	20.01 \pm 1.88	1.479	0.141
AIIS-PIIS	113.74 \pm 7.32	112.07 \pm 8.62	1.267	0.207
PSIS-AIIS	135.79 \pm 7.39	134.78 \pm 8.68	0.756	0.451
AIIS-ASIS	35.50 \pm 12.76	34.44 \pm 4.27	0.675	0.501
PSIS-UEA	118.69 \pm 7.74	119.59 \pm 7.96	0.706	0.481
ASIS-NEA	55.43 \pm 6.11	55.99 \pm 5.03	0.618	0.538

There is a significant positive linear relationship between the depth of the acetabulum and the diameter of the acetabulum ($r [148] = 0.266, p < 0.001$).

Table 3 shows the regression model for prediction of diameter of the acetabulum using the depth of the acetabulum. It shows that for every 1 mm increase in the acetabular depth, the acetabular diameter increases by 0.312 times. The equation is "Y = bx + a" where Y =

Acetabular diameter, x = Acetabular depth, b = margin of error = 0.312, a = constant determined to be 43.919.

Table 3a. The regression model for prediction of the diameter of the acetabulum using the depth of the acetabulum (model summary)

Model	R	R square	Adjusted R square	Std. error of the estimate	Change statistics				
					R square change	F change	df1	df2	Sig. F change
1	0.266 ^a	0.071	0.064	2.65396	0.071	11.087	1	146	0.001

a. predictors: (constant), acetabular depth

Table 3b. The regression model for prediction of the diameter of the acetabulum using the depth of the acetabulum (ANOVA)

Model	Sum of Squares	Df	Mean Square	F	Sig.
1 Regression	78.090	1	78.090	11.087	0.001 ^b
Residual	1028.350	146	7.043		
Total	1106.439	147			

a. dependent variable: acetabular diameter
b. predictors: (constant), acetabular depth

Table 3c. The regression model for prediction of the diameter of the acetabulum using the depth of the acetabulum (coefficients – the equation is “ $Y = bx + a$ ” where Y = acetabular diameter, x = acetabular depth, b = margin of error = 0.312, a = constant = 43.919)

Model	Unstandardized coefficients		Standardized coefficients	T	Sig.
	B	Std. error	Beta		
1 (Constant)	43.919	3.020		14.541	0.000
acetabular depth	0.312	0.094	0.266	3.330	0.001

a. dependent variable: acetabular diameter

Discussion

The difference between the mean values of right and left sides for the depth, diameter, wall thickness and width of the acetabulum including the mean distances of bony landmarks on the iliac bones from this study are not statistically significant, this is similar to findings from other studies.^{1,3,6,18,19-21} This is important especially for patients that will require a second total hip replacement surgery or implant surgery on the opposite side as similar sizes of implants can be kept for the opposite side.

The mean acetabular depth of dry bones in South East Nigeria was higher than what was reported in studies carried out in Indian, Portuguese, Canadian, Chinese and Turkish populations. Gangavarapu et al. and Yuges et al. in separate studies in Indian population obtained values of 24.09±2.69 mm 29.9±0.21 mm respectively, Arsuaga et al. documented a value of 25.1 mm in Portugal.^{8,17,18} Lang documented an acetabular depth of 29±3.8 mm in dry bones in Canada.⁷ Bagei et al. in an acetabular study in Turkey reported mean

depth of 24.87–22.85 mm.²² Zheng et al. documented 19.4±2.21 mm as the value of acetabular depth in Chinese population.²³ A study by Ukoha et al. in Nigeria obtained value of 29.7 mm for the acetabular depth.⁶ These differences may be as a result of the peculiarities in body build/sizes of the different populations involved. For the Chinese, it may be because the study was a CT scan-based study which may not be as accurate as the value obtained by direct measurement. Similar study done in Nigeria also had lower mean value, this may be attributed to the fact that this study is more encompassing, it involved 6 centers in 3 states with more hip bones measured.⁶ This finding is important because it will motivate our local bioengineers to look at the possibility of manufacturing acetabular cups with dimensions similar to acetabulum of the local population.

The mean acetabular diameter of dry hip bones in South East Nigeria is higher than the values reported in 2 separate Indian studies, with reported values of 49.4±3.5 mm and 47.4±0.23 mm.^{17,18} However mean acetabular diameter from this study is lower than 55.2±3.11 reported in China and 55.8±3.7 reported by a previous Nigerian study.^{6,23} Chauhan et al. opined that smaller values of the acetabulum parameters seen in Indians are due to short stature of Indians when compared to Africans.¹² Knowledge of the acetabular diameter of our local population will aid pre-operative surgical planning, by ensuring that sizes of acetabular cups matching the values of native acetabulum of the local population are available.

The mean value of acetabular notch width of acetabulum of dry hip bones in south-east Nigeria was more than that reported for Indian and South Africa populations with mean values of 22.25±2.97 mm and 21.85±3.42 mm respectively.^{17,24} This may be due to racial variations in sizes of bones.

The acetabular wall thickness from this study follows similar pattern as was documented by Wasielewski et al. and Varodompun et al. showing the posterior-superior wall is the thickest with the highest value followed by posterior-inferior wall, then anterior-superior wall and anterior-inferior wall in that order.^{3,15} However, the acetabular wall thickness of hip bones in south-east Nigeria is thicker than what was documented for the Thai population only at the antero-inferior quadrant. The mean value from the other 3 quadrants is less than that of the Thai population. The reported mean values for the Thai population include; 15.16 mm, 3.49 mm, 29.12 mm, and 26.63 mm for the antero-superior, antero-inferior, postero-superior, and postero-inferior quadrants respectively.¹⁵ This finding could be as a result of racial variations, it could also be because the wall thickness from this study was measured 2 cm from the rim while those of the Thais measured from any point on a middle segment between the rim and the center of

the acetabulum. Knowledge of the wall thickness pattern will enable orthopedic surgeons that do hip replacement surgeries to always direct acetabular screws to the postero-superior quadrant.

The mean distances between the AIIS and the PIIS, the AIIS and ASIS, and the ASIS and the nearest edge of acetabulum (NEA) for the Turkish population are 117.51 ± 7.7 mm, 41.46 ± 5.02 mm, and 60.3 ± 5.46 mm respectively.¹⁶ These values are more than that obtained from the present study. However, the mean distance between the PSIS and the UEA of hip bones in south east Nigeria are more than the mean value (106.24 ± 12.63 mm) reported for Turkish population and less than the value (124.9 ± 7.1 mm) documented for USA population.^{16,20} A Chinese study obtained 139.1 ± 5.6 mm as the mean distance between the PSIS and AIIS, this is greater than the value obtained in this study.²¹ All these differences may be as a result of racial variation in the dimensions of the hip bone. Bony landmarks of the iliac bone usually serve as guides to Surgeons. Knowledge of the mean values of distances between these landmarks is very important because it helps surgeons to select and use screws of appropriate length, this is of immense benefit to centers that do not have a C-arm machine for real-time imaging.

There is a significant positive linear relationship between acetabular depth and diameter. Devi et al. reported positive and significant correlation between the depth and diameter of the acetabulum ($r=0.416$ $p<0.001$) in India.²⁵ Aksu et al. in another study on Turkish people reported positive and significant correlation between the depth and diameter of the acetabulum ($r=0.498$ $p<0.001$).²⁶ Mahmut et al. in a radiological study reported a positive significant correlation between the acetabular diameter and depth in males and females.²⁷ However, none of these previous authors proposed a model for predicting acetabular diameter from depth and vice versa. The model equation proposed in this study can be used to predict to some degree of accuracy the acetabular diameter using the depth, especially in some cases of arthroplasty surgeries where the diameter cannot be measured directly due to bony defects in diseased acetabulum. Estimation of the acetabular diameter from the depth will assist the surgeon to have an idea of the size of the acetabular reamer he/she will start with. And the array of sizes of acetabular cups that will be kept in the inventory for the surgery.

Recommendations

The morphometric values obtained in this study will assist Surgeons that do hip and acetabular surgeries in our environment to stock the sizes of implants that matches that of the local population in their inventory. The values obtained can also assist bioengineers that manufacture the implants and prosthesis for hip surgeries to

produce the ones that will match our local population. Surgeons can use the model obtained from this study to predict the diameter of the acetabulum from the depth of the acetabulum and vice versa.

Study limitations

The bones are not stored separately based on sex, hence being unable to discriminate between genders of the bones limited our study.

Conclusion

This study was able to establish the mean acetabular depth, acetabular diameter, acetabular notch width, acetabular wall thickness and distances between bony landmarks of hip bones in south-east Nigeria. There is no statistically significant difference between the mean values of right and left sides. There are variations between the majority of the study means and that from other regions.

There is statistically significant relationship between the depth of the acetabulum and the diameter of the acetabulum. The diameter of the acetabulum can be used to significantly predict the depth of the acetabulum and vice versa.

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Declarations

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Author contributions

Conceptualization, H.C.E., E.E., A.U.K., U.E.A., I.I.O., R.T.E. and C.V.A.; Methodology, H.C.E., E.E., A.U.K., U.E.A., I.I.O., R.T.E. and C.V.A.; Software, A.U.K.; Validation, H.C.E., E.E., A.U.K., U.E.A., I.I.O., R.T.E. and C.V.A.; Formal Analysis, A.U.K. and H.C.E.; Investigation, H.C.E. and C.V.A.; Resources, H.C.E., E.E., A.U.K., U.E.A., I.I.O., R.T.E. and C.V.A.; Data Curation, H.C.E.; Writing – Original Draft Preparation, H.C.E.; Writing – Review & Editing, H.C.E., E.E., A.U.K., U.E.A., I.I.O., R.T.E. and C.V.A.; Visualization, H.C.E., E.E., A.U.K., U.E.A., I.I.O., R.T.E. and C.V.A.; Supervision, E.E.; Project Administration, H.C.E., E.E., A.U.K., U.E.A., I.I.O., R.T.E. and C.V.A.

Conflicts of interest

No conflicts of interest

Data availability

Data was readily available during the work hours and work days.

Ethics approval

Ethical approval was obtained from the Research and Ethics Committee of Enugu State University of Science and Technology. Ethical right permission number is ES-UCOM/FBMS/ETR/2022/015.

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The relationship between the consumption of traditional Turkish brewing style coffee and other brewing style coffee and HbA1c levels in patients with type 2 diabetes

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ABSTRACT

Introduction and aim. To investigate the relationship between the consumption of traditional Turkish brewing style coffee and other brewing style coffee and hemoglobin A1c:glycated hemoglobin (HbA1c) levels in patients with type-2 diabetes mellitus.

Material and methods. One hundred fifty patients were included in the study. The research sample was obtained from a family health center that called İzmir Karşıyaka 16 -Family Health Center in İzmir-Türkiye. Sociodemographic characteristics, medications, diet, nutritional status, self-reported health status, and consumption of Turkish and other coffee consumption style and other beverages of the patients were recorded. HbA1c, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, total cholesterol, and fasting plasma glucose levels in the previous year were obtained from medical records. All patients had their HbA1c readings taken on quarterly basis. In addition, routine blood tests, including HbA1c measurements, were conducted every six months as part of the regular follow up at the family health center. The International Physical Activity Questionnaire (IPAQ) was used to evaluate the daily physical activities of the patients.

Results. The median HbA1c value of the group taking oral antidiabetic drugs was significantly lower than the median HbA1c value of the group using insulin ($p=0.012$). There was no significant difference in HbA1c levels regarding missing a meal, drinking coffee (sugar-free or not), and physical activity ($p>0.05$). Correlation analysis showed a significant weak relationship between the amount of Turkish coffee consumption per week and fasting blood glucose level ($p=0.041$, $r=-0.088$). There was a negative weak and significant relationship ($p<0.05$) between HbA1c levels and the amount of Turkish coffee consumption per week ($p=0.014$). In the exponential regression model, coffee consumption per week explained the HbA1c level in proportion to 2.9% ($F=4.386$; $p=0.038$).

Conclusion. Consumption of Turkish coffee was inversely correlated with fasting glucose and HbA1c levels. Future studies are needed to determine the effect of coffee in the treatment of diabetes mellitus.

Keywords. blood glucose, coffee, diabetes mellitus, fasting glucose level, glycated hemoglobin A, physical activity

Introduction

This study investigates the consumption of coffee prepared in the traditional Turkish style, which involves a unique brewing method, and the consumption of other types of coffee. The term ‘Turkish and other coffee

consumption style’ refers to these distinctions. The effects of these different coffee consumption styles on hemoglobin A1c:glycated hemoglobin (HbA1c) levels in patients with type 2 diabetes are examined. Traditional Turkish coffee is prepared using finely ground Arabica

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beans boiled in a special pot called a cezve, resulting in a distinctive flavor and higher levels of bioactive compounds compared to other brewing methods.¹

Diabetes mellitus (DM) is one of the most important health problems and it is one of the leading causes of death. Nearly half a billion people have DM worldwide and the global diabetes prevalence is estimated to be 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045.² Factors that cause an increase in the frequency of diabetes mellitus are rapid population growth and prolongation of life expectancy, increase in the prevalence of the obesity due to urbanization and decrease in physical activity. Due to the risk factors like age, lacking physical activity and obesity diet-based strategies are an important part of medical treatment. Therefore, the use of natural compounds for diet regimens in DM has gained much attention for a long time. In this manner, evidence-based literature data has been expanded in recent years.^{3,4}

Coffee is one of the most consumed drinks in the world and coffee consumption increases gradually due to suggested beneficial effects. Along with protective effects on the cardiovascular system, favorable outcomes on some diseases including type 2 diabetes mellitus, non-alcoholic fatty liver disease, liver cancer, gout, kidney stones, and Parkinson's disease were investigated in the literature.^{5,6} Although the biological mechanism of the inverse relationship between diabetes and coffee consumption is not fully known, several different mechanisms are proposed.^{6,7} Some studies suggest that its positive effects on chronic diseases may be due to the antioxidant effects via various pathways.⁸ It contains vitamins and minerals such as ascorbic acid, riboflavin, niacin, folic acid, pantothenic acid, magnesium, potassium, manganese, and fluoride.⁹ Also, it contains polyphenols (hydroxycinnamic acids); which are supposed to be the best candidate for the beneficial effects of coffee and tea consumption on various metabolic disorders.¹⁰ The effects of polyphenols (active dietary chlorogenic acid) in the prevention of chronic diseases are attributed to their ability to improve endothelial function and suppress vascular endothelial cell expression of proinflammatory cytokines.¹¹

Many studies have shown that coffee consumption can reduce the risk of developing type-2 diabetes in a dose-dependent manner.^{5,12,13} High coffee consumption is associated with higher insulin secretion, insulin sensitivity, and β -cell function.^{5,14} Chlorogenic acid, one of the most important phenolic components in coffee was shown to have antihyperglycemic effects.⁶ Another possible mechanism related to the antihyperglycemic effect is the inhibition of sodium-dependent glucose transporters in the gut. Another hypothesis to explain the relationship between coffee and type 2 DM is the idea that chlorogenic acid competes with the glucose-6-phos-

phate translocase enzyme, inhibiting its activity and reducing intestinal glucose absorption.¹⁵ Another possible mechanism is explained by adiponectin. Intake of caffeine, polyphenols, and metabolites is significantly associated with levels of adiponectin, an important regulator of insulin sensitivity and tissue inflammation.¹⁶ It is suggested that magnesium, one of the components of coffee, also contributes to this effect by increasing insulin sensitivity.¹⁷ Coffee may also affect the secretion of gastrointestinal peptides such as glucagon-like peptide 1 and gastric inhibitory polypeptide.^{18,19}

Aim

There are a lot of studies about coffee intake that have beneficial effects on type-2 DM prevalence and glycaemic control. So, we aimed to investigate whether Turkish coffee consumption would affect the HbA1c levels in patients with type-2 DM.

Material and methods

Our research was a cross-sectional-descriptive type. The sample size was 80% power with, a 5% margin of error and 50% unknown frequency since no similar study was conducted in our country before. With a 5% deviation, it was calculated that at least 150 patients should be recruited to study. Patients with type-2 DM whose medical records included HbA1c, high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol, triglyceride, and fasting plasma glucose values in the previous year were included in the study. Type 1 DM patients, patients who reject to study, pregnant and, <18 years of age were excluded from the study.

Before the study approval of the local ethical committee was obtained (Clinical Research Ethics Committee of the Faculty of Medicine at İzmir Katip Çelebi University in June 2021 with the code 2605). The participants were first informed about the study, and verbal and written consent of the volunteers was obtained. A 34-question sociodemographic data questionnaire, liquid consumption questionnaire, and international physical activity test prepared by the researchers in line with the relevant literature, were applied to the participants by face-to-face interview technique. Participants' age, gender, employment status, marital status, income status, smoking, chronic diseases, health status, medications, the time of diagnosis with DM, diet, nutritional status, and the number of meals were examined. Height and weight measurements of the participants and body mass index (BMI) were recorded. Consumption of Turkish coffee, granulated coffee, espresso, coffee with milk, black tea, green tea, cold tea, cola, and energy drink with their amount and frequency were questioned, which was formed by the researchers in line with the relevant literature. The International Physical Activity Questionnaire (IPAQ) was used to evaluate the dai-

ly physical activities of the patients. This questionnaire provided information about sitting, walking, moderately vigorous activities, time spent in vigorous activities, and physical activity levels are determined by grouping individuals according to this information. International validity and reliability studies were carried out by Craig et al.²⁰ The Turkish versions of the IPAQ short and long forms are reliable and valid in the assessment of physical activity.²¹ The biochemical values and HbA1c levels of the patients in the previous year were screened from the medical records.

Statistical analysis

All data were analyzed using SPSS 23.0 package program (IBM, Armonk, NY, USA). The numeric variables were presented as mean and standard deviations; categorical variables were presented as numbers (n) and percentages (%). Normality assumption was tested with Kolmogorov-Smirnov and Shapiro-Wilk test. Continuous data that did not show normal distribution were tested for their conformity to the normal distribution by data transformation. Mann-Whitney U test was used for pairwise comparisons. The Kruskal-Wallis test was used to compare more than two independent groups. Post hoc Dunn's test was used for within-group comparisons when a significant difference was detected in the comparison of multiple groups. While examining the relationship between measurement-based numerical data, Pearson and Spearman's correlation analysis was carried out according to whether or not conditions were met or not for Pearson correlation. ETS exponential regression analysis was conducted to evaluate the extent of the relationship between continuous variables. For all analysis results, the significance level of $p < 0.05$ was accepted.

Results

Sociodemographic features of the patients are presented in Table 1.

Self-declaration of the health status of the patients is shown in Table 2. Most of them (73.3%) thought that their health status was fair. 84% of patients have been using only oral antidiabetic. Adaption to the diet list was only 29.3%. The prevalence of following a diet recommended by a doctor or a dietician perpetually was 20%. Regular medication use for DM was 70.3% (Table 2).

59.3% of the patients said that they consumed three main meals a day, and 72.7% had consumed less than three snacks a day, 48% did not miss a meal. 57% of the patients said they consumed fruit every day, and 66% of them said they consumed vegetable food every day. The consumption of black Turkish coffee ratio was 60.7% among patients. It was observed that 75.3% of the patients consumed other kinds of black coffee. The prevalence of drinking sugar-free tea was 66.7. Water consumption of less than 3 L/per day among patients

was 80%. The nutritional habit of the patients is presented in Table 3.

Table 1. Distribution of sociodemographic characteristics of individuals

		Mean±SD
Age		61.69±12
Height (cm)		166.5±8.59
Weight (kg)		83.23±15.95
BMI (kg/m ²)		30.05±5.6
		n (%)
Gender	Male	61 (40.7)
	Woman	89 (59.3)
What is your education status?	Illiterate	12 (8)
	literate	13 (8.7)
	Primary school	73 (48.7)
	Middle school	19 (12.7)
	High school	21 (14)
What is your working status?	University	12 (8)
	Working	23 (15.3)
	Retired	87 (58)
What is your marital status?	Not working	40 (26.7)
	Married	114 (76)
	Single	1 (0.7)
Your income status?	Divorced	10 (6.7)
	Widow	25 (16.7)
	Equal to my expenses	127 (84.7)
Smoking	Less than my expenses	19 (12.7)
	More than my expenses	4 (2.7)
Total	Yes (1 or more per day)	34 (22.7)
	I am a social drinker (less than 1 per day)	10 (6.7)
	No	106 (70.7)
Total		150 (100)

Table 2. Information about the health status of individuals

		n (%)
How do you think your health is?	Very good	1 (0.7)
	Good	31 (20.7)
	Fair	110 (73.3)
	Poor	7 (4.7)
What do you use to treat diabetes?	Very poor	1 (0.7)
	Oral antidiabetic	126 (84)
	Oral antidiabetic + insulin	20 (13.3)
Duration of diagnosis of DM	Insulin	4 (2.7)
	≤9 years	89 (59.3)
Have you received a diet list before?	≥10 years	61 (40.7)
	Yes	102 (68)
Do you follow your diet list?	No	48 (32)
	Yes	44 (29.3)
Do you follow a diet recommended by your doctor or dietician in the treatment of diabetes?	No	106 (70.7)
	Yes	30 (20)
	Sometimes	86 (57.3)
Do you use your medications regularly in the treatment of diabetes?	None	34 (22.7)
	Yes	119 (70.3)
	Sometimes	26 (17.3)
Total	none	5 (3.3)
Total		150 (100)

Table 3. Nutritional habits of individuals

		n (%)
How many main meals do you have a day?	Less than 3 meals	53 (35.3)
	3 meals	89 (59.3)
	More than 3 meals	8 (5.3)
How many snacks do you have a day?	Less than 3 meals	109 (72.7)
	3 meals	31 (20.7)
	More than 3 meals	10 (6.7)
If you miss a meal, which meal do you miss?	Morning	6 (4)
	Noon	68 (45.3)
	Evening	4 (2.7)
	None	72 (48)
Do you consume fruits every day?	Yes	86 (57.3)
	Sometimes	59 (39.3)
	None	5 (3.3)
Do you consume vegetables every day?	Yes	99 (66)
	Sometimes	47 (31.3)
	None	4 (2.7)
How do you consume your Turkish coffee?	Simple	91 (60.7)
	Low sugar	28 (18.7)
	Middle	21 (14)
	Sugary	10 (6.7)
How do you consume your coffee (other kinds of coffee)	Simple	113 (75.3)
	Sugary	37 (24.7)
How do you consume your tea?	Simple	100 (66.7)
	Sugary	50 (33.3)
How many liters of water do you consume per day?	Less than 3 liters	120 (80)
	3 liters	24 (16)
	More than 3 liters	6 (4)
Total		150

The HbA1c levels of the individuals were compared according to the duration of DM, having a diet list, compliance with the diet list, following a diet list recommended by a doctor or a dietician, and individual perception of their health. There was no difference between the groups ($p>0.05$) (Table 4). However, when we compared HbA1c levels were compared according to the medication used in the treatment of DM; diabetes, it was seen that the median HbA1c value of the group taking oral antidiabetic drugs was significantly lower than the median HbA1c value of the group using insulin ($p=0.012$). The International Physical Activity Questionnaire (IPAQ) was used to evaluate the daily physical activities of the patients. This questionnaire provided information about sitting, walking, moderately vigorous activities, time spent in vigorous activities, and physical activity levels are determined by grouping individuals according to this information. International validity and reliability studies were carried out by Craig et al.¹⁹ The Turkish versions of the IPAQ short and long forms are reliable and valid in the assessment of physical activity.²¹

HbA1c levels according to the nutritional habits of individuals were shown in Table 6. There was no significant difference in terms of missing a meal, drinking coffee sugar-free or not, and physical activity questionnaire ($p>0.05$).

Table 4. List of how many people consume Turkish coffee and how many people consume other coffee consumption style

Beverage consumption	n	%
Turkish brewing style coffee (plain)	91	60.7
Other brewing styles coffee (plain)	113	75.3
Tea (plain)	100	66.7
Less than three liters of water per day	120	80

Table 5. Comparison of HbA1c levels of individuals according to their health status, diet status, and medications, physical activity

Category	n	Median (min–max)	p
Duration of diabetes diagnosis			
≤9 years	89	7.2 (5.3–13.8)	0.242
≥10 years	61	7.5 (5.3–15.4)	
Regular medication use for DM			
Yes	119	7.1 (5.3–13.8)	0.383
No	31	7.7 (5.9–15.4)	
Received a diet list			
Yes	102	7.5 (5.3–15.4)	0.283
No	48	6.9 (5.8–10.6)	
Physical activity			
Inactive	109	7.5 (5.3–15.4)	0.333
Minimally active	37	6.9 (5.3–11.6)	
Very active	4	7.55 (5.9–8.4)	

Table 6. HbA1c levels according to the nutritional habits of the patients (Kruskal-Wallis test)

	n	Median (min–max)	p
If you miss a meal, which meal do you miss?	Morning	6 7.45 (6.2–9.4)	0.384
	Noon	68 7.50 (5.7–15.4)	
	Evening	4 8.15 (6.3–9.4)	
	I'm not jumping	72 7 (5.3–13.5)	
How do you consume Turkish coffee?	Simple	91 7.6 (5.3–15.4)	0.311
	Low sugar	28 7.4 (5.7–11.6)	
	Middle	21 6.5 (5.6–9.9)	
	Sugary	10 6.9 (6–12.4)	
Physical activity questionnaire	Inactive	109 7.5 (5.3–15.4)	0.333
	Minimally active	37 6.9 (5.3–11.6)	
	Very active	4 7.55 (5.9–8.4)	

We used correlation analysis with the amount of Turkish coffee and BMI, fasting blood glucose, triglyceride, and physical activity score. It was found that there was a significant weak relationship between the amount of consumption of Turkish coffee/per week and fasting blood glucose level. ($p=0.041$, $r=-0.088$) (Table 7).

There was a negative, weak and significant relationship ($p<0.05$) between HbA1c levels and the amount of consumption of Turkish coffee per week ($p=0.014$). No significant relationship was found between HbA1c lev-

els and consumption of the amount of black tea, other coffees, other beverages per week, and physical activity levels ($p>0.05$) (Table 8).

Table 7. Correlation analysis of coffee consumption and health metrics*

Variable	BMI	Fasting blood glucose	HDL	Triglyceride	Physical activity
Turkish coffee (per week)	-0.088	-0.167	0.073	-0.018	0.056
p	0.284	0.041	0.377	0.828	0.493

* Spearman correlation analysis, BMI – body mass index, HDL – high-density lipoprotein

Table 8. Correlation analysis between HbA1c and the beverages consumed by individuals and weekly physical activities*

	Turkish coffee per week	Black tea per week	Other kinds of coffees per week	Other drinks per week	Physical activity
HbA1c level	r -0.200	-0.071	-0.065	0.105	-0.107
	p 0.014	0.388	0.430	0.201	0.193

* Spearman correlation analysis

In the exponential regression model between Turkish coffee consumption amount (mL)/per week which has a negative relationship with the HbA1c level, coffee consumption amount/per week explained the HbA1c level in proportion to 2.9% ($F=4.386$; $p=0.038$) (Table 9).

Table 9. The role of Turkish coffee consumption in explaining the change in HbA1c level*

	B	Std. Error	β	t	F	R2_	p
Turkish coffee consumption/per week (mL)	8,065	0.263		30.633	4.386	0.029	0.038
	0.0003	0.000143	-0.170	-2.094			

* ETS exponential regression analysis

The relationship between HbA1c levels of the patients and coffee consumption/per week was demonstrated in Figure 1.

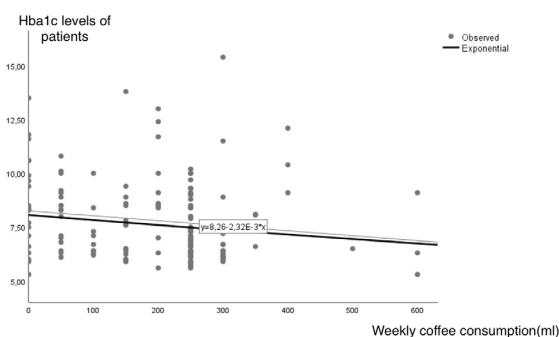


Fig. 1. Weekly coffee consumption (mL) (x), HbA1c levels of the patients (y)

Discussion

DM is one of the most important health problems today. Treatment of DM includes medications, diet, and lifestyle changes. Nutritional support especially has begun to be considered mainly in recent years become a rising field of interest for the treatment of DM.

Coffee is one of the most consumed beverages in our country as well as all over the world. Turkish coffee which is cooked with traditional techniques in our country takes an important place in the daily diet and social life of individuals in people of all ages in our society.

Many studies have been conducted about the relationship between coffee and chronic diseases. It is known that coffee contains many bio-phytochemicals that affect serum lipids and metabolism. The phytochemicals in coffee are affected by the cooking technique. Boiled coffee such as Turkish coffee, contains more phytochemicals than filter coffees.²² When we searched the literature we found a few studies that investigated the relationship between Turkish coffee and type-2 DM.

In a study conducted by Grosso et al. it was shown that coffee and tea consumption was inversely related to the metabolic syndrome and its components.⁶ In our study, there was a significant negative correlation between Turkish coffee consumption and HbA1c level, but no significant relationship was found between HbA1c levels and other types of coffee types and tea. The lower consumption rate of other coffee types in our country compared to Turkish coffee may have affected the results. As for tea, the high amount of consumption and generally drinking with sugar may cause high glucose intake. Future studies comparing sugar-free tea with coffee in large equal groups will reveal more informative results. In a cross-sectional study with 300 participants conducted by Peksever et al. in our country, no significant relationship was found between type-2 DM and coffee, but the normal body fat ratio was found to be significantly higher in those who consumed more than 800 mL of black tea per day compared to those who consumed less than 800 mL.²³ But in contrast to this study, the percentage of participants who consumed Turkish coffee without sugar was higher in our study at 48.3% and 73% percentages. The Japan Public Health Center-based Prospective Diabetes study by Kabeya et al.²⁵ found that coffee consumption of ≥ 240 mL/day was significantly associated with a change in the FPG level by -1.9 mg/dL in men ($p=0.013$) and -1.4 mg/dL in women ($p=0.015$), as compared to coffee consumption of 0 mL/day in contrast to other beverages. But unlike our study, their study showed some conflicting relationships between the HbA1c levels and consumption of some types of beverages. Therefore, in a study by Albar et al. no association was found between average caffeine intake and HbA1C levels.²⁴ Sar-

riá et al. showed that regular consumption of the green/roasted coffee blend produces positive effects on blood pressure, glucose and triglyceride levels.²⁶ In their meta-analysis Kondo et al., suggested that green tea, but not caffeinated/decaffeinated coffee or black tea, may reduce FBG levels, compared with placebo/water.²⁷ So there are conflicting findings on the relationship between glycemic control and coffee consumption despite the beneficial effects of coffee being mentioned in DM, HT, and many various diseases.^{5,6,26,28,29}

When we compared the HbA1c levels according to the individuals' perception of their health and their diet status, no difference was observed between the groups ($p>0.05$). However, HbA1c levels were significantly higher in the group using insulin treatment. This situation may be due to the fact insulin treatment was usually kept for patients with unregulated glucose levels and high HbA1c values

In the study, no correlation was found between the amount of Turkish coffee consumption per week and HDL, LDL, triglyceride, or total cholesterol values contrary to Sarriá et al. Karabudak et al. found no significant differences in DL, VLDL, TG, or total cholesterol levels between groups when participants were assigned to 3 groups as who do not drink coffee, who drink Turkish coffee, and who drink granulated coffee.^{26,30} Therefore the review by Grosso et al. also found conflicting results about serum lipids associated to coffee consumption.⁶

There are some limitations of our study. The incomplete questioning of the amount of sugar added to coffee and tea, the high mean average, and the low physical activity level may affect study outcomes. Increased sample size with equal distribution and lower mean age will provide beneficial findings.

Conclusion

According to the results of our study consumption of Turkish coffee was inversely correlated with fasting glucose and HbA1c level. Future studies with a higher sample size will give more information about the effect of coffee in DM and whether coffee may be nutritional support for treatment.

Declarations

Funding

None

Author contributions

Conceptualization, R.Ö.T.; Methodology, E.M.K.; Software, R.Ö.T.; Validation, R.Ö.T.; Formal Analysis, R.Ö.T.; Investigation, R.Ö.T.; Resources, R.Ö.T.; Data Curation, R.Ö.T.; Writing – Original Draft Preparation, R.Ö.T.; Writing – Review & Editing, R.Ö.T.; Visualization, R.Ö.T.; Supervision, R.Ö.T.; Project Administration, R.Ö.T.; Funding Acquisition, R.Ö.T.

Conflicts of interest

The authors have no conflicts of interest to disclose.

Data availability

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

Ethics approval

The protocol for the study was approved by a suitably constituted Ethics Committee (June 2021 with the code 2605) of the institution in which them work was undertaken and the study conformed to the provisions of the Declaration of Helsinki.


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Effect of sensory retraining program on latency, amplitude and conduction velocity of sensory nerves of lower limb in type 2 diabetic peripheral neuropathy

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ABSTRACT

Introduction and aim. India is known as the world's diabetes capital. Diabetic neuropathy is the most common complication of diabetes, and if not managed properly, leads to diabetic foot complications like ulcers and amputations. Physiotherapy interventions addressing these complications are very limited. Hence, this study was performed to access to what extent a sensory retraining program affects the latency, amplitude, and conduction velocity of the sural nerve in lower limbs. The study aims to improve the nerve conduction velocity of the sural nerve in diabetic neuropathy with a sensory retraining program.

Material and methods. One-hundred individuals who met the inclusion criteria were randomly allocated into 2 groups. The control group had received routine medical care. The experimental group individuals were managed with a sensory retraining program 5 days a week for 16 weeks. Latency, amplitude, and conduction velocity values were recorded before and after the study.

Results. Results conclude that the sensory retraining program is effective in decreasing the latency and improving conduction velocity. It did not have any effect on the amplitude.

Conclusion. Hence, we conclude that a sensory retraining program can be incorporated into routine physical therapy intervention in subjects with diabetic peripheral neuropathy.

Keywords. amplitude, conduction velocity, diabetic neuropathy, latency, sensory retraining, sural nerve

Introduction

The increasing prevalence of diabetes mellitus (DM) is particularly alarming, and the World Health Organization predicts it will become the sixth leading cause of death by 2030. With 6.7 million deaths attributed to diabetes in 2021, it underscores the ur-

gent need for comprehensive strategies to address this chronic condition.¹ Individuals living with type 2 diabetes mellitus (T2DM) are indeed at risk of experiencing both short-term and long-term complications if the condition is not effectively managed. These complications can significantly impact quality of life

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and increase the risk of premature death if not managed effectively.²

In diabetic individuals, anomalous changes in blood glucose levels can damage nerves.³ Sixty to seventy percent of DM individuals have nervous system involvement.⁴ Peripheral neuropathy is the most common type of neuropathy in DM.⁵ Even the state of “Impaired Glucose Tolerance” has a negative influence on the conduction velocity of nerves.⁶ Every 20 seconds, a lower limb is lost due to diabetic neuropathy complications. Eighty-five percent of lower limb amputations are preceded by diabetic foot ulcers.⁷ Diabetic peripheral neuropathy (DPN) increases the amputation risk 1.7 times.⁸ The sural nerve innervates the major weight accepting areas of the sole of the foot. Due to this length dependent relation, it is commonly involved in DPN. The electro-physiological evaluation of the sural nerve has a high diagnostic value in detecting DPN. Gurinder Mohan et al. in their study on type 2 diabetic neuropathy patients have concluded that in 80% of the patients, the sural nerve is involved followed by 62% tibial and 50% deep peroneal nerves.⁹

The treatment protocols for DPN present a challenge as reported in the current literature given the multifaceted nature of the condition and its associated complications, which require careful consideration during physiotherapy interventions. The ongoing debate regarding the potential reversibility of nerve damage highlights the intricate nature of addressing DPN and necessitating a diverse array of treatment strategies. While some research studies have suggested improvements in nerve conduction velocity through pharmacological interventions like aldose reductase inhibitors, others have demonstrated the efficacy of physical exercise modalities such as Tai Chi and aerobic exercises in enhancing nerve function. Nevertheless, there remains a scarcity of empirical evidence regarding the effectiveness of sensory retraining programs in improving key neurophysiological parameters such as latency, amplitude, and conduction velocity of sensory nerves in the lower limb. Given the pivotal role of the sural nerve which is a significant nerve in the lower limb responsible for supplying essential regions of the sole and is closely associated with DPN, this research aims to investigate the potential efficacy of sensory retraining programs in enhancing these parameters.

Aim

The aim of this study is to improve nerve conduction velocity of the sural nerve in diabetic neuropathy by implementation of a sensory retraining program.

Material and methods

Ethical clearance

Approval was obtained from the Ethics Committee (Reg.No.No.ECR/477/Inst/AP/2013.). Patients were informed about the procedure of the study. A written

informed consent was obtained from all the patients before their participation in the study.

Inclusion criteria

Type 2 diabetic neuropathy, both genders, Age: 45–60 years, TCSS – moderate neuropathy score 9–11, non-obese individuals.

Exclusion criteria

Individuals having following conditions were excluded: plantar ulcers, spinal deformities, amputations, arthritis of lower limb joints, radiculopathy, severe visual and vestibular disorders, history of angina and CABG, neurological disorders like stroke, cerebellar ataxia, myelopathy, postural hypotension, other causes of neuropathy like severe B12 deficiency, liver and kidney diseases, thyroid disorders, connective tissue diseases and other metabolic or hereditary neuropathies, skin lesions that interfere with the procedure, alcoholics, smokers, patients using medicines that interfere with nerve function like isoniazid, and furaxone.

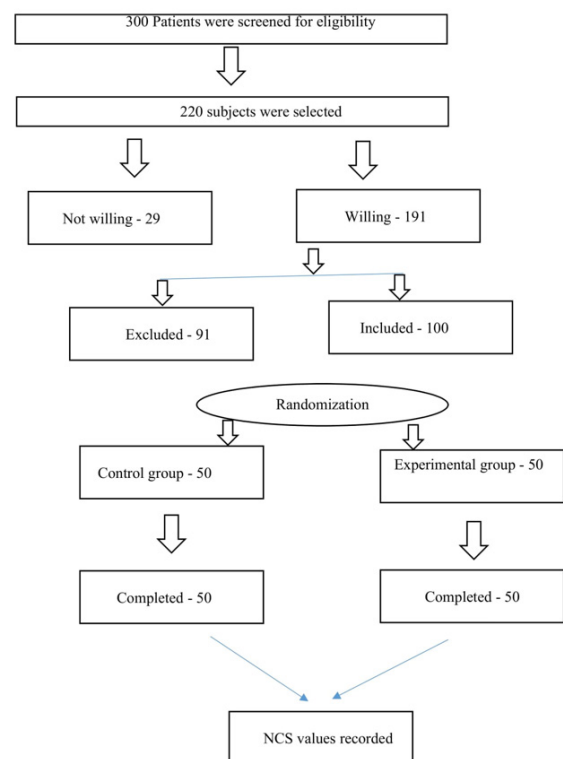


Fig. 1. CONSORT flow diagram

Methodology

In this study, we enrolled 100 subjects. They were randomly split into 2 groups: a control group and an experimental group of 50 each. Once the informed consent was signed, base line recordings were taken and a procedure for sural NCS recording.^{10,11} The procedure was carried out by using an Octopus clarity EMG-NCV machine. Based on our previous work, we have recorded

and measured unilateral values as there are no right left differences in the NCS parameters in DPN patients.¹² An anti-dromic method of sensory nerve conduction of the sural nerve was followed.

Machine Settings: Frequency – 20 Hz to 3 kHz, Sweep speed – 2 ms/division, Gain – 20 μ V/division.

The Microsoft Excel and GraphPad prism software (Graphstats Technologies, Karnataka, India) were used for analysis of the data. The p values less than 0.05 were regarded as statistically significant.

Treatment protocol

Treatment protocol for control group

Continuing their regular medical care, participants in the control group benefit from ongoing foot examinations, ensuring their health is carefully monitored throughout the study. Upon completion of the study period, they receive exercises tailored to support their well-being further.

Treatment protocol for experimental group

Embarking on a transformative journey spanning 16 weeks, participants in the experimental group engage in a meticulously crafted exercise regimen, designed to enhance sensory perception.^{13,14} With dedicated sessions five days a week. The exercises were done sequentially starting with warm-up and ending with cool-down, with frequent rest periods in between.

Warm-up exercises (5 minutes)

Relaxed deep breathing exercises: 2 minutes

Bilateral knee and ankle free range of motion exercises: 2 minutes, stretching: 1 minute and 1 – minute rest.

Static Balance Exercises – the following exercises were done on firm (floor) surface progressed to foam (soft mattress) surface.

On both the surfaces individuals should practice with the following variations - with eyes open, eyes closed, head neutral, head extended (slightly between 10–20 degrees).

Exercises – normal double limb stance, closed stance, tandem stance, single limb stance – on both legs, heel standing, toe standing. 2 mins rest is allowed.

Dynamic activities – the following exercises were done on firm (floor) surface progressed to foam (soft mattress) Surface. On both the surfaces individuals should practice with the following variations -with eyes open, eyes closed, head neutral, head extended (slightly between 10–20 degrees).

Walking forwards – 4 mins, walking backwards – 4mins. One minute rest is allowed after completing the tasks. Walking sideways – 4 mins, tandem gait – 4 mins. Again 1min rest is allowed.

Exercises on stability disc – Participants may use support initially for confidence. Exercises on stability

disc are as following: standing stable – 2 mins, squatting – 2 mins, standing on one leg – 1 min, heel rise – 1min. Upon completing the 4 exercise variants. 2 mins rest is incorporated.

Cool-down (5 minutes)

As the session draws to a close, participants gone through a phase of cool-down exercises which are similar to warm-up exercises. With the completion of the study, participants Sural NCS values are once again meticulously recorded.

Results

There is no significant difference between control group and experimental groups, for the parameters age (years), height (cm), weight (kg), BMI – body mass index (kg/m^2), DM (years), Toronto clinical scoring system (TCSS).

Table 1. Baseline values of control group and experimental group

Parameters	Groups	n	Minimum	Maximum	Mean	SD	p
Age (years)	Control group	50	45	59	51.26	4.29	0.653
	Experimental group	50	45	59	50.88	4.12	
Height (cm)	Control group	50	147	173	159.8	5.77	0.615
	Experimental group	50	147	173	159.2	6.49	
Weight (kg)	Control group	50	50	74	60.54	6.04	0.269
	Experimental group	50	50	72	59.22	5.83	
BMI (kg/m^2)	Control group	50	21.1	25	23.65	1.14	0.220
	Experimental group	50	20.2	25	23.34	1.39	
DM (years)	Control group	50	10	15	11.82	1.64	0.635
	Experimental group	50	10	15	11.98	1.72	
TCSS	Control group	50	9	11	9.82	0.80	0.721
	Experimental group	50	9	11	9.88	0.87	

Table 2. Latency, amplitude and conduction velocity values of control group and experimental group – pre and post intervention.

Parameters	Experimental group (n=50)		p	Control group (n=50) Mean Value		p
	Mean Value			Value		
	Pre	Post	Pre	Post		
Latency	2.92	2.65	<0.0001	2.98	3.00	0.132
Amplitude	7.88	7.76	0.858	7.56	7.42	0.524
Conduction velocity	35.11	38.78	<0.0001	35.22	35.02	0.074

After completion of the treatment period latency, amplitude and conduction velocity values of sural nerve were recorded among all individuals in both the groups.

Table 2 shows in experimental group latency was decreased, amplitude is decreased and CV is increased. This infers the given exercise is effective only on latency and CV. On the other hand in control group - latency was increased, amplitude is decreased and CV also decreased. This infers that the disease is progressing.

Discussion

In diabetes hyperglycemia stimulates the production of neurotrophin like nerve growth factor (NGF) and accelerates the neuropathy by interfering with the normal axonal repair and regeneration.¹⁵ Neuropathy robs the individual's ability to recognize about detrimental pressure and pain. It is also associated with decreased activity levels, sensory impairments and arterial diseases that results in lower limb complications like ulcers, infections etc.¹⁶ In DPN the touch and thermal sensations are decreased in the heels, which are innervated by sural nerve. This is the nerve to be damaged first in the progression of the disease.¹⁷ There is no effective medical management for enhancing the peripheral nerve function.¹⁸ As the peripheral nerves have the ability to regenerate, early diagnosis and early intervention reduces the morbidity, mortality and increases the QOL.¹⁹

After completion of the study (Table 2) there observed decreased latency and improved conduction velocity which states that sensory retraining program is effective to improve the peripheral nerve function, means peripheral nerve is responsive to our intervention.

Very few studies were done to know the effect of exercise on NCV. Our study results co insides with the work done by Hung et al. who have proved that 12 weeks of Tai Chi Chuan exercise showed increase in MNCV of bilateral median and tibial nerves.²⁰ Snehil Dixit et al. have given 8 weeks of aerobic training for DPN patients and proved that both sural and peroneal nerves have shown improved conduction.²¹ Sukhee Ahn et al. have proved that 1 hour of Tai Chi per session, twice a week for 12 weeks, improved glucose control, balance, neuropathic symptoms, and some dimensions of quality of life in diabetic patients with neuropathy.²² Zakaria et al., treated DPN patients with LASER and exercises. The results of this study showed significant improvement in sensory and motor nerve conduction velocity due to increase in nerve regeneration which leads to improvement in superficial sensation.¹⁷ Balduccia et al., in their study of 4 years of mild aerobic exercise can arrest the development of DPN. Sural NCV in control group decreased and in experimental group remained the same.²³ Amplitude is not increased after the intervention as exercise can never change the number of nerve fibers. This has been supported by Balduccia et al.²³ Exercise can influence the micro vascular factors by facilitating the blood flow to the peripheral nerves. So the improvement in the NCS measures could be due to vascular adaptations in the nerves.²⁴ While doing exercise there will be increased temperature. With increase in the skin temperature the sodium channels will undergo hyper polarisation and there will be faster impulse propagation. Sodium influx is increased and the latency is decreased. Per 1 degree Celsius raise in the temperature there is 5% increase in the nerve conduction.²⁵

With exercise there will be a decrease in oxidative stress and reduced inflammation is seen. Even the neurotrophin growth factor levels are restored. The demyelination process can be arrested and myelin damage is prevented. Physical activity will decrease Ca^{2+} channel dysfunction and help to improve nerve conduction.²⁶

Study limitations

The exploration of sensory retraining-induced conduction increment mechanism was not conducted in this study. A follow-up to ascertain the extent of treatment benefits was not conducted as part of this study. Future research can be done to know the reversibility of nerve function that can be thoroughly analyzed through the utilization of invasive and quantitative techniques such as intraepidermal nerve fiber density (I E2N3 FD) etc. Follow-up trials can provide valuable insights into the duration for which the treatment effects are sustained, offering a comprehensive understanding of the long-term efficacy of the intervention.

Clinical implications

The implementation of a sensory retraining program emerges as a promising avenue within rehabilitation, offering tangible benefits for nerve function enhancement. Clinicians can strongly recommend this treatment protocol for betterment of the patients. The sensory retraining program holds promise not only as a treatment technique but also as a proactive preventive therapy. By incorporating it into preventive care strategies, individuals may proactively fortify their sensory functions, potentially averting the onset or progression of conditions like diabetic peripheral neuropathy.

Conclusion

Routine balance training can be incorporated with sensory specific exercises to get promising benefits to DPN individuals. The findings of this study conclude that there is significant improvement in latency, conduction velocity with sensory specific retraining in DPN individuals. Thus these exercises are recommended for reversing the pathological process of the distal nerves of the lower limbs.

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Declarations

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Author contributions

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

Conflicts of interest

No potential conflict of interest was reported by the author(s).

Data availability

The data related to the current study is available with the corresponding author.

Ethics approval

Approval was obtained from the Ethics Committee (Reg.No.No.ECR/477/Inst/AP/2013.)

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ORIGINAL PAPER

Social vulnerability and medication adherence in individuals with chronic obstructive pulmonary disease

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ABSTRACT

Introduction and aim. The association between social vulnerability and medication adherence in individuals with chronic obstructive pulmonary disease is unclear. The aim of this study was to show the correlation of social vulnerability and medication adherence in individuals with chronic obstructive pulmonary disease.

Material and methods. Data collection was conducted by face to face interview in November 2022 and May 2023. Social vulnerability and medication adherence were evaluated using the Social Vulnerability Scale and the Medication Adherence Report Scale respectively.

Results. The mean scores of individuals with chronic obstructive pulmonary disease for social vulnerability and medication adherence were 20.02 ± 6.69 and 22.76 ± 3.29 respectively. Levels of social vulnerability varied according to comorbidity, hypertension, heart failure, visit to emergency service in the last three months due to COPD exacerbation, and using herbal products instead of medication ($p < 0.05$). Mean scores for medication adherence were lower in women, never smokers, and those who used herbal products instead of medication ($p < 0.05$).

Conclusions. Having a higher level of social vulnerability is associated with worse medication adherence ($r = -0.31$, $p < 0.01$). Individuals using herbal products are found to be at risk for social vulnerability and non-adherence to medication ($p < 0.05$).

Keywords. chronic obstructive pulmonary disease, frailty, medication adherence, social vulnerability

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic disease in which effective management requires long-term adherence to medication.¹ The increased symptom burden and recurrent hospitalizations negatively impact the Health-Related Quality of Life (HRQOL) in COPD.² Pharmacological treatment for COPD patients can alleviate symptoms, improve lung function, and reduce the risk of exacerbations.³ The findings of a systematic review show that non-adherence to inhaler therapy is common in COPD patients.⁴ Achieving good adherence involves a process including the initiation, implementation, and persistence of

a treatment plan. In COPD, this means obtaining and taking the first inhaled dose followed by obtaining and taking refills of the medication over time.⁵ Medication adherence, defined as the degree to which a patient follows the prescribed timing, dosage, and duration of their medication, is notably linked to various factors.⁶ These factors include diminished treatment expectations, perceptions of illness, comorbidities, depression, aging, smoking, an individuals' lack of confidence, and insufficient knowledge, all contributing to non-adherence.⁷⁻⁹ Difficulty in medication adherence adversely affects health-related quality of life,¹⁰ leading to unfavorable clinical outcomes, increased healthcare utilization,

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and overall healthcare costs.⁶ Frailty is also a significant factor affecting the quality of life in COPD patients.¹¹⁻¹³ Frailty can lead to worsening respiratory function and dyspnea in individuals with COPD, idiopathic pulmonary fibrosis, and asthma.¹⁴ Moreover, it increases mortality and hospitalizations.^{15,16} Marengoni et al. identified that individuals with COPD are twice as vulnerable to frailty compared to those without COPD.¹⁷ While the literature predominantly addresses physical frailty in individuals with COPD,^{11-13,15,18,19} limited research has explored the social aspects of frailty.^{20,21} Social elements like social activities, social support, social networks, loneliness, and solitary living emerge as the predominant social factors across diverse frailty assessment tools.²² Van Oostrom et al. define social frailty by utilizing measures such as loneliness, inadequate social support, and restricted social engagement.²³

Social vulnerability is a tendency towards credulity and gullibility. It causes stress exposure in communities and individuals.²⁴ At the same time, social vulnerability is a significant risk factor for physical frailty; thus, assessing social vulnerability is crucial.²⁵ Early intervention for fragile individuals can prevent potentially risky situations, improve quality of life, and reduce care costs.²⁶ Social vulnerability has a strong impact on the future risk of physical disability among elderly individuals in the community.²⁷ Frailty and COPD are strongly correlated in the elderly population.^{19,28,29} Social vulnerability in older individuals can pose a risk of mortality and morbidity.³⁰ Despite emerging evidence suggesting that frailty begins much earlier than age 65, most studies on frailty have focused solely on individuals over this age threshold.^{11,15,20} In individuals with COPD, social vulnerability is a significant risk factor for severe acute exacerbation and unexpected hospitalization.²⁰ Identification of social vulnerability presents an opportunity to intervene to improve the lives of individuals and communities following an adverse health.³¹ As far as we are aware, no existing literature investigates the correlation between social vulnerability and medication adherence in individuals below the age of 65 with COPD. Simultaneously addressing social vulnerability and medication adherence may provide a new framework for improving conditions that could affect individuals at an early stage. Therefore, this study explores the relationship between social vulnerability and medication adherence in individuals under the age of 65 with COPD.

Aim

The following research questions were proposed for this purpose: What are social vulnerability and medication adherence scores in persons with COPD? Do social vulnerability and medication adherence differ by sociodemographic and disease-related characteristics? Is there a relation between social vulnerability and medication adherence scores in persons with COPD?

Material and methods

Design and sample

The research had a descriptive and relational design. The study population consisted of patients presenting to a university hospital's chest disease outpatient clinic. The minimum sample size in the population was determined by power analysis with the program G*Power (v3.1.9.7). In the calculation performed to obtain a .05 significance level (α), and an 95% statistical test power ($1-\beta$), effect size was calculated as 0.3 and a minimum sample size of 134 was found according to the standard deviation value.

Inclusion criteria

Eligible for participation in this study were:

- age between 40 and 65,
- diagnosis of COPD for at least one year (The participants with hospital data confirming a post-bronchodilator forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) ratio of less than 70% for COPD according to the GOLD)¹,
- use of inhalers in accordance with their COPD diagnosis,
- no exacerbation or respiratory tract infection at the time of data collection
- ability to speak, read, and write in Turkish,
- having the cognitive capacity to provide informed consent (no psychiatric or cognitive problems as confirmed by medical record review),
- volunteering to participate in the study,

Exclusion criteria

Excluded from participation in this study were:

- patients were excluded from the study who had had COPD for less than one year,
- who answered the questions incompletely,
- who were over 65 years old,
- who had an exacerbation and/or a respiratory tract infection when data was collected,
- who did not have the cognitive capacity to provide informed consent,
- or who were unwilling to participate in the study.

Data collection and instruments

Data were collected between November 2022 and May 2023. The patients who met the inclusion criteria were first explained the content (the aim of the research, what it covered, and its duration) of the study and asked to sign an informed consent form. The patients were told that they could withdraw from the study at any time. 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 participants were asked to answer the questions in the data collection instruments in a way that they thought suitable

and to answer all the questions. All data were collected following the instructions given by the first author of the study. Socio-demographic and clinical characteristics of patients were obtained through interviews with researchers, patient records, and the hospital's electronic medical record system. The data collection tools included:

Data gathering form

The data-gathering form was prepared by the researchers in light of the literature review. The form consists of 20 questions encompassing participants' socio-demographic and disease-related characteristics.^{7,18,20,21,24,27,30,32,33}

Social vulnerability scale

The Social Vulnerability Scale was developed by Pinsker et al. and adapted to the Turkish language by Saricam.^{24,34} This scale, consisting of 15 items on a 5-point Likert scale, encompasses two subscales: "gullibility" and "credulity." Credulity is an inclination to believe things that are untrustworthy or unlikely to be true or real. Gullibility relates to a tendency to being manipulated or deceived, either over and over again or in the presence of obvious warning signs. The scores obtained from the scale range from 15 to 75. The scores on the subdimensions of the scale are 8-40 for gullibility (items 1-8.), and 7-35 for credulity (items 9-15.). The scale has no cutoff point. Higher scores indicate greater social vulnerability. Cronbach's alpha for Turkish version was 0.94.²⁴ The Cronbach's alpha for the current total sample was 0.85.

Medication Adherence Report Scale (MARS)

The Medication Adherence Report Scale was developed by Horne and Weinman to assess drug compliance in chronic diseases and was adapted to the Turkish language by Temeloglu Sen et al.^{33,35} Medication adherence was assessed using the MARS, which is a five-item, structured, self-reported questionnaire, to identify a variety of non-adherent behaviors, including a tendency to avoid, forget or stop taking medication and a tendency to adjust or alter the dose prescribed by the clinician. The participants were asked about their adherence to inhaler drugs, prescribed for COPD treatment. This 5-item Likert scale was employed to assess medication adherence. The total test score is determined by summing the scores obtained from the items. The scores obtained from the scale range from 5 to 25. The scale has no cutoff point. Higher total scores indicate better medication adherence and lower total scores indicate poor medication adherence. Cronbach's alpha for Turkish version was 0.78.³³ The Cronbach's alpha for the current total sample was 0.87.

Statistical analysis

The data in this study were analyzed using SPSS 22.0 (IBM Corp., Released 2013, IBM SPSS Statistics for Win-

dows, Version 22.0, Armonk, NY: IBM Corp.). Categorical variables were expressed as numerical values and percentages, and continuous variables were expressed as means, standard deviations, medians, and maxima and minima. The Kolmogorov-Smirnov test was used for conformity to normal distribution. The Mann-Whitney and Kruskal-Wallis tests were used to compare variables which did not show normal distribution. The association between numerical variables was assessed by Spearman correlation analysis. Statistical significance was taken as $p < 0.05$.

Ethics approval

This research was conducted in conformity with ethical principles and the Helsinki Declaration. Before beginning the study, permission was obtained from the Ethics Committee of the Postgraduate Education Institute of Çanakkale Onsekiz Mart University, decision No. 20/16, dated 24 November 2022. Appropriate permissions were obtained from the institution where the study was conducted (dated 29 November 2022, No. E-78179085-199-2200284102). Written permission was obtained by e-mail from the researchers to use the scales in the collection of data. Informed written approval was obtained from the participants concerning their willing and voluntary participation.

Results

The eligible sample size was 134. Thirty-one were excluded based on the exclusion criteria. Patients were excluded from the study who had had COPD for less than one year ($n=4$), who answered the questions incompletely ($n=7$), who over 65 years old ($n=12$), and who did not agree to participate in the study ($n=8$). After applying the inclusion and exclusion criteria, the study included 136 voluntary participants.

The mean age of the patients included in the study was 59.29 (SD=5.42) years, and the duration of the COPD was 8.02 (SD=5.20) years. The mean scores of individuals with COPD for social vulnerability and medication adherence were 20.02 (SD=6.69) and 22.76 (SD=3.29) respectively (Table 1). Of the participants, 41.2% scored 25 points on the Medication Adherence Scale, while 58.8% scored less than 25.

Average medication adherence scores were lower in females ($p=0.02$), non-smokers ($p=0.01$), and those using herbal products instead of medication ($p=0.01$) (Table 1, Table 3).

Average social vulnerability scores were higher in individuals with comorbidities ($p=0.01$), hypertension ($p=0.01$), heart failure ($p=0.03$), those who visited the emergency service in the last three months once due to COPD exacerbation ($p=0.03$), and those using herbal products instead of medication ($p=0.00$) (Table 2, Table 3).

Social vulnerability was higher in individuals using herbal products instead of medication to expectorate mucus ($p=0.04$) (Table 4). The herbal products used in-

stead of medication were chamomile (64.4%), sage tea (59.3%), ginger (59.3%), garlic (28.8%), pine cone syrup (20.3%), and licorice root (16.9%).

Table 1. Comparison of social vulnerability scale and MARS scores according to socio-demographic characteristics*

n=136	Mean±SD (Min–Max)							
Age	59.29±5.42 (42–65)							
Time since COPD diagnosis	8.02±5.2 (1–20)							
Total number of medications used	5.10±2.88 (1–15)							
Social vulnerability	20.02±6.69 (15–43)							
Gullibility	9.43±2.26 (8–18)							
Credulity	10.57±5.10 (7–30)							
Medication adherence	22.76±3.29 (5–25)							
	Gullibility		Credulity		Social vulnerability		Medication adherence	
	Mean±SD (Min–Max)	P	Mean±SD (Min–Max)	P	Mean±SD (Min–Max)	P	Mean±SD (Min–Max)	P
MW Gender, n (%)								
Female, 45 (33.1)	9.96±2.31 (8–16)	0.01	11.04±5.66 (7–30)	0.39	21±7.34 (15–43)	0.21	21.73±3.83 (10–25)	0.02
Male, 91 (66.9)	9.16±2.21 (8–18)		10.34±4.82 (7–28)		19.54±6.33 (15–41)		23.26±2.88 (5–25)	
KW Age, n (%)								
40–49 Years, 12 (8.8)	10.25±2.73 (8–16)		12.17±6.9 (7–30)		22.42±9.10 (15–43)		22.08±5.7 (5–25)	
50–59 Years, 41 (30.1)	9.44±2.42 (8–18)	0.52	9.66±3.61 (7–21)	0.62	19.10±5.32 (15–36)	0.70	22.39±3 (14–25)	0.25
60–65 Years, 83 (61)	9.30±2.11 (8–18)		10.80±5.41 (7–28)		20.13±6.89 (15–41)		23.04±2.98 (10–25)	
MW Marital status, n (%)								
Married, 97 (71.3)	9.32±2.21 (8–18)	0.34	10.72±5.52 (7–30)	0.60	20.07±7.17 (15–43)	0.40	22.57±3.63 (5–25)	0.76
Single, 39 (28.7)	9.69±2.40 (8–18)		10.21±3.92 (7–24)		19.90±5.38 (15–36)		23.23±2.23 (15–25)	
KW Education level, n (%)								
Primary school, 12 (8.8)	9.70±2.58 (8–18)		11.36±6.17 (7–30)		21.07±7.94 (15–43)		22.53±4.04 (5–25)	
Middle school, 41 (30.1)	9.33±2.06 (8–18)	0.49	10.18±3.25 (7–20)	0.24	19.58±4.61 (15–38)	0.18	23.06±2.22 (16–25)	0.69
High school/university, 83 (61.0)	8.94±1.6 (8–13)		9.3±3.75 (7–24)		18.24±5.05 (15–36)		22.94±2.32 (17–25)	
MW Residential area, n (%)								
Rural area, 44 (32.4)	9.48±2.73 (8–18)	0.42	10.3±4.2 (7–26)	0.84	19.82±6.27 (15–41)	0.99	23.18±2.55 (14–25)	0.52
Urban area, 92 (67.6)	9.40±2.02 (8–18)		10.71±5.5 (7–30)		20.12±6.91 (15–43)		22.55±3.59 (5–25)	
KW Employment status, n (%)								
Employed, 30 (22.1)	9.27±2.6 (8–18)		10.07±4 (7–22)		19.33±5.82 (15–37)		22±4.36 (5–25)	
Unemployed, 37 (27.2)	9.62±2.16 (8–16)	0.25	11.00±5.76 (7–30)	0.85	20.62±7.25 (15–43)	0.88	22.57±3.51 (10–25)	0.88
Retired, 69 (50.7)	9.39±2.18 (8–18)		10.57±5.20 (7–28)		20.00±6.79 (15–41)		23.19±2.54 (15–25)	

* MW – Mann-Whitney U test, KW – Kruskal-Wallis H test

A weak negative correlation was observed between medication adherence and gullibility scores ($r=-0.31$,

$p<0.01$), while a moderate negative correlation was found between social vulnerability ($r=-0.43$, $p<0.01$) and credulity scores ($r=-0.43$, $p<0.001$) (Table 5).

Table 2. Comparison of social vulnerability scale and MARS scores according to clinical characteristics*

n=136	Gullibility		Credulity		Social vulnerability		Medication adherence	
	Mean±SD (Min–Max)	P	Mean±SD (Min–Max)	P	Mean±SD (Min–Max)	P	Mean±SD (Min–Max)	P
KW COPD stage, n (%)								
GOLD 1, 19 (14)	9.79±2.44 (8–16)		11.05±5.81 (7–30)		20.84±7.57 (15–43)		22.47±4.77 (5–25)	
GOLD 2, 62 (45.6)	9.21±2.1 (8–18)	0.65	9.69±4.07 (7–24)	0.2	18.92±5.77 (15–37)	0.24	22.89±3.04 (12–25)	0.8
GOLD 3, 55 (40.4)	9.55±2.39 (8–18)		11.40±5.79 (7–28)		20.98±7.25 (15–41)		22.71±3 (10–25)	
MW Coexisting diseases, n (%)								
Yes, 87 (64)	9.79±2.43 (8–18)	0.01	11.22±5.25 (7–30)	<0.001	21.05±6.88 (15–43)	<0.001	22.53±3.21 (10–25)	0.13
No, 49 (36)	8.78±1.77 (8–18)		9.43±4.66 (7–28)		18.20±5.97 (15–38)		23.16±3.44 (5–25)	
MW Hypertension, n (%)								
Yes, 60 (44.1)	9.98±2.47 (8–18)	<0.001	11.6±5.92 (7–30)	0.04	21.63±7.52 (15–43)	0.01	22.45±3.52 (10–25)	0.52
No, 76 (55.9)	8.99±2 (8–18)		9.76±4.22 (7–28)		18.75±5.68 (15–38)		23±3.1 (5–25)	
MW Coronary artery disease (CAD), n (%)								
Yes, 22 (16.2)	8.82±1.74 (8–15)	0.13	10.55±5.99 (7–27)	0.40	19.36±7.10 (15–37)	0.27	21.86±4.57 (10–25)	0.92
No, 114 (83.8)	9.54±2.34 (8–18)		10.58±4.94 (7–30)		20.15±6.63 (15–43)		22.93±2.98 (5–25)	
MW Heart failure, n (%)								
Yes, 20 (14.7)	10.25±2.47 (8–18)	0.02	11.15±4.04 (7–24)	0.051	21.45±5.92 (15–36)	0.03	22.95±2.63 (15–25)	0.85
No, 116 (85.3)	9.28±2.21 (8–18)		10.47±5.27 (7–30)		19.78±6.81 (15–43)		22.72±3.4 (5–25)	
MW Diabetes, n (%)								
Yes, 34 (25)	9.71±2.02 (8–15)	0.12	11.76±5.76 (7–30)	0.06	21.74±7.17 (15–43)	0.07	22.68±2.79 (16–25)	0.54
No, 102 (75)	9.33±2.34 (8–18)		10.18±4.83 (7–28)		19.54±6.49 (15–41)		22.78±3.45 (5–25)	

* MW – Mann-Whitney U test, KW – Kruskal-Wallis H test

Discussion

As far as our knowledge extends, this study marks the inaugural attempt to explore the correlation between social vulnerability and medication adherence in individuals under the age of 65 diagnosed with COPD. The findings reveal that higher levels of social vulnerability are associated with poorer medication adherence. Medication adherence is an integral part of chronic disease management, and it has been defined by the World Health Organization as the degree to which the patients accept and follow the treatment organized by health care professionals. Advanced age has been shown to be an important factor for poor adherence to medical therapies.¹ Advanced age has been shown to be an important factor for poor adherence to medical therapies.³⁶ Social

vulnerability is also known to increase with age.³⁰ Frailty represents an increased vulnerability to adverse health outcomes.^{12,16} Vulnerable patients experience frequent exacerbation and/or admissions to hospital, which limits their rehabilitation.^{15,19} It is known that in COPD patients with dyspnea, widespread vulnerability not only does not affect health-related quality of life, but also increases the frequency of use of medical services.¹¹ Difficulty in medication adherence among individuals with COPD can adversely impact disease management.⁷ Similarly, this study reports that the majority of individuals with COPD struggle to achieve optimal levels of medication adherence. COPD and aging can increase the risk and severity of frailty.^{17,19}

Table 3. Comparison of social vulnerability scale and MARS scores according to descriptive characteristics*

n=136	Gullibility		Credulity		Social vulnerability		Medication adherence	
	Mean±SD (Min–Max)	p	Mean±SD (Min–Max)	p	Mean±SD (Min–Max)	p	Mean±SD (Min–Max)	p
KW Smoking status, n (%)								
a. Never smoked, 21 (15.4)	9.95±2.42 (8–16)		10.95±5.39 (7–27)		20.9±6.71 (15–36)		21.1±3.95 (10–25)	a<b
b. Former smoker, 82 (60.3)	9.32±2.41 (8–18)	0.22	10.40±4.89 (7–26)	0.64	19.76±6.75 (15–41)	0.41	23.24±2.79 (12–25)	0.01
c. Currently smoking, 33 (24.3)	9.36±1.73 (8–13)		10.76±5.56 (7–30)		20.12±6.67 (15–43)		22.61±3.72 (5–25)	
KW Visit to emergency service in the last three months due to COPD exacerbation, n (%)								
a. Once, 39 (28.7)	10.13±2.75 (8–18)		11.69±5.06 (7–26)		21.87±7.10 (15–41)		22.49±4.19 (5–25)	a>d
b. Twice, 31 (22.8)	9.35±1.82 (8–13)	0.06	10.58±4.86 (7–30)	0.03	19.94±6.16 (15–43)	0.03	22.42±2.51 (16–25)	0.23
c. Three times and more, 22 (16.2)	9.09±2.41 (8–18)		11.36±6.69 (7–27)		20.45±8.31 (15–38)		22.68±3.99 (10–25)	
d. Never, 44 (32.4)	9.02±1.89 (8–16)		9.18±4.16 (7–28)		18.23±5.41 (15–38)		23.27±2.43 (16–25)	a>d
KW Hospitalization in the last three months due to COPD exacerbation, n (%)								
Once, 35 (25.7)	9.83±2.67 (8–18)		12.49±7.00 (7–30)		22.37±8.71 (15–43)		22.97±2.70 (15–25)	
Twice, 14 (10.3)	8.64±1.15 (8–11)	0.35	9.29±4.12 (7–19)	0.19	17.93±5.26 (15–30)	0.17	23.43±3.06 (14–25)	0.41
Three times and more, 11 (8.1)	9.55±3.21 (8–18)		10.36±4.82 (7–20)		19.91±7.77 (15–38)		23.55±2.07 (19–25)	
Never, 76 (55.9)	9.37±2.05 (8–16)		9.96±4.03 (7–27)		19.34±5.43 (15–37)		22.42±3.70 (5–25)	
KW Home oxygen therapy, n (%)								
Yes, 39 (28.7)	9.62±2.76 (8–18)	0.98	10.56±4.82 (7–26)	0.88	20.23±6.93 (15–41)	0.88	22.95±3.02 (14–25)	0.47
No, 97 (71.3)	9.35±2.04 (8–18)		10.58±5.24 (7–30)		19.94±6.63 (15–43)		22.68±3.41 (5–25)	
MW Use of herbal products instead of medication, n (%)								
Yes, 59 (43.4)	9.63±2.12 (8–16)	0.09	11.27±4.58 (7–27)	<0.001	20.93±5.97 (15–37)	<0.001	21.42±4.20 (5–25)	<0.001
No, 77 (56.6)	9.27±2.37 (8–18)		10.04±5.44 (7–30)		19.32±7.15 (15–43)		23.78±1.83 (17–25)	

* MW – Mann-Whitney U test, KW – Kruskal-Wallis H test

Table 4. Comparison of social vulnerability scale and MARS scores according to frequency and reasons for herbal product use

n=59	Gullibility		Credulity		Social vulnerability		Medication adherence	
	Mean±SD (Min–Max)	p	Mean±SD (Min–Max)	p	Mean±SD (Min–Max)	p	Mean±SD (Min–Max)	p
KW Herbal product use frequency, n (%)								
a. Every day, 8 (13.6)	10.13±2.36 (8–15)		13.13±6.56 (7–24)		23.25±8.4 (15–37)		21.38±3.96 (12–24)	
b. Once a week, 13 (22.0)	9.62±1.76 (8–14)	0.75	12.54±5.77 (7–27)	0.36	22.31±6.29 (15–35)	0.34	20.54±5.09 (10–25)	0.86
c. Two or more times a week, 26 (44.1)	9.73±2.49 (8–16)		11.00±3.81 (7–21)		20.73±5.77 (15–36)		21.38±4.41 (5–25)	
d. Occasionally/Rarely, 12 (20.3)	9.08±1.51 (8–12)		9.25±2.26 (7–14)		18.33±3.23 (15–25)		22.5±2.91 (15–25)	
MW To prevent the progression of the disease, n (%)								
Yes, 24 (40.7)	9.96±2.54 (8–16)	0.64	10.63±4.41 (7–22)	0.20	20.58±6.64 (15–37)	0.32	20.92±5.32 (5–25)	0.69
No, 35 (59.3)	9.40±1.79 (8–14)		11.71±4.71 (7–27)		21.17±5.55 (15–35)		21.77±3.26 (10–25)	
MW Because they believe it is beneficial with minimal side effects, n (%)								
Yes, 24 (40.7)	9.75±2.42 (8–16)	0.96	11.00±3.79 (7–21)	0.99	20.75±5.89 (15–36)	0.84	20.63±4.83 (5–25)	0.29
No, 35 (59.3)	9.54±1.93 (8–15)		11.46±5.10 (7–27)		21.06±6.10 (15–37)		21.97±3.69 (10–25)	
MW Recommended by trusted individuals, n (%)								
Yes, 10 (16.9)	9.80±1.99 (8–13)	0.6	11.20±2.62 (7–15)	0.4	21.00±4.27 (15–28)	0.51	20.80±6.07 (5–25)	0.81
No, 49 (83.1)	9.59±2.17 (8–16)		11.29±4.91 (7–27)		20.92±6.29 (15–37)		21.55±3.79 (10–25)	
MW To avoid respiratory difficulties, n (%)								
Yes, 30 (50.8)	9.73±2.13 (8–15)	0.65	10.53±3.96 (7–21)	0.21	20.33±5.58 (15–36)	0.50	21.93±4.48 (5–25)	0.08
No, 29 (49.2)	9.52±2.15 (8–16)		12.03±5.10 (7–27)		21.55±6.38 (15–37)		20.90±3.90 (10–25)	
MW To alleviate cough, n (%)								
Yes, 40 (67.8)	9.60±2.07 (8–15)	0.93	11.53±4.86 (7–27)	0.54	21.18±6.05 (15–37)	0.50	21.6±4.5 (5–25)	0.29
No, 19 (32.2)	9.68±2.29 (8–16)		10.74±4 (7–21)		20.42±5.93 (15–35)		21.05±3.58 (14–25)	
MW To facilitate the removal of phlegm, n (%)								
Yes, 29 (49.2)	9.97±2.23 (8–16)	0.14	11.9±3.89 (7–24)	0.03	21.86±5.38 (15–36)	0.04	20.9±4.25 (5–25)	0.08
No, 30 (50.8)	9.3±2 (8–15)		10.67±5.16 (7–27)		20.03±6.45 (15–37)		21.93±4.17 (10–25)	

* MW – Mann-Whitney U Test, KW – Kruskal-Wallis H Test

Table 5. Relationship between social vulnerability scale and MARS*

n=136		MARS
Social vulnerability	r	-0.43
	p	<0.001
Gullibility	r	-0.31
	p	<0.001
Credulity	r	-0.43
	p	<0.001

* r – Spearman correlation analysis

Determining social vulnerability and non-adherence to medication before old age can provide the possibility of intervention in the early period. In this study, medication adherence was also not optimal for those using herbal products instead of medication. Additionally, medication adherence was poor in females and non-smokers. Similarly, Vetrano et al. found that females exhibited lower levels of medication adherence than males.³⁷ Humenberger et al. determined no relationship between age and medication adherence.³⁸ The study's findings support the literature regarding age and gender. In contrast, Müllerová et al. found that individuals aged 40 to 59, currently smoking, and having ≥ 2 comorbidities experienced difficulties in medication adherence.³⁹ This discrepancy may be related to non-smokers and those using herbal products having experienced more severe health issues. Also, patients may think that there is no need for prescribed medicines. Adherence to treatment is strongly related to patients' beliefs concerning the need for prescribed drugs and the functional severity of the disease.⁴⁰ In contrast to these findings, there are studies which report no correlation between smoking and medication adherence.^{10,38}

Although the study did not find a very high average score for social vulnerability, it suggests that individuals aged 42–65 with COPD may experience social vulnerability. Similarly, Hirai et al. found a high incidence of social vulnerability, even at ages 40–59, at a rate of 23.5%.²⁰ Middle-aged individuals with COPD may be vulnerable to social frailty. Makizako et al. noted that among non-physically frail adults, social frailty has the potential to precipitate physical frailty rapidly.²⁵ Social vulnerability contributes to the factors which increase the risk of adverse health conditions. Regmi et al. found that social vulnerability factors are associated with an increased risk of 30-day readmission in patients with heart failure.⁴¹ Cognitive impairments have been shown to be an important factor for poor adherence to medical therapies.³⁶ There have been several studies reporting the impact of social vulnerability on adverse health outcomes, such as cognitive impairment, disability, and mortality.^{42,43} Early recognition of frailty is crucial, as interventions introduced at this stage are likely to prevent or delay functional decline, reduce healthcare costs, and minimize the risks of hospitalization, disability, and mortality.^{19,44} Tsutsumimoto et al. found that social frailty was associated with cognitive and physical function in older Japanese adults.³⁰ Hirai et al. found a higher risk of decreased appetite, more severe shortness of breath, severe acute exacerbation, and unexpected hospitalization in individuals with COPD and social vulnerability.²⁰ Similarly, in this study, the social vulnerability was higher in individuals with comorbidities, hypertension, heart failure, those who visited the hospital/emergency department once due to COPD exacerbation, and those

using herbal products instead of medication. Individuals with accompanying chronic illnesses are known to be more vulnerable in terms of social frailty.^{25,45} Nguyen et al. found that obesity, depression/anxiety, and cardiovascular diseases were significantly associated with the most socially vulnerable patients with multimorbidity (having at least three chronic conditions).⁴⁵

Bunt et al. characterized social frailty as a complex concept, signifying a spectrum of susceptibility to the potential loss of social resources, deficiency in social behaviors, social activities, and self-management skills necessary to meet fundamental social requirements.⁴⁶ Various other studies also have explicitly defined social vulnerability as insufficient participation in social networks (or no participation at all) and the perception of a lack of contacts and support.^{20,23,27,46} A low level of social relationships in those with COPD may constitute a risk for solving problems relating to the disease.⁴⁷ Inadequate social support can render individuals socially vulnerable and fragile. Chen et al. found that individuals not living alone with COPD perceived a good level of social support, and perceived social support was associated with increased participation in pulmonary rehabilitation.⁴⁸ Limpawattana et al. identified determinants of frailty as having accompanying cancer, being hospitalized at least twice in the previous year, higher waist circumference measurement, and the presence of sarcopenia.²⁸ Chen et al. observed that frailty in individuals with COPD experiencing dyspnea affected health-related quality of life and increased medical service utilization.¹¹ Van Oostrom et al. found that being female, older age, lower education level, being married, working in a paid job, current smoking, healthy eating, and comorbidity were associated with one or more areas of frailty.²³ In this study, being female was related to a higher risk of gullibility. While socio-demographic factors are essential for frailty, their impact varies across different frailty domains. Unlike findings in the literature, marital status, education level, residential area and employment status were not found to be related to social vulnerability. Also, in this study, no significant difference in social vulnerability and medication adherence was observed based on the stage of COPD. This discrepancy may be attributed to the absence of individuals in the very severe stage and older individuals with COPD in this study. The frailty state is closely linked to the extent of airflow blockage and the frequency of exacerbations. Hence, individuals experiencing severe COPD are at a greater risk of frailty in comparison to those with less severe manifestations of the condition.¹⁵

Study limitations

The present study has several limitations that should be acknowledged. A limitation of this study is that this was a survey, and as such was prone to selection bias. For

this reason, some participants may have responded in a socially expected way, particularly in measurements related to social vulnerability. Our study is limited by its single-center design and small sample size, which may affect its generalizability. The present study excluded patients with cognitive mental disorder. Therefore, our findings could be affected by the exclusion of patients with cognitive mental disorder. Because studies on social vulnerability in individuals with COPD could not be accessed, it was not possible to conduct a multi-directional discussion of the relation between social vulnerability and medication adherence. Nevertheless, all questionnaires selected in this study had strong validity and reliability that is thoroughly documented in the scientific literature.

Conclusion

This study can provide valuable insights into the early detection of social vulnerability and associated factors in individuals with COPD. The study's results revealed that an increase in social vulnerability level is related to poorer medication adherence in patients with COPD. Social vulnerability has been identified in non-elderly individuals with COPD. Individuals using herbal products are found to be at risk for social vulnerability and non-adherence to medication.

Evaluating social vulnerability in individuals with COPD is crucial for health care professionals this can contribute to enhancing patient medication adherence and reducing complications associated with the disease. Identifying psychosocial factors that may lead to social vulnerability in patients, reducing social vulnerability, and promoting providing adequate social support among the public should be targeted to achieve this goal. While it is clear that adherence to treatment in COPD is a critical issue, and that improving it will certainly take time and require major efforts, it is also obvious that raising awareness of the disease and improving cooperation among specialists, general practitioners, pharmacists, nurses, social workers and patients is the starting point at which this change should immediately begin.

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Author contributions

Conceptualization, S.E., C.M.B. and E.O.; Methodology, S.E., C.M.B. and E.O.; Software, S.E., C.M.B. and E.O.; Validation, S.E., C.M.B. and E.O.; Formal Analysis, S.E.; Investigation, S.E., C.M.B. and E.O.; Resources, S.E., C.M.B. and E.O.; Data Curation, S.E., C.M.B. and E.O.; Writing – Original Draft Preparation, S.E.; Writ-

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Conflicts of interest

All authors declare that they have no conflicts 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

This research was conducted in conformity with ethical principles and the Helsinki Declaration. The Scientific Research Ethics Committee approved this research under the decision numbered 20/16, dated 24 November 2022. 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.

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Low depression scores among pet dog owners – a comparative cross-sectional study from Anuradhapura Sri Lanka

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ABSTRACT

Introduction and aim. The role of canine companionship in psychosocial and cardiovascular health is increasingly explored. However, such studies are scarce in South Asia. Hence, we aim to compare psychosocial and cardiovascular indices between pet dog owners and age and sex-matched non-pet owners in Anuradhapura, Sri Lanka.

Material and methods. A community-based, comparative, cross-sectional study was done among 52 pet dog owners and 52 age, sex-matched non-pet owners. An allostatic load was calculated using predefined cutoffs. Chi-square (or Fisher's exact), Mann Whitney U, and Spearman's rho were performed ($p < 0.05$).

Results. The pet dog owners' group had a significantly lower mean (SD) [7.8 (7.2)], and median (interquartile range) [6 (2–12.5)] depression score when compared to the non-pet owners [11.2 (8.6)], 10 (6–14.5) respectively ($p = 0.03$). Also, the pet dog owners showed a significant negative correlation between the pet bond scale score and cortisol ($r = -0.36$, $p = 0.01$).

Conclusion. A significantly lower depression score in the pet dog owners' group than in the non-pet owners' group was observed. And, the pet bond score had a significant negative correlation with the stress hormone cortisol. Thus, pet dog ownership could improve psychosocial health and will guide towards one-health interventions and research among South Asian communities.

Keywords. canine-companionship, cortisol, depression, income, one health, stress

Introduction

Humans and dogs have had an emotional bond for thousands of years.¹ Canine companionship is identified as an integral part of a family.² Also, pet ownership contributes to a healthy neighbourhood relationship.³ Further, dog ownership contributes towards human health and well-being.⁴ Variable results such as positive, mixed, negative, and no impact of pet ownership on mental health are reported in prior literature.⁵ Thus, an objective as-

essment of the association between dog ownership and mental well-being is warranted. Moreover, canine companionship is associated with reduced mortality possibly due to decreased cardiovascular mortality.⁶ Dog owners are more likely to achieve optimum cardiovascular health metrics.⁷ And, ownership is protective against coronary artery disease.⁴ Animal companionship regulates the autonomic responses in patients with non-communicable diseases.⁸ Dog owners had a significantly higher

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chance of survival within one year after acute myocardial infarction than those who did not own dogs.⁹ Also, pet ownership reduces the risk of high blood pressure and improves the control of hypertension among owners.¹⁰ Further, owners who regularly walked their dogs are significantly less likely to have self-reported diabetes, hypertension, hypercholesterolemia, and depression than those who did not own dogs.¹¹ Moreover, dog owners had higher high-density lipoprotein, lower cholesterol, lower triglycerides and lower low-density lipoprotein.^{7,12,13} The cardiovascular benefits of pet ownership are explained via many possible mechanisms including increased physical activity, reduced sympathetic response to stress, and improved physiological parameters, mental well-being and social interaction. And, the American Heart Association finds a probable association and a causal role of dog ownership with a decreased cardiovascular disease risk but, recommends against pet adoption, rescue, or purchase for the primary purpose of reducing cardiovascular disease risk.¹⁴ The role of canine companionship in psychosocial and cardiovascular health is increasingly explored. However, such studies are scarce in South Asia.

Stress is identified as a pervasive risk factor for cardiovascular disease.¹⁵ Work-related stress and social isolation have an increased risk of coronary heart disease.¹⁶ Stress is associated with cardiovascular disease independent of familial background, history of somatic or psychiatric diseases, and psychiatric comorbidity.¹⁷ Stress-related biomarkers like cortisol have been analysed to find an association between canine companionship and cardiovascular health.^{18–20} A significant reduction of cortisol in canine companionship and dog-assisted therapy along with a significant reduction in heart rate, systolic blood pressure, mean arterial blood pressure or total cholesterol was reported in a systematic review.²¹ However, the precise mechanism underlining the above phenomenon is unclear. A hypothesis states that “canine companionship reduces stress and could decrease: (A) neuropeptide Y (NPY) level, (B) cortisol level, and (C) NPY-induced potentiation of cortisol levels. The net effect would be a reduction in the end processes that contribute to cardiovascular disease.”²² A high level of cortisol is associated with hypertension, diabetes, dyslipidaemia, and immune modulation.^{23–26} Also, body physique correlates with cortisol.²⁷ Further, NPY stimulates cortisol secretion and plays a key role in cardiovascular disease.^{28,29} NPY plays an important role in regulating emotional-affective behaviour, stress coping and feeding.³⁰ NPY is implicated in hypertension, arrhythmia, immune modulation, and dyslipidaemia.^{29,31} Hence, both cortisol and NPY regulations have an essential role in cardiovascular therapeutics.^{26,29} And, NPY is a prospect for the treatment of metabolic syndrome.³² Moreover, NPY regulates neuroprotection, restores bone marrow dysfunction, and regulates the composition of the bone marrow microenvironment.³³

Hence, the modulation of NPY has a significant role in various diseases.³⁴

Natural stressors are associated with an increased level of cortisol.³⁵ And, cortisol is a potential diagnostic biomarker of stress.³⁶ Therefore, the analysis of cortisol is helpful in stress-related assessments.³⁷ Also, cortisol has a positive association with incident cardiovascular disease.³⁸ Cortisol has an important role in the circadian rhythm and the regulation of cardiac function.³⁹ An Indian study showed a stronger association between cortisol and cardiovascular risk factors.⁴⁰ Further, a study from Scotland revealed cortisol excretion rate to be positively correlated with anthropometry.²⁷ Hence, cortisol regulation has a vital role in cardiovascular therapeutics.²⁶ The role of NPY is extensively explored for the treatment of metabolic syndrome.³² Therefore, the assessment of NPY has a pivotal role in research. Also, assessing stress and emotional-affective behaviour involves the measurement of biomarkers like NPY. Hence, the NPY levels among healthy individuals are essential in interpreting values related to disorders. A study from the United Kingdom (UK) reported a *mean* NPY of 55 pmol/l in 18 healthy controls by using specific radioimmunoassay to measure NPY concentrations.⁴¹ Also, a study from the United States of America (USA) reported a mean (SD) plasma NPY of 79.8 (34.9) pmol/l among 100 sedentary healthy controls by using a competitive radioimmunoassay.⁴² Further, a Chinese study reported a mean (SD) serum NPY of 478.89 (145.53) pg/mL among 71 metabolically healthy obese participants by using a specific enzyme-linked immunosorbent assay (ELISA).⁴³ The interpretation of biomarkers like NPY and cortisol requires baseline levels for the region, which is deficient in prior research, especially for the South Asian region.

Aim

Initially, we aim to determine the measures of central tendency, variation for NPY and cortisol, and its relationship with socio-demographic factors, anthropometry, blood pressure, scale scores, and biochemical tests among healthy dwellers of Anuradhapura, Sri Lanka. Then the study intends to compare socio-demographic factors, anthropometry, blood pressure, psychosocial scale scores, NPY, cortisol, and other biochemical tests related to cardiovascular health between pet dog owners and age, sex-matched individuals who do not own a pet in Anuradhapura, Sri Lanka. Moreover, it intends to sort significant differences or correlations for the variables of interest with the pet bond score among pet dog owners.

Material and methods

Study design, setting, and population

We report a community-based, comparative, cross-sectional study (pet dog owners and non-pet owners) with laboratory analysis in the Anuradhapura district, Sri

Lanka. Since baseline data on NPY and cortisol were not available in Sri Lankan literature, the present study was also designed to identify the natural variation for NPY and cortisol among healthy dwellers of Anuradhapura. Data collection was initiated in May 2022. The dwellers in the district during the study period were considered the study population. Anuradhapura is a predominantly rural, agrarian district of Sri Lanka.^{44,45} Anuradhapura is the largest district by surface area in Sri Lanka. However, the district's population density (per 131 km²) is much lower than the country's density (per 350 km²).^{46,47} Fifty-seven per cent of households in Anuradhapura owned a pet, the dog was the most common pet owned by 41% of the households.⁴⁸ And, the dog owners of Anuradhapura experienced companionship with their pets.⁴⁹

Sample size

Pet dog owners and non-pet owners

Previous data on NPY among dog and non-dog owners was unavailable in the literature. The data for serum cortisol levels among dog and non-dog owners were available in only a Mexican study and were used for sample size calculation.²⁰ The following formula was used for sample size calculation: $n_B = (1 + 1/k) [\sigma \times (Z_{1-\alpha/2} + Z_{1-\beta}) / (\mu_A - \mu_B)]^2$. Where n_A and n_B are the calculated sample size for the non-pet owners' and pet dog owners' groups respectively, k is n_A/n_B (matching ratio) (=01), σ is the standard deviation (=4.73), $Z_{1-\alpha/2}$ is the type I error (=1.96), $Z_{1-\beta}$ is the power (=0.80), μ_A is the non-pet owners' group mean (=14.77) and μ_B is the pet dog owners' group mean (=12.12). Accordingly, the sample size was 50 for each group. Fifty-two individuals were recruited for each group while maintaining a male-to-female ratio of 1:1.

Healthy dwellers

Forty healthy individuals were recruited from the district. Ten participants were recruited from each of the following age groups while maintaining a male-to-female ratio of 1:1: 18 to ≤30, 31 to ≤40, 41 to ≤50, and 51 to ≤60 years.

Participants, selection criteria and sampling method

Pet dog owners and non-pet owners

A community-based convenience sampling was done to recruit suitable participants. The experience gained by the investigators during a previous project conducted in Anuradhapura, Sri Lanka, on the prevalence of pet ownership was used to recruit the participants.⁴⁸ Individuals who owned ≤3 dog(s) as the only pet for the last 1 year or more and had a pet bond score of >50 were included in the pet dog owners' group. And, individuals who did not own any pets for the last 1 year or more were included in the non-pet owners' group. Participants aged ≥41 to ≤65 years, Sinhala Buddhist, permanently residing in Anuradhapura district for ≥5 years, having an estimated glomerular filtration rate ≥60 ml/min/1.73 m² according

to Chronic Kidney Disease Epidemiology Collaboration equation, and having a Body Mass Index of 18.5 to 29.9 kg/m² were included for both the groups. Individuals with the following were excluded from both groups: acute illness, on medications, history of chronic diseases (organic or psychiatric), history of long-term drug treatment, history of immunosuppression (steroid treatment or chemotherapy), pregnancy, everyday smoking, and heavy alcohol use.^{50,51} Age-matched non-pet owners were recruited by selecting participants with the same age ±2 years. A male-to-female ratio of 1:1 was maintained.

Healthy dwellers

A community-based convenience sampling was done to recruit suitable participants. Participants aged ≥18 to ≤60 years, Sinhala Buddhist, permanently residing in Anuradhapura district for ≥5 years, having an estimated glomerular filtration rate (eGFR) ≥60 ml/min/1.73 m² according to Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, and having a body mass index (BMI) of 18.5 to 29.9 kg/m² were included. Individuals with the following were excluded: acute illness, on medications, history of chronic diseases (organic or psychiatric), history of long-term drug treatment, history of immunosuppression (steroid treatment or chemotherapy), pregnancy, everyday smoking, and heavy alcohol use.^{50,51}

Study instruments

The questionnaire on (i) socio-demographic factors was interviewer-administered by the first author while the (ii) Depression Anxiety Stress Scale-21 (DASS-21) translated into the Sinhala language and (iii) Multi-dimensional Scale of Perceived Social Support (MSPSS) translated into the Sinhala language were self-administered by the participants with instructions from the first author.⁵²⁻⁵⁵ Also, the (iv) pet bond scale was self-administered among pet dog owners in the Sinhala language.^{56,57} The following socio-demographic factors were considered: years residing in Anuradhapura, age, sex, whether the participant was the head of household, education level, employment, marital status, whether the participant was living alone, number of adults and children at home (males and females), sector, household income, alcohol consumption, smoking, betel chewing, and sleeping hours per day. The face validity of the questionnaire on socio-demographic factors was established. Subsequently, the questionnaire on socio-demographic factors and the pet bond scale were pre-tested in 05 subjects to improve language and sequence. The content validity of the pet bond scale was acceptable as it is a 25-item 5-point Likert scale ranging from 0 (strongly disagree) to 4 (strongly agree) which assesses the bonding between the owner and pet.⁵⁶ A total score of 0 to 100 can be achieved on the pet bond scale. The corresponding author has provided permission to translate and use the pet bond scale.⁵⁶

Anthropometric measurements of weight, height, waist circumference and hip circumference were measured using a standard digital bathroom scale (SECA robusta 813 CE), stadiometer (SECA 213 CE 0123) and measuring tape respectively. Two blood pressure measurements within a minute interval were measured using a standard aneroid desk model sphygmomanometer (ACCOSON CE 0413) after a 5-minute rest in the seated position. The average of the two measurements was used.

A blood sample of 6 mL for fasting blood sugar, lipid profile, serum creatinine, c-reactive protein, NPY and cortisol was obtained at 9 am following overnight fasting of 12 hours. Blood samples for fasting blood sugar, lipid profile, serum creatinine, and c-reactive protein were analysed at the Durdans Hospital laboratory, Anuradhapura.⁵⁸ It is a Joint Commission International-accredited hospital in Sri Lanka. Procedures for measurement of the above investigations were well established and routinely done at the above laboratory. The method used for the analysis of fasting blood sugar, total cholesterol, triglyceride, high-density lipoprotein (HDL) and serum creatinine is enzymatic colorimetric assay; for low-density lipoprotein (LDL), if triglyceride is ≥ 400 mg/dL, is enzymatic end-point; for c-reactive protein is turbidimetric method. Quality control for fasting blood sugar, total cholesterol, triglyceride and serum creatinine was done using Bio-Rad lyphochek assay chemistry control; for HDL and LDL using Gernorm and Gerpath Quality control; for c-reactive protein using Diagam Protein C Reactive High and Low Control. eGFR was calculated according to the CKD-EPI equation. Duplicate measurements of NPY and cortisol were analysed using ELISA at the Department of Animal and Food Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka. For NPY, ELISA kits and quality controls from Elabscience®, Elabscience Biotechnology Inc., USA were used (E-EL-H1893).⁵⁹ For cortisol, ELISA kits and quality controls from Abia Cortisol, AB Diagnostic Systems, Germany were used (DK.038.01.3).⁶⁰ ELISA reading was done using the Thermo Scientific Spectrophotometer Multiskan Sky, Singapore (51119700DP).

Data collection

Ethical clearance was obtained from the Ethics Review Committee of the Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka (ERC/2020/76). Informed written consent was obtained from all participants. Prior permission was obtained from the Regional Director of Health Services, Anuradhapura. Further, all Medical Officers of Health in the Anuradhapura district were informed by the Regional Director of Health Services to facilitate data collection in their areas. Recruited participants, were invited to the Durdans Hospital laboratory, Anuradhapura for data and sample collection. The first author was involved in the following: de-

scribing the study, obtaining informed written consent, collecting data, and collecting anthropometric measurements. All necessary measures were taken to preserve the participant's privacy and confidentiality. The process lasted for about 30 minutes.

Data description and analysis

Collected data were entered into a Microsoft Excel spreadsheet. Mean with SD was reported for continuous variables and frequency with percentages for categorical variables. Also, the World Health Organization/ International Society of Hypertension (WHO/ISH) risk prediction chart for Southeastern Asian Region B was used to predict the 10-year risk of a fatal or non-fatal cardiovascular event.⁶¹ Further, the following were used to calculate the allostatic load: (A) For the cardiovascular system, systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, total cholesterol, HDL, triglyceride, and LDL; (B) For the metabolic system, body mass index, waist circumference, waist-to-hip ratio, fasting blood sugar, and estimated glomerular filtration rate; (C) For the immune system, c-reactive protein; (D) For the neuroendocrine system, NPY and cortisol. Cut-off values for the parameters used to calculate the allostatic overload are shown in Table S1. Scores for depression, anxiety and stress in DASS-21 were calculated by summing the scores for the relevant items. Scores on the DASS-21 were multiplied by 2 to calculate the final score.⁵² A total score for Perceived Social Support was calculated by adding all values across the twelve items and subsequently, a mean score was obtained. Moreover, separate scores for social support from a significant other, family and friends were analysed. The analysis was performed using Microsoft Excel and add-ins. Continuous variables with a $p < 0.2$ were included in a multivariable model and a backward stepwise multiple linear regression was used to identify continuous variables with a $p < 0.05$. The stopping rule was satisfied when all remaining variables in the model had a $p < 0.05$. The Cronbach's alpha of questions representing pet bond scale, depression, anxiety, and stress of DASS-21 and social support from a significant other, family and friends of MSPSS was derived for pet dog owners' group, non-pet owners' group and healthy dwellers group.

Pet dog owners and non-pet owners

The chi-square test (or Fisher's exact) and Mann-Whitney U test were performed to determine a significant difference in the proportions and distributions of variables ($p < 0.05$). Spearman's rho was sorted for the pet bond score against the continuous variables of interest ($p < 0.05$).

Healthy dwellers

Measures of the central tendency of NPY and cortisol were described. Mann Whitney U test was performed to determine a significant difference in the distribution of NPY

and cortisol between the two groups of the categorical variables of interest ($p < 0.05$). Spearman's rho was sorted for NPY and cortisol against the continuous variables of interest ($p < 0.05$). Kruskal-Wallis test (and when significant post hoc Dunn's multiple comparisons test) was performed to determine significant differences between the age groups in relation to the NPY and cortisol levels ($p < 0.05$).

Ethics approval

Ethical clearance was obtained from the Ethics Review Committee of the Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka (ERC/2020/76). Informed written consent was obtained from all participants. The study was performed in accordance with the Declaration of Helsinki. All methods were carried out in accordance with relevant guidelines and regulations. All necessary measures were taken to preserve the participant's privacy and confidentiality.

Results

Demographic data

Pet dog owners and non-pet owners

Fifty-two participants (male: female=1:1) were recruited each for the pet dog owners' and non-pet owners' groups. Most of the participants have completed the general certificate of education (advanced level; the highest level of examination sat within the Sri Lankan high-school education system) or above (pet dog owners=71%, non-pet owners=69%), were employed (pet dog owners=79%, non-pet owners=92%), currently married (pet dog owners=98%, non-pet owners=90%), not living alone (pet dog owners=100%, non-pet owners=98%), had >Rs 100,000 monthly household income (pet dog owners=69%, non-pet owners=56%), never consumed alcohol (pet dog owners=56%, non-pet owners=67%), never smoked (pet dog owners=92%, non-pet owners=88%), never chewed betel (pet dog owners=92%, non-pet owners=94%), and had <10% 10-year risk of a fatal or non-fatal cardiovascular event (pet dog owners=98%, non-pet owners=100%). The Cronbach's alpha of questions representing pet bond scale, depression, anxiety, and stress of DASS-21 and social support from a significant other, family and friends of MSPSS in the pet dog owners' group were 0.91, 0.78, 0.76, 0.83, 0.82, 0.88, 0.92 respectively, indicating good internal consistency in the responses. The Cronbach's alpha of questions representing depression, anxiety, and stress of DASS-21 and social support from a significant other, family and friends of MSPSS in the non-pet owners' group were 0.81, 0.79, 0.80, 0.65, 0.86, 0.79 respectively, indicating good internal consistency in the responses.

Healthy dwellers

Most of the study participants were non-head of household (70% – 28/40), completed the general certificate of education (advanced level; the highest level of examina-

tion sat within the Sri Lankan high-school education system) or above (75% – 30/40), employed (90% – 36/40), currently married (73% – 29/40), not living alone at the household (100% – 40/40), rural residents (53% – 21/40), and having a monthly household income of more than 100,000 Sri Lankan rupees (53% – 21/40). Also, most had ≥ 2 adult males in the household (53% – 21/40), ≥ 2 adult females in the household (58% – 23/40), no male children in the household (65% – 26/40), no female children in the household (60% – 24/40), never consumed alcohol (68% – 27/40), never smoked (83% – 33/40), and never chewed betel (95% – 38/40). All participants above the age of 40 years had <10% 10-year risk of a fatal or non-fatal cardiovascular event according to the World Health Organization/ International Society of Hypertension (WHO/ISH) risk prediction chart for Southeastern Asian Region B. The Cronbach's alpha of questions representing depression, anxiety, and stress of DASS-21 and social support from a significant other, family and friends of MSPSS in the healthy dwellers were 0.90, 0.84, 0.85, 0.78, 0.82, and 0.86 respectively, indicating good internal consistency in the responses.

Comparison of the variables of interest among pet dog owners and non-pet owners

The categorical and continuous variables of the pet dog owners and non-pet owners' groups are compared in Table S2 and Table 1 respectively. Proportions related to the sector, being the head of household, education level, employment, marital status, living alone, number of adults and children at home (males and females), monthly household income, alcohol consumption, smoking, betel chewing, and 10-year risk of a fatal or non-fatal cardiovascular event were not significantly different between the two groups.

The pet dog owners' group had a significantly lower mean (SD) [7.8 (7.2)], and median (interquartile range) [6 (2–12.5)] depression score in DASS-21 when compared to the non-pet owners' group [11.2 (8.6)], 10 (6–14.5) respectively ($p = 0.03$). However, the distributions were not significantly different in the two groups for age, duration of stay at Anuradhapura, hours of sleep per day, weight, height, body mass index, waist circumference, hip circumference, waist/hip ratio, heart rate, systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, DASS-21 scores for anxiety and stress, MSPSS scores (total, significant other, family, friend), fasting blood sugar, c-reactive protein, total cholesterol, HDL, triglyceride, LDL, very-low-density lipoprotein, total cholesterol/HDL ratio, triglyceride/HDL ratio, serum creatinine, estimated glomerular filtration rate, and allostatic load.

The pet dog owners' group had a mean (SD) of 388.2 (225.4) pg/mL, and a median (interquartile range) of 341.1 (223–512.8) pg/mL for NPY when compared to 408 (215) pg/mL and 347.6 (211.2–592.1) pg/mL for the non-

Table 1. Comparison of continuous variables between the pet dog owners' group and the non-pet owners' group of the biochemical tests

No	Item	Pet dog owners (n=52)		Non-pet owners' (n=52)		p of Mann Whitney U test
		Mean (SD) and median (Interquartile range)	Mean (SD) and median (Interquartile range)	Mean (SD) and median (Interquartile range)	Mean (SD) and median (Interquartile range)	
1	Age (years)	46 (5) 46 (42.8–48)	46 (6.1) 44 (42–48)			0.50
2	Duration of stay at Anuradhapura (years)	35.9 (13.3) 40.0 (22.8–46)	36.7 (15.3) 41.5 (26–45.3)			0.77
3	Hours of sleep per day	6.9 (1) 7 (6–8)	6.9 (1.1) 7 (6–8)			0.85
4	Weight (kg)	66.9 (9) 64.6 (60.8–71.8)	64.1 (10.8) 62.6 (56.7–72.8)			0.20
5	Height (cm)	163 (9.3) 163.8 (155.8–168.9)	161 (8.8) 160.5 (155–166.6)			0.90
6	BMI (kg/m ²)	25.2 (2.8) 24.9 (22.9–27.4)	24.7 (3.3) 25.1 (22.4–27)			0.57
7	Waist circumference (cm)	82.6 (6.9) 82.0 (77.5–87.3)	82.1 (7.8) 82.5 (75–89)			0.97
8	Hip circumference (cm)	97.5 (6.4) 96.5 (93–101.3)	96.4 (7.5) 97.0 (92–101)			0.62
9	Waist/Hip ratio	0.9 (0) 0.9 (0.8–0.9)	0.9 (0.1) 0.9 (0.8–0.9)			0.40
10	Heart rate (per minute)	67 (4.6) 66.0 (64.0–69.0)	67.4 (6.9) 65 (62–70)			0.56
11	Systolic blood pressure (mmHg)	118.7 (5.7) 119.0 (115.5–123)	117.8 (8) 119.5 (111–122.3)			0.78
12	Diastolic blood pressure (mmHg)	75.5 (4.8) 75.0 (71–79)	75.2 (5.8) 73.0 (71–79)			0.71
13	Mean arterial blood pressure (mmHg)	89.9 (4.5) 89.7 (85.9–94.1)	89.4 (6) 89.2 (86.5–92.3)			0.57
14	Depression score (Depression Anxiety Stress Scale-21)	7.8 (7.2) 6 (2–12.5)	11.2 (8.6) 10.0 (6–14.5)			0.03
15	Anxiety score (Depression Anxiety Stress Scale-21)	7 (6.7) 4 (2–12)	8.5 (7.5) 6 (10–14.5)			0.23
16	Stress score (Depression Anxiety Stress Scale-21)	13.8 (8.9) 12 (8–20)	15.5 (8.6) 14 (10–20)			0.26
17	Total score for perceived social support (Multi-dimensional Scale of Perceived Social Support)	5.2 (0.8) 5.1 (4.7–5.6)	5 (0.5) 4.9 (4.7–5.2)			0.14
18	Perceived social support from the significant other (Multi-dimensional Scale of Perceived Social Support)	5.3 (0.9) 5.1 (4.8–6)	5.1 (0.8) 5.0 (4.8–5.5)			0.20
19	Perceived social support from the family (Multi-dimensional Scale of Perceived Social Support)	5.4 (1) 5.3 (4.9–6)	5.2 (0.9) 5.0 (4.5–5.8)			0.10
20	Perceived social support from friends (Multi-dimensional Scale of Perceived Social Support)	4.9 (0.9) 4.8 (4.4–5.3)	4.8 (0.7) 4.8 (4.5–5)			0.42
21	Fasting blood sugar (mg/dL)	95.0 (16.1) 91.5 (85.0–97.5)	94.9 (12.9) 93.5 (86.0–99.3)			0.52
22	C-reactive protein (mg/l)	0.7 (1.2) 0.1 (0.1–0.6)	1.1 (2.5) 0.1 (0.1–0.3)			0.82
23	Total cholesterol (mg/dL)	200.2 (32.9) 205.5 (176–220.3)	203.8 (3.7) 204.5 (177–223)			0.80
24	HDL (mg/dL)	38.4 (7.8) 36.0 (32–42)	40.2 (7.6) 38.0 (34.8–45.3)			0.13
25	Triglyceride (mg/dL)	170.0 (79.7) 147.0 (115–192)	164.1 (87.1) 138.0 (97–214.3)			0.42
26	LDL (mg/dL)	126.7 (31.5) 125.1 (107.9–148)	132.7 (27.9) 131.7 (112.2–152.9)			0.35
27	Very-low-density lipoprotein (mg/dL)	33.3 (13.8) 29.4 (23–38.4)	30.9 (13.5) 27.6 (19.4–40)			0.32
28	Total cholesterol/HDL	5.3 (1) 5.5 (4.6–6.1)	5.2 (1.1) 5.1 (4.5–6)			0.49
29	Triglyceride/HDL	4.6 (2.4) 4.4 (3–5.4)	4.3 (2.5) 3.4 (2.4–5.5)			0.24
30	Serum creatinine (mg/dL)	0.8 (0.2) 0.8 (0.6–0.9)	0.8 (0.2) 0.8 (0.6–0.9)			1.00
31	Estimated glomerular filtration rate (ml/min/1.73m ²)	103.3 (13.8) 105.0 (95–112.3)	100.1 (13.8) 100.5 (91–110)			0.20
32	NPY (pg/mL)	388.2 (225.4) 341.1 (223–512.8)	408 (215) 347.6 (211.2–592.1)			0.61
33	Cortisol (nmol/L)	234.4 (73.1) 235.3 (188.9–292.2)	234.8 (122.2) 193.0 (149.1–286.9)			0.31
34	Allostatic load	2.4 (1.4) 2 (1–3)	2.4 (1.5) 2 (1–3)			0.96

Table 2. Comparison of categorical variables between the high pet bond score group and the low pet bond score group among the pet dog owners^a

No	Variable	Description	Percentage in the high pet bond group (Score 70 to 100) (n=21)	Percentage in the low pet bond group (Score 50 to 69) (n=31)	p
1	Gender	Male	33	61	<0.05*
		Female	67	39	
2	Sector	Rural	33	52	0.19*
		Urban	67	48	
3	Head of the household	Yes	33	58	0.08*
		No	67	42	
4	Education	Completed or above the general certificate of education (advanced level; the highest level of examination sat within the Sri Lankan high-school education system)	81	65	0.20*
		Below or up to general certificate of education (advanced level)	19	35	
5	Employment	Employed	86	74	0.52 [#]
		Unemployed	14	26	
6	Marital status	Currently married	95	100	0.80 [#]
		Never married or separated	5	0	
7	Living alone	Yes	0	0	Not performed
		No	100	100	
8	No of adult males (≥12 years) at home	≥1	100	100	Not performed
		None	0	0	
9	No of adult females (≥12 years) at home	≥1	100	100	Not performed
		None	0	0	
10	No of male children (<12 years) at home	≥1	19	32	0.29*
		None	81	68	
11	No of female children (<12 years) at home	≥1	57	32	0.08*
		None	43	68	
12	Monthly household income (Rs)	>100,000	86	58	0.03*
		≤100,000	14	42	
13	Alcohol consumption	Never	67	48	0.19*
		Moderation	33	52	
14	Smoking	Never	100	87	0.23 [#]
		Somedays or former	0	13	
15	Betel chewing	Never	91	94	0.99 [#]
		Somedays or former	9	6	
16	10-year risk of a fatal or non-fatal cardiovascular event according to the WHO/ISH risk prediction chart for the Southeastern Asian region B	<10%	100	97	0.99 [#]
		10% to <20%	0	3	
17	No of pet dogs	>1	38	23	0.23*
		=1	62	77	

a * – Chi-square test was performed, # – Fisher's exact was performed

pet owners' group. However, the difference was not significant ($p=0.61$). Further, the pet dog owners' group had a mean (SD) of 234.4 (73.1) nmol/L, and a median (interquartile range) of 235.3 (188.9–292.2) nmol/L of cortisol when compared to 234.8 (122.2) nmol/L and 193 (149.1–286.9) nmol/L for the non-pet owners' group. However, the difference was not significant ($p=0.31$).

Pet bond score and the variables of interest among pet dog owners

The mean pet bond score of the pet dog owners' group was 68.4 (11.2) with a median (interquartile range) of 66 (60.8–79.3). The distribution of the pet bond score was not sig-

nificantly different in relation to sex, sector, whether the participant was the head of household, education level, employment, number of children at home (males and females), monthly household income, alcohol consumption, and number of pet dogs at home. Table S3 summarises the p-values for the categorical variables of interest against the pet bond score. The categorical variables of the high (scores 70 to 100) and low (scores 50 to 69) pet bond groups are compared in Table 2. The high pet bond group had a significantly higher percentage of females (67%) when compared to the low pet bond group (39%) ($p=0.047$). The high pet bond group had a significantly higher percentage of participants having a monthly household income of

more than Rs 100,000 (86%) when compared to the low pet bond group (58%) ($p=0.03$). However, proportions related to the sector, being the head of household, education level, marital status, number of children at home (males and females), alcohol consumption, smoking, betel chewing, the 10-year risk of a fatal or non-fatal cardiovascular event, and number of pet dogs at home were not significantly different between the high and low pet bond groups. Cortisol ($r=-0.36$, $p=0.01$), perceived social support from a significant other ($r=0.34$, $p=0.01$), and height ($r=-0.31$, $p=0.02$) were significantly correlated with the pet bond score. A backward stepwise regression found only the cortisol to have a $p<0.05$ (Figure 1). The R^2 of the single variable regression was 0.13 with the predictive model, pet bond score; + 81.43 - 0.06 (cortisol). However, age, duration of stay at Anuradhapura, hours of sleep per day, weight, body mass index, waist circumference, hip circumference, waist/hip ratio, heart rate, systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, DASS-21 scores for depression, anxiety and stress, MSPSS score (total, family, friend), fasting blood sugar, c-reactive protein, total cholesterol, HDL, triglyceride, LDL, very-low-density lipoprotein, total cholesterol / HDL ratio, triglyceride / HDL ratio, serum creatinine, estimated glomerular filtration rate, NPY, and allostatic load did not have a significant correlation with the pet bond score. Table 3 summarises the p-values for the continuous variables of interest against the pet bond score.

Cortisol and its association with the variables of interest among healthy dwellers

The mean (SD) of cortisol was 317.3 (132.5) nmol/L with a lower and upper 95% CI of 274.9 and 359.7 respectively. The cortisol ranged from 153.9 to 713.7 nmol/L. The median was 275.4 nmol/L with an interquartile range of 217.5 to 362.7. Skewness and kurtosis for the distribution were + 1.14 and + 0.95 respectively showing asymmetrical and potentially mesokurtic shape. The post hoc power was 0.99 for the distribution of cortisol. The following parameters were used to calculate the power: study mean (317.3 nmol/L), mean (SD) from previous literature among healthy controls [381.9 (97.7) nmol/L]⁶², sample size (40) and type I error (0.05). Participants who were never married had a significantly higher mean (SD) [426.8 (167.9) nmol/L], and median (390.4 nmol/L) of cortisol when compared to those who were married [275.8 (88.7) nmol/L], (272.8 nmol/L) respectively ($p=0.01$). Participants who consumed alcohol in moderation had a significantly higher mean (SD) [381.8 (133.7) nmol/L], and median (361.5 nmol/L) of cortisol when compared to those who never consumed alcohol [286.2 (122.4) nmol/L], (272.8 nmol/L) respectively ($p=0.04$). However, the distribution of cortisol was not significantly different between the two groups related to sex, sector, whether the participant was the

head of a household, education level, number of adults and children at home (males and females), and household income. Table 4 summarises the p-values for the categorical variables of interest against cortisol.

Table 3. Continuous variables of interest against the pet bond score among pet dog owners

No	Variables	Spearman's rho	p
1	Age (years)	0.18	0.20
2	Duration of stay at Anuradhapura (years)	-0.04	0.77
3	Hours of sleep per day	-0.10	0.50
4	Weight (kg)	-0.20	0.16
5	Height (cm)	-0.31	0.02
6	BMI (kg/m ²)	0.09	0.54
7	Waist circumference (cm)	0	0.98
8	Hip circumference (cm)	0.12	0.42
9	Waist/Hip ratio	-0.06	0.67
10	Heart rate (per minute)	-0.06	0.66
11	Systolic blood pressure (mmHg)	0.02	0.91
12	Diastolic blood pressure (mmHg)	-0.08	0.56
13	Mean arterial blood pressure (mmHg)	-0.07	0.61
14	Depression Anxiety Stress Scale-21 – depression score	0.05	0.75
15	Depression Anxiety Stress Scale-21 – anxiety score	0.07	0.63
16	Depression Anxiety Stress Scale-21 – stress score	-0.06	0.67
17	Multi-dimensional Scale of Perceived Social Support – total score	0.18	0.20
18	Multi-dimensional Scale of Perceived Social Support – score for significant other	0.34	0.01
19	Multi-dimensional Scale of Perceived Social Support – score for family	0.16	0.26
20	Multi-dimensional Scale of Perceived Social Support – score friend	0.16	0.26
21	Fasting blood sugar (mg/dL)	-0.08	0.58
22	C-reactive protein (mg/l)	-0.19	0.19
23	Total cholesterol (mg/dL)	-0.10	0.50
24	HDL (mg/dL)	0.10	0.50
25	Triglyceride (mg/dL)	-0.13	0.38
26	LDL (mg/dL)	-0.13	0.38
27	Very-low-density lipoprotein (mg/dL)	-0.12	0.39
28	Total cholesterol/High-density lipoprotein	-0.13	0.34
29	Triglyceride/High-density lipoprotein	-0.12	0.39
30	Serum creatinine (mg/dL)	-0.10	0.50
31	Estimated glomerular filtration rate (ml/min/1.73m ²)	-0.22	0.12
32	Cortisol (nmol/L)	-0.36	0.01
33	Neuropeptide Y (pg/mL)	0.10	0.50
34	Allostatic load	0.10	0.48

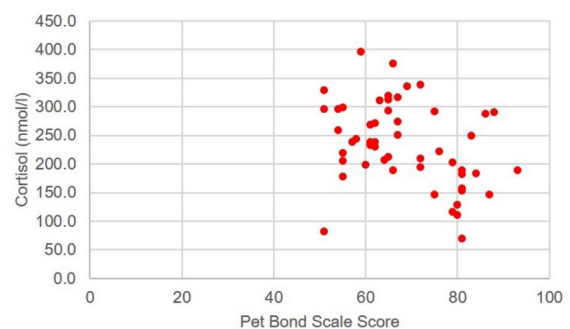


Fig. 1. Cortisol against pet bond scale score among pet dog owners ($r=-0.36$, $p=0.01$)

Table 4. Categorical variables of interest against NPY and cortisol among healthy dwellers

No	Variable	Groups	n (%) (Total=40)	NPY		Cortisol	
				Mean (SD) and median (Interquartile range) pg/mL	p of Mann Whitney U test	Mean (SD) and median (Interquartile range) nmol/L	p of Mann Whitney U test
1	Sex	Male	20 (50)	434.9 (243.3) 361.3 (226.4–610.7)	0.96	334.1 (132.9) 299.3 (247.7–395.8)	0.41
		Female	20 (50)	428 (221.6) 428.1 (280.8–577.5)		300.5 (133.3) 275.4 (210.8–328.8)	
2	Sector	Rural	21 (53)	417.8 (219.5) 385.5 (227–580.6)	0.72	331.7 (153.9) 275.9 (218.5–370.4)	0.87
		Urban	19 (47)	446.5 (245.7) 431.4 (252.6–661.9)		301.3 (105.8) 274.9 (225.9–353.7)	
3	Head of household	No	28 (70)	436.1 (222.5) 428.1 (255.1–628.1)	0.79	337.1 (142.5) 320.7 (237.1–375.4)	0.14
		Yes	12 (30)	420.6 (255.7) 313.6 (223.1–601.8)		271.1 (95) 269.4 (212.8–273.7)	
4	Education level	completed the general certificate of education (advanced level) or above	30 (75)	395 (222.9) 348.5 (225.1–567.9)	0.08	325 (122.5) 296.8 (247.8–365.0)	0.33
		up to or below the general certificate of education (advanced level)	10 (25)	540.8 (225.5) 578.3 (360.6–674.4)		294.2 (164) 262.4 (184.4–309.7)	
5	Employed	Yes	36 (90)	422.1 (238.6) 348.5 (226.4–610.7)	Test not done	322.5 (137.6) 274.0 (217.5–367.3)	Test not done
		No	4 (10)	515.2 (107.4) 493.9 (429.7–579.3)		270.3 (62.5) 289.8 (252.1–307.9)	
6	Marital status	Currently married	29 (73)	412.1 (237.0) 340.9 (224.4–600.2)	0.32	275.8 (88.7) 272.8 (200.0–320.9)	0.01
		Never married	11 (27)	482.5 (211) 518.2 (342.2–653.5)		426.8 (167.9) 390.4 (297.2–551.3)	
7	No of adult males (≥12 years)	≥2	21 (53)	413.7 (207.7) 431.4 (224.4–580.6)	0.71	329.5 (135.7) 320.9 (214.4–370.4)	0.39
		<2	19 (47)	451.1 (256.2) 356.0 (270.2–644.8)		303.8 (131.1) 271.5 (248.2–336.5)	
8	No of adult females (≥12 years)	≥2	23 (58)	412 (207.9) 385.5 (256.5–543.1)	0.58	332.8 (142.3) 275.9 (231.6–378.3)	0.46
		<2	17 (42)	457.7 (260.6) 424.8 (247.5–687.2)		296.3 (118.8) 273.0 (195.5–352.5)	
9	No of male children (<12 years)	None	26 (65)	441.4 (229.2) 408.4 (250–619.5)	0.63	318.1 (126.7) 296.8 (225–363.4)	0.88
		≥1	14 (35)	413.0 (238.1) 376.6 (240.9–542.7)		315.8 (147.6) 275.4 (228–347)	
10	No of female children (<12 years)	None	24 (60)	472.5 (219.1) 497.7 (324.9–630)	0.14	314.5 (137.1) 275.4 (198.9–375.4)	0.86
		≥1	16 (40)	369.9 (238.4) 205.5 (286.5–549)		321.5 (129.5) 288.2 (264.8–356.5)	
11	Monthly household income	>Rs 100,000	21 (53)	428.9 (212.8) 424.8 (247.5–623.9)	0.98	313.7 (134) 303.6 (195.5–361.5)	1.00
		≤Rs 100,000	19 (47)	434.3 (253) 356 (257.7–603.3)		321.2 (134.3) 272.8 (254.6–343.6)	
12	Alcohol consumption	Never	27 (68)	389.8 (227) 340.9 (186.5–537.3)	0.10	286.2 (122.4) 272.8 (197.8–323.2)	0.04
		Moderation	13 (32)	517.9 (218.6) 580.6 (290.2–623.9)		381.8 (133.7) 361.5 (271.5–519.7)	
13	Smoking	Never	33 (83)	433.5 (237.8) 424.8 (247.5–623.9)	Test not done	313 (133.5) 275.9 (214.4–361.5)	Test not done
		Former and somedays	7 (17)	559.8 (205.6) 337 (258.6–565.0)		337.7 (135.9) 273 (269.4–403.4)	
14	Betel chewing	Never	38 (95)	418.5 (227.8) 370.7 (232.1–599.9)	Test not done	321.7 (134.2) 282.9 (225–365)	Test not done
		Somedays	2 (5)	677.1 (108.8) 677.1 (638.7–715.5)		234.3 (54.8) 234.3 (214.9–253.6)	

Table 5. Continuous variables of interest against NPY and cortisol among healthy dwellers

No	Variable	Mean (SD)	NPY		Cortisol	
			Spearman's rho	p-value	Spearman's rho	p-value
1	Age (years)	39.5 (11.8)	-0.12	0.46	-0.38	0.02
2	Duration of stay at Anuradhapura (years)	33.0 (13.6)	-0.07	0.69	-0.09	0.59
3	Hours of sleep per day	7.2 (1.2)	0.28	0.08	0.34	0.03
4	Weight (kg)	62.6 (11.0)	0	0.99	0.04	0.79
5	Height (cm)	163.7 (10.2)	0.11	0.49	0.27	0.09
6	BMI (kg/m ²)	23.3 (3.0)	-0.12	0.47	-0.19	0.25
7	Waist circumference (cm)	79.8 (9.0)	-0.10	0.54	-0.24	0.14
8	Hip circumference (cm)	95.9 (6.5)	-0.07	0.67	-0.06	0.72
9	Waist/Hip ratio	0.8 (0.1)	0.01	0.96	-0.16	0.34
10	Systolic blood pressure (mmHg)	113.6 (10.1)	-0.10	0.56	-0.11	0.51
11	Diastolic blood pressure (mmHg)	74.4 (7.9)	-0.23	0.15	0.08	0.65
12	Mean arterial blood pressure (mmHg)	87.5 (8.2)	-0.18	0.26	-0.05	0.78
13	Depression Anxiety Stress Scale-21 – Depression score	9.9 (10.4)	-0.02	0.89	0.29	0.07
14	Depression Anxiety Stress Scale-21 – anxiety score	8.2 (8.6)	-0.13	0.41	0.19	0.25
15	Depression Anxiety Stress Scale-21 – stress score	14.5 (9.9)	-0.16	0.31	0.33	0.04
16	Multi-dimensional Scale of Perceived Social Support – total score	5 (0.8)	0.02	0.88	0.08	0.61
17	Multi-dimensional Scale of Perceived Social Support – score for significant other	5 (1)	-0.06	0.71	-0.00	0.99
18	Multi-dimensional Scale of Perceived Social Support – score for family	5.1 (0.9)	0	1.00	0.08	0.62
19	Multi-dimensional Scale of Perceived Social Support – Score friend	4.8 (0.9)	0.03	0.84	0.11	0.50
20	Fasting blood sugar (mg/dL)	91.6 (7.9)	0.09	0.58	0.06	0.71
21	C-reactive protein (mg/l)	2.5 (2.6)	-0.01	0.94	0.15	0.37
22	Total cholesterol (mg/dL)	210.2 (30.5)	-0.06	0.71	-0.20	0.21
23	HDL (mg/dL)	42.2 (9.8)	0.19	0.25	0.09	0.58
24	Triglyceride (mg/dL)	130.8 (66.4)	0.01	0.96	-0.09	0.59
25	LDL (mg/dL)	143.2 (28)	-0.15	0.34	-0.23	0.15
26	Very-low-density lipoprotein (mg/dL)	24.8 (9.9)	0.07	0.69	-0.08	0.61
27	Total cholesterol/HDL	5.2 (1.2)	-0.16	0.33	-0.13	0.43
28	Serum creatinine (mg/dL)	0.9 (0.2)	0.12	0.48	0.07	0.67
29	Estimated glomerular filtration rate (ml/min/1.73m ²)	96.1 (14.5)	-0.05	0.78	0.30	0.06
30	Cortisol (nmol/L)	317.3 (132.5)	0.14	0.38	Not applicable	Not applicable
31	NPY (pg/mL)	431.4 (229.7)	Not applicable	Not applicable	0.14	0.38
32	Allostatic load against neuropeptide Y	1.7 (1.4)	-0.15	0.37	Not applicable	Not applicable
33	Allostatic load against cortisol	1.7 (1.5)	Not applicable	Not applicable	-0.18	0.27

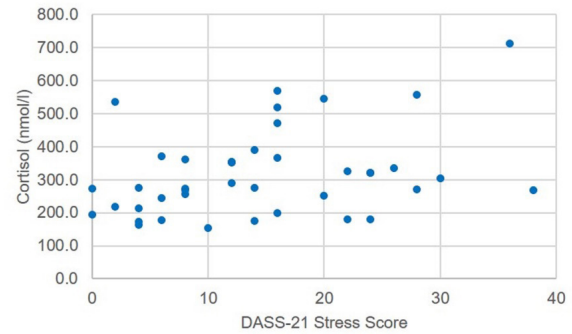


Fig. 2. Cortisol against DASS-21 stress score among healthy dwellers ($r=0.33$; $p=0.04$)

Age ($r=-0.38$, $p=0.02$), hours of sleep per day ($r=0.34$, $p=0.03$) and DASS-21 scores for stress ($r=0.33$, $p=0.04$) were significantly correlated with cortisol (Fig. 2). A backward stepwise regression found age, height, and stress to have a $p<0.05$. The R^2 of the three variable regression was 0.38 with the predictive model, cortisol level; $-452.84 - 3.39$ (age) $+ 5.08$ (height) $+ 4.98$ (stress). Spearman's rho for the following combinations was performed: age vs. hours of sleep, age vs. stress score, and hours of sleep vs. stress score. Out of the above combinations, age had a significant negative correlation with stress score ($r=-0.37$, $p=0.02$). Also, cortisol levels showed a significant difference between the age groups of 18 to ≤ 30 (median 380.4 nmol/L), 31 to ≤ 40 (median 285.5 nmol/L), 41 to ≤ 50 (median 273.2 nmol/L), and 51 to ≤ 60 (median 245.7 nmol/L) years ($p=0.04$). The significant pair was 18 to ≤ 30 vs 51 to ≤ 60 years. However, duration of stay at Anuradhapura, weight, height, BMI, waist circumference, hip circumference, waist/hip ratio, systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, DASS-21 scores for depression and anxiety, MSPSS score (total, significant other, family, friend), fasting blood sugar, c-reactive protein, total cholesterol, HDL, triglyceride, LDL, very-low-density lipoprotein, total cholesterol/HDL ratio, serum creatinine, estimated glomerular filtration rate, NPY and allostatic load did not have a significant correlation with cortisol. Table 5 summarises the p-values for the continuous variables of interest against cortisol.

NPY and its association with the variables of interest among healthy dwellers

The mean (SD) of NPY was 431.4 (229.7) pg/mL with a lower and upper 95% CI of 360.2 and 502.6 respectively. The NPY ranged from 106.7 to 907.2 pg/mL. The median was 405.1 pg/mL with an interquartile range of 242.4 to 610.7. Skewness and kurtosis for the distribution were +0.31 and -0.92 respectively showing a potentially symmetrical and mesokurtic shape. The post hoc power was 0.98 for the distribution of NPY. The following parameters were used to calculate the pow-

er: study mean (101.4 pmol/l), mean (SD) from previous literature among healthy controls from the USA [79.8 (34.9) pmol/l], sample size (40) and type I error (0.05).⁴² The study mean NPY in pg/mL was multiplied by 0.235 to convert to pmol/l.⁶³ The distribution of NPY was not significantly different in relation to sex, sector, whether the participant was the head of household, education level, marital status, number of adults and children at home (males and females), household income, and alcohol consumption. Table 4 summarises the p-values for the categorical variables of interest against NPY. Also, age, duration of stay at Anuradhapura, hours of sleep per day, weight, height, BMI, waist circumference, hip circumference, waist/hip ratio, systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, DASS-21 scores for depression, anxiety and stress, MSPSS score (total, significant other, family, friend), fasting blood sugar, c-reactive protein, total cholesterol, HDL, triglyceride, LDL, very-low-density lipoprotein, total cholesterol/HDL ratio, serum creatinine, estimated glomerular filtration rate, cortisol, and allostatic load did not have a significant correlation with NPY. Table 5 summarises the p-values for the continuous variables of interest against NPY. However, a backward stepwise regression found only the hours of sleep per day to have a $p < 0.05$. The R^2 of the single variable regression was 0.11 with the predictive model, NPY level; $-27.71 + 63.99$ (hours of sleep/day). NPY levels did not show a significant difference between the age groups of 18 to ≤ 30 (median 549.4 pmol/l), 31 to ≤ 40 (median 463.1 pmol/l), 41 to ≤ 50 (median 257.7 pmol/l), and 51 to ≤ 60 (median 386.1 pmol/l) years ($p = 0.41$).

Comparison of NPY and cortisol of healthy dwellers against the pet dog owners and non-pet owners

The healthy dwellers between the age of 41 to ≤ 60 years had a non-significant mean (SD) [268.4 (83.4) nmol/L], and median (interquartile range) [272.2 (209.7–297.6) nmol/L] of cortisol when compared to the pet dog owners' group [234.4 (73.1) nmol/L, 235.3 (188.9–292.2) nmol/L] ($p = 0.22$) and non-pet owners' group [234.8 (122.2) nmol/L, 193 (149.1–286.9) nmol/L] ($p = 0.08$) respectively. Also, the healthy dwellers between the ages of 41 to ≤ 60 years had a non-significant mean (SD) [378 (237) pg/mL], and median (interquartile range) [339 (158.3–567.4) pg/mL] of NPY when compared to the pet dog owners' group [388.2 (225.4) pg/mL, 341.1 (223–512.8) pg/mL] ($p = 0.71$) and non-pet owners' group [408 (215) pg/mL, 347.6 (211.2–592.1) pg/mL] ($p = 0.49$) respectively.

Discussion

To the best of our knowledge, it is the first study from the South Asian region to compare NPY, cortisol, and other biochemical tests related to psychosocial and car-

diovascular health between pet dog owners and age, sex-matched non-pet owners. The study outcomes are helpful for one-health research in low-middle income and South Asian regions. Our study did find a significantly lower depression score in the pet dog owners' group when compared to the non-pet owners' group. Depression scores of pet owners have been significantly lower than non-pet owners in prior literature as well.⁶⁴ Also, pet owners were less depressed than non-pet owners.⁶⁵ Similar findings have been reported in other settings during the COVID-19 pandemic.⁶⁶ Thus, the above evidence suggests a lower depression level among pet owners when compared to non-pet owners. Further, a community-based three-arm controlled study concludes that pet dog acquisition may reduce loneliness among owners.⁶⁷ Moreover, pet ownership has been found to provide companionship, give a sense of purpose, reduce loneliness and increase socialisation among community-dwelling older adults which may increase resilience against mental illnesses.⁶⁸ However, few studies did not find an association between pet ownership and depression in adolescents and older adults.^{69,70}

Owners perceived stress reduction when interacting with their pet dogs.⁴⁹ Dog ownership is associated with significant decreases in stress and post-traumatic stress symptoms among veterans.⁷¹ A systematic review revealed a significant reduction of the stress hormone cortisol in dog companionship and dog-assisted therapy showing both long-term (5.5 years) and short-term (15 to 30 mins) influence respectively.²¹ A session with a therapy dog significantly reduced systolic blood pressure, pulse rate, and salivary cortisol levels among nursing students.¹⁸ Also, a session with a facility dog and a palliative care psychologist significantly reduced the pulse rate and salivary cortisol among veterans of the armed forces receiving palliative care.¹⁹ Further, the canine companionship group had significantly lower total cholesterol and serum cortisol when compared to the control group.²⁰ Cortisol is implicated in hypertension, type 2 diabetes mellitus, dyslipidaemia, and immune modulation.^{23–26} And, high cortisol level is associated with metabolic syndrome.⁷² Although the cortisol levels were not significantly different between the pet dog owners and the non-pet owners of the present study, the pet bond score among pet dog owners had a significant negative correlation with cortisol. More the bond with the pet dog lower was level of the stress hormone cortisol. Also, the present study among healthy dwellers showed a significant positive correlation between stress scores and cortisol. Further, the hormone cortisol is known to have a positive association with incident cardiovascular disease.³⁸ Nevertheless, the loss of a pet dog is also associated with stress in the bereaved owner.⁷³ The present study used the pet bond score to measure the bond between owners and pet dogs. Such

studies are scarce in the literature. The high pet bond group had a significantly higher percentage of females and participants having a monthly household income of more than Rs 100,000. In prior literature, pet ownership was significantly associated with the number of adult females at home.⁴⁸ Also, households with an adult female are more likely to own a dog.⁷⁴ Further, households with high incomes are more likely to own a dog.⁷⁵

To the best of our knowledge, it is the first, community-based study among healthy volunteers from the region to report the measures of central tendency, variation for NPY and cortisol, and its relationship with socio-demographic factors, anthropometry, blood pressure, scale scores, and biochemical tests. The study mean (SD) of cortisol [317.3 (132.5) nmol/L] among the healthy dwellers of the present study was comparable to an Asian study among healthy participants from Thailand [381.93 (97.74) nmol/L].⁶² Also, the study among healthy volunteers showed a significant positive correlation between stress scores and cortisol. A meta-analysis provides evidence for an association between natural stressors and elevated cortisol levels.³⁵ An elevation of salivary cortisol during stressful periods compared to relaxed periods was reported.⁷⁶ However, prior literature has also shown a significant association for cortisol with depression and anxiety. A significant association between current anxiety disorder and higher awakening salivary cortisol was reported.⁷⁷ And, another study found higher stress and serum cortisol levels among individuals with major depressive disorder.⁷⁸

The study among the healthy dwellers showed that the distribution of cortisol in those who were never married was significantly higher compared to those who were currently married. A study has shown that married individuals had lower salivary cortisol levels than never married or previously married. It further revealed that married had a rapid decline in cortisol during the afternoon hours compared to never married (but not with previously married). And, higher stress was associated with higher cortisol levels in previously married compared to the married and never married groups. Thus, cortisol is a candidate mechanism for the association of marital status and health.⁷⁹ Also, the study among healthy dwellers showed that the distribution of cortisol in those who consumed alcohol in moderation was significantly higher compared to those who never consumed alcohol. Among men and women alcohol consumption was positively associated with serum cortisol.⁸⁰ Also, acute binge intoxication is associated with increased blood cortisol.⁸¹ Further, a positive relationship between different indices of alcohol intake and daily release of salivary cortisol was found in an ageing cohort.⁸² Our study showed a significant negative correlation between age and cortisol. A study also found that the cortisol peak is advanced by 24 minutes per de-

cade concerning the age of healthy adults.⁸³ However, another study showed that age above 60 years was associated with an increased morning serum cortisol.⁸⁴ Our study included only participants aged 18 to 60 years. The present study showed a significant positive correlation between hours of sleep per day and cortisol. A prior clinical trial has shown elevated plasma cortisol the next evening due to loss of sleep.⁸⁵ Greater sleep-wake behaviour problems were associated with decreased cortisol responses among children and adolescents.⁸⁶ Also, the present study's backward stepwise regression model found a positive association between height and cortisol levels among healthy dwellers. However, prior literature showed a negative association between fasting plasma cortisol and adult height which shows that adult height may be affected by physiological variations in adrenocortical glucocorticoid secretion.⁸⁷

The study mean (SD) of NPY [431.4 (229.7) pg/mL] was comparable to an Asian study among metabolically healthy obese participants from China [478.89±145.53 pg/mL].⁴³ However, NPY from the study was higher when compared to the findings from the UK and the USA which used radioimmunoassay.^{41,42} Hence, the present study may hint towards a difference in NPY between the Global North and South. However, future research is necessary for confirmation. The NPY did not show a significant association with any of the possible determinants among healthy dwellers selected based on predetermined selection criteria. However, a backward stepwise regression found hours of sleep per day to be significantly associated with NPY. NPY has hypnotic properties and regulates sleep in humans.⁸⁸ And, NPY inhibits noradrenergic signalling to promote sleep.⁸⁹

The findings of the comparative study are unique, as it has compared demographic and laboratory data between pet dog owners and age, sex-matched non-pet owners of a low-middle-income South Asian region. The study produced exclusive data about the effect of canine companionship on psychosocial and cardiovascular health from a South Asian country where similar studies were scarce. Future studies among participants with non-communicable diseases and on different treatment options are methodologically challenging yet, would yield further details on the role of canine companionship in psychosocial and cardiovascular health. Also, it is crucial to assess the cost-effectiveness of canine companionship and its health benefits among socioeconomically deprived families. A positive finding on the effect of pet dog ownership on human psychosocial health will guide towards one-health interventions among the dwellers of this community. Also, the findings will help plan future research on canine companionship and human psychosocial health. Further, the study found the baseline values of NPY and cortisol among healthy dwellers of a low-middle-income South

Asian country. It will help interpret such biomarkers in future research and clinical practice in the region.

Study limitations

The study among healthy dwellers was conducted in one of 25 districts in Sri Lanka which cannot be generalized. Also, the study lacks adequate comparison due to the unavailability of previous similar local or regional data. Further, the cost of laboratory investigations limited the sample size to 40 healthy dwellers. However, it produced valuable baseline data on NPY and cortisol levels among healthy dwellers of a district in a low-middle-income South Asian country where similar studies were scarce. Thus, the findings will be helpful for future research in South Asia. Geographic, sociocultural, and other demographic differences could have led to the negative findings in the present study. Publishing negative results could lead to a new understanding and interpretation of prior positive results. Further, it would help adjust future research plans and increase the chance of positive results.⁹⁰

Conclusion

A significantly lower depression score in the pet dog owners' group than in the non-pet owners' group was observed. And, the pet bond score had a significant negative correlation with the stress hormone cortisol. Taken together, our results suggest that pet dog ownership may have improved psychosocial health by reducing depression and stress. Thus, canine companionship could be an option to reduce depression and stress and will guide towards one-health interventions among South Asian communities. The findings will help plan future research on canine companionship and human psychosocial health. Also, the study found the baseline values of NPY and cortisol among healthy dwellers of a low-middle-income South Asian country which will help interpret such biomarkers in future research and clinical practice in the region.

Supplementary materials

Table S1 file format – .docx. Cut-off and points for allostatic load.

Table S2 file format – .docx. Comparison of categorical variables between the pet dog owners' group and the non-pet owners' group.

Table S3 file format – .docx. Categorical variables of interest against the pet bond score among pet dog owners.

Declarations

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Author contributions

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

Conflicts of interest

The author(s) declare no competing interests.

Data availability

All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).

Ethics approval

Ethical clearance was obtained from the Ethics Review Committee of the Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka (ERC/2020/76).

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Relationship between adolescent smartphone use and peer attachment and nomophobia

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ABSTRACT

Introduction and aim. Nomophobia is fear of being without a cell phone or being unable to use one. This paper investigated the effect of friendship attachment on nomophobia in adolescents.

Material and methods. This descriptive cross-sectional study was conducted in five high schools in the Central Anatolia Region of Türkiye. The sample consisted of 1033 adolescents. Data were collected using a personal information form, the Adolescent Friendship Attachment Scale, and the Nomophobia Questionnaire. Data analysis was conducted using parametric tests such as One-Way ANOVA and Independent Sample t-Test, with Bonferroni tests for post-hoc comparisons.

Results. The majority of participants were girls (58.3%), lived in nuclear families (76.2%). Most students used their smartphones for 2-4 hours daily (40.3%), and were active on social media (86%). The study found significant differences in phone usage time and friend attachment scores among different age groups. Women had higher friend attachment scores and nomophobia scores compared to men. Social media use was associated with higher nomophobia and friend attachment scores.

Conclusion. Adolescents' smartphone uses characteristics influence their nomophobia levels and friendship attachment styles. Professionals should plan interventions to raise adolescents' awareness of nomophobia.

Keywords. adolescent, attachment style, nomophobia

Introduction

Mobile phones have become integral to our lives, providing access to the latest information and services thanks to the ever-evolving mobile ecosystem. Mobile phones have introduced a new term: nomophobia, an acronym for “No Mobile Phobia.” Nomophobia is the fear of not being able to communicate via a cell phone. This fear can lead to various addictive behaviors and symptoms.¹ Nomophobia affects youth because they are more likely than adults to communicate, interact, and play online games on their smartphones and the Internet.²

Nomophobic adolescents cannot stay away from their phones.^{3,4} Although nomophobia allows adolescents to socialize in the virtual world, it causes them

to become dependent on their smartphones or exhibit avoidance behaviors.⁴ Today, adolescents are inseparable from their mobile devices as they always carry them with them. However, smartphones can lead to addiction at a later stage.⁵ Adolescents who are always online on their smartphones face social, psychological, and physiological problems. Research shows that nomophobia is closely related to personality changes, loneliness, anxiety, and depression.⁵⁻⁸ Additionally, nomophobic students are more likely to experience emotional, personality, and mental health disorders.²

The psychosocial development of adolescents is significantly influenced by their friendships and peer relationships. Adolescents tend to be more oriented to

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wards their friends and peers, seeking emotional connection and support rather than solely focusing on physical intimacy.^{9,10} Attachment anxiety and avoidance, which are related to individuals' attachment styles, have been associated with neuroticism.^{10,11} Loneliness and the desire for social interaction are factors that contribute to excessive smartphone use and higher levels of nomophobia.² Insecure attachment styles have been found to be associated with various social difficulties and negative outcomes in adolescents, such as problems with social relationships, engagement in socially dangerous behaviors, low self-esteem, difficulties in forming close relationships, feelings of loneliness, and high levels of social anxiety.¹² Despite the growing recognition of nomophobia as a prevalent issue among adolescents, few studies have specifically examined the relationship between nomophobia and friendship attachment styles. Understanding how adolescents with high levels of nomophobia, who frequently use their cell phones, perceive and experience their friendship attachments is crucial. Therefore, the aim of this study is to investigate the relationship between friendship attachment styles and nomophobia in adolescents. In this study, the term "insecure attachments" refers to attachment styles characterized by either attachment anxiety or attachment avoidance. Attachment anxiety refers to an individual's tendency to worry about being rejected, abandoned, or unloved, while attachment avoidance refers to a tendency to avoid close emotional relationships and to be uncomfortable with emotional intimacy.^{10,11}

The study aims to enhance understanding of how friendship attachment styles and nomophobia intersect in adolescent development, potentially revealing the role of friendship attachments in influencing nomophobic behaviors. By addressing this gap in research, the study contributes to both attachment theory and nomophobia literature, offering theoretical insights and practical implications for interventions targeting healthier attachment patterns and reduced reliance on mobile technology among adolescents. This study delves into the intersection of friendship attachment styles and nomophobia in adolescents, a crucial yet underexplored area in the context of adolescent development. It highlights the profound influence of mobile technology on adolescent relationships and the emergence of nomophobia as a complex emotional issue. By positioning friendship attachments as a central focus, the study aims to unveil the nuanced mechanisms underlying nomophobic behaviors. Unlike previous research, which has often overlooked friendship attachments, this study sheds light on their pivotal role in shaping adolescents' relationship with mobile technology. The findings hold theoretical significance by enriching our understanding of adolescent development in the digital era and offer

practical implications for interventions aimed at promoting healthier attachment patterns and mitigating nomophobic tendencies among adolescents.

Aim

This paper investigated the effect of friendship attachment on nomophobia in adolescents.

Research questions

1. Is there a relationship between friendship attachment styles and mobile phone addiction in adolescents?
2. Is there a relationship between friendship attachment styles and nomophobia in adolescents?

Material and methods

Study design

This descriptive cross-sectional study was conducted in Türkiye during the spring semester of the 2021–2022 academic year, involving five public high schools in a district of Central Anatolia. The study population comprised 2358 high school students who met the inclusion criteria of owning a smartphone, being enrolled in high school, and volunteering to participate. Smartphone use was deemed necessary to accurately assess nomophobia, the primary focus of the research. High school students were targeted to examine adolescents, the study's intended demographic. Voluntary participation was emphasized to ensure participants' willingness and consent. Additionally, schools with similar family types and income levels were selected to control for potential confounding variables. The study did not explore the effects of culture, diet, or environmental events on anxiety control, as its focus was specifically on nomophobia and attachment styles in adolescents. The random sample selection procedure involved two stages: selecting high schools and then selecting students within those schools. In the first stage, the study randomly selected five high schools from a pool of 12 general high schools in the district center, considering economic status and family structure similarities among students. This random selection minimized bias and enhanced the study's generalizability. In the second stage, students were selected from the population of 2358 students across ninth to twelfth grades in the five chosen high schools using simple random sampling, ensuring each student had an equal chance of selection. The intended sample size was 331 students, determined through power analysis, but the actual sample comprised 1033 students due to unexpectedly high participation rates and support from the schools. Although exceeding the predetermined sample size, this larger sample offers opportunities for more detailed exploration of research questions and potentially increases statistical power, while recognizing the

importance of maintaining methodological rigor and generalizability.

Ethics approval

The study was approved by the ethics committee of the faculty of medicine of Selçuk University (20.01.2022-E.212504). Permission was obtained from the Directorate of National Konya (04.03.2022-247174). Authorization was obtained from the developers of the scales. Students and parents were briefed on the research purpose and procedure. Informed consent was obtained from those who agreed to participate. In the research, specific procedures were followed to protect the ethical rights of the participants. The informed consent process was designed to ensure that participants understood the purpose, procedures, potential risks and benefits of the research. During this process, all participants were provided with detailed information about the research and their voluntary participation was ensured. In the case of underage participants, written informed consent was first obtained from their parents. During this process, parents were given detailed information about the purpose, duration, expected outcomes and potential effects on their children. Parents had the opportunity to ask any questions and obtain information before giving consent for their children to participate in the study. After consent was obtained from the parents, assent was also obtained from the child participants. In this process, children were informed about the research using a language appropriate to their age and level of understanding and asked if they wanted to participate. Children's consent was obtained when they voluntarily expressed their willingness to participate in the research. Children were informed that they had the right to refuse to participate in the research or to leave at any stage.

Implementation of the study

All students, parents, and school administrators were informed about the purpose and procedure of the study. Informed consent was obtained from those who agreed to participate. School permission was obtained. Students who refused to participate and participants who filled out the forms incompletely were excluded from the study. Sample selection was based on simple random sampling. The population consists of 2358 students studying in ninth, 10th, 11th and 12th grades in five high schools selected by simple random sampling from 12 general high schools in the district center. Although the sample size was determined as 331 students by power analysis (95% confidence interval, 95% representativeness of the universe and 0.5% alpha level) to adequately represent the universe. The sample consisted of 1033 students. Data were collected face-to-face in classrooms. Each participant took 20 minutes to complete the data collection forms.

Data collection tools

The data were collected using a personal information form, the Adolescent Friendship Attachment Scale (AFAS), and the Nomophobia Questionnaire (NMP-Q).

Personal information form

The personal information form was based on a literature review conducted by the researcher.^{3,13} The form consisted of 20 items on age, gender, grade level, family type, smartphone use status, etc.

Adolescent friendship attachment scale (AFAS)

The AFAS was developed by Wilkinson and adapted to Turkish by Ercan.^{14,15} The instrument consists of 23 items rated on a five-point Likert scale. It has three subscales: secure attachment, avoidant attachment, and anxious/ambivalent attachment. In secure attachment, children perceive their mothers as caring and responsive, fostering a sense of security and trust. They feel confident that their mother will be available when needed, promoting emotional well-being and positive social development. Conversely, avoidant attachment arises when mothers demonstrate insensitivity, leading children to distance themselves emotionally and develop a sense of detachment. When separated from their mothers, they display indifference upon reunion. Anxious-ambivalent attachment occurs when mothers are inconsistently responsive, causing children to experience anxiety and insecurity. These children may exhibit clingy behavior and struggle with regulating their emotions. Overall, the quality of maternal responsiveness shapes the attachment style, influencing children's emotional adjustment and social interactions. When children are separated from their mothers, they are more cry and react more, cannot be calmed by other adults, experience anxiety in unfamiliar situations.¹⁵ Higher scores on a subscale indicate the dominance of that attachment style. The scale has a Cronbach's alpha of 0.88.¹⁵ In the present study, the questionnaire had a Cronbach's alpha of .79. As a result of the analyses, it was found that the item-total score correlations for the sub-dimensions of the scale ranged between 0.21-0.71, the Cronbach Alpha internal consistency coefficient ranged between 0.60-0.89, and the test-retest reliability values ranged between 0.81-0.83. As a result of the confirmatory factor analysis using LISREL 8.7 program, the fit index values were χ^2/sd (648.27/227)=2.856, NNFI=0.91, CFI=0.95, GFI=0.88, AGFI=0.86, RMSEA=0.067, SRMR=0.059, and the three-factor structure (secure attachment, avoidant attachment and anxious/ambivalent attachment) overlapping with the original scale was confirmed. Criterion validity analyses revealed that there were correlations in the expected direction between the scores of the Attachment to Friends Scale

for Adolescents and the scores of the Relationship Scales Questionnaire.

Nomophobia questionnaire (NMP-Q)

The NMP-Q was developed by Yildirim and Correia and adapted to Turkish by Yildirim et al.^{16,17}The questionnaire consists of 20 items rated on a seven-point Likert scale. It has four subscales: not being able to access information (four items), giving up convenience (five items), not being able to communicate (six items), and losing connectedness (five items). Total scores range from 20 to 140. Scores are rated on a scale. 0–20 points indicate no nomophobia, 21–60 low, 61–100 moderate, and 101–140 high nomophobia.¹⁷A pretest was conducted to assess the validity and reliability of the Turkish NMP-Q, a measure of nomophobia among Turkish college students. Confirmatory factor analysis (CFA) was performed using AMOS 22 software to validate the underlying structure of the items. The results of the CFA indicated a valid model fit, with acceptable thresholds for normed χ^2 , CFI, and RMSEA. The reliability of the NMP-Q was found to be high (Cronbach's alpha = 0.92), with satisfactory alpha values for the four factors (ranging from .74 to .94). In the present study, the questionnaire had a Cronbach's alpha of 0.85.

Data analysis

The data analysis for this study was conducted using the Statistical Package for Social Sciences (IBM SPSS Corp; Armonk, NY, USA, v. 24.0). Statistical significance was assessed at both the 0.05 and 0.001 levels, which is a common practice in social science research to balance the risk of Type I and Type II errors. Descriptive statistics, including means and standard deviations, were reported to provide a concise summary of the variables. To examine the underlying distribution of the data, the Shapiro-Wilk test for normality was performed. The results of this test indicated that the data exhibited a normally distributed structure (p-values >0.05), satisfying the key assumption for the use of parametric statistical tests. Consequently, parametric methods were employed, including One-Way Analysis of Variance (ANOVA) and Independent Sample t-Tests. One-Way ANOVA was utilized to determine if the means of the dependent variables differed significantly across multiple groups, while the Independent Sample t-Test was used to assess differences in means between two independent groups. Furthermore, post-hoc Bonferroni analyses were conducted to allow for a more detailed examination of the differences between specific groups. The robustness of the parametric tests to moderate deviations from normality, given the large sample size, provided additional confidence in the appropriateness of the selected statistical approaches.

Table 1. Distribution and relationship of demographic and social variables related to nomophobia and friend attachment total scores*

Variables	n	Average daily mobile phone time used			NMP-Q total score			AFAS total score		
		Mean±SD	t/F	p	Mean±SD	t/F	p	Mean±SD	t/F	p
Age	14 (n=119) ¹	2.29±1.01	2.338	0.054 5>2>1	72.95±26.92	0.817	0.514	74.78±7.81	4.281	0.002 1>4 2>4
	15 (n=294) ²	2.23±0.91			69.77±22.68			73.87±8.81		
	16 (n=351) ³	2.31±0.97			70.00±24.66			71.97±9.27		
	17 (n=230) ⁴	2.32±0.87			67.95±25.21			71.27±9.27		
	18 (n=39) ⁵	2.71±1.05			70.58±31.28			72.56±13.51		
	Gender	Female (n=602)			2.28±0.91			0.769		
Male (n=431)		2.33±0.99	67.75±25.24	71.28±10.76						
Family type	Nuclear family (n=787) ¹	2.30±0.94	3.231	0.040 3>2>1	69.89±25.10	0.237	0.789	72.65±9.70	0.389	0.678
	Extended family (n=199) ²	2.37±0.93			69.16±23.63			72.62±9.41		
	Fractured family (n=47) ³	2.59±0.92			71.89±24.52			73.91±9.31		
Family income level	Income is less than expenses (n=168)	2.30±1.04	1.225	0.294	66.29±23.81	2.063	0.128	73.85±9.83	2.128	0.120
	Income equal to expenses (n=623)	2.33±0.92			70.52±24.96			72.72±8.86		
	Income exceeds expenses (n=242)	2.22±0.92			70.56±24.86			71.85±11.18		
Place of residence	Village (n=243) ¹	2.34±0.91	1.993	0.137	70.69±21.58	3.213	0.041 1>3	72.90±9.05	1.914	0.148
	District (n=763) ²	2.28±0.96			69.98±25.38			72.76±9.72		
	Province (n=27) ³	2.62±0.88			58.07±31.69			69.14±11.42		
Number of siblings	Only child (n=44)	2.34±1.05	1.533	0.204	68.09±26.98	0.622	0.601	72.59±12.89	0.635	0.593
	One sibling (n=404)	2.23±0.93			69.01±24.80			72.27±8.93		
	Two siblings (n=248)	2.32±0.94			71.56±25.52			72.67±9.99		
	Three siblings and above (n=337)	2.37±0.95			69.79±23.94			73.25±9.67		

* one-way ANOVA, independent sample t test, SD – standard deviation

Results

The study involved mostly girls (58.3%) from nuclear families (76.2%), with 39.1% having no siblings and 73.9% living in districts. Less than half used smartphones for 2-4 hours daily (40.3%) and 17.9% checked them every 20 minutes. Most participants (78.2%) used their smartphones immediately upon waking, and 86% were social media users. Significant differences were found in phone usage times among 14, 15, and 18-year-olds ($p=0.054$), with 18-year-olds using phones more. Friend attachment scores varied significantly between 14, 15, and 17-year-olds ($p=0.002$), with lower scores for 17-year-olds. Women had higher friend attachment and nomophobia scores than men ($p<0.05$). Broken families had higher phone usage ($p=0.04$). No significant relationships were found between family type and friend attachment or nomophobia scores, or between family income and phone usage, nomophobia, or friend attachment scores ($p>0.05$). However, village residents had higher nomophobia scores than those in provinces ($p=0.041$). No significant relationships were found between the number of siblings and phone usage, friend attachment, or nomophobia scores ($p>0.05$).

Table 2 reveals significant differences in nomophobia scores based on phone checking frequency, with higher scores for those who check their phones more often. Notably, individuals who check their phones immediately upon waking show higher nomophobia scores, suggesting a link between early phone use and intense nomophobia. Social media users also exhibit higher nomophobia and friend attachment scores, indicating that social media use increases both nomophobia and social connection. The analysis shows that individuals using fewer applications (0–3) have higher attachment scores compared to those using more applications (4–6 or 7–9). Furthermore, phone use for social media is associated with higher nomophobia and attachment scores compared to using phones for music, texting, or gaming, highlighting the significant impact of social media on these metrics.

Table 3 indicates that women have significantly higher secure attachment (38.64) and lower avoidant attachment (15.53) scores than men ($p<0.001$ for both). Women also have slightly higher anxious-fearful attachment scores (19.49) than men ($p=0.018$). In nomophobia sub-dimensions, women score slightly higher than men in not being able to access information (13.98, $p=0.002$), sacrificing comfort (18.66, $p=0.02$), and not being able to communicate (23.37, $p=0.038$), but there is no significant gender difference in the fear of losing online connection ($p=0.896$). Additionally, there are no significant differences in secure, avoidant, and anxious-fearful attachment sub-dimensions across nuclear, extended, and fragmented fam-

Table 2. The effect of phone usage habits on nomophobia and attachment to friends: comparison of variables*

Variables	n	NMP-Q total score			AFAS total score		
		Mean±SD	t/F	p	Mean±SD	t/F	p
Frequency of checking the smartphone	I do not check (n=138) ¹	62.42±30.53			72.63±11.02		
	Every five minutes (n=167) ²	74.86±26.56			73.55±8.56		
	Every ten minutes (n=182) ³	76.33±22.49			72.95±8.41		
	Every twenty minutes (n=185) ⁴	67.84±21.83	6.111	$p<0.001$	73.50±9.17	1.490	0.167
	Every thirty minutes (n=153) ⁵	70.83±22.62		$2>1$	72.40±10.07		
	Once an hour (n=147) ⁶	67.04±22.07		$3>1$	70.85±10.74		
	Every two hours (n=29) ⁷	58.34±22.11		$3>2$	74.55±7.39		
	Every three hours (n=32) ⁸	68.78±26.42			70.93±10.87		
Checking the smartphone as soon as you wake up in the morning	Yes (n=808)	71.91±25.18			72.78±9.59		
	No (n=225)	62.4±21.78	26.577	$p<0.001$	72.41±9.75	0.260	0.610
Social media use	Yes (n=887)	71.15±24.52	9.044	$p<0.001$	73.02±9.32	4.490	0.011
	No (n=145)	61.91±25.01			70.64±11.12		
Number of applications used on the smartphone	0–3 applications (n=462) ¹	67.9±23.23			73.06±9.54		
	4–6 applications (n=377) ²	71.88±25.64	2.161	0.091	73.55±8.77	5.707	0.001
	7–9 applications (n=95) ³	68.69±22.45			69.80±9.39		$1>3$
	10 or more applications (n=99) ⁴	72.2±29.69			70.55±12.31		$2>1$
	Listening to music (n=252) ¹	72.4±9.68			72.78±9.68		
Purpose of smartphone use	Messaging (n=207) ²	66.53±25.06			70.57±11		
	Talking (n=160) ³	65.41±23.82			72.9±8.89		
	Studying (n=67) ⁴	67.38±23.20	5.208	$p<0.001$	73.92±10.28	4.987	$p<0.001$
	Spending time on social media (n=247) ⁵	75.03±23.29		$5>1>2$	74.63±8.3		$5>6>2$
	Playing games (n=96) ⁶	65.9±28.16			70.92±9.14		

* one-way ANOVA, independent sample t test, SD – standard deviation

ilies ($p>0.05$). Similarly, no significant differences are found in nomophobia sub-dimensions related to family type ($p>0.05$).

Table 3. Comparison of friend attachment and nomophobia sub-dimensions by gender and family type*

Variables	Gender	Mean±SD	t	p
Sub-dimensions of attachment to friends				
Secure connecting	Female	38.64±7.59	27.377	$p<0.001$
	Male	35.88±9.36		
Avoidant attachment	Female	15.53±4.66	18.269	$p<0.001$
	Male	16.77±4.56		
Anxious fearful attachment	Female	19.49±5.97	5.636	0.018
	Male	18.62±5.63		
Nomophobia sub-dimensions				
Inability to access information	Female	13.98±6.06	9.882	0.002
	Male	12.76±6.27		
Don't give up on comfort	Female	18.66±7.40	5.445	0.020
	Male	17.56±7.54		
Inability to communicate	Female	23.37±9.14	4.337	0.038
	Male	22.16±9.23		
Lose online connection	Female	15.30±7.16	0.017	0.896
	Male	15.24±7.60		
	Family type	Mean±SD	F	p
Sub-dimensions of attachment to friends				
Secure connecting	Nuclear family	37.36±8.52	2.056	0.128
	Extended family	38.38±8.43		
	Fractured family	35.87±7.87		
Avoidant attachment	Nuclear family	16.02±4.72	4.916	0.08
	Extended family	15.69±4.34		
	Fractured family	18.04±4.63		
Anxious fearful attachment	Nuclear family	19.23±5.88	1.623	0.198
	Extended family	18.54±5.63		
	Fractured family	20±6.12		
Nomophobia Sub-Dimensions				
Inability to access information	Nuclear family	13.39±6.28	0.612	0.543
	Extended family	13.9±5.68		
	Fractured family	13.17±6.5		
Don't give up on comfort	Nuclear family	18.19±7.53	0.988	0.373
	Extended family	17.93±7.29		
	Fractured family	19.63±7.33		
Inability to communicate	Nuclear family	23.01±9.39	0.412	0.663
	Extended family	22.49±8.57		
	Fractured family	22.12±9.19		
Lose online connection	Nuclear family	15.29±7.39	1.613	0.200
	Extended family	14.82±7.06		
	Fractured family	16.95±7.59		

* one-way ANOVA, independent sample t test, SD – standard deviation

Discussion

In the study, it was observed that 18-year-olds spent more time on their phones compared to 14-year-olds and 15-year-olds, albeit not statistically significant ($p=0.054$). This finding aligns with existing research indicating an increase in phone usage among adolescents and young adults.¹⁸⁻²⁰ Factors such as height-

ened independence, social networking, and access to diverse applications and online content likely contribute to this trend in older age groups.^{21,22} Regarding friend attachment scores, it was found that 14-year-olds and 15-year-olds scored higher than 17-year-olds ($p=0.002$). This decline in peer attachment among 17-year-olds may be influenced by various factors, including shifting priorities, changing social dynamics, and increased focus on academic and future goals. Attachment to friends is a dynamic process shaped by individual values, social influences, and environmental factors.^{23,24} Studies by Kindschi et al. and Chang and Wu underscore the significance of values and peer relationships in adolescents' attachment dynamics.^{23,25} Moreover, De Meulenaere et al. emphasize the role of both internal cognitive models and environmental factors in shaping attachment relationships during adolescence.²⁶

In the study, women's friend attachment scores and nomophobia scores were higher than men ($p<0.05$). Research consistently shows that women tend to have higher levels of nomophobia, or fear of being without their smartphones, compared to men.^{27,28} This gender difference is attributed to women's greater use of smartphones for social connection and their stronger emotional attachment to these devices.²⁷ These findings are in line with broader gender differences in technology use and psychological attachment.²⁸

The study shows higher rates of phone use among adolescents from fractured homes compared to other family types, potentially influenced by reduced parental supervision and communication. Studies support this finding.²⁹⁻³¹ However, in this context, excessive phone use is associated with negative health consequences, highlighting the need for strategies to encourage healthier phone use habits in all family structures.

In the study, the total nomophobia score of those living in the village was higher than those living in the city ($p=0.041$). Research indicates that nomophobia is prevalent among young people, especially females and those who view their phone use as harmful to their nutrition.²⁸ Higher nomophobia levels in rural areas may be influenced by differences in technology access, social networks, psychological factors, and cultural aspects like vertical collectivism.^{32,33}

In the study, no statistically significant relationship was found between family income level, number of siblings, phone use, friend attachment scores and total nomophobia scores ($p>0.05$). The lack of a significant relationship between family income level, number of siblings, phone use, friend attachment scores, and total nomophobia scores is consistent with previous study.² This suggests that the influence of these factors on phone use and engagement with technology may be more subtle and context-dependent.¹ Further research is

needed to explore the complex interplay between these factors and the development of nomophobia.¹⁶

In the study, individuals who check their phones more frequently have higher nomophobia total scores ($p < 0.001$). Studies have shown a strong positive correlation between high phone use frequency and increased nomophobia, with frequent checking and dependence on phones linked to higher nomophobia scores.^{2,34,35} Research by Rodríguez-García et al. and Humood et al. highlights the prevalence of severe nomophobia and its negative impact on mental health, especially among university students.^{2,34}

In the study, checking the phone immediately in the morning is associated with higher total nomophobia scores. Research indicates that increased smartphone usage is linked to higher levels of loneliness, anxiety, and nomophobia, especially among individuals with social anxiety and loneliness who frequently use system and social media apps.^{36,37} The fear of missing out significantly contributes to problematic smartphone use and nomophobia, with habits like checking phones first thing in the morning reflecting a desire for social connection and fear of missing out.³⁸

In the study, individuals who use social media have higher nomophobia scores and friend attachment scores. Research shows that social media use is linked to higher levels of nomophobia and friend attachment, with smartphone use and feelings of personal inadequacy being strong predictors of nomophobia.^{27,39} Additionally, online social connections positively predict Facebook communication and relational closeness, suggesting that social media use can both increase nomophobia and strengthen social connections.⁴⁰

Those who use fewer and medium numbers of applications have higher total attachment scores ($p < 0.05$). Tam et al. and Mehra et al. both explore factors influencing the continued use and adoption of mobile apps, with satisfaction, habit, and perceived usefulness being key drivers.^{41,42} These studies provide a foundation for understanding the potential link between the number of actively used applications and attachment scores, suggesting that individuals who use fewer apps may have a stronger attachment to specific ones. However, further research is needed to confirm this relationship.

Spending time on social media is associated with higher nomophobia scores and friend attachment scores, compared to other activities like playing games and texting. The purpose of phone use can have an impact on nomophobia and attachment, with social media use showing significant effects. Research has shown that the purpose of phone use, particularly social media use, is a significant factor in understanding the psychological implications of smartphone usage.² Spending time on social media has been associated with higher nomophobia scores and friend attachment scores com-

pared to other activities like playing games and texting.³⁶ This is further supported by Elhai, who found that non-social smartphone use, which includes social media, is linked to problematic smartphone use and fear of missing out.⁴³

In Table 3, in the secure attachment subscale, the mean of women (38.64) is higher than that of men and there is a statistically significant difference ($p < 0.001$). Research indicates that women have lower levels of avoidant attachment but slightly higher levels of anxious-fearful attachment compared to men, suggesting gender differences in attachment styles with women tending towards secure attachment and men towards avoidant attachment.⁴⁴

The study suggests that women experience slightly higher levels of specific nomophobia sub-dimensions compared to men, particularly in areas of inability to access information, sacrificing comfort, and not being able to communicate, while no significant gender difference was found in losing online connection. A systematic review by Rodríguez-García et al. found nomophobia prevalent in the general adult population, especially among university students, with further studies by Humood et al. and León-Mejía et al. highlighting high prevalence in this group and greater vulnerability among women and younger individuals.^{2,34,45}

In the study, no significant differences regarding attachment sub-dimensions (secure attachment, avoidant attachment, and anxious-fearful attachment) were found among nuclear family, extended family, and fragmented family ($p > 0.05$). No significant differences regarding nomophobia sub-dimensions (not being able to access information, sacrificing comfort, not being able to communicate, and losing online connection) were found among different family types ($p > 0.05$). Research indicates no significant differences in attachment and nomophobia sub-dimensions among different family types, suggesting that individual experiences and interactions, rather than family structure alone, play a stronger role in influencing these factors.^{46,47}

In the cultural context of Türkiye, these findings are particularly relevant. The strong family bonds and social structures in Turkish society might influence attachment styles and smartphone usage behaviors differently than in more individualistic cultures.⁴⁸⁻⁵⁰ However, the similarities with global patterns suggest that nomophobia and attachment issues transcend cultural boundaries, driven more by the ubiquitous presence of technology and the universal human need for social connection. Nomophobia, the fear of being without a mobile phone, is a growing concern in Türkiye, particularly among the younger generation. Sarı et al. and Alini both highlight the psychological and religious aspects of this issue, with Sarı et al., suggesting psychotherapy and Islamic therapy as potential treatments.^{48,51} Bernar-

dini and Bak further emphasize the global nature of nomophobia, with Bernardini noting its prevalence in Italy and Bak finding it to be a common issue across different generations in Türkiye.^{52,53} These studies collectively underscore the need for culturally sensitive interventions to address nomophobia and excessive smartphone use in Türkiye. Thus, this study underscores the importance of considering both individual and cultural factors in addressing attachment and nomophobia. It highlights the need for culturally sensitive approaches in interventions and educational programs aimed at reducing excessive smartphone use and its psychological impacts in Türkiye.

Study limitations

The cross-sectional nature of our research design represents an important limitation, as it precludes our ability to establish causal relationships between the variables examined. We can only identify associations, rather than determine the direction of causation. The primary limitation of this study is its regional focus on Central Anatolia, which may affect the generalizability of the results to other geographic areas due to unique socioeconomic and cultural characteristics. To enhance generalizability, future research should expand to include multi-regional or nationwide studies and international comparisons to understand how these phenomena vary across different contexts.

Study implications

To address the effects of excessive smartphone use and nomophobia, schools and parents should collaborate on educational initiatives. Schools can implement workshops and counseling services focused on healthy friendships and coping strategies to reduce smartphone dependence, while also promoting responsible social media use and regulating screen time during school hours. Parents need to be educated on nomophobia and encouraged to support balanced phone use in their children by setting boundaries and modeling healthy behaviors. Mental health professionals can provide assessments, cognitive behavioral therapy, and coping skills training. Practical interventions such as digital literacy programs, digital detox, mindfulness practices, and apps to monitor screen time can significantly reduce nomophobia and improve adolescents' well-being and emotional regulation.

Conclusion

Friendship attachment styles influence nomophobia in adolescents, alongside smartphone use characteristics, indicating the importance of interventions by health professionals, educators, and parents to mitigate nomophobia. Future research should focus on testing the effectiveness of educational programs, exploring the im-

port of different types of attachment (parental, romantic, friendship), and investigating how attachment styles in these relationships affect nomophobia levels. Friendship attachment styles significantly impact nomophobia in adolescents, influenced by smartphone use characteristics, prompting the need for interventions by health professionals, educators, and parents to mitigate nomophobia through targeted educational programs. Understanding the mediating and moderating roles of psychological, social, and environmental factors in the relationship between friendship attachment styles and nomophobia can provide valuable insights for developing effective interventions and promoting healthy smartphone use habits among adolescents.

Declarations

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Author contributions

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

Conflicts of interest

The author(s) declare no competing interests.

Data availability

To increase the transparency and reproducibility of this research, data sets generated and/or analyzed during the study may be made available from the corresponding author for further analysis or replication by other researchers.

Ethics approval

The study was approved by the ethics committee of the faculty of medicine of Selçuk University (20.01.2022-E.212504). Permission was obtained from the Directorate of National Konya (04.03.2022-247174).

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Phytochemical analysis, stability, and antimicrobial activity of eighteen medicinal plants studied against five multi-drug resistant human pathogens

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ABSTRACT

Introduction and aim. Several medicinal plants from India have been reported to be effective against human pathogens, but comprehensive comparative studies are scarce. The aim of this study has been to evaluate and compare the antimicrobial activity, phytochemical composition, and stability at different temperatures and pH of ethanolic extracts of eighteen Indian medicinal plants which are prevalent in North-east India and thrive abundantly.

Material and methods. These plants included *Syzygium cumini*, *Cannabis sativa*, *Camellia sinensis*, *Murraya koenigii*, *Alstonia scholaris*, *Terminalia chebula*, *Flemingia strobilifera*, *Azadirachta indica*, *Prunus persica*, *Euphorbia thymifolia*, *Averrhoa carambola*, *Paderia foetida*, *Psidium guajava*, *Spondias pinnata*, *Garcinia cowa*, *Litsea cubeba*, *Micania macrantha*, and *Phlogocanthus thyrsoflorus*. Their potent medicinal properties made them the ideal choice for this study which included the use of agar well diffusion method and phytochemical analysis.

Results. Agar well diffusion has been used to test their antibacterial activity, which demonstrated higher antibacterial activity of *G. cowa* extract against *Vibrio cholerae* and *Staphylococcus aureus*, whereas *S. pinnata* extract was most effective against *V. cholerae* and *Enterococcus faecium*.

Conclusion. Phytochemical analysis revealed the presence of tannins, alkaloids, saponins, glycosides, steroids, terpenoids, flavonoids, and phenols. The antimicrobial activity of these plant extracts remained stable at higher temperatures and varying pH levels.

Keywords. antibiotic resistance, antimicrobial activity, extracts, medicinal plants, phytochemicals

Introduction

Infectious diseases are a major cause of increased morbidity and mortality and one of the irresistible threats to the human race. Treatments of infectious diseases are mostly carried out by antibiotics. Thus, the success of curing an infectious disease relies on the availability of

effective antibiotic. The problem mounts up due to the development of antibiotic resistance by the microbial pathogens.¹⁻³ The use and misuse of antimicrobial drugs accelerate the emergence of drug-resistant microbes.^{4,5} Poor infection control practices, inadequate sanitary conditions, and improper food-handling, etc. encourage

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the further spread of multidrug-resistant pathogens.^{6,7} Development of plant-based antibiotic formulation is an alternative to such situations.⁸

In recent years, the growing interest in the exploration of the medicinal properties of plant extracts due to their diverse array of bioactive compounds has promoted investigations into enhanced extractions of phytochemicals and the potential use of the various plant extracts to address several health conditions including treatment against microbial infections and autoimmune diseases like Rheumatoid arthritis.⁹ For this, the utilization of enzyme complexes for the recovery of plant extracts has proved to be a successful methodology. Enzymes like zympex-014 have been proven to effectively enhance phenolic recovery along with antioxidant capacity in *Pongamia pinnata* leaf extract.¹⁰

Azadirachta indica, a widely recognized medicinal plant has been studied extensively for its anti-inflammatory effects where it has shown to reduce inflammation in carrageenan-induced rats.¹¹ Plants such as *Citrus paradisi*, *Thymus vulgaris* and *Caltha palustris* var. *alba* have also been reported to reduce inflammation.¹²⁻¹⁴ In the same way, *T. vulgaris* and *C. palustris* var. *alba* have been revealed to showcase other health benefits too such as antioxidant, antibacterial, and antidiabetic properties thereby implying their suitability for medicinal use.^{12,13}

Similarly, investigations into phytochemicals obtained from plants such as *Phyllanthus emblica*, *Teucrium stocksianum* and *Salvia hispanica* have shown significant antioxidant properties.¹⁵⁻¹⁸ Furthermore, studies have indicated the ability of phytochemicals extracted from several medicinal plants such as *Melia azedarach*, *P. emblica*, *T. vulgaris*, and *C. palustris* var. *alba* to act as antimicrobial agents thereby displaying their capability to be used in the treatment of microbial infections.^{12,13,15,19} *M. azedarach* has been identified to possess the potential to have therapeutic applications against diseases such as tuberculosis.²⁰ This therefore has shown the immense potential of plant extracts as valuable sources of therapeutic agents and emphasized the need for research in this field.

While phytochemicals have become a prime area of investigation to develop antimicrobial agents, studies portraying microbial production of antimicrobial compounds and the development of biomaterials with integration of plant science have also become emerging topics of research. Bioactive polar and non-polar metabolites extracted from bacteria such as *Bacillus* sp. have exhibited antimicrobial properties apart from other significant biological activities, including antidiabetic, anti-inflammatory, antioxidant, and anti-hemolytic effects. Compounds isolated from the bacteria like 1,2-benzenedicarboxylic acid have shown potential anticancer properties against epithelial glioblastoma cancer genes too.²¹

The exploration of plant extracts in modern medicine has focused on plant-derived compounds and their synthesized nanoparticles which have displayed potential as a promising alternative to traditional therapies. One significant area of research has involved the synthesis of silver nanoparticles (AgNPs) using plant extracts that show antimicrobial properties. For example, inhibition zones in agar well diffusion assays have been observed for AgNPs synthesised using fenugreek leaf extract against the gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*.²² Similarly, research on *Calliandra harrisii* leaf extract synthesized AgNPs has displayed substantial activity against multidrug-resistant *Staphylococcus* species and has even been reported to outperform conventional antibiotics such as vancomycin and cefoxitin. Docking studies conducted reveal the efficacy of these nanoparticles against virulent genes of the multi-drug resistant bacteria thereby suggesting their potential in addressing the growing issue of antibiotic resistance.²⁰

Several researchers are now exploring plant-derived substances as potential alternatives or complement to antibiotics. Studies such as those on *Crocus sativus*, have demonstrated the effectiveness of their modified compounds as plant-based antimicrobials.²³

Also since plant-based formulations contain a large number of molecules that work in synergy to cause an antimicrobial effect, development of resistance is much slower. North-eastern India is mostly inhabited by a large number of tribes, who prefer using plant-based cures rather than bottled medicines.^{24,25} There have been studies on the antimicrobial activity of medicinal plants against pathogens. However, comparative analyses of different medicinal plants and their effects on various pathogens are lacking. The novelty and rationale of this study lie in comparing the antimicrobial activity of these plants and assessing their stability under different temperatures and pH.

Aim

In this study, we compared the efficacy of eighteen such medicinal plants against five human pathogens (*Vibrio cholerae*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium* and *Pseudomonas aeruginosa*). The plants used were *S. cumini*, *C. sativa*, *C. sinensis*, *M. koenigii*, *A. scholaris*, *T. chebula*, *F. strobilifera*, *A. indica*, *P. persica*, *E. thymifolia*, *A. carambola*, *P. foitida*, *P. guajava*, *S. pinnata*, *G. cowa*, *L. cubeba*, *M. macrantha*, and *P. thyrsoiflorus*.

Material and methods

Collection of plant materials

Plant materials were collected from villages named Lekai gaon, Jokai gaon near Assam Medical College and Hospital Campus of Dibrugarh District of Assam which are found to occur in abundance throughout the North-east

ern region of India. The collected Plants were *S. cumini* (1), *C. sativa* (2), *C. sinensis* (3), *M. koenigii* (4), *A. scholaris* (5), *T. chebula* (6), *F. strobilifera* (7), *A. indica* (8), *P. persica* (9), *E. thymifolia* (10), *A. carambola* (11), *P. foitida* (12), *P. guajava* (13), *S. pinnata* (14), *G. cowa* (15), *L. cubeba* (16), *M. macrantha* (17), *P. thyrsoiflorus* (18). It may be noted that, throughout this article, these numbers of plants were maintained for convenience in discussion and data presentation. Collected plant materials were washed with tap water twice and finally washed with distilled water. Washed materials were dried at room temperature under shade. The dried plant materials were then pounded in mortar and pestle and used for extraction.

Preparation of the plant extract

Plant extracts were prepared using the maceration technique with ethanol as the solvent.²⁶ Initially, 10 grams of dried plant material were weighed and soaked in 40 milliliters of ethanol, agitated at 80 rpm for 24 hours at room temperature. The resulting mixture was filtered through Whatman no. 1 filter paper, and the plant residue was re-soaked in another 40 milliliters of ethanol. This process was repeated twice to ensure thorough extraction. The combined filtrate was then concentrated using a rotary evaporator and reconstituted with ethanol. The final extracts were stored at 4°C for subsequent use.

Microorganisms

Gram-negative and gram-positive bacteria were used in the study. Five clinical strains were obtained from the Department of Microbiology, Assam Medical College, and Hospital of Assam. The isolates were *V. cholerae*, *S. aureus*, *E. faecalis*, *E. faecium* and *P. aeruginosa*. The isolates were maintained using nutrient agar media by streaking technique.²⁷

Determination of antibacterial activity

The antibacterial assay was conducted with modifications to previously described methods.²⁸⁻³⁰ Bacterial broth cultures were grown for 18 hours, and the turbidity was adjusted to 0.5 McFarland standard (1×10^8 CFU/mL). Then, 100 μ L of each bacterial culture was spread onto sterile agar media plates. For the agar well diffusion assay, wells were punched into the agar plates using a sterile 5 mm cork borer. Each well was loaded with 100 μ L of plant extract (100 mg/mL) and allowed to diffuse into the media at room temperature for 30 minutes. The plates were then incubated overnight at 37°C. The following day, the zones of inhibition (ZOI) were measured and recorded. This assay was repeated three times to ensure consistency.

Determination of minimum inhibitory concentration

The Minimum Inhibitory Concentration (MIC) was determined using the micro-dilution method.³¹ The pro-

cedure was conducted in 96-well U-bottom plates. Plant extracts were diluted with sterile peptone water, creating a series of concentrations: 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.125 mg/mL, 1.56 mg/mL, 0.78 mg/mL, and 0.39 mg/mL. For each assay, 100 μ L of bacterial culture at 0.5 McFarland standard (1×10^8 CFU/mL) was used. The plates were incubated at 37°C, and the MIC was determined by visually assessing the turbidity in each well. The lowest concentration at which no bacterial growth (i.e., no turbidity) was observed was recorded as the MIC value for that extract.

Effect of temperature and pH

To assess temperature tolerance, plant extracts were heated at 4°C, 60°C, and 100°C for one hour before performing the antibiotic activity assay.³² For pH tolerance, each plant extract was exposed to acidic (pH 2) and basic (pH 10) conditions for one hour. The basic pH was achieved by adding 1M NaOH dropwise until the pH reached 10, and the acidic pH was achieved using 1N HCl.⁶ After treatment, both acid- and base-treated extracts were neutralized before being used in the bioassay.

Phytochemical screening of plant materials

Phytochemicals were detected as described earlier³³ and outlined below

Tannins: 200 mg of plant material was soaked in 10 mL of distilled water and filtered. 2 mL of the filtrate was added to 2 mL of dilute FeCl₃. The appearance of a blue-black or violet precipitate indicated the presence of tannins and phenols.

Alkaloids: 2 mL of ethanolic extract (10 gms/40 mL) was mixed with a few drops of diluted HCl and filtered. 2 mL of the filtrate was treated with Mayer's reagent and Wagner's reagent. A creamy white precipitate with Mayer's reagent and a reddish-brown precipitate with Wagner's reagent indicated the presence of alkaloids.

Saponins: 2 mL of ethanolic extract was diluted with 20 mL of distilled water and shaken vigorously. The presence of a stable, persistent froth indicated the presence of saponins.

Glycosides: 1 g of dried leaf powder was extracted with 10 mL of 70% ethanol for a few minutes and filtered. 5 mL of the filtrate, 10 mL of water, and 0.5 mL of a strong lead acetate solution were added and filtered again. The filtrate was shaken with 5 mL of chloroform, and the chloroform layer was separated. This was dissolved in glacial acetic acid, and a drop of ferric chloride solution was added, followed by sulfuric acid, which formed the lower layer. A reddish-brown color between the two layers and a bluish-green upper layer indicated the presence of glycosides.

Flavonoids: The extracts were treated with 80% sulfuric acid. A yellowish-orange color indicated the presence of flavonoids.

Steroids and Terpenoids: The Liebermann-Burchard reaction was used. In a test tube, 2 mL of chloroform extract prepared from the leaves of the eighteen different plants selected for the study was mixed with 2 mL of acetic anhydride and a few drops of concentrated sulfuric acid. A blue-green ring between the layers indicated the presence of steroids, while a pink-purple ring indicated the presence of terpenoids.

Results

Antimicrobial activity of plant extracts

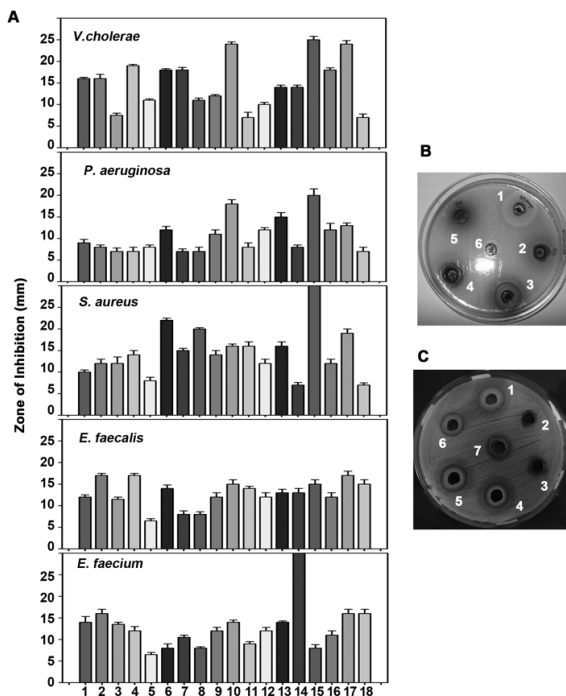


Fig. 1. Comparative study of antibacterial assay of ethanolic extract of plants (A: Zone of Inhibition. Numbers in X-axis indicate plants as mentioned in the text. Each bar indicates diameter of zone of inhibition after deducting the cup diameter ± S.D. of three replicates, B and C: Representative photos of agar well diffusion assay. B: against *V. cholerae*, with the following plant extracts. 1 – *M. macrantha*, 2 – *P. persica*, 3 – *E. thymifolia*, 4 – *A. carambola*, 5 – *P. foitida*, 6 – *P. guajava*. C: against *S. aureus* with 1 – *A. indica*, 2 – *P. persica*, 3 – *E. thymifolia*, 4 – *A. carambola*, 5 – *P. foitida*, 6 – *P. guajava*, 7 – *S. pinnata*)

To study the antimicrobial activity of ethanolic extract of medicinal plants, standard agar well diffusion method was used. A total of eighteen different plants were used in the study. 1-18 extracts were tested against five pathogens – *V. cholerae*, *S. aureus*, *E. faecalis*, *E. faecium*, and *P. aeruginosa*. The zone of inhibition for all the plant extracts against all pathogens is plotted in the same scale for comparative evaluation (Fig. 1A), and representative bioassay plates are shown in Figures 1B and 1C. Also, we have determined the minimum inhib-

itory concentration for all the extracts against all the pathogens (Table 1). As shown in Figure 1, *E. thymifolia* (10), *G. cowa* (15), and *M. macrantha* (17) showed higher anti-vibrio activity.

Table 1. MIC values of ethanolic extract od plants*

Sl.No	Plant extracts	<i>V. cholerae</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>E. faecium</i>
1.	<i>Syzygium cumini</i>	12.5	>100	50	50	50
2.	<i>Cannabis sativa</i>	6.25	50	25	25	25
3.	<i>Camellia sinensis</i>	25	50	25	100	50
4.	<i>Murraya koenigii</i>	6.25	>100	12.5	25	100
5.	<i>Alstonia scholaris</i>	100	>100	100	100	100
6.	<i>Terminalia chebula</i>	6.25	50	6.25	25	50
7.	<i>Flemingia strobilifera</i>	12.5	>100	12.5	50	100
8.	<i>Azadirachta indica</i>	25	>100	6.25	50	50
9.	<i>Prunus persica</i>	12.5	>100	50	100	100
10.	<i>Euphorbia thymifolia</i>	3.125	25	50	50	100
11.	<i>Averrhoa carambola</i>	6.25	50	50	50	50
12.	<i>Paderia foetida</i>	25	50	100	100	100
13.	<i>Psidium guajava</i>	25	25	50	100	100
14.	<i>Spondias pinnata</i>	3.125	50	50	12.5	3.125
15.	<i>Garcinia cowa</i>	3.125	12.5	1.78	50	50
16.	<i>Litsea cubeba</i>	50	50	50	100	100
17.	<i>Micania macrantha</i>	3.125	50	25	25	50
18.	<i>Phlogocanthus thyriflorus</i>	>100	>100	>100	50	50

* > – indicates MIC is more than 100 mg/mL

Interestingly, plants *E. thymifolia* (10) and *G. cowa* (15) also have high anti-*Pseudomonas* activity (Fig. 1 and Table 1). All the plant extracts in our study showed modest antimicrobial activity against *E. faecalis*, showing 5-15mm zone of inhibition. Almost similar results were obtained against *E. faecium* except for *S. pinnata* (14). *S. pinnata* (14) extract is highly antibiotic against this pathogen (3.12 mg/mL). The results altogether suggest that though all traditionally used medicinal plants possess antimicrobial activity, they do differ in their strength and specificity.

MIC was determined as the lowest concentration of extract at which growth of tested bacteria is inhibited, i.e. the concentration at which no turbidity was visible after overnight incubation (Table 1). All the pathogens were resistant to the plant extract at a concentration below the MIC. For this study, resistant strains i.e., with higher MIC were chosen. MIC of *E. thymifolia* (10), *S. pinnata* (14), *G. cowa* (15), and *M. macrantha* (17) against *V. cholerae* was 3.125 mg/mL, and highest MIC values were obtained from *A. scholaris* (5) (100 mg/mL) and *P. thyriflorus* (18) (>100 mg/mL). In the case of *P. aeruginosa*, the MIC of eighteen plants ranged from 50mg/mL to greater 100 mg/mL, except *G. cowa* (15) which exhibited MIC value 12.5 mg/mL. The lowest MIC value was obtained for *S. aureus* by the extract of *G. cowa* (15) (1.78 mg/mL). MIC obtained for *E. faecalis* was between 12.5

mg/mL to 100 mg/mL. For *E. faecium*, ethanolic extract of *S. pinnata* (14) showed the lowest MIC value (3.125 mg/mL).

Temperature and pH tolerance by plant extracts

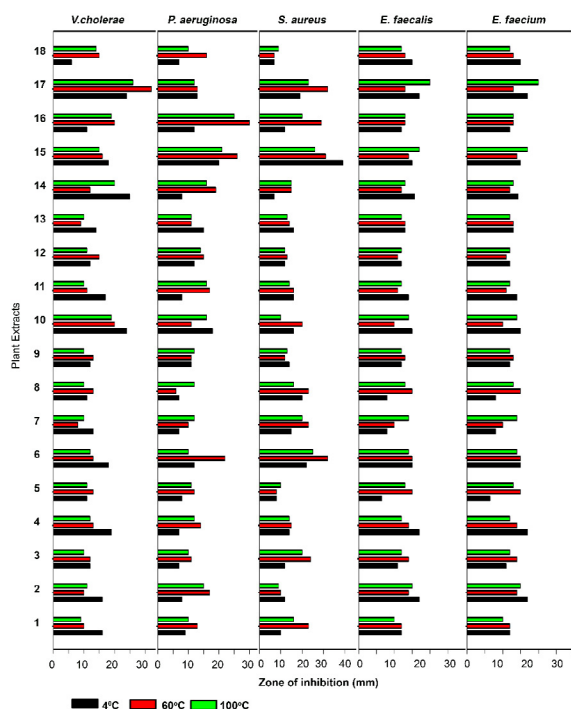


Fig. 2. Effect of different temperatures on plant extracts in terms of antibiotic activity. Plant extracts were treated at the indicated temperature for 1 hour before conducting bioassay by agar-well diffusion assay. The zone of inhibition was measured as mentioned in Figure 1. Plant numbers are in the same order as mentioned in the text

Different temperature treatments of plant extracts showed that all the extracts were stable at higher temperatures i.e. retained their inhibitory property even after treating at 60°C and 100°C (Fig. 2). Plant extracts were not only stable at a higher temperature, for instance, exhibited improvement in their inhibition activity, which was observed with *L. cubeba* (16) against all the bacterial isolates. Similarly, *M. macrantha* (17) and *P. thyrsoiflorus* (18) also showed higher ZOI at higher temperature against *V.cholerae* and *S.aureus*. *S. cumini* (1), *C. sativa* (2), *M. koenigii* (4), *T. chebula* (6), *A. carambola* (11) and *G. cowa* (15) showed a modest reduction in antimicrobial activity against *V.cholerae*. Similarly, *C. sativa* (2), *P. guajava* (13), *G. cowa* (15) also exhibited a decrease in inhibition activity against *S. aureus* with increase in temperature. Higher temperatures also reduced the antibacterial activity of *M. koenigii* (4) and *P. thyrsoiflorus* (18) against *E. faecalis* and *E. faecium*. While the reduction in antimicrobial activity may suggest a partial loss of structural integrity of the

bioactive molecules; enhancement in activity may be attributed to better extraction.

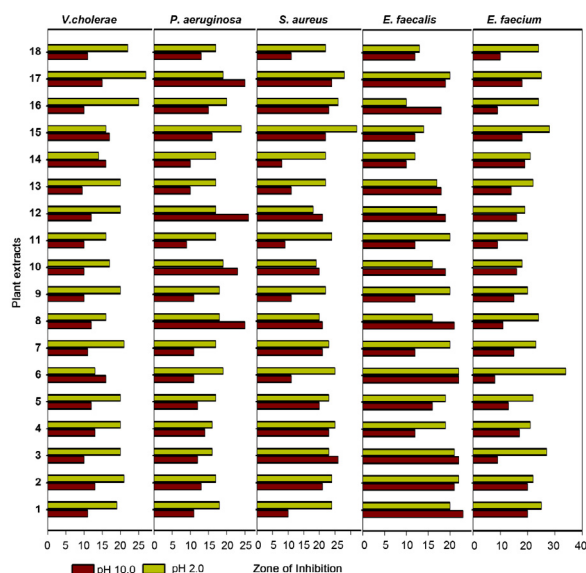


Fig. 3. Effect of different pH on plant extracts in terms of antibiotic activity. The plant extracts were treated with HCl and NaOH to adjust the pH to 2 and 10 respectively. After 30 mins pH was neutralized for bioassay. Zone of inhibition was measured as mentioned in Figure 1. Plant numbers are in the same order as mentioned in the text

To determine the extreme pH tolerance, plant extracts were treated with acid and alkali to get a final pH of 2 and 10 respectively. The extracts were further neutralized and activity was checked against the tested pathogens. Significant antibiotic activity was retained in all the extracts against all the pathogens (Fig. 3). However, in general, we observed that treating with alkaline pH (10) was more detrimental than the acidic pH (2) with certain exceptions. For example, alkaline treated extracts of *M. macrantha* (17), *P. foitida* (12), *E. thymifolia* (10) and *A.indica* (8) showed higher activity against *Paeruginosa* (Fig. 3). In other cases, the acid treated samples were more or equal to the alkaline treated samples against all the pathogens.

Phytochemical analysis

The presence of phytochemicals in medicinal plants imparts medicinal values and led to the discovery of phytodrugs of immense importance. Studies of predominant phytochemicals from the ethanolic extract of eighteen plants revealed the presence of different phytochemicals like tannins, alkaloids, saponins, glycosides, steroids, terpenoids, flavonoids, and phenols (Table 2). Flavonoids were detected in the ethanolic extract of all the plants except *S. cumini* (1), *P. foitida* (12), *L. cubeba* (16) and *M. macrantha* (17). Tannins, alkaloids, and flavonoids largely contribute to medicinal properties of plants. They were detected in *A. scholars* (5), *P. persica*

(9), and *E. thymifolia* (10). Besides these plants, extracts of *P. thyrsoiflorus* (18), *S. pinnata* (14), *M. koenigii* (4) are also rich in various phytochemicals (Table 2).

Table 2. Phytochemical analysis of ethanolic extract of plants*

Plants	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Tannins	-	+	+	-	+	+	-	+	+	+	-	-	-	-	-	-	+	+
Alkaloids	-	-	-	-	+	-	-	-	+	+	-	+	+	+	-	-	+	-
Saponins	-	-	+	-	+	-	-	+	-	+	-	-	-	+	-	-	-	+
Glycosides	-	-	-	+	-	+	-	-	+	-	-	+	-	+	-	-	-	-
Steroids	-	-	-	+	+	+	+	+	-	+	-	-	-	+	+	-	+	+
Terpenoids	+	+	-	+	-	+	-	-	-	-	-	-	+	-	+	+	+	+
Flavonoids	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+
Phenols	+	+	+	+	+	+	-	-	+	+	+	-	-	-	-	-	-	+

* + – presence, - - absence, Plant No. 1 – 18 as mentioned: *S. cumini* (1), *C. sativa* (2), *C. sinensis* (3), *M. koenigii* (4), *A. scholaris* (5), *T. chebula* (6), *F. strobilifera* (7), *A. indica* (8), *P. persica* (9), *E. thymifolia* (10), *A. carambola* (11), *P. foitida* (12), *P. guajava* (13), *S. pinnata* (14), *G. cowa* (15), *L. cubeba* (16), *M. macrantha* (17), *P. thyrsoiflorus* (18)

Discussion

The evolution of antibiotic resistance in pathogenic microorganisms is a natural phenomenon. However, certain human activities, such as the misuse and overuse of antibiotics, accelerate the development and spread of multidrug resistance. The use of medicinal plant-based formulations may offer an effective approach to mitigating the emergence of multidrug-resistant pathogens. Ethnic tribes around the world extensively use plants for medicinal purposes, including those in the biodiversity-rich northeastern region of India, where local populations frequently utilize medicinal plants for common ailments.^{24,34,35} Numerous research papers describe the antimicrobial properties of these medicinal plants yet given the vast diversity of both medicinal plants and microbial pathogens, the number of studies remains insufficient. Moreover, the comparison of the efficacy of various medicinal plants as antimicrobial agents against pathogens underscores the rationale and novelty of this research. This study aims to partially address this knowledge gap by providing a comprehensive and comparative evaluation of eighteen medicinal plants against five different human bacterial pathogens (Table 1).

To conduct this extensive study, we focused on ethanolic extracts of the plants. Ethanol, with a polarity index of 5.1, effectively solubilizes a wide range of biomolecules, bridging the solubility spectrum between water (9) and hexane (0).³⁶ Previous reports indicate that ethanol extracts of medicinal plants contain flavonoids, phenolics, alkaloids, and other substances.²⁸ Our analysis confirmed the presence of tannins, alkaloids, saponins, glycosides, terpenoids, flavonoids, and

phenols in varying degrees across the extracts (Table 2). This table also compares the abundance of secondary metabolites among the eighteen plants; for instance, *S. cumini* (1) is rich in terpenoids and phenols, whereas these compounds are less abundant in *F. strobilifera* (7), *A. indica* (8), and *P. persica* (9). Flavonoids are crucial components of several medicinal plant extracts; the flavonoid fraction of *P. guajava* leaves, for example, inhibits the growth of *P. aeruginosa*³⁷⁻⁴¹ and may counter biofilm formation.⁴² We found an abundance of flavonoids in most ethanolic extracts, supporting their role as bioactive components along with other metabolites.

The most significant finding of our study is the comparison of the antimicrobial efficacy of the selected medicinal plant extracts against the different pathogens. A plant extract with high inhibitory activity against one pathogen may have little effect on another. For example, *E. thymifolia* (10) extract strongly inhibits the growth of *V. cholerae* with a MIC of 3.125 mg/mL but is less effective against *P. aeruginosa* (MIC 25 mg/mL) and nearly ineffective against *S. aureus*, *E. faecalis*, and *E. faecium*. Like pharmaceutical compounds that require extensive validation, the bioactivity of medicinal plant extracts should also be confirmed through multiple independent studies. For instance, our findings on the bioactivity of *S. cumini* against *V. cholerae* corroborate previous reports by Sharma et al. and Islam.^{43,44} However, most of the interactions between plant extracts and pathogens reported here are novel. To our knowledge, there are no previous studies on the interactions between extracts of the selected plants and *E. faecalis* or *E. faecium*. Our results show that these two pathogens are generally resistant to most extracts, but is susceptible to the extract of *S. pinnata* (14) (Table 1).

Additionally, there are no prior reports on the susceptibility of *V. cholerae*, *S. aureus*, *E. faecalis*, *E. faecium*, and *P. aeruginosa* to extracts of *F. strobilifera* (7), *E. thymifolia* (10), *A. carambola* (11), *P. foitida* (12), *S. pinnata* (14), *M. macrantha* (17), *P. thyrsoiflorus* (18), and *L. cubeba* (16). Besides antimicrobial properties and phytochemical constituents, this study also evaluates and compares the efficacy of the plant extracts under extreme pH and temperature conditions. The pH analysis conducted aimed to determine the impact on the extracts upon exposure to acidic (pH=2) and basic (pH=10) conditions, to understand how the extracts react to different pH conditions of the human gut. The temperature analysis involved exposure of the extracts to various temperatures (4°C, 60°C, and 100°C), which was performed to determine the adequate temperature for their transportation and storage.

Our research shows that the growth inhibition properties of the extracts are retained at both pH 2 and pH 10 (Fig. 3), with higher efficacy observed at alkaline pH. Although the reason for reduced activity at low pH is unclear, it is possible that certain components precipitate

and become unavailable in the assay. This hypothesis is supported by the significant loss of antibiotic activity observed when extracts are incubated at low temperatures, such as 4°C (Table 4). The bioactive ingredients are highly thermostable, retaining activity even at 100°C, making it unlikely that their bioactivity would be lost during storage at 4°C. However, our findings indicate that storage at 4°C significantly reduces the activity of the samples.

Nonetheless, the study has limitations which gives the scope for further research. The study acknowledges the presence of different bioactive components. It does not, however, explore the synergistic or antagonistic effects of these compounds in different combinations. Furthermore, the study focuses on plants from the north-eastern region of India. This region is rich in biodiversity, however, the findings may vary significantly depending on the location of the medicinal plants. Further research into these areas will help answer these questions.

Conclusion

Our work demonstrated the efficacy of eighteen medicinal plants towards five microbial pathogens. The medicinal plants and the pathogens were taken from the same geographical regions, which are from the state of Assam in India. The ethanolic extracts of the medicinal plants selected were observed to possess significant antimicrobial properties which had been tested using agar well diffusion. They also tend to possess a wide range of varying MIC values for the pathogens which range from 1.78 mg/mL to >100 mg/mL. These results suggest that the effects of compounds are variable and specific against certain pathogens. A combinatorial approach may be considered for better results and for making broad-spectrum activity. The presence of phytochemicals such as tannins, alkaloids, saponins, glycosides, steroids, terpenoids, flavonoids, and phenols in plant extracts was determined which indicated the medicinal values of these plants and their potential to be used in the development of medications. Also, the plant extracts showed stability at extreme temperatures and pH by retaining their antimicrobial activity even though certain variations in their effectiveness were observed. While exposure to higher temperatures displayed improved inhibition activity in some plant extracts and reduced activity in some, alkaline pH showed more detrimental antimicrobial activity of the extracts compared to acidic pH.

Based on further research on these phytochemicals, the local tribals who rely on these medicines can be guided about the disease-specific use of plants.

Declarations

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Author contributions

Conceptualization, S.P.N.; Methodology, G.D.M. and J.B.; Software, G.D.M., J.B. and S.M.; Validation, G.D.M., J.B. and S.M.; Formal Analysis, G.D.M., J.B. and S.M.; Investigation, G.D.M., J.B. and S.M.; Resources, S.D., L.S. and S.P.N.; Data Curation, G.D.M. and J.B.; Writing – Original Draft Preparation, G.D.M. and J.B.; Writing – Review & Editing, G.D.M., J.B. and S.M.; Visualization, S.P.N.; Supervision, S.P.N.; Project Administration, S.P.N.; Funding Acquisition, S.P.N..

Conflicts of interest

Authors declare no conflict of interest.

Data availability

All data generated or analyzed during this study are included in this article.

Ethics approval

Not applicable.

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Perceived COVID-19 anxiety, fear and depression levels among university students during the decreased restrictions of COVID-19 pandemic

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ABSTRACT

Introduction and aim. The COVID-19 pandemic has negatively impacted human life. It is observed that a significant group affected by this situation are university students. Evaluating the psychological states of students during the transition to normal life from the pandemic period is important. This study was conducted to examine the perceived levels of COVID-19 anxiety, fear, and depression among university students during the decreased restrictions of the COVID-19 pandemic.

Material and methods. This cross-sectional study was conducted with 1170 university students. The data were collected using “the Introductory Information Form, the Fear of COVID-19 Scale, the Coronavirus Anxiety Scale – Short Form, and the Depression Scale.

Results. It was observed that there was a difference between gender, class, education level, income level, having had COVID-19, and having a deceased relative due to COVID-19 and perceived COVID-19 anxiety and fear and depression levels among university students ($p < 0.05$).

Conclusion. During the COVID-19 pandemic, although restrictions have been lifted, students still experience moderate levels of COVID-19 anxiety, fear, and depression. Therefore, considering the factors that affect university students’ mental state, there is a need for services that support students’ mental well-being.

Keywords. anxiety, depression, fear, pandemic, university students

Introduction

The coronavirus is a pandemic disease that affects many areas of life, including physical, social, and economic aspects, and has caused deaths.¹ According to the World Health Organization’s report from March 2022, COVID-19 has led to more than 5.9 million deaths.² This disease not only poses a threat to the health of middle-aged and elderly individuals but also affects children.³ It has been reported that COVID-19 has resulted in deaths among individuals aged 20 and under, accounting for 4% of total number of COVID-19 deaths.⁴

COVID-19, with its rapid spread and deadly consequences, has affected people of all ages. University stu-

dents, who are in a period of their lives where they need to be independent and self-sufficient, have been particularly negatively affected in both their physical and mental health.⁵ When reviewing the literature, it can be seen that the COVID-19 pandemic has brought about various challenges such as restrictions on disease prevention, disruption to educational processes, fear of the disease, living with parents, and increased use of digital devices.^{6,7} It has been reported that the mental health of university students was negatively affected. During the COVID-19 process, mental problems such as fear, anxiety, depression, and addiction are reported to be experienced by students.⁸⁻¹⁰ In one study, it was reported that

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approximately one-third of adults experienced anxiety or depression, and this rate doubled especially at young age. It has been reported that young people aged 18–24 are at risk of depression and anxiety.¹¹ These psychological problems are also associated with gender, place of residence, medical history, substance use, weak and moderate levels of social support, lack of knowledge, and income status.^{7,12} During the period when the Omicron variant of the COVID-19 virus was detected, pandemic restrictions were being relaxed and the world was beginning to move towards normalization. However, it has been reported that the Omicron virus is more prevalent among young people and spreads rapidly. Therefore, the study was designed to assess the impact of COVID-19 on university students during the decreased restrictions of the COVID-19 pandemic.

Aim

The aims of the study were to assess the perceived COVID-19 anxiety and fear, as well as depression levels among university students and identify the factors associated with perceived COVID-19 anxiety, fear, and depression levels among university students. Moreover, the aim was to determine the impact of COVID-19 on university students.

Material and methods

Design, sample and setting

This is a descriptive and cross-sectional study. The research was conducted between February 23 and March 31, 2022 at a university located in the eastern region of Türkiye. The population of the research consisted of 4116 undergraduate and associate degree students who registered for the 2022 Spring Semester at the university. During the period when the research was conducted, a sample of 1170 undergraduate and associate degree students voluntarily participated in the research, were reached, and filled out the data forms completely.

Data collection tools

In this study, university students were administered the Introductory Information Form, the Fear of COVID-19 Scale, the Coronavirus Anxiety Scale – Short Form, and the Depression Scale as data collection instruments.

University students' introductory information form

This form includes a total of 24 items that cover descriptive characteristics of university students such as age, gender, and educational status.

Fear of COVID-19 Scale

The Fear of COVID-19 Scale is a 7-item scale that was originally developed by Ahorsu et al.¹³ It was adapted to Turkish by Bakioğlu et al. in 2020, and in that study, it was found to have good reliability with a Cronbach's al-

pha coefficient of 0.82. The scale is based on a 5-point Likert-type response format, with no reverse-coded items. Scores on the scale range from 7 to 35, and the total score reflects an individual's level of fear of COVID-19, with higher scores indicating greater levels of fear.¹⁴ In the current study, the Cronbach's alpha coefficient of the scale was calculated to be 0.78.

The Coronavirus Anxiety Scale – Short form

The Coronavirus Anxiety Scale – Short form is a 5-point Likert-type scale with five items and one dimension. It was originally developed by Lee in 2020 and later adapted to Turkish by Biçer et al.^{15,16} The scale measures anxiety related to the COVID-19 pandemic and has a total score range of 0-20, with each item being scored on a scale of 0-5.

Beck Depression Scale

The Beck Depression Inventory is a scale developed by Beck et al. to measure the behavioral symptoms of depression in adolescents and adults. It consists of 21 items and was translated into Turkish and validated for reliability by Hisli. The items are statements that assess self-evaluation of depression on a scale from 0 to 3, depending on the severity of the depression. A higher score on the inventory indicates a higher level of depression symptoms. Scores on the inventory can be interpreted as follows: 0-9 indicates low levels of depression, 10-16 indicates mild levels, 17-29 indicates moderate levels, and 30-40 indicates severe levels of depression.¹⁷

Ethical approval

In order for the research to be conducted, permission was first obtained from the Ministry of Health (date and number: 2022-02-10T19-12-05). Then, ethical approval was obtained from a university's Non-Invasive Ethics Committee (decision no. 03 dated January 28, 2022 and letter no. 42594 dated February 7, 2022). Legal permission was obtained from the university rectorate (letter no. E.44209 dated February 17, 2022) to conduct the research on students at the university. The principles of "Informed Consent," "Voluntariness," and "Protection of Privacy," which require the protection of individual rights, were fulfilled in the research. The Helsinki Declaration was adhered to during the research process.

Data collection and analysis

The data were collected between February 23rd and March 31st, 2022. After obtaining the necessary permissions, data forms were created using Google Forms. The data forms were then shared with the university students' advisors, who shared the links to the forms in WhatsApp groups they had created within the Provincial Directorate of National Education. Only completely filled out questionnaires were considered for assessment. During the specified period, parents were

asked to fill out the data forms online. Data analysis was performed using SPSS software version 22. normality test was conducted on the data. The data was analyzed using descriptive statistics such as numbers, percentages, means, t-tests, Mann-Whitney U tests, Kruskal-Wallis tests, and correlation tests.

Results

The average perceived COVID-19 anxiety score for female university students (75.8%) was 5.11 ± 4.62 , the perceived COVID-19 fear score was 16.86 ± 7.18 , and the depression score was 20.72 ± 11.48 . The perceived COVID-19 anxiety, fear, and depression scores of female university students were found to be statistically significantly higher than those of male students (24.2%) ($p < 0.05$). The average perceived COVID-19 anxiety score of undergraduate students (69%) was 4.86 ± 4.57 , and the average perceived COVID-19 fear score was 16.34 ± 7.06 . The perceived COVID-19 fear level among undergraduate students was higher than that of associate degree students (31%), and this difference was statistically significant ($p < 0.05$). On the other hand, the average depression score of associate degree students (21.29 ± 12.60) was higher than that of undergraduate students, and this difference was also statistically significant ($p < 0.05$).

The average perceived COVID-19 anxiety score for students living in dormitories was 4.89 ± 4.58 , the perceived COVID-19 fear score was 16.32 ± 7.36 , and the depression score was 20.54 ± 11.74 , with a percentage of 87.6%. The perceived COVID-19 anxiety, fear, and depression scores of students living in dormitories were found to be statistically more significant and higher than those living at home (12.4%) ($p < 0.05$). The average perceived COVID-19 anxiety score for students (82.4%) with insufficient income to meet their needs was 4.94 ± 4.63 , the perceived COVID-19 fear score was 16.21 ± 7.39 , and the depression score was 21.13 ± 11.88 . The perceived COVID-19 anxiety, fear, and depression scores of students with insufficient income to meet their needs were statistically significant and higher than those of students with sufficient income (17.6%) ($p < 0.05$). The average perceived COVID-19 anxiety score for students who have had COVID-19 (34.4%) was 5.36 ± 4.89 , the perceived COVID-19 fear score was 16.50 ± 7.42 , and the depression score was 21.88 ± 12.3 . The COVID-19 anxiety and depression scores for students who have had COVID-19 were found to be statistically significant and higher compared to students who have not had COVID-19 (65.6%) ($p < 0.05$). The perceived COVID-19 anxiety score of students who have lost a close relative due to COVID-19 (46.5%) was found to be 5.38 ± 4.74 , the perceived COVID-19 fear score was 17.07 ± 7.44 , and the depression score was 21.32 ± 11.73 . The COVID-19 anxiety, fear, and depression scores of students who have

lost a close relative to COVID-19 were statistically significant and higher than those who did not lose a close relative (53.5%) to the disease ($p < 0.05$). The average perceived COVID-19 anxiety score of students who have received the COVID-19 vaccine (95.3%) was 4.77 ± 4.59 , the perceived COVID-19 fear score was 16.11 ± 7.24 , and the depression score was 20.31 ± 11.7 . The COVID-19 anxiety, fear, and depression scores of students who have received the vaccine were found to be statistically significant and higher than those who have not received the vaccine (4.7%) ($p < 0.05$) (Table 1).

Table 1. Mean scores of Perceived Fear, Anxiety, and Depression according to the descriptive characteristics of university students

Characteristics	n	%	COVID-19 Anxiety M±SD	COVID-19 Fear M±SD	Beck Depression M±SD
Age	21.20±2.05 (Min=18, Max= 41)				
Gender					
Female	886	75.8	5.11±4.62	16.86±7.18	20.72±11.48
Male	284	24.2	3.54±4.38	13.26±6.88	19.01±12.98
Test/p			t=5.020 p<0.001	t=7.410 p<0.001	t=2.117 p=0.03
Education status					
Associate degree	363	31.0	4.44±4.69	15.23±7.67	21.29±12.60
Undergraduate	807	69.0	4.86±4.57	16.34±7.06	19.87±11.51
Test/p			t=-1.442 p=0.15	t=-2.433 p=0.01	t=1.896 p=0.05
Class					
1	400	34.2	3.97±4.09	15.38±7.09	20.36±11.88
2	493	42.1	5.10±4.99	16.48±7.57	20.58±12.36
3	226	19.3	5.37±4.49	16.30±7.00	20.14±10.98
4	51	4.4	4.25±4.57	14.84±6.55	18.03±10.73
Test/p			KW=17.644 p=0.001	KW=6.000 p=0.11	KW=1.555 p=0.67
Accommodation					
Dormitory	1025	87.6	4.89±4.58	16.32±7.36	20.54±11.74
Home	45	12.4	3.60±4.71	13.68±6.16	18.68±12.68
Test/p			MU=58592.0 p<0.001	MU=59009.5 p<0.001	MU=67298.0 p=0.05
Perceived average income					
Insufficient income	963	82.4	4.94±4.63	16.21±7.39	21.13±11.88
sufficient income	207	17.6	3.75±4.43	15.00±6.60	16.43±11.04
Test/p			t=3.371 p=0.001	t=2.159 p=0.03	t=5.214 p<0.001
COVID-19 infection status					
Yes	403	34.4	5.36±4.89	16.50±7.42	21.88±12.31
No	767	65.6	4.38±4.42	15.73±7.19	19.48±11.56
Test/p			t=3.454 p=0.001	t=1.729 p=0.08	t=3.293 p=0.001
Having a relative dying due to COVID-19					
Yes	544	46.5	5.38±4.74	17.07±7.44	21.32±11.73
No	626	53.5	4.16±4.43	15.07±7.00	19.42±11.93
Test/p			t=4.526 p<0.001	t=4.739 p<0.001	t=2.731 p=0.006
Being vaccinated COVID-19					
Yes	1115	95.3	4.77±4.59	16.11±7.24	20.31±11.70
No	55	4.7	3.82±5.13	13.60±7.65	19.90±14.93
Test/p			MU=22974.0 p=0.03	MU=21337.0 p=0.005	MU=25996.50 p=0.41

Table 2. Levels of Perceived Anxiety, Fear, and Depression According to the Impact of COVID-19

Characteristics	n	%	COVID-19 Anxiety	COVID-19 Fear	Beck Depression
The situation that negatively affect life					
Yes	1082	92.5	4.89±4.60	16.37±7.26	20.46±18.38
No	88	7.5	2.76±4.36	11.43±5.8	18.38±11.4
Test/p			MU=31848.5 p<0.001	MU=27141.5 p<0.001	MU=42491.5 p=0.09
The situation that affect the economic situation					
Yes	1095	93.6	4.83±4.62	16.19±7.26	20.56±11.80
No	75	6.4	3.18±4.20	13.25±6.89	16.66±12.36
Test/p			MU=31167.0 p<0.001	MU=30340.5 p<0.001	MU=32382.0 p=0.002
The situation that affect the education status					
Yes	1039	88.8	4.92±4.62	16.30±7.30	20.58±11.91
No	131	11.2	3.18±4.24	13.62±6.63	18.15±11.34
Test/p			MU=50761.5 p<0.001	MU=52772.0 p<0.001	MU=59474.5 p=0.01
The situation that follow COVID-19 news					
Yes	872	74.5	5.12±4.63	16.81±7.27	20.08±11.81
No	298	25.5	3.58±4.49	13.61±6.73	20.96±12.06
Test/p			MU=101511.0 p<0.001	MU=94545.0 p<0.001	MU=124416.0 p=0.27

The perceived COVID-19 anxiety score average among students who reported that COVID-19 had a negative impact on their lives was 4.89±4.60, the perceived COVID-19 fear score average was 16.37±7.26, and the depression score average was 20.46±18.38. The COVID-19 anxiety, fear, and depression scores were statistically significantly higher among students who reported that COVID-19 had a negative impact on their lives compared to those who reported no impact (p<0.05). The perceived COVID-19 anxiety score of students (93.6%) who were negatively affected by the COVID-19 pandemic on the economic situation was 4.83±4.62, perceived COVID-19 fear score was 16.19±7.26, and depression score was 20.56±11.80. It was observed that the perceived COVID-19 anxiety, fear, and depression scores of students who were negatively affected by COVID-19 were statistically significantly higher than those who were not affected (6.4%) (p<0.05). The average perceived COVID-19 anxiety score for students (88.8%) affected by the COVID-19 education disruption was 4.92±4.62, the perceived COVID-19 fear score was 16.30±7.30, and the depression score was 20.58±11.91. It was found that the perceived COVID-19 anxiety, fear, and depression scores of students affected by COVID-19 education disruption were statistically significantly higher than those who were not affected (11.2%) (p<0.05). The perceived COVID-19 anxiety score average is 5.12±4.63 and the COVID-19 fear score average is 16.81±7.27 among students who follow COVID-19 news (74.5%). The

COVID-19 anxiety and fear score averages of students who follow COVID-19 news were found to be statistically significant and higher than those who do not follow COVID-19 news (25.5%) (p<0.05) (Table 2).

The average perceived COVID-19 anxiety score for university students was calculated as 4.73±4.61, the COVID-19 fear score as 16.00±7.27, and the Beck depression score as 20.31±11.87 (Table 3).

Table 3. Distribution of total scale point averages of COVID-19 Anxiety, Fear, and Beck Depression perceived by university students

Scale	Mean	±SD	Min	Max
COVID-19 Anxiety	4.73	4.61	0	20
COVID-19 Fear	16.00	7.27	7	34
Beck Depression	20.31	11.87	0	60

Positive and statistically significant correlation was found between COVID-19 anxiety, fear, and Beck depression mean scores among university students (p<0.05) (Table 4).

Table 4. The Correlation between COVID-19 Anxiety, Fear, and Depression mean scores^a

Factors	COVID-19 Anxiety	COVID-19 Fear	Beck Depression
COVID-19 Anxiety		0.630**	0.350**
COVID-19 Fear	0.630**		0.303**
Beck Depression	0.350**	0.303**	

a**– p<0.001

Discussion

COVID-19 continues to affect life in many areas. In addition to being a physical health emergency, COVID-19 is described as a crisis related to an unnatural trauma that also affects mental health.¹⁸ To control the disease during the COVID-19 pandemic, many countries have implemented rules such as isolation and quarantine for protection purposes. However, this situation has led to negative effects on human relationships, causing mental health problems such as loneliness, fatigue, anger, anxiety, fear, and depression.^{16,19} Yang et al. have reported that stressors related to COVID-19 will have a long-term impact on adolescents.²⁰

In this study conducted during a period when quarantine was not mandatory and restrictions were relaxed, a positive relationship was found between perceived COVID-19 anxiety, fear levels, and depression levels among university students. When similar studies on the subject were examined, it was observed that the average anxiety scores of students ranged from 3.34, 4.72±4.45, 7.74 to 8.36; average fear score ranges from 14.90, 14.95, 16.87 to 22.42 and average depression scores were 5.92, 6.59±5.44, 9.24 and 12.58.^{5,8,10,18,21-24} The score ranges indicated on the scales represent moderate levels of anxiety and depression in students. The COVID-19

quarantine period and its aftermath have shown that levels of anxiety and depression are still moderate.

In this study, it was found that female university students who reside in dormitories, have insufficient income to meet their needs, have received the COVID-19 vaccine, and have lost a loved one due to COVID-19, had high levels of perceived COVID-19 anxiety, fear, and depression. COVID-19 anxiety and depression scores were also higher for university students who had recovered from the disease, while fear levels were higher for undergraduate students. Similar studies have shown that being female, being in quarantine or isolation, starting school later, experiencing financial difficulties, having low income, and having a family member with COVID-19 are all associated with anxiety, fear, and depression.^{5-7,20} Concern about the disease, uncertainty about the future, and feelings of loneliness have also been reported to be related to fear and anxiety.^{9,24} Infection fear, protective measures, monotony, lack of knowledge, decreased family income, or loss experienced have been reported to cause fluctuations in adolescent mental health and have negative effects on mental health.²⁵ Hu and Quian reported an increase in behavioral problems and peer relationship problems, but a decrease in psychosocial tendencies.²⁶

In this study, it was found that students who reported that COVID-19 had a negative impact on their daily lives, economic situation, and education also had higher levels of COVID-19 anxiety, fear, and depression. Students who followed COVID-19 news more closely had lower levels of COVID-19 anxiety and fear. University students are more susceptible to psychosocial problems compared to other individuals. Akin-odeyne et al. reported that 26.4% of students experienced financial difficulties during the COVID-19 pandemic, 55.8% had negative effects on their friendships, and more than 40% reported negative impacts on their academic life.²⁵ Piya et al. found that 13.5% of students were unable to focus on their studies and 54% spent most of their time on social media.²¹ Ünal et al. found that university students had a high level of knowledge about COVID-19, and the majority of participants experienced normal levels of depression, anxiety, and stress.²⁷ This was attributed to the high level of knowledge about the disease, low risk perception, and living with parents. Visser et al. reported that during the quarantine period, individuals experienced significant distress, had difficulty adapting academically, and felt socially isolated, leading to emotional challenges.²⁸ Leroy et al. showed that students studying in health and non-health fields had a lower likelihood of showing poor mental health outcomes and lower risks of depression.²⁹

Conclusion

It has been observed that university students have moderate levels of COVID-19 anxiety, fear, and depression,

despite the relaxation of pandemic restrictions. These mental health issues were found to be more prevalent among female students, those living in dormitories, those with low incomes, those who have lost a relative, those whose lives have been negatively impacted by the disease, those experiencing economic difficulties, those whose education has been affected, and those who follow news about the disease. It was also determined that COVID-19 anxiety, fear, and depression levels trigger each other. Therefore, there is a need for support services to reduce these mental health problems among students. In this context, it is important for groups that provide services specifically for university students to actively use integrated counseling services with healthcare services.

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Declarations

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Author contributions

Conceptualization, K.B.; Methodology, K.B.; Software, K.B.; Validation, K.B.; Formal Analysis, K.B.; Investigation, K.B.; Resources, K.B.; Data Curation, K.B.; Writing – Original Draft Preparation, K.B.; Writing – Review & Editing, K.B.; Visualization, K.B.; Supervision, K.B.; Project Administration, K.B.

Conflicts of interest

No potential conflict of interest was reported by the author.

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

Written permissions were obtained from the Non-Interventional Research Ethics Committee of a University (decision no. 03 dated January 28, 2022 and letter no. 42594 dated February 7, 2022).

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ORIGINAL PAPER

Assessment of the quality of life in type 2 diabetes patients – a comparative study of WHOQOL-BREF and DQOL instruments

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ABSTRACT

Introduction and aim. Type 2 diabetes mellitus (T2DM) is a prevalent health issue that significantly impacts the quality of life of patients. Evaluating quality of life in T2DM patients is crucial for understanding the broader effects of the disease and improving patient care. This study aims to evaluate and compare the effectiveness of the WHOQOL-BREF and DQOL instruments in measuring the quality of life of T2DM patients.

Material and methods. A descriptive quantitative study was conducted from March to June 2023 at Puskesmas Wanadadi 1 Banjarnegara. The sample consisted of 195 T2DM patients selected using purposive sampling based on the following inclusion criteria: diagnosed with T2DM for at least one year, aged 30-70 years, willing to participate, and able to complete the questionnaires. Data were collected using the WHOQOL-BREF and DQOL questionnaires and analyzed descriptively.

Results. The analysis revealed that the WHOQOL-BREF and DQOL instruments provided comprehensive insights into the quality of life of T2DM patients. The WHOQOL-BREF was particularly effective in assessing physical and environmental domains ($p < 0.05$), while the DQOL was more sensitive to diabetes-specific concerns and psychological well-being ($p < 0.05$).

Conclusion. Both the WHOQOL-BREF and DQOL are effective in evaluating the quality of life of T2DM patients, each offering unique strengths in different domains. The findings suggest that a combined use of these instruments could provide a more holistic understanding of the quality of life impacts in T2DM patients, guiding more targeted interventions to improve patient outcomes.

Keywords. DQOL, quality of life, type 2 diabetes mellitus, WHOQOL-BREF

Introduction

Type 2 diabetes mellitus (T2DM) is a growing health issue commonly encountered in society and is one of the four major non-communicable diseases of global concern. Over the past decade, there has been a significant increase in the prevalence of this disease, highlighting its seriousness.¹ T2DM, also known to the public as a blood sugar disease, is a chronic condition character-

ized by elevated blood sugar levels due to a dysfunctional metabolic system and the inability to produce insulin as needed.²

According to the World Health Organization (WHO), quality of life is defined as an individual's perception of their position in life within the context of the culture and value systems in which they live, and in relation to their goals, expectations, standards, and con-

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cerns.³ Generally, there are six aspects used to measure quality of life: physical health, psychological well-being, level of independence, social relationships, environment, and spirituality.^{4,5} Living with T2DM can negatively impact the quality of life of patients, whether or not complications are present.^{6,7} Spousal support encompasses all forms of positive behavior and attitudes provided to individuals who are ill or experiencing health problems, offering physical and psychological comfort, accelerating recovery, enhancing immunity, and reducing stress and psychological disorders.^{8,9}

Improving the quality of life for T2DM patients requires consistent motivation and support from close relatives and good social interactions. Tangible support for DM patients is also crucial to achieving the desired health outcomes.^{10,11} One study reports that among 59% of respondents who had suffered from T2DM for more than five years, 53% had a moderate quality of life.¹² Meanwhile, members of the diabetes community at Ngrambe Health Center had a better quality of life compared to non-members, with 70% of community members having a good quality of life compared to 52.5% of non-members with poor quality of life.¹³ Another study also showed a relationship between social support and the quality of life of T2DM patients.¹⁴ The results revealed a negative relationship between emotional distress and quality of life, and a positive relationship between social support and the quality of life of elderly T2DM patients.

Although numerous studies have investigated the quality of life in T2DM patients, there is a notable gap in the literature regarding a direct comparison between the two most commonly used quality of life measurement instruments, WHOQOL-BREF and DQOL.¹⁵ Most studies employ only one of these instruments without comparing their effectiveness and reliability in assessing the quality of life among the T2DM population.¹ Additionally, existing research often fails to provide an in-depth understanding of how each instrument identifies specific aspects of quality of life most affected by T2DM.^{16,17}

This study aims to fill this knowledge gap by directly comparing WHOQOL-BREF and DQOL in assessing the quality of life in T2DM patients. By evaluating these two instruments comprehensively, this research hopes to reveal valuable information about the strengths and weaknesses of each instrument, as well as their relative contributions to understanding the quality of life in T2DM patients. The results of this study can offer clearer guidance for healthcare practitioners in choosing the most appropriate instrument for measuring quality of life in the T2DM population. Furthermore, this research focuses on the T2DM patient population in a specific region, providing important local perspectives within a global context.

Aim

This study aims to evaluate and compare the effectiveness of two commonly used quality of life instruments, WHOQOL-BREF and DQOL, in measuring the quality of life in patients with type 2 diabetes mellitus (T2DM). Additionally, this research seeks to identify specific aspects of quality of life that are most affected by T2DM, and how these two instruments measure and reflect changes in these aspects.

Material and methods

Study design

This study uses a descriptive quantitative approach, aimed at providing a more detailed description of certain symptoms or phenomena. The research design is a predetermined strategy to achieve the research objectives and serves as a guideline in the implementation of the study. This design is used to determine the quality of life of patients with T2DM in Banjarnegara, Central Java.

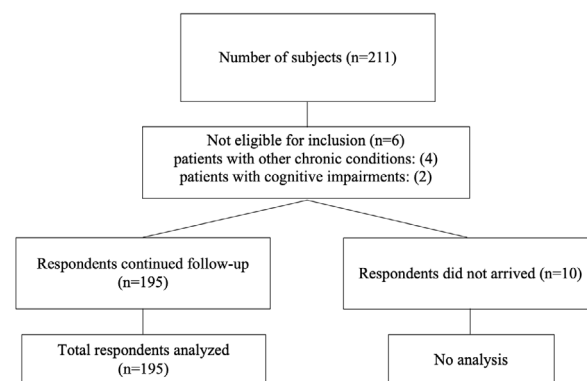


Fig. 1. Flow diagram for participant assignment in this study

This study was conducted from March to June 2023, with the sample criteria being patients with T2DM. Some of the inclusion criteria for determining the sample are patients diagnosed with T2DM for at least one year, aged 30-70 years, willing to participate and provide written consent, patients actively participate in activities and health checks at routine health services every month, are able to read and write, and patients who have stable diabetes conditions without major changes in treatment or complications in the last three months, and able to understand and complete the questionnaires. The exclusion criteria include patients with severe diabetes complications (e.g., severe diabetic neuropathy, diabetic ketoacidosis, or retinopathy leading to blindness), patients with other chronic conditions that significantly affect quality of life (e.g., advanced cancer, chronic heart failure), patients who have recently experienced an acute condition or undergone major surgery in the last three months, female patients who are pregnant, and patients with cognitive impairments or mental health conditions that interfere

with their ability to complete the questionnaires. The total number of respondents was 195 patients

Instruments

The instruments used in this study are the WHOQOL-BREF questionnaire and the DQOL questionnaire, which are used to measure the quality of life of T2DM patients. Primary data were obtained directly from T2DM patients in the form of quality of life data. Secondary data were taken from medical records, including information on gender, occupation, age, and duration of T2DM. WHOQOL-BREF can be completed by the patient himself (self-administered) or through an interview guided by researchers. Each item is rated on a 5-point Likert scale, from 1 (very dissatisfied) to 5 (very satisfied). A total score was calculated for each domain, which was then converted to a 0-100 scale for ease of interpretation. Higher scores indicate better quality of life. Statistical analysis can be performed to compare scores between groups, such as in your study comparing patients with T2DM. The Cronbach's alpha coefficient value for the WHOQOL-BREF instrument consists of the Physical Domain 0.82, the Psychological Domain 0.78, the Social Relations Domain 0.76, and the Environmental Domain 0.79. Overall, Cronbach's alpha values for the WHOQOL-BREF instrument are often above 0.70, indicating a good level of reliability.

The WHOQOL-BREF is a shortened version of the WHOQOL-100, designed to broadly assess the quality of life in four domains: physical, psychological, social relationships, and environment. Although not exclusively measuring sleep quality, the questionnaire includes relevant questions about sleep within the physical and psychological domains. The questionnaire encompasses several parameters, including questions about satisfaction levels with various aspects of life, evaluations of physical and psychological health, and satisfaction with social relationships and the environment. The final score or assessment results of the WHOQOL-BREF can be calculated by combining the responses to all questions. Domains are not given a score, if $\geq 20\%$ of questions were not answered by respondents.¹⁸

The Diabetes Quality of Life Questionnaire (DQOL) is an assessment tool used to measure the impact of diabetes on an individual's quality of life. The DQOL questionnaire consists of several parameters including Feelings about diabetes management, impact of diabetes on daily life, discomfort caused by diabetes, concerns about the development of diabetes complications, and concerns about dependence on others in managing diabetes. The Diabetes Quality of Life (DQOL) instrument is an assessment tool specifically designed to measure the quality of life in diabetes patients. This instrument was developed by the Diabetes Control and Complications Trial (DCCT) and is widely used in research and clinical

practice to understand the impact of diabetes and its treatment on patients' quality of life. DQOL consists of 46 items divided into four main domains, namely satisfaction, impact, worry, and social/vocational concerns. DQOL can be filled in by the patient himself (self-administered) or through interviews guided by researchers. Each item is rated on a 5-point Likert scale, from 1 (never) to 5 (always). A total score was calculated for each domain, with lower scores indicating better quality of life. DQOL scores allow researchers to identify specific areas of a patient's life most affected by diabetes, so that more appropriate interventions can be designed.¹² The Cronbach's alpha coefficient values for the DQOL instrument are satisfaction 0.82, impact 0.76, worry 0.77, and social/vocational concerns 0.78. Overall, Cronbach's alpha values for the DQOL are usually above 0.75, indicating that this instrument has good internal consistency.

Data analysis

The sample in this study was taken using purposive sampling, a technique with criteria determined by the researcher. Data obtained from the questionnaires will be analyzed descriptively to provide a detailed overview of the quality of life of T2DM patients at Puskesmas Wanadadi 1 Banjarnegara. The analysis is performed to observe the frequency distribution and relationships between the variables studied, such as age, gender, occupation, and duration of the disease, with quality of life. We used IBM SPSS version 26 software (Armonk, NY, USA) for the statistical analysis of the research results.

Ethical approval

This study has received ethical approval from the Health Research Ethics Committee with number 037.6/II.3.AU/F/KEPK/II/2023. All respondents were given complete information about the study's purpose and procedures, and their participation was voluntary. The researcher ensures the confidentiality of respondents' personal data and that it is only used for the purposes of this study. Written consent from each respondent was obtained before data collection began.

Results

The study involved 195 respondents who met the criteria. The data collection process took a considerable amount of time given the large number of respondents, and the questionnaires were administered twice, using both the WHOQOL-BREF and DQOL. All respondents actively participated. Data such as age, body mass index, and duration of diabetes were tested for normality, and the results indicated non-normal distribution with the Kolmogorov-Smirnov test ($p < 0.05$). The majority of respondents were unemployed women with a monthly income of less than 500,000 IDR, as shown in Table 1. Statistical analysis using the Spearman rank

test revealed differences in the analysis using WHOQOL-BREF and DQOL, particularly in respondents' body mass index and schedule blood sugar checks. Other variables showed significant correlations, such as monthly income, living at home, age, and duration of diabetes ($p < 0.05$), as shown in Table 2.

Table 1. Characteristic of respondent (n=195)

	n	%	Median (Min-Max)	Mean±SD
Sex				
Male	65	33.3		
Female	130	66.7		
Work				
Yes	101	51.8		
No	94	48.2		
Income or salary (IDR)				
< 500.000	118	60.5		
500.000–1.000.000	61	31.3		
> 1.000.000	16	8.2		
Stay at home				
Alone	80	41.4		
With family	115	58.6		
Schedule blood sugar checks				
Yes	72	36.9		
No	123	63.1		
Age			55 (42–63)	53.5±9.62
Body mass index			22 (17–27)	21.8±3.31
Long duration of DM (Month)			7 (1–25)	8.5±4.74
WHOQOL-BREF			72 (54–92)	71.6±10.8
DQOL			35 (22–48)	33.2±9.29

Table 2. Results of spearman test (n=195)

	WHOQOL-BREF		DQOL	
	r	p	r	p
Sex	0.057	0.430	0.029	0.692
Work	0.071	0.323	0.010	0.890
Income or salary (IDR)	0.230	0.017	0.030	0.019
Stay at home	0.170	0.032	0.289	0.014
Schedule blood sugar checks	0.058	0.422	0.122	0.008
Age	0.171	0.015	0.294	0.018
Body mass index	0.027	0.706	0.170	0.033
Long duration of DM (month)	0.277	0.028	0.185	0.023

Table 3. Results of Comparative Analysis WHOQOL-BREF and DQOL instruments (n=195)

	Mean ± SD		Std Error Mean	t	p
	WHOQOL-BREF	DQOL			
Physical	65.3±10.2	72.1±9.8	6.8	8.12	<0.001
Psychological	63.7±11	70.5±10.5	6.7	7.54	0.001
Social relation	60.4±12.5	68.3±11.7	7.9	6.89	<0.001
Environment	62.9±10.8	69.8±10.2	6.9	7.22	<0.001

Discussion

The higher prevalence of T2DM in women can be explained by several biological and social factors. Biologically, women tend to have different body fat distribution compared to men, which can increase insulin resistance and the risk of type 2 diabetes.¹⁹ Additionally, hormonal changes that occur during pregnancy (gestational dia-

betes) can increase the risk of developing T2DM later in life. Socially, women often bear more responsibility for household management and family care, which can limit the time and resources available for maintaining their own health.^{20,21} These factors can contribute to an unhealthy lifestyle and an increased risk of T2DM.

Low income in T2DM patients is often associated with decreased physical ability and activity as they age. Economic status plays a crucial role in determining individuals' access to the resources needed to maintain health, such as nutritious food, medications, and medical care. Low income limits patients' ability to obtain the necessary care to manage their diabetes effectively. Dependence on uncertain support from children and family adds a layer of uncertainty to patients' lives.^{22,23} Support from family and loved ones is essential in managing chronic diseases like diabetes. However, uncertain dependence can create additional stress and uncertainty, negatively impacting patients' quality of life. Low income and dependence on family assistance have a direct impact on various aspects of quality of life measured by WHOQOL-BREF and DQOL. WHOQOL-BREF encompasses dimensions of physical health, psychological well-being, social relationships, and the environment.²⁴ Low income can affect all these dimensions by limiting access to healthcare, increasing psychological stress, reducing opportunities to participate in social activities, and restricting the ability to create a healthy and safe living environment.²⁵

The variables of age and duration of T2DM have been proven to be relevant and correlated with the quality of life of patients, both when measured using WHOQOL-BREF and DQOL. This finding can be explained through several theoretical concepts relevant to the management of chronic diseases and quality of life.²⁶ As individuals age, various aspects of physical and mental health tend to decline. In DM patients, aging is usually accompanied by an increased risk of diabetes-related complications such as cardiovascular disease, neuropathy, nephropathy, and retinopathy. The theory of biological aging explains that as individuals age, bodily functions decline, which affects their ability to manage chronic diseases such as diabetes.²⁷

The WHOQOL-BREF questionnaire includes dimensions of physical and psychological health that are highly relevant to the effects of aging. In older patients, decreased physical function can lead to limited mobility, pain, and increased dependence on others, all of which have a negative impact on quality of life. Psychologically, aging is also often associated with an increased risk of depression and anxiety, which can further worsen diabetes management and quality of life.^{15,28}

The duration of suffering from DM is also an important variable that affects quality of life. The longer someone lives with diabetes, the greater the likelihood

of experiencing chronic complications that can worsen quality of life. The theory of adaptation to chronic illness suggests that while individuals may learn to adapt to their illness over time, long-term exposure to the physical and psychological stressors of diabetes can gradually decrease quality of life.²⁹

DQOL is more specific in capturing the nuances of living with diabetes over time. For example, variables such as the time required for blood sugar checks and the frequency of routine monitoring are highly relevant to patients who have lived with diabetes for a long time.³⁰ The longer time spent managing diabetes, the greater the burden felt by the patient, which can affect aspects of quality of life measured by DQOL, including physical discomfort, concerns about complications, and the burden of daily diabetes management.¹⁰

Research findings indicate significant differences in correlation tests measuring quality of life using WHOQOL-BREF and DQOL, particularly in the variables of blood sugar monitoring status and body mass index. In the DQOL questionnaire, these variables have a greater influence, which becomes even more relevant when further analyzed in the context of the time required to measure blood sugar levels. This item is not found in the WHOQOL-BREF questionnaire. Additionally, DQOL includes supportive items such as the frequency of routine check-ups or monitoring, which are not integrated into WHOQOL-BREF.¹⁷

The DQOL questionnaire is specifically designed to measure aspects of quality of life unique to diabetes patients. Blood sugar monitoring status and body mass index are crucial variables affecting daily diabetes management. Regular blood sugar monitoring and body mass index tracking are key components in effective diabetes management, as they are directly related to the risk of diabetes complications and the stability of the patient's health condition.³¹

In DQOL, items related to the time required to measure blood sugar levels are highly relevant because regular and rapid measurements provide the necessary information for immediate adjustments in diabetes management. For instance, patients can take corrective actions for hypoglycemia or hyperglycemia based on quick blood sugar test results. This is vital in preventing acute and chronic complications that can impact quality of life.

In this study, we compared two main instruments, WHOQOL-BREF and DQOL, to assess the quality of life of T2DM patients. Each instrument has advantages and disadvantages that are described based on the domains measured. The WHOQOL-BREF instrument has the advantage that in the physical domain it provides a comprehensive picture of daily activities, dependence on drugs and treatments, as well as other physical aspects such as energy, mobility and sleep.

This is important for understanding the impact of diabetes on general physical health. In the psychological domain, this instrument covers emotional aspects such as self-image, emotional status, and self-esteem, which are especially relevant for patients with chronic conditions such as diabetes. The social domain measures social support and the quality of personal relationships, which are important aspects of chronic disease management. While the environmental domain examines external factors such as financial resources, safety, and access to health services, providing a holistic view of quality of life. The disadvantage of the WHOQOL-BREF is that the instrument is designed for the general population and does not specifically capture the impact of diabetes on quality of life. Does not focus on specific aspects of diabetes such as concerns about complications or day-to-day diabetes management.

The advantage of the DQOL instrument is that it specifically assesses patient satisfaction with diabetes management, including medical care and glucose control, providing in-depth insight into the effectiveness of care. The impact domain, already focused on the impact of diabetes on daily activities and social functioning, provides specific information about how diabetes affects the patient's life. The worry domain, measures patient concerns about diabetes complications and related health problems, which is important for understanding the psychological burden of diabetes, and the social/vocational Concerns domain assesses the impact of diabetes on social interactions and work opportunities, important aspects for the quality of life of patients who are still actively working or interacting in society. Based on these advantages, DQOL still has disadvantages, namely that it does not cover several aspects measured by WHOQOL-BREF, such as environmental factors and physical safety, and although it is very detailed in the diabetes aspect, DQOL does not provide a holistic picture of overall quality of life.

Based on Table 3, it can be concluded that the DQOL instrument provides better results in assessing the quality of life of T2DM patients compared to the WHOQOL-BREF. The significant differences between these two instruments indicate that the DQOL is more sensitive in capturing specific aspects of quality of life that are affected by diabetes. Therefore, DQOL can be considered a more effective tool to evaluate the quality of life of T2DM in the context of this study.

DQOL better assesses physical aspects relevant to T2M conditions, such as glucose management and the physical impact of diabetes complications. Higher scores on the DQOL reflect that patients feel their quality of life is better in physical terms when assessed with this instrument. The DQOL is also more sensitive

in measuring the psychological impact of diabetes, including anxiety, depression, and stress related to managing chronic disease. The DQOL's ability to capture these psychological nuances makes it a better tool for evaluating patients' emotional well-being.

In the social domain, the DQOL appears to be more effective in evaluating the impact of diabetes on social interactions and the support patients receive from family and friends. This is important because social support is a key factor in managing chronic disease. DQOL also better measures specific environmental aspects related to diabetes patients' quality of life, such as access to health care, financial resources, and physical environments that support disease management.

DQOL also includes items regarding the frequency of routine check-ups or monitoring, which is an essential aspect of diabetes management. Routine check-ups allow for early detection of complications, adjustment of treatment, and evaluation of the effectiveness of diabetes management. The absence of this item in WHOQOL-BREF suggests that this questionnaire may be less sensitive in capturing the nuances of quality of life influenced by the frequency and efficiency of medical monitoring in diabetes patients.

Conclusion

The WHOQOL-BREF quality of life questionnaire is more comprehensive as it covers various aspects of quality of life, including physical health, psychological well-being, social relationships, and the environment. However, it is not specifically designed for patients with diabetes. On the other hand, the DQOL is recommended for measuring the quality of life in diabetes patients, as its name suggests. It is specifically designed for diabetes patients, making it better suited to capture the unique aspects of living with diabetes. This questionnaire covers various relevant aspects such as diabetes management, discomfort caused by diabetes, and concerns about complications.

Declarations

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Author contributions

Conceptualization, A.R. and K.N.; Methodology, A.R. and P.A.W.S.; Validation, S.W. and K.N.; Formal Analysis, P.A.W.S. and K.N.; Investigation, A.R.; Resources, A.R. and P.A.W.S.; Data Curation, P.A.W.S.; Writing – Preparation of Original Draft, A.R. and P.A.W.S.; Writing – Review & Editing, A.R. and P.A.W.S.; Visualization, P.A.W.S.; Supervision, A.R. and S.W.

Conflicts of interest

All author declare have no conflict of interest.

Ethics approval

This study was approved by the local ethics committee (Health Research Ethics Committee in Universitas Muhammadiyah Gombong, date: February 9, 2023 decision number: 037.6/II.3.AU/F/KEPK/II/2023).

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Comparison of novel bioactive, bioceramic materials in vital pulp therapy in deciduous teeth – a clinical in vivo study

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ABSTRACT

Introduction and aim. The use of modern bioceramic materials has enhanced the predictability of vital pulp therapy. This study aimed to assess the clinical success of Biodentine, modified NeoPutty mineral trioxide aggregate (MTA), and calcium hydroxide Ca(OH)₂ as pulp capping materials for indirect pulp capping in carious primary teeth.

Material and methods. Indirect pulp treatment (IPT) was performed on 36 deciduous molars in 36 patients who were presented with deep carious lesions. The teeth were randomly assigned to three groups: Biodentine (12 teeth), modified NeoPuttyMTA (12 teeth), and Ca(OH)₂ (12 teeth).

Results. A statistical analysis was conducted using SPSS software version 21.0. Pearson's Chi-square test was employed to compare success and failure rates among Biodentine, modified Neoputty MTA, and Ca(OH)₂ at three different time intervals (30, 90, and 180 days) and overall success and failure rates regardless of the time intervals. In the statistical analysis, different pulp capping materials yielded varying success rates. The modified NeoPutty MTA group demonstrated a success rate of 91.67%, the Biodentine group 83.33%, and the Ca(OH)₂ group 58.33% after 6 months. However, these differences were not statistically significant.

Conclusion. IPT with calcium silicate-based materials, such as Biodentine and modified NeoPutty MTA, showed superior results when compared to the use of calcium hydroxide Ca(OH)₂. Although differences in success rates were observed among the materials, they did not reach statistical significance.

Keywords. calcium hydroxide, children, deciduous teeth, pulp capping

Introduction

Vital pulp therapy represents a conservative and patient-friendly approach to addressing teeth with compromised pulp tissue, often resulting from dental caries, trauma, or restorative procedures.¹ In recent times, there has been ongoing debate among dentists regarding the best course of action for treating deep carious lesions, challenging the conventional belief in complete caries removal.² Traditionally, the emphasis was on complete-

ly removing all caries-affected dentin. However, current thinking advocates for a more conservative approach. It is now recognized that complete dentin removal may not always be necessary to halt the progression of caries effectively. In fact, striving to entirely excavate carious lesions carries the risk of inadvertently exposing the pulp, which can lead to the direct infiltration of harmful microorganisms into the pulp chamber, potentially compromising the treatment's success.³

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Recent studies have shed light on the fact that even after superficial caries removal, some residual microorganisms may persist within the dentin.⁴ Surprisingly, this isn't necessarily detrimental; in fact, it can trigger a mild inflammatory response within the pulp. This subtle inflammation is considered beneficial, as it can contribute to the natural regeneration of pulp tissue. Therefore, the management of deep carious lesions is now geared towards achieving a different goal. Rather than solely focusing on complete eradication of all carious tissue, the primary objective is to resolve the inflammation within the pulp and, critically, to preserve its vitality. This approach recognizes the potential for the pulp's inherent healing mechanisms to repair and regenerate tissue when given the opportunity without unnecessary intervention.⁵ The focus has shifted towards preserving pulp vitality and harnessing the body's own mechanisms for healing and repair. Indirect pulp therapy (IPT) is a valuable approach in pediatric dentistry, particularly for treating carious lesions in deciduous teeth. This conservative procedure involves selectively removing infected dentin while preserving a thin layer of affected dentin to avoid pulp exposure. After careful excavation, a biocompatible material, often mineral trioxide aggregate (MTA) or calcium hydroxide (CH), is placed over the remaining affected dentin to create a protective barrier and promote healing. IPT aims to halt the progression of caries, eliminate microbial activity, and encourage the natural healing processes within the pulp, ultimately preserving the vitality of the deciduous tooth. This approach is especially crucial in pediatric dentistry to maintain primary teeth's function, space for permanent teeth, and overall oral health in young patients. The choice of material plays a crucial role in the success of this procedure. Very few studies have reported on the clinical outcome of indirect pulp capping with Biodentine, CH, and MTA in deciduous teeth.⁶ There are some drawbacks of MTA and to overcome this we used the newer MTA, namely NeoPutty MTA (Nusmile, USA). The objective of indirect IPT in primary teeth is to treat deep carious lesions while preserving the vitality of the dental pulp. This conservative approach aims to prevent the progression of caries, avoid pulpal exposure, and stimulate the formation of reparative dentin. Among the materials used for IPT, Mineral Trioxide Aggregate (MTA), Biodentine, and Calcium Hydroxide are commonly compared due to their distinctive properties and clinical outcomes. MTA is renowned for its excellent sealing ability and biocompatibility. Biodentine is appreciated for its quick setting time and bioactivity, and calcium hydroxide is valued for its long history of use and cost-effectiveness. NeoPutty MTA was launched in 2020 by NuSmile (Houston, Texas) to minimize most of MTA's drawbacks. This material was said to be premixed, bioceramic, nonstaining, resin-free, and it does not dry out when used. NeoPutty MTA has been

shown to have similar physical and mechanical properties to traditional MTA materials, including good sealing ability, biocompatibility, and radiopacity. It has also been shown to have a shorter setting time than traditional MTA materials, which can reduce treatment time and improve patient comfort. This new material has antibacterial properties because of the alkaline pH of the material. Ideal endodontic cement should have bacteriostatic or bactericidal properties. MTA has the drawback of minimal antibacterial effect, especially against *Enterococcus faecalis*, which is the major microorganism responsible for the progression of pulpal and periradicular diseases and endodontic failures. *E. faecalis* forms a minor part of the microbial flora in uninstrumented canals, while it is a main etiologic factor for periradicular lesions that develop following the endodontic treatment. In various studies, adding certain additives, such as metallic silver, chlorhexidine, and calcium fluoride, has reduced the antibacterial counts. This comparison seeks to evaluate their effectiveness in achieving the primary objective of IPT - maintaining pulp vitality and promoting dentinogenesis in primary teeth.

Aim

This study reported the clinical success of three materials, Biodentine, CH, and modified NeoPutty MTA, used for indirect pulp capping of carious molars.

Material and methods

A clinical study was conducted, involving three experimental groups between March 2023 and August 2023. The trial received ethical approval and followed the guidelines outlined in CONSORT 2010. The study included participants aged between 4 and 7 years. Prior to enrollment, all parents were provided with detailed information about the treatment procedure, its potential benefits and drawbacks, as well as alternative treatment options. Signed Informed consent was obtained from all the parents of the children, before the procedure commenced. The inclusion and exclusion criteria were:

Inclusion criteria

1. Mild discomfort experienced in response to chemical and thermal stimuli.
2. Presence of active carious lesions affecting either the occlusal or proximal surfaces of primary molars.
3. Extent of carious lesion such that complete removal of caries would pose a risk of exposing the pulp.
4. Cooperative children and parents who are willing to adhere to instructions and attend scheduled follow-up appointments.

Exclusion criteria

1. History of spontaneous sharp, penetrating pain, or tenderness upon percussion.

2. Presence of abnormal tooth mobility, fistula formation, interrupted lamina dura, internal or external root resorption, interradicular or periapical pathosis, or an enlarged periodontal ligament space.
3. Presence of chronic systemic illnesses such as congenital or rheumatic heart disease, hepatitis, or leukemia.
4. Patients currently undergoing long-term medication regimens, particularly corticosteroid therapy.
5. Patients who are physically or mentally challenged.

The Institutional Ethical Clearance (IHEC/SDC/Pedo/2204/22/004) was obtained and children's parents were informed about the procedure and a written consent was obtained both in English and local language. The sample size for this study was determined to be 12 participants per group, following the guidelines outlined by Steven A. Julious, who recommends a minimum sample size of 12 for a pilot design. In total, 36 participants were enrolled in the study.⁷

In this clinical procedure, a total of 40 children initially diagnosed with deep dental caries were assessed, and 36 of them were selected to participate in the study. The assessment of pulp vitality included tooth sensitivity tests like thermal tests and pulse oximetry. Preoperative radiographs were taken to evaluate the condition of the periodontium and hard tissues. Local anesthesia, specifically 2% lidocaine hydrochloride with epinephrine 1:80,000 (Lignospan, Septodont, France), was administered either buccally via infiltration for maxillary teeth or by the infra-alveolar nerve block technique for mandibular teeth selected for the experiment. Isolation was achieved using a rubber dam (Hygienic; Coltene/Whaledent, USA). Caries removal was performed manually with a spoon excavator, followed by the use of a sterile BR 31 ball round bur (Mani Inc., Japan) mounted to a handpiece. Caries was removed until resistance was encountered during hand excavation or with the bur. If bleeding occurred during this process due to pulp exposure, the tooth with pulp exposure was excluded from the trial. To allocate each tooth after caries excavation to one of the three experimental groups, a simple randomization technique was employed.

The study involved three experimental groups, each with a distinct treatment approach:

Group 1: Dycal (Dentsply, USA) was prepared by mixing it on a manufacturer-provided paper pad. The resulting mixture was then placed in the base of the prepared cavity using a plastic filling instrument.

Group 2: Biodentine (Septodont, France) was manipulated following the manufacturer's guidelines. The prepared mixture was then placed in the base of the cavity.

Group 3: Modified NeoPuttyMTA (Nusmile, USA) was directly applied over the cavity base. Neoputty MTA was mixed with antibiotics (clindamycin 300 mg, ciprofloxacin 500 mg, and metronidazole 400 mg).

These distinct treatments were administered to the respective groups as part of the experimental procedure. The experimental materials were applied within the cavity floor with a thickness approximately ranging from 1 to 2 mm. Following this, the cavity was filled with direct glass-ionomer cement (GC Fuji IX, GC Japan). Occlusion was assessed during the one-month recall visit. Children were scheduled for follow-up appointments at 1, 3, and 6 months after the initial procedure. During these follow-up visits, the pulp-capped tooth was carefully examined and assessed using pulp sensitivity tests, which included cold and electrical tests. Clinical evaluation was also conducted to determine if there were any symptoms or signs of disease. For a tooth to be considered clinically successful in this study, it had to exhibit no symptoms of disease and respond within normal limits to sensitivity tests.

Statistical analysis

The statistical analysis was done using SPSS version 22.0 (IBM, Armonk, NY, USA) and the results have been given with each table below.

Results

A total of 36 children were included in this study, comprising 20 males and 16 females. The patient selection process and the treatment procedures performed are illustrated in Figure 1. Specifically, 12 molar teeth were subjected to indirect pulp capping with CH, another 12 with Modified NeoPutty MTA, and 12 with Biodentine. Among the 36 molars included in the trial, 8 were maxillary molars, and 28 were mandibular molars.

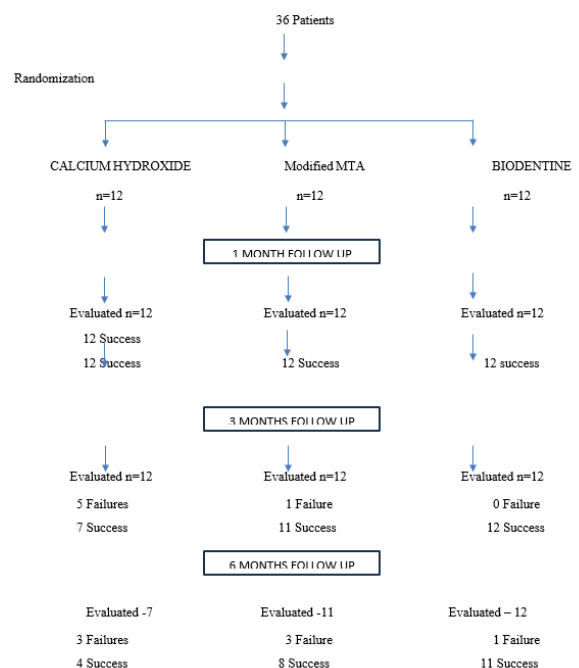


Fig. 1. Flowchart showing randomization of selected cases and review done

The success rates of the experimental materials at different intervals are outlined in Table 1. Biodentine exhibited a success rate of 91.67% at 3 months and 66.67% at 6 months. Modified NeoPutty MTA had a success rate of 100% at 3 months and 91.67% at 6 months, while CH showed a success rate of 58.33% at 3 months and 33.33% at 6 months.

Table 1. Clinical success of materials at different time periods*

	CH (n=12)	Biodentine (n=12)	Modified NeoPutty MTA (n=12)
1 month follow up	12/12 (100%)	12/12 (100%)	12/12 (100%)
3 months follow up	7/12 (58.33%)	11/12 (91.67%)	12/12 (100%)
6 months follow up	4/12 (33.33%)	8/12 (66.67%)	11/12 (91.67%)

* Chi square value=0.297; p=0.681

Table 2. Comparison between calcium hydroxide and Biodentine

	CH	Biodentine
1month	12	12
3 month	7	11
6 month	4	8
Chi square value; p	1.06; 0.585	

Table 3. Comparison between calcium hydroxide and modified NeoPutty MTA

	CH	Modified NeoPutty MTA
1 month	12	12
3 month	7	12
6 month	4	11
Chi square value; p	2.19; 0.33	

Table 4. Comparison between Biodentine and Modified NeoPutty MTA

	Biodentine	Modified NeoPutty MTA
1 month	12	12
3 month	11	12
6 month	8	11
Chi square value; p	0.275; 0.871	

The inter group comparisons between calcium hydroxide and Biodentine (Table 2), calcium hydroxide and modified Neoputty MTA (Table 3) and Biodentine and modified NeoPutty MTA (Table 4) did not show any statistically significant result.

Discussion

The objective of conservative dental treatment is to maintain the vitality of the pulp while ensuring its normal functions. This approach recognizes that only a pulp that is both vital and functional has the capacity to naturally heal itself by stimulating the formation of reparative dentin and resolving any inflammation.⁸ Our study focused on implementing a conservative approach for

the treatment of carious molar teeth by employing novel materials for indirect pulp capping. The primary objective of this trial was to assess the effectiveness of three different materials - CH, modified NeoPutty MTA, and Biodentine - in the context of indirect pulp capping for primary molars. This assessment was conducted through a comprehensive evaluation of pulp vitality and clinical observations over a period of six months. CH was chosen as one of the materials in the trial due to its historical use as the gold standard for pulp capping procedures over nearly a century. However, it is essential to note that CH is associated with several limitations, such as poor adhesion to dentin, susceptibility to dissolution, and the formation of tunnel-like defects in the resulting dentin bridge.⁹ However, there has been a noticeable shift in the preferences of clinicians when it comes to choosing pulp capping materials, transitioning from CH to MTA due to the more reliable and consistent outcomes associated with MTA.¹⁰ MTA is a material that was developed by modifying Portland cement and was introduced to the field in 1993.¹¹ Over time, it has gained significant recognition in the management of deep carious lesions. MTA is composed of several key components, including tricalcium silicate, tricalcium oxide, tricalcium aluminate, bismuth oxide, and silicate oxide. This calcium silicate-based pulp capping material plays a crucial role in initiating the formation of reparative dentin by promoting the release of growth factors and cytokines. However, it is essential to acknowledge that MTA does have certain limitations. These limitations include an extended setting time, the potential for tooth discoloration, and challenges associated with its handling, all of which can present obstacles to its ideal utilization in dental procedures.¹² To overcome these drawbacks NeoPutty MTA (Nusmile) was produced. The manufacturers claim that this material has better properties than traditional MTA, even better than the newer MTA available like ProRoot MTA (Dentsply, Tulsa, USA), MTA Angelus (Angelus, Brazil). NuSmile NeoPUTTY® is a premixed bioactive, bio-ceramic MTA that triggers hydroxyapatite and supports healing. NeoPUTTY is the preferable choice among pediatric dentists recently due to its firm, non-tacky consistency, resistance to washout, and notable bioactivity.¹³ This versatile material meets various pulp-related needs in pediatric dentistry. Moreover, NeoPUTTY offers the advantage of being ready-to-use, requiring no additional preparation, thus minimizing wastage. This not only saves costs but also reduces chair time, making it an efficient and practical choice for dental procedures. There has not been much clinical studies on Neoputty MTA. The modified NeoPutty was made with triple antibiotics to enhance the efficacy.

Studies conducted by Aeinehchi et al. have reported that MTA demonstrates a superior dentinogenic in-

duction rate when compared to CH.¹⁴ Two additional studies have conducted comparisons between calcium hydroxide and MTA, revealing significant findings. In one study involving a sample of 109 teeth, it was observed that the MTA group achieved a 100% success rate, the calcium hydroxide group achieved a 93.5% success rate, and the Glass Ionomer Cement (GIC) group achieved a 97% success rate. In another study conducted by George et al., which included a total sample size of 40, a notable difference was observed between MTA and Dycal in terms of their ability to promote reparative dentin formation. These findings highlight the distinct outcomes associated with different pulp capping materials.¹⁵ Biodentine, introduced in 2009, represents a significant advancement in dental materials. It offers enhanced biocompatibility, a clinically acceptable setting time, improved mechanical strength, better bonding with dentin surfaces, and greater ease of handling.¹⁶ Biodentine offers several distinct advantages compared to other products, including a shorter setting time of approximately 12 minutes, superior mechanical properties, and excellent sealing capabilities. In a study by Arora et al., it was noted that Biodentine's sealing ability closely resembled that of apatite crystals when observed under a scanning electron microscope.¹⁷ This characteristic positions Biodentine as a promising agent for vital pulp therapy (VPT) applications. Biodentine has the drawback of poor washout resistance and poor radiopacity, two properties which are essential for an Indirect pulp procedure.¹⁸ NeoPutty MTA has better radiopacity and washout resistance than Biodentine.

A clinical trial was conducted to assess the efficacy of IPT using CH, MTA, and Biodentine in primary molars. The study comprised a sample size of 45 primary molars, distributed evenly into three groups, with each group consisting of 15 teeth. The clinical outcomes revealed a 100% success rate across all three groups with a 6 month follow up. However, on radiographic evaluation, Biodentine demonstrated superiority compared to the other materials.⁶

In our trial, the modified NeoPutty MTA group demonstrated a remarkable success rate of 91.67%, while the CH group achieved a success rate of 58.33%, and the Biodentine group achieved an 83.33% success rate. These findings underscore the favorable attributes and clinical performance of NeoPutty MTA compared to the other materials tested in the study. The better results for modified NeoPutty MTA might be due to better washout resistance than CH and Biodentine and the antibiotics. In 2017, Saber, Meligy, and Alaki examined the use of Biodentine in the IPT (indirect pulp treatment) of primary molars.¹⁹ The study included 60 patients, and a split-mouth design was employed, with one side receiving IPT using Biodentine and the other side treated with calcium hydroxide. The 12-month

follow-up results revealed no statistically significant difference in success rates between the two groups. Notably, Biodentine's reduced cost compared to MTA has increased its accessibility for clinical use. Our study too showed a better clinical success of Biodentine in comparison to calcium hydroxide. In another study comparing Biodentine and Theracal for indirect pulp treatment for young permanent teeth, by the end of 24 months, 54 teeth presented for follow up with overall success rate of 100% in Theracal, 94.44% in Biodentine, and 77.78% in Dycal. Overall success of Theracal was statistically significant in comparison to Biodentine and Dycal at 24 months follow up ($p=0.03$).²⁰

Another factor that influences the success of IPT is the final restorative material. The bacterial leakage through the final restoration is considered to be highly detrimental. Neoputty MTA being a premixed material has the advantage of the placement of the final restoration immediately rather than waiting for the material to set. Neoputty MTA is a promising new material which has overcome many disadvantages of MTA. The modified NeoPutty we made with triple antibiotics is still better than NeoPuttyMTA.²¹ This is one of the first studies to compare golden standard for IPT, CH with newer materials Biodentine and modified Neoputty MTA in primary teeth.

Study limitations

The sample size although small is appropriate as derived by appropriate statistical tools. Follow up is less. The follow up could have been more to get better results in the study for a new material being tested.

Conclusion

According to the outcomes of our study, a better clinical success rate was observed with modified NeoPutty MTA than CH group and Biodentine group. Nonetheless, it is imperative to emphasize the need for subsequent research involving larger sample sizes and extended follow-up periods. Moreover, additional histological investigations are essential to provide robust support for these conclusions. In conclusion, while each material has its advantages and limitations, Biodentine and MTA generally provide better outcomes for IPC in primary teeth compared to CH. Biodentine's ease of use and quick setting time, combined with its biological properties, make it a favorable option. MTA's superior sealing and biocompatibility make it an excellent choice, albeit with higher cost and handling challenges. CH, while still effective, is often outperformed by the newer materials in terms of long-term success and pulp vitality preservation. Clinicians should consider these factors alongside patient-specific needs to choose the most appropriate material for IPC in primary teeth.

Declarations

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Author contributions

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

Conflicts of interest

The authors have no conflicts of interest to declare.

Data availability

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

Ethics approval

The Institutional Ethical Clearance (IHEC/SDC/Pedo/2204/22/004) was obtained.







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ORIGINAL PAPER

Assessment of behavior and barriers of post-exposure prophylaxis for prevention of rabies in patients attending a tertiary care center in eastern India – a mixed method approach

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ABSTRACT

Introduction and aim. Rabies from animal bites, although preventable, is almost 100% fatal, with the dog being the most common infected animal. Several factors influence the timely initiation of post-exposure prophylaxis (PEP) which needs to be explored. This study aimed to determine the facilitating and inhibiting factors in initiating PEP among animal bite victims attending a tertiary care center in Eastern India.

Material and methods. A cross-sectional study was conducted among 150 consenting animal bite victims attending casualty and medicine as out-patients in a tertiary care hospital. A mixed-method study approach was undertaken to explore the facilitating and inhibiting factors for the timely initiation of PEP using a pre-tested and semi-structured questionnaire and in-depth interviews. Data were analyzed using SPSS software version 20.

Results. The most common biting animal were dogs (96.2%). Around 102 patients (68%) were males and 48 (32%) were females. Timely initiation of PEP was seen in 112 (74.7%) of the study sample while delay was seen in 38 (25.3%). The inhibiting factors were the absence of an accompanying person (54.67%), the absence of vaccines and immunoglobulins in the nearest health facility (50.67%), referral from other health facilities (44%) and preferred other modalities of treatment (24.67%). Delays in initiation of PEP was significantly associated with gender ($p=0.018$), place of residence ($p<0.001$), those living more than 20 km from a health facility ($p=0.014$), educational status ($p<0.001$), and those with monthly income <10000 INR ($p<0.01$).

Conclusion. Lower economic status, education, and accessibility to health facilities were major inhibiting factors causing a delay in the initiation of PEP. Further, community-based studies are warranted.

Keywords. animal bite, behavior, cost, delay, post-exposure prophylaxis, rabies

Introduction

India is endemic for rabies. Rabies is almost 100% fatal, with the dog being the most common biting animal.¹ Globally, dog-mediated rabies causes about 59,000 deaths, out of which 35% occur in India, thereby making it the country with the highest fatality in Asia and the second highest in the world.² Although cases of dog bites

constitute a problem of considerable magnitude, not all victims of a dog bite seek timely medical help. Unquestionably, the community's level of knowledge and concern about dog bite injuries has an important role in dealing with this problem.³ People have very basic knowledge or have a misconception about rabies and its prevention. There are many myths and false beliefs associated

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with wound management. These include the application of oils, herbs and red chilies on the wounds inflicted by a rabid animal, and faith in indigenous medicines that have unproven efficiency.⁴ Rabies is a costly disease mainly because of the costs of post-exposure treatment (PET) in humans and vaccination programs in animals. Post-exposure treatment in humans accounts for the highest proportion of the costs of rabies control measures.⁵ Though in our institution vaccines as well as immunoglobulin are given free of cost, still, it is presumed that multiple visits by the victims to the health center for vaccination may impose a financial burden. Although many articles were found regarding the awareness and knowledge of wound management and rabies, there is a lack of literature about the factors influencing the timely initiation of post-exposure prophylaxis (PEP) in animal bite victims. Also, there are very few studies on the indirect cost involved in PEP.

Aim

This study was undertaken to determine the various factors influencing the timely initiation of anti-rabies PEP. We have also tried to ascertain the first aid measures adopted to the victim's post-animal bite and the indirect cost involved in PEP.

Material and methods

This study was a hospital-based cross-sectional study conducted in the casualty and medicine department of Kalinga Institute of Medical Sciences (KIMS), a tertiary care center in Bhubaneswar, Odisha. The World Health Organization (WHO) has categorized contact with suspected rabies-infected animals into three categories: category I (no exposure) – animal licks on intact skin, contact while feeding or touching the animal, category II (exposure) – nibbling of exposed skin, small, non-bleeding scratches or abrasions, and category III (severe exposure) – one or more transdermal bites or scratches, animal lick saliva contaminating mucous membranes or broken skin, and exposures from direct bat contact. The intramuscular 5-dose regimen is followed for anti-rabies PEP at KIMS. According to the regimen, 0.5 mL of anti-rabies is administered intramuscularly (over the deltoid) on days 0, 3, 7, 14, and 28.⁶ The victims are treated in casualty during the first visit and in the medicine department on an outpatient basis during subsequent follow-up visits. The cost of the anti-rabies vaccine is Rs. 400/dose and that of immunoglobulin is Rs. 300. The anti-rabies vaccine and immunoglobulin are provided to animal bite victims at the casualty and subsequent doses at General Medicine on an outpatient basis. All 150 animal bite victims presented to the hospital between April and June 2023 were included in the study through the universal sampling method. The selection of the study setting was a hospital keeping accessibility and follow-up of the animal bite patients in mind. Inclusion criteria: animal bite victims providing informed

consent and patients available for follow-up. Exclusion criteria: re-exposure cases of animal bites for PEP. Before the study commenced, ethical clearance was obtained from the Institutional Ethical Committee (approval number: KIMS/KIIT/IEC/1325/2023). Informed consent was taken from all study participants and consent from parents if the victim was less than 18. A mixed-method study approach was undertaken to explore the facilitating and inhibiting factors for the timely initiation of PEP using a pre-tested and semi-structured questionnaire and in-depth interviews. For our study, the delay in the initiation of anti-rabies PEP was defined as starting the PEP schedule greater than or equal to 24 hours post-animal bite. Data were compiled into Microsoft Excel and analyzed using SPSS version 20 (IBM, Armonk, NY, USA). Descriptive data were interpreted as frequencies and percentages. The chi-square test was applied to determine the association between factors associated with delay in initiation of PEP. A p-value less than 0.05 was considered to be statistically significant.

Results

A total of 150 animal bite victims were interviewed. There were 126 adults and 24 children. Among the study populations, 74.7% (112) were males (97 adults and 15 children) and 25.3% (38) were females (29 adults and 09 children). The most common biting animal were dogs (96.2%). Around 67.3% were from urban areas and 83.3% were literate. Timely initiation (treatment initiation within 24 hours) of PEP was seen in 74.7% of the study sample while delay was seen in 25.3% of the study population. The inhibiting factors were the absence of an accompanying person (54.67%), the absence of vaccines and immunoglobulins in the nearest health facility (50.67%), referred from other health facilities (44%), and preferred other modalities of treatment (24.67%). Table 1 depicts the various inhibiting and facilitating factors associated with the timely initiation of PEP.

The most common site of the bite was the lower limb (56, 37.33%), in both adults and children. The most common category of bite was category II (81, 54%). Delay in initiation of PEP was significantly associated with gender ($p=0.018$), place of residence ($p<0.001$), those living more than 20 km from a health facility ($p=0.014$), educational status ($p<0.001$), and those with monthly income <10000 INR ($p<0.01$). Table 2 depicts the association between socio-demographic and clinical variables with timely initiation of PEP.

Concerning first-aid measures, the majority of the victims washed their wounds with soap (142, 94.67%), followed by washing with running tap water (136, 90.67%) and applying anti-septic cream (112, 74.67%). Around 19 (12.67%) applied turmeric paste while 6 (4%) applied chilly-oil paste. Table 3 further lists the different first aid measures undertaken by the animal bite victims.

Table 1. Factors influencing timely initiation of anti-rabies PEP

Inhibiting factors delaying timely initiation of PEP	Frequency, n (%)
Work pressure	31 (20.67)
Unaware of PEP	27 (18)
Lack of money	48 (32)
Lack of transport	22 (14.67)
Lack of drugs in the nearby health facilities	76 (50.67)
Referred to other health facilities	66 (44)
Lack of accompanying person	82 (54.67)
Confidence in alternate modalities of treatment	37 (24.67)
Facilitating factors favoring timely initiation of PEP	
Aware of the PEP	123 (82)
Easy access to health facilities	101 (67.34)
Peer pressure	27 (18)
Unknown status of previous vaccination	16 (10.67)

Table 2. Association between sociodemographic and clinical variables with timely initiation of PEP*

Factors affecting acceptance of early treatment		< 24 hours (n=112)	≥ 24 hours (n=38)	Chi-square (p) ^a
Gender	Male	82	20	5.524 (0.018)
	Female	30	18	
Residence	Urban	92	09	44.083 (0.00001)
	Rural	20	29	
Distance from health facility	<20 km	22	15	6.0047 (0.014)
	≥ 20 km	90	23	
Educational status	Illiterate	09	16	23.7124 (0.00001)
	Literate	103	22	
Monthly income	<10000 INR	11	18	25.6484 (0.00001)
	≥10000 INR	101	20	
Site of bite	Head/neck	10	11	9.6473 (0.022)
	Upper limb	43	12	
	Trunk	15	03	
	Lower limb	44	12	
Type of animal bite	Dog	105	35	0.1485 (0.928)
	Cat	05	02	
	Others	02	01	
Category of bite	I	02	04	30.3743 (0.00001)
	II	49	32	
	III	61	02	

* ^a – Chi-square test used to measure association

Table 3. First aid measures undertaken by the animal bite victims*

First aid measures after an animal bite	Frequency n (%) ^a
Applied nothing	25 (16.67)
Washed with stagnant water	14 (9.34)
Washed with running tap water	136 (90.67)
Washed with soap	142 (94.67)
Applied antiseptic cream	112 (74.67)
Applied chilly-oil paste	06 (4)
Applied turmeric powder/ paste	19 (12.67)
Any tablets (antibiotics, analgesics, etc.)	34 (22.67)

* ^a – total % age may be >100% because of multiple responses by the subjects

The indirect cost involved in anti-rabies PEP ranged between 650–3600 Indian Rupees in total. The factors responsible were mainly wage loss, transport, cost due to other medications, and cost of indigenous treatments. Table 4 shows the median cost and range of cost for each factor involved.

Table 4. Indirect Cost involved in anti-rabies PEP

Factor	The median cost in INR	Range
Cost for transport	400	200–1400
Cost of wage loss	700	600–2100
Cost of any other medications	300	150–900
Cost of traditional/indigenous treatments	200	100–250
Total cost	1100	650–3600

Discussion

The study was conducted among 150 animal bite victims visiting a tertiary care center for anti-rabies PEP. There were 74.7% males and 25.3% females. A preponderance of males was also seen in a study done by Puwar.⁷ The cause could be attributed to males staying outdoors and taking risks more often. The most common biting animal was a dog, seen in 96.2% of victims. The less common biting animals were cats and monkeys. A similar prevalence was also reported by Joseph et al. with dogs being the most common biting animal.⁸ Around 67.3% were from urban areas and 83.3% were literate. A majority of the victims, i.e., 75.33% resided more than 20 km from any health facility, which made timely access to PEP difficult. The most common site of the bite was the lower limb (56, 37.33%) followed by the upper limb (55, 36.67%). This was consistent with the findings of a study done by Khan et al., Esmaeilzadeh et al., and Puwar.^{7,9,10} The most common category of bite was category II (81, 54%). A study by Wani et al. also reported category II as the most common type of bite.⁵ A study by Joseph et al. however, reported category III as the most common type of bite.⁸

Prompt initiation of PEP is extremely essential because rabies is a 100% fatal disease. In our study, around 74.7% of the victims started PEP within the first 24 hours, while a delay was seen in about 25.3% of victims. Inferior findings were reported by studies done by Puwar, and Liu et al. with only 58% and 35.3% of animal bite victims reported to the health facility within 24 hours respectively.^{7,11} Studies by Esmaeilzadeh et al. and Ganasva et al. reported superior findings where 85.9% and 80% of animal bite victims received PEP within 24 hours respectively.^{10,12}

The inhibiting factors delaying prompt initiation of PEP in our study were the absence of an accompanying person (54.67%), the absence of vaccines and immunoglobulins in the nearest health facility (50.67%), victims referred from other health facilities (44%), and preference for modalities of treatment (24.67%). Referral to

other health centers because of the non-availability of PEP in the nearest health facility not only caused a delay in prompt initiation of PEP but also indirectly increased the overall expenditure due to added time and travel. A report by an anti-rabies clinic of a government hospital in Chennai has mentioned reasons like loss of wages, forgotten dates, distance from the health center, and incurred expenditure for the delay in initiation of PEP.¹³ A similar study in the urban slums of Chennai reported wage loss and overlapping of school timings as reasons for delays in initiating PEP.¹⁴ Prompt and timely initiation of PEP is essential for neutralizing the rabies virus and preventing its spread to the central nervous system.¹⁵ The delay in initiation of timely PEP was significantly associated with gender ($p=0.018$), place of residence ($p<0.001$), distance more than 20 km from a health facility ($p=0.014$), educational status ($p<0.001$) and monthly income <10000 INR ($p<0.01$). Similar statistical significance was reported in various other studies.^{5,16,17}

The majority of the victims in our study washed their wounds with soap (142, 94.67%), followed by washing with running tap water (136, 90.67%) and applying anti-septic cream (112, 74.67%). Around 19 (12.67%) applied turmeric paste while 6 (4%) applied chilly-oil paste. Around 16.67% of victims did not apply any first-aid measures. Similar findings were reported by Singh A. et al. who reported that 67% of animal bite victims washed their wounds with soap and water while 26% of victims believed in the application of herbal medicines (turmeric, chilly, etc.).¹⁸ Puwar, in their study, also found that only 39.4% of animal bite victims washed their wounds correctly with soap and water. Around 60% of victims applied items like snuff, chili powder, tobacco, tea, and toothpaste over the wounds, which was quite alarming.⁷ A study by Venkatesan et al. also reported around 36% of victims cleaned their wounds with soap and water and 21% applied irritants like lime, ash, onion, etc.¹⁹ Several other studies in pan-India have also reported the application of irritants like turmeric, chilly, oil, etc. over bite wounds by victims.²⁰⁻²²

The indirect cost involved in anti-rabies PEP was Rs. 1100 ranging between Rs. 650–3600. The factors responsible were mainly wage loss, transport, cost due to other medications, and cost of indigenous treatments. A study by Ramya reported similar findings in their study where the indirect cost involved in PEP was around Rs. 1350 (IQR Rs. 1050–1750). The factors involved were travel expenses, loss of wages, and other expenses like food, etc.²³ Another study was done by Ravish in Bangalore who compared the economic burden of PEP in a government and private hospital and reported that the total median cost incurred by the victims at the Government hospital was Rs. 585 (IQR Rs. 444–725) and that in Private hospital was Rs. 5200 (IQR Rs. 4900–5701).

This included both direct and indirect costs of PEP.²⁴ The economic burden affects compliance with PEP and hence indirectly causes delays in the timely initiation of PEP. Establishing more anti-rabies clinics at all levels of health care and ensuring the availability of vaccines and immunoglobulin is warranted. Conversion of the intramuscular route of the anti-rabies PEP schedule to the intradermal route can also reduce the number of visits to the health facility, thus reducing the indirect cost.

Study limitations

Since our study was hospital-based, the findings cannot be generalized to the whole community. Using these findings as a baseline, further robust community-based studies need to be planned to get more clarity on the research question. Also, animal bite victims who did not visit the hospital for vaccination could not be assessed and the reasons for no vaccination could not be evaluated. The selection of the study setting was a hospital keeping accessibility and follow-up of the animal bite patients in mind.

Conclusion

Despite the availability of affordable anti-rabies vaccination in India, several socio-demographic, economic (both direct and indirect cost), and accessibility-related issues hamper the timely and prompt initiation of PEP. The groups most likely to experience delay are female gender, rural habitats, those residing >20 km from a health facility, those illiterate, and lower socio-economic status. The application of turmeric powder and chilly oil paste over bite wounds shows the prevalence of myths and misconceptions about first-aid measures for animal bite wounds. Increasing awareness regarding the importance of timely PEP, debunking myths and misconceptions about wound management, and increasing the availability of more anti-rabies clinics at all levels of healthcare are warranted to reduce delay in seeking PEP and to reduce the economic burden for poor communities. Cost-effective and sustainable strategies like mass dog vaccination can also aid in curbing rabies at source.

Declarations

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Author contributions

Conceptualization, I.D. and P.S.P.; Methodology, I.D., R.N. and P.S.P.; Software, I.D. and P.S.P.; Validation, I.D. and P.S.P.; Formal Analysis, I.D. and D.M.; Investigation, I.D. and P.S.P.; Resources, I.D. and D.M.; Data Curation, I.D., R.N. and P.S.P.; Writing – Original Draft Preparation, I.D. and R.N.; Writing – Review & Editing, P.S.P.,

B.K.B. and S.N.; Visualization, I.D. and P.S.P.; Supervision, B.K.B. and S.N.; Project Administration, I.D., R.N., P.S.P., B.K.B. and S.N.

Conflicts of interest

The author(s) declare no competing interests.

Data availability

Since it is sensitive data, it can be shared with the journal and reviewers upon request.

Ethics approval

All subjects gave informed consent for inclusion before participating in the study. The study was conducted under the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Kalinga Institute of Medical Sciences (KIIT/KIMS/IEC/1325/2023).

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ORIGINAL PAPER

The impact of baby care education provided to mothers in the early postpartum period on the maternal role and postpartum anxiety

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ABSTRACT

Introduction and aim. Baby care training given to mothers can help them feel more secure and make their motherhood role more effective. The aim was to determine the effect of early postnatal infant care training on the maternal role and anxiety in first-time mothers.

Material and methods. The study involved 60 mothers of which n=30 were in a control group with treatment as usual and n=30 were in the intervention group receiving special training on infant feeding, basic hygiene practices, baby's sleep patterns and basic care skills. Training took place in the first 8 and 24 hours after birth and mothers were followed up 5 weeks later. The "Barkin Maternal Function Inventory (BMFI)" and "Postpartum Anxiety Scale (PAS)" were used to measure maternal roles and postpartum anxiety at 6 weeks after birth.

Results. Participants were usually between 28–32 years of age and were first-time mothers. Six weeks after birth, there was a statistically significant difference in BMFI total scores between the experimental and control groups ($p < 0.001$), while the experimental group scored higher (89.8 ± 11.01). There was no significant difference between the groups in terms of postpartum anxiety.

Conclusion. Significant improvements were observed in the anxiety levels and mothering skills of mothers who received training. It is recommended that similar training programs be implemented on a larger scale and their effects be monitored long-term.

Keywords. anxiety, baby care, education, maternal role, postpartum

Introduction

The postpartum period is an important turning point in the lives of mothers and a critical period that requires them to cope with the care and needs of their newborn babies.¹ Among the difficulties encountered in this period, issues such as postpartum anxiety and fulfilling the role of motherhood come to the forefront. It is thought that infant care training given to mothers in the postpartum period can reduce postpartum anxiety levels by enabling mothers to cope with these new roles and responsibilities more effectively. Such training can help mothers understand their babies better and be better equipped to care for them. It can also contribute to the

prevention of mental problems such as postpartum anxiety. Thus, mothers can feel safer and care for their babies more consciously. The dissemination and support of infant care education given to mothers in the early postpartum period is of great importance for the health of mothers and the development of infants. Further research on this subject and the development of practices in this direction may contribute positively to the health of mothers and infants.²

Infant care education given to mothers in the early postnatal period usually covers topics such as breastfeeding, infant feeding, infant care and infant sleep patterns. In this way, mothers feel better equipped to take care of

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their babies, which helps the mother to fulfil her role better.^{3,4} In addition, baby care training also helps mothers to establish a healthier communication with their babies. This allows the mother to fulfil her role more effectively.⁵ A study has been shown that mothers can improve their skills in baby care by increasing their attachment, motherhood self-efficacy and self-confidence to their babies.⁵ Literature reviews on the effect of infant care training given to mothers in the postpartum period on maternal role and postpartum anxiety reveal the existence of various studies.⁵⁻⁹ These studies generally address issues such as mothers' education levels, postpartum anxiety levels, maternal role perceptions and infant care skills. However, most of the existing research focuses on the effects of a specific training program and usually evaluates short-term outcomes. Studies examining longer-term effects and the sustainability of training programs are limited. In addition, it is observed that most of the research on the effects of infant care training on the role of motherhood and postpartum anxiety focuses on a specific region or cultural group and is usually conducted with small samples. This situation shows that generalizations and studies involving participants from different cultures are needed. In this context, it is necessary to conduct studies that are comprehensive and include participants from different cultures in future research. And it shows that generalizations and studies involving participants from different cultures are needed. In this way, the effects of infant care training given to mothers can be evaluated more comprehensively and contribute to understanding the experiences of mothers in the postpartum period. Therefore, the effect of infant care training provided to mothers contributes to the emotional, social, and physical development of the baby by strengthening the mother-infant relationship.^{3,4}

Postpartum anxiety is a common problem seen in mothers in the postpartum period.⁶ This can cause mothers to worry excessively about their babies and this anxiety can negatively affect their daily lives.⁷ At this point, the importance of baby care education emerges. Because these training programs help mothers reduce their worries about their babies and make them more aware of baby care. Mothers' lack of trust can negatively affect their experience of maternity and their ability to care for their babies adequately. Training given to mothers about caring for their babies effectively increases their sense of competence.³ This, in turn, helps reduce postpartum anxiety.

In addition, it is observed that most of the research on the effects of infant care training on the role of motherhood and postpartum anxiety focuses on a specific region or cultural group and is usually conducted with small samples. This situation shows that generalizations and studies involving participants from different cultures are needed. In this context, it is necessary

to conduct studies that are comprehensive and include participants from different cultures in future research. In this way, the effects of infant care training given to mothers can be evaluated more comprehensively and contribute to understanding the experiences of mothers in the postpartum period. Therefore, the effect of infant care training given to mothers in the early postpartum period on the role of the mother and postpartum anxiety is very important.^{3,4} In this way, the effects of infant care training given to mothers can be evaluated more comprehensively and contribute to understanding the experiences of mothers of different cultures in the postpartum period. Therefore, studying the effect of infant care training on the maternal role perception and postpartum anxiety in Turkish mothers in the early postpartum period is very important. The significance of this is underscored by the fact that infant care training provided to mothers of premature babies contributes to the emotional, social, and physical development of the infant by strengthening the mother-infant relationship.^{6,7}

According to the findings of a meta-analysis on a similar topic, educational interventions during pregnancy and postpartum periods increased the maternal competence scores of first-time mothers.¹⁰ However, there is no specific study in the literature on the effect of infant care education given to mothers in the early postpartum period on the maternal role and postpartum anxiety. In this way, mothers can establish a healthier relationship with their babies and spend the postpartum period in a more positive way.

Aim

Our aim is to provide evidence of the effectiveness of a training that helps mothers and babies in terms of its effect on the maternal role and postpartum anxiety in the postpartum period. Based on the lack of studies examining a similar topic in the literature, this study aims to fill the gap in this field and to investigate the effects on the maternal role and anxiety levels of first-time mothers.

Material and methods

This randomized controlled study was conducted between September and November 2022. This decision was aimed at improving the accuracy and reliability of the research.

The sample size of this study was calculated using G*Power (v3.1.7) software. Yurtsal ve Eroğlu was taken as reference.¹³ This inventory is an important measurement tool for understanding mothers' attitudes and behaviors related to infant care. It is also useful for understanding mothers' psychological states and determining how their maternal roles affect them. The effect size obtained from the previous study was obtained as 0.05=1.07 (1) as a result of the analysis, the minimum sample size was calculated as 48, 24 in the

trial group and 24 in the control group, for a total of 80, and the theoretical power was calculated as 0.80. Participants were selected from among women with a gestation period between 37 and 41 weeks, who had their first pregnancy with a single fetus, any risk factors during pregnancy, nor attended any special parenting education during pregnancy, and were at least literate. In total, 60 primiparous pregnant women were recruited for this study. Age, sex, race/ethnicity, and financial status of participants were recorded as part of the demographic data collection process. The inclusion standards were established based on the objectives of this study. The exclusion criteria in the study who could not speak Turkish, had a baby in an incubator, and received infant care training were excluded from the study, considering communication difficulties, safety and well-being of the participants, data reliability and consistency. After randomly selecting participants, they were assigned to either the education or control group. Randomization ensured that the participants were evenly distributed into two groups. Researchers were required to know who these individuals belonged to, because of the nature of the study, whereas those responsible for the data remained anonymous. All pregnant women assigned to the trial or control group stayed in separate rooms where communication between them was not allowed.

The study's registration, assignment, monitoring, and analysis steps were performed using CONSORT 2018 (Fig. 1).

The trial group participated in the study knowing that they were being treated, so the researcher (MÖ) could not ensure that the application remained confidential. Randomization and data collection were performed by another investigator (ÖT). Data collection forms were administered on the online platform 6 weeks after birth. To reduce bias, the researcher who provided the training (ÖT) was not included in the data collection phase.

Experimental-control group work process

In the first stage of the research, mothers who were eligible for the sample 8 hours after birth were randomly assigned to the trial and control groups. "Personal Information Form" and "Postpartum Anxiety Scale" (developed by Fallon et al.) were applied to the mothers. The trial group underwent intervention approximately 8 and 24 hours after birth. Each session lasted 30 minutes (60 minutes) and included practical baby care training on the baby model prepared by the researchers and a baby care book. Mothers in the control group continued their routine care. No application has been received.

Baby Care Training Booklet Content: This booklet has been created by taking expert opinions on baby care and the content has been enriched. It contains de-

tailed information on topics such as baby skin care, eye care, security problems. The content prepared by the researchers is aimed at guiding new parents. This booklet aims to provide new parents with accurate information on baby care and has been prepared by researchers with the recommendations of experts.

In the second phase, baby care counseling was provided to the mothers in the trial group for 6 weeks by the researcher (CR), who provided baby care education via online conversation about baby care problems. In the third step, "Barkin Maternal Function Inventory" and "Postpartum Anxiety Scale" were applied online to both groups six weeks later.

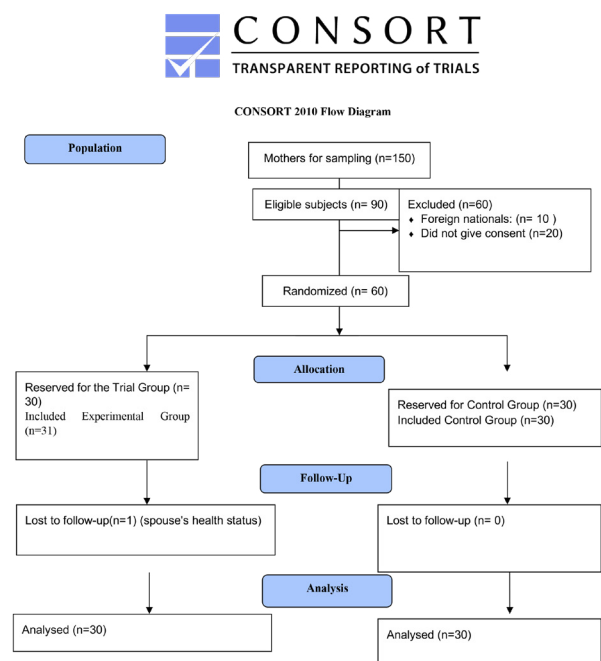


Fig. 1. CONSORT flow diagram

Measures

Barkin Inventory of Maternal Function (BIMF)

Jennifer Lynn Barkin developed the Barkin Maternal Function Inventory in Atlanta. The Barkin Maternal Function Inventory has 5 sub-dimensions: Self-Care, Maternal Psychology, Infant Care, Social Support, and Adaptation to Maternity. BIMF consists of 20 questions. The answers on the scale are numbered from 0 to 6. While the lowest score in the scoring of the scale is 0, the highest score is 120.¹⁴ It was observed that the higher the scoring, the higher the functional status. The overall Cronbach alpha reliability coefficient of the scale was 0.73 and from the scale sub-dimensions; It was calculated that the self-care dimension was 0.66, the maternal psychology sub-dimension was 0.71, the infant care sub-dimension was 0.62, the social support sub-dimension was 0.69, and the motherhood adaptation sub-dimension was 0.50. Barkin found the Cronbach Alpha coefficient of the scale to be 0.87. The validity and re-

liability study of the Turkish form of the BAF Inventory was conducted by Aydın and Kukulu.¹⁵ The reliability coefficient of the BAF Inventory was found to be Cronbach Alpha 0.73.

Postpartum Anxiety Scale (PAS)

The scale Fallon et al. developed has 51 items and is used to assess postpartum anxiety symptoms. Maternal skills and attachment, newborn well-being and safety, and infant care practices relate to psychosocial maternity compliance. Each item in the scale is scored as never=1, not very often=2, often=3 and almost always=4. The lowest score that can be obtained from the scale is 44 and the highest score is 176. Higher scores indicate more anxiety symptoms.¹⁵ In this study, the internal consistency reliability coefficient of the PAS sub-dimensions: $\alpha=0.89$ for maternal skills and attachment; $\alpha=0.89$ for newborn well-being and safety; $\alpha=0.87$ for infant care practices; $\alpha=0.81$ for psychosocial adjustment to motherhood and $\alpha=0.91$ for the whole scale. The adaptation of the scale to Turkish was made by Duran in 2019 and the Cronbach alpha coefficient was found to be $r=0.91$.¹⁷ In this study, Cronbach's alpha coefficient of the scale was found to be $r=0.88$.

Statistical Analysis

Data analysis was performed using the SPSS 24.0 software package (IBM, Armonk, NY, USA). The conformity of the data to the normal distribution was evaluated by Shapiro Wilk test. Independent sample T test and paired sample T test were used for the analysis of normally distributed data, and Mann Whitney U test was used for the analysis of normally dispersed data. Scale, society, size and statistical significance level were accepted as $p<0.05$.

Results

The distribution of sociodemographic and obstetric characteristics of the pregnant women included in the study according to the groups is given in Table 1. There is no significant difference between the two groups in terms of educational status ($p>0.05$). The majority are at university level or above. When the education level of the spouses is examined, it is seen that the control group has a higher education level of high school and below. The sociodemographic and obstetric characteristics of the women included in the study are largely similar.

The mean BIMF total score of the mothers was 89.80 ± 11.01 in the trial group and 71.86 ± 20.49 in the control group at 6 weeks postpartum, and the difference between them was found to be statistically significant ($p<0.001$; Table 2). There was a statistical difference between the trial and control groups in all sub-dimensions (Self-care sub-dimension, $p=0.002$; maternal psychology sub-dimension: $p=0.002$; infant care sub-dimension:

$p=0.008$; social support sub-dimension: $p=0.002$; adjustment to maternity subscale, $p=0.002$).

Table 1. Comparison of socio-demographic and obstetric characteristics of women*

		Groups				Test1	p
		Trial		Control			
		(n=30)		(n=30)			
	n	%	n	%			
Age	23–27	5	16.7	11	36.7	$\chi^2=3.293$	0.193
	28–32	14	46.7	12	40		
	33–37	11	36.7	7	23.3		
Income status	Income less than expenditure	4	13.3	8	26.6	$\chi^2=1.778$	0.411
	Income matches expenditure	19	63.3	17	56.7		
	Income more than expenditure	7	23.3	5	16.7		
Education status	High school	3	10	10	33.3	$\chi^2=3.535$	0.060
	University and higher education	27	90	20	66.7		
Spouse education status	Middle school	2	6.7	17	56.7	$\chi^2=28.893$	<0.001
	High school	5	16.7	10	33.3		
	University and higher education	23	76.7	3	10.0		
Delivery method	Caesarean	27	88.4	28	93.1	$\chi^2=1.080$	0.299
	Normal	3	11.6	2	6.9		
Ability to cope with challenges	Very adequate	18	60	19	63.3	$\chi^2=0.000$	>0.999
	Moderate	12	40	11	36.7		
Assessing the pregnancy process	Occasionally troubled	15	50	11	36.7	$\chi^2=0.0611$	0.434
	Comfortable	15	50	19	63.3		
Total		30	100	30	100		

* significant at $p<0.001$, ¹ – Chi-square test

Table 2. Comparison of Barkin Maternal Function Inventory Total and Subscale Scores of women in the trial and control groups after education*

Barkin Maternal Function Inventory Subscales	Groups		Test1	p
	Trial	Control		
	(n=30) Mean±sd	(n=30) Mean±sd		
Self-care subscale	12.50±4.67	8.60±4.63	t=3.245	0.002
Maternal psychology sub-dimension	11.66±1.72	9.56±3.08	t=3.255	0.002
Baby care sub-dimension	25.00±2.18	21.56±5.58	t=-2.254	0.008
Social support sub-dimension	15.93±3.88	12.06±5.82	t=3.025	0.004
Compliance with maternity subscale	24.70±2.86	20.06±5.39	t=-3.916	<0.001
Total score	89.80±11.01	71.86±20.49	t=4.222	<0.001

* significant at $p<0.001$ ¹ – independent-sample t test

There was no significant difference between the trial group and the mothers after the training (6 weeks after birth) in terms of mothering skills, attachment anxiety, (37.39 ± 2.27 ; 34.10 ± 2.36 , respectively), infant care practices anxiety, (30.86 ± 2.16 ; 29.53 ± 2.44), adaptation to psychosocial motherhood and total postpar-

tum anxiety scale (17.33 ± 1.24 ; 15.83 ± 1.62 ; 117.83 ± 3.98 ; 112.20 ± 3.53 , respectively ($p > 0.05$; Table 3).

In the control group, maternal skills attachment anxiety scores were higher than the control group before the training. The post-training scores of the experimental group decreased more than the control group (Table 3). Neonatal well-being and safety anxiety scores did not differ in the trial and control groups before and after the training; the difference between them was insignificant ($p = 0.092$; Table 3).

Table 3. Trial and control groups comparison of Postpartum Anxiety Scale Subscale Scores and Total Averages After Weeks*

Postpartum Anxiety Scale		Groups		Test1	p
		Trial (n=30) Mean \pm ss	Control (n=30) Mean \pm ss		
Maternity Skills Attachment Anxiety	Pre-education	37.30 \pm 2.27	38.23 \pm 2.41	t=-1.540	0.129
	6 weeks after birth	34.10 \pm 2.36	34.83 \pm 2.92	t=-1.067	0.290
	Test ²	t=5.158	t=4.581		
	p	0.000	0.000		
Neonatal Well-Being and Safety Anxiety	Pre-education	32.33 \pm 1.60	31.96 \pm 2.02	t=0.777	0.440
	6 weeks After birth	32.73 \pm 1.98	32.06 \pm 1.94	t=1.315	0.194
	Test ²	t=-0.858	t=-0.204		
	p	0.398	0.840		
Baby Care Practices Anxiety	Pre-education	30.86 \pm 2.16	29.93 \pm 2.82	t=1.436	0.156
	6 weeks After birth	29.53 \pm 2.44	28.63 \pm 1.51	t=1.712	0.092
	Test ²	t=2.143	t=2.282		
	p	0.041	0.030		
Psychosocial Compliance with Maternity	Pre-education	17.33 \pm 1.24	16.66 \pm 1.44	t=1.916	0.060
	6 weeks after birth	15.83 \pm 1.62	15.16 \pm 1.91	t=1.456	0.151
	Test ²	t=3.801	t=3.503		
	p	0.001	0.002		
Postpartum Anxiety Scale total score	Pre-education	117.83 \pm 3.98	116.80 \pm 4.17	t=0.981	0.381
	6 weeks after Birth	112.20 \pm 3.53	110.70 \pm 3.77	t=1.587	0.118
	Test ²	t=6.127	t=5.556		
	p	0.000	0.000		

* significant at $p < 0.001$ ¹ – independent-sample t test,

² – paired Sample t test

Discussion

This study observed that infant care provided to mothers in the early postpartum period effectively influenced their maternal role. Maternal competence is shaped by the dynamic interplay between mothers' ongoing success in caring for infants.

Primiparous mothers' lack of knowledge about infant care, typical infant characteristics or illnesses creates anxiety in mothers and reduces their enjoyment of maternity.¹⁸ Studies have shown that all mothers need training in newborn care, and primiparous mothers with first-time infant care responsibilities experience more difficulties in newborn care than multiparous mothers.^{18,19} The more prepared a mother feels to care for her baby, the higher her feelings of success in her

maternity journey. In this study, infant care training in early postpartum increased women's maternal competence. They may feel more confident and competent in their new role. It has been shown that women whose lack of knowledge is completed and who are supported in caring for their babies increase their maternal competence.¹⁸ In similar study, it was recommended that mothers who gave birth for the first time should receive training in infant care.¹⁹ The study emphasizes the importance of training mothers on infant care. It is emphasized that infant care training can positively affect mothers' feelings of maternal attachment and self-confidence and thus help mothers to establish a healthier relationship with their babies.

Regardless of skill or competence, positive maternal behaviors are vital to a successful transition to single maternity. Transitioning to the role of maternity is a stressful process, especially for primiparous women. In addition, anxiety is one of the most common mental disorders of the postpartum period and can affect the quality of life of mothers and the health and development of their children. Babysitting and getting used to the role of a new mother can also cause anxiety and anxiety in some women. In one study, postpartum anxiety was found in 23% of new mothers who participated in the study.²⁰ In this study, we measured anxiety levels in women 24 hours after delivery. In the research conducted in both groups, we found that anxiety levels were high. We predicted that first-time mothers would have high levels of anxiety, because we know that this is inherent in being a first-time mother. The results of the research confirm this prediction. The anxieties experienced by new mothers are a natural reaction to stepping into a new period in their lives. These findings suggest that appropriate strategies should be developed to support new mothers and reduce their anxiety levels. However, we observed that the women who received infant care had lower levels of anxiety after 6 weeks, suggesting that women who received infant care had lower levels of anxiety over time. The findings of our study provide important insight into the effectiveness of support programs for coping with postpartum anxiety. Our results emphasize the importance of early intervention and support services in combating postpartum anxiety. These findings are expected to contribute to the development of counselling policies to support women's mental health in the postpartum period. Observing that anxiety levels of women receiving infant care decreased over time provides an important insight into the effectiveness of support programs in combating postpartum anxiety.²¹ This finding shows how important early intervention and support services are in coping with postpartum anxiety.

In the study, it was stated that most of the mothers gave birth by caesarean section. It is known that cae-

sarean section rates in private hospitals in Türkiye are high.²² It is known that mothers who give birth by caesarean section have more difficulties in adapting to the role of motherhood and have higher anxiety levels than those who give birth normally.^{23,24} It is thought that caesarean section makes it difficult for mothers to adapt to the care of their babies and to adopt their maternal roles. Mothers who give birth by caesarean section have a longer recovery period and can start caring for their babies later than those who give birth normally. This situation may negatively affect mothers' attachment to their babies and their adoption of maternal roles.²⁵ In addition, it is known that mothers who give birth by caesarean section have higher anxiety levels than those who give birth normally.²⁶ Caesarean section is thought to increase mothers' concerns about the health and safety of their babies, which in turn increases their anxiety levels. However, the study did not measure the effect of caesarean section on mothers' anxiety levels. The reason for this may be that the sample of the study consisted largely of mothers who had caesarean section, which made it difficult to make comparisons with the control group. In addition, the fact that the relationship between the mode of delivery and anxiety levels of mothers was not examined in the study can be considered as a limitation. In conclusion, the negative effects of caesarean section on mothers' adaptation to the role of motherhood and anxiety levels need to be examined in more detail. In future studies, evaluating the relationship between mode of delivery and anxiety levels will increase the knowledge on this subject.

In the study, all women's concerns about the safety of their babies persisted at almost the same level immediately after birth or at later periods. This can be attributed to the constant worry for their babies that is inherent in motherhood. Mothers tend to worry about their babies all the time, and this worry appears to be a constant reality. This reflects mothers' sensitivity and responsibility for their babies' safety. Therefore, the persistence of concerns about the safety of infants can be considered a fundamental feature of the experience of motherhood. This requires us to understand and support mothers' sensitivities about the health and safety of their babies.²⁷ At the same time, it is important to support mothers and raise awareness about this issue, because these concerns have become an integral part of their lives. In this context, understanding and supporting mothers' concerns about the safety of their babies is also of great importance for the overall health and well-being of society. Therefore, providing support and information to mothers who are concerned about the safety of their babies is an important factor that can positively affect the motherhood experience. In a similar study, it was recommended that the addition of qualitative methods such as interviews can help to gain in-depth information.

These methods allow us to understand participants' experiences and thoughts in more detail and can broaden the scope of the research.

Study limitations

In this study, it was determined that mothers who met the criteria for participating in the study could not be included in the study in cases where the researcher was not in the hospital. This may adversely affect the study's ability to generalize. Because the sample participating in the study represents a certain group of mothers, this makes it difficult to make generalizations. Uncertainties may arise as to whether the results of the study apply to all mothers. This is the limitation of the study. In addition, it has been determined that the majority of women give birth by cesarean section. Caesarean section rates in Türkiye are known to be quite high and it is observed that it is difficult to find women who give birth almost normally. This study was conducted in a private hospital, and it is known that caesarean section rates are high in private hospitals in Türkiye. Therefore, most mothers had a caesarean section, and this may have increased their anxiety levels. They may also have had more difficulty adjusting to their role as mothers. Considering all these factors, it can be considered that cesarean delivery may have increased mothers' anxiety levels by making it difficult for them to adapt to their maternal roles. This, in turn, can limit the effectiveness of training programs. In addition, the measurement tools consist of two different questionnaires to assess changes in the role of motherhood, and it is unclear whether these questionnaires measure changes in the role of motherhood well enough. Therefore, it is a limitation of this study to say that interviews can certainly make a useful contribution to the research process. In this way, the data can be re-investigated more comprehensively and in more detail.

In this study, the selection criteria of the participants and the conduct of the study included some factors that limit the generalizability of the study results. First, the women included in the study were selected from a group with specific characteristics such as duration of pregnancy, number of pregnancies, pregnancy complications and educational status. Therefore, it may be difficult for the study results to represent all mothers. In the study conducted in a private hospital, the high rate of cesarean delivery may make it difficult for mothers to adapt to the role of motherhood. This may limit the effectiveness of the training programs.

Conclusion

As a result of the research, it was determined that the baby care training given to mothers increased the role of motherhood and adaptation to the role of motherhood. It has been observed that the education given to wom-

en who do not receive any training on baby care during pregnancy helps them to get through this process more easily, even in the early maturation period.

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Declarations

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Author contributions

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

Conflicts of interest

The author(s) declare no competing interests.

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 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 Arel university in Istanbul. (Date: 06.08.2022/Decision No: 227158).

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Determination of the effect of gender perception among university students on attitudes towards disabled women

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ABSTRACT

Introduction and aim. This study investigates the effect of gender perception among university students on attitudes towards disabled women.

Material and methods. The target population of this descriptive and cross-sectional study consisted of university students enrolled in the Faculty of Health Sciences in a province located in southern Türkiye. The Socio-demographic Form, the Gender Perception Scale, and the Attitudes towards Disabled Women Scale were administered to the participants through Google Forms. The sample of the study consisted of 644 students registered at the Faculty of Health Sciences and meeting the inclusion criteria.

Results. A positive and moderately significant correlation was found between gender perception and attitudes towards disabled women among students who were enrolled in the Faculty of Health Sciences. The variables explaining 26.3% of the total variance for the attitudes towards disabled women included the presence of a disabled individual in the family, education of disabled individuals together with individuals with no disabilities, views about the marriage of disabled women, kinship with the disabled relative, and gender perception scale score.

Conclusion. It was concluded that gender perception had a significant effect on the attitudes of students enrolled in the Faculty of Health Sciences towards disabled women.

Keywords. disabled women, gender, university student

Introduction

Disability is a physical, mental, or neurological dysfunction that affects all or some parts of an individual's life.¹ Disability can be affected by cultural sociocultural, demographic, and environmental factors such as age, chronic diseases, socioeconomic conditions, wars, migration, natural disasters, social beliefs, perceptions, and social roles.^{2,3} Disabled individuals have the same rights as other individuals by law, yet they face problems such as stigmatization, lack of access to health

services, limited access to education and employment opportunities, and exclusion from daily life activities, and thus they are considered as a disadvantaged group.^{1,3-5} According to the World Health Organization, there are 1.3 billion disabled people in the world; one out of every six people has a disability and disabled people constitute 16% of the world population.⁶ As for Turkey, the proportion of disabled population in the total population is reported to be 6.9%, which is 5.9% in men and 7.9% in women.⁶

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In the global gender equality report published by the United Nations Entity for Gender Equality and the Empowerment of Women, Turkey ranks 129th out of 146 countries, indicating that women in Turkey face serious inequalities in areas such as education, health, economic opportunities, and political participation.⁷ This low ranking indicates that gender inequality is still a major problem in Turkey, and women face discrimination in many areas of society. This situation creates even more severe challenges, especially for disabled women, and the awareness and education of health sciences students on this issue plays a critical role in combating these inequalities. Women are exposed to disabilities more than men due to factors such as higher life expectancy than men, gender perception, social roles and norms, taboo beliefs and thoughts, poverty, culture, status, lack of education and employment, and difficulties in accessing health care services.⁸⁻¹⁰ Society reportedly displays positive attitudes and behaviors towards disabled women, yet these women are exposed to negative and exclusionary attitudes and behaviors.⁷ Negative attitudes towards disabled women have been found to negatively affect their self-confidence, productivity, and participation in work and school life and cause them to experience poverty and social avoidance.¹¹ Social judgment and negative attitudes cause women to experience conditions such as frustration, unhappiness, anxiety, deterioration in body image, shame, decreased self-esteem, and isolation.^{9,12}

Negative attitudes towards disabled women also lead to problems such as inadequate access to reproductive and sexual health services, violence, rape, being deprived of family planning methods, lack of menstrual hygiene, experiencing chronic diseases and recurrence of existing diseases, increased alcohol and cigarette use, and increased sexually transmitted diseases.^{5,13,14} Factors negatively affecting attitudes towards disabled women include variables such as individuals' age, gender, income level, education level, and their parents' education level.¹⁵⁻¹⁸ The increase in the individual's education level positively affects attitudes toward disabled women. Iwakuma et al. found that the training given to university students for disabled individuals decreased students' perception of disability as a difficulty and enabled them to feel closer to disabled individuals.¹⁸ Disabled women are affected mostly by the health personnel's negative and judgmental attitudes and behaviors.¹⁹ Determination of the gender perceptions and attitudes towards disabled women among Faculty of Health Sciences students, who are the health personnel candidates of the future, is highly important in terms of contributing to the work, education, family life, and health status of disabled women.

Although the literature includes studies on health workers and disability, no studies have been found to have examined attitudes towards disabled women in the context of gender. The present study is believed to contribute to the field in this aspect.

Research questions

Q1) Is the perception of gender equality among university students related to attitudes towards disabled women?

Q2) Do sociodemographic features and some variables affect the perception of gender equality among university students?

Q3) Do sociodemographic features and some variables affect attitudes towards disabled women among university students?

Aim

In this regard, this study aims to determine the effect of gender perception among Faculty of Health Sciences students on attitudes toward disabled women.

Material and methods

Design

This study was conducted between January 1 and May 20, 2023 and utilized a descriptive and cross-sectional design.

Sample

The study data were collected online (Google Forms). The form started with a question indicating the respondent's consent to participate in the study. While the target population of the study consisted of 1134 students enrolled in the Faculty of Health Sciences in a province located in southern Turkey, the sample was determined as 419 students using the sample calculation with a known population method (99% confidence interval, 5% margin of error) (<https://www.calculator.net/sample-size-calculator.html>). Considering the possible losses, 50% more than the planned sample size was included. As a result, 644 students who were enrolled in the Faculty of Health Sciences and met the inclusion criteria were included. The inclusion criteria were being over 18 years of age and being a student at the Faculty of Health Sciences. Not being an active student and having any disability were determined as the exclusion criteria.

Data collection

Data were collected using the socio-demographic form, the Gender Perception Scale, and the Attitudes Towards Disabled Women Scale.

Socio-demographic form

The Socio-demographic form, which was developed by the authors based on the literature, consists of 19 questions collecting data about the socio-demographic char-

acteristics of the students (gender, class year, department, income level, family type, etc.) as well as their disability-related condition (the presence of a disabled individual in the family, education of disabled individuals together with individuals with no disabilities, etc.).^{13,19}

Gender Perception Scale (GPS)

The 25-item scale was developed by Altınova and Duyan in 2013, who also performed the validity and reliability of the scale, to measure how gender roles are perceived.²¹ The scale is responded on a five-point Likert scale (1=strongly disagree, 5=strongly agree). While 10 items on the scale are positive statements, 15 items are negative statements. The scores to be obtained from the scale range between 25 and 125, with higher scores indicating egalitarian gender perception. Cronbach's alpha reliability coefficient of the scale was reported to be 0.87.²¹ This study found Cronbach's alpha value of the scale as 0.93.

Attitudes towards Disabled Women Scale (ATDWS)

The 17-item scale, which aims to determine attitudes towards disabled women, was developed by Yılmaz and Ören in 2021.¹⁴ The scale is responded to on a five-point Likert scale (1= Strongly agree, 5= Strongly disagree) and includes four sub-scales: disabled women and their private lives, disabled women and disadvantages, disabled women and social support, and disabled women and gender. Scores to be obtained from the scale range from 17 to 85 points. The scale has no reverse items, and higher scores indicate higher positive attitudes toward disabled people. Cronbach's alpha values of the sub-scales were determined as 0.873, 0.736, 0.639, and 0.679, respectively. Cronbach's alpha value was determined as 0.817 for the whole scale. In this study, Cronbach's alpha values of the sub-scale were determined as 0.924, 0.834, 0.750, and 0.768, respectively. Cronbach's alpha value of the scale was determined as 0.901 for the total scale in this study.

Statistical analysis

Data were analyzed using the SPSS 25.0 package program (IBM, Armonk, NY, USA). Data analysis included descriptive statistics (number, percentage, median, mean, standard deviation), Skewness and Kurtosis for normality distribution, t-test, One-Way ANOVA, Mann Whitney U test, and Kruskal Wallis test. Bonferroni correction was used to determine which groups indicated the difference in the analysis of multiple groups.

Pearson correlation analysis was used for the comparison of the Attitudes towards Disabled Women Scale with disability-related variables. Multiple Linear Regression analysis was used to see the effects of variables related to the Attitudes towards Disabled Women Scale. Statistical significance was determined as $p < 0.05$.

Ethical approval

Before the study was conducted, ethics committee approval (dated 28.12.2022 and decision number 2022/10/12) was obtained from the Scientific Research and Publication Ethics Board of a state university. The participants were informed before the study was started, and their informed consent was obtained online (Google Forms).

Results

Table 1 demonstrates students' sociodemographic characteristics and the relationship between some variables and GPS and ATDWS. Students' average age was found 20.74 ± 1.93 years. While 75.5% of the students were female, 30.9% were enrolled in the second year, 77.8% were enrolled in the nursing department, 63.2% had income equal to expenses, 82% lived in a nuclear family, mothers of 37.9% graduated from primary school, fathers of 28.9% graduated from high school, the longest place of residence was a district or village for 50.2%, 21.6% lived in the Black Sea region, 55.4% had three or more siblings, 74.1% had a sister, and 86.8% had parents together.

The Gender Perception Scale median score was found to be significantly higher in females compared to males; those enrolled in the departments of Nursing and Nutrition and Dietetics compared to those in the Department of Social Service; those living in nuclear families compared to those living in extended families; those whose mothers are primary school graduates compared to those whose mothers are illiterate; those whose fathers are high school and university graduates compared to those whose fathers are literate; those whose fathers are primary school, secondary school, high school, and university graduates compared to those whose fathers are illiterate; those living in a city compared to those living in a district; those living in the Black Sea region compared to those living in Eastern and Southeastern Anatolia region; those who had one or two siblings compared to those who had three or more siblings; and those who had sisters compared to those who did not have sisters.

Attitudes towards Disabled Women Scale mean score was found to be higher in females compared to males; those who were in the first and second year than those who were in the third year; those who were in the Department of Nursing and Nutrition and Dietetics than those who were in Social Service; and those who had two siblings than those who had three or more siblings. The Bonferroni test, which was conducted to determine which group differed in terms of the geographical region of residence, indicated no differences between the groups, yet the Attitudes towards Disabled Women Scale mean score was found to be higher in students living in the Aegean region.

The Gender Perception Scale total median score was found to be significantly higher in those who did not

Table 1. Students' Socio-demographic Features and the Relationship of some variables with GPS and ATDWS*

Socio-demographic features	n (%)	GPS		ATDWS	
		Median (Min–Max)	Test and statistics	Mean±SD	Test and statistics
Gender					
Female	486 (75.5)	110 (65–125)	MU=17083.000	62.9±10.54	t=5.129
Male	158 (24.5)	87 (54–125)	p<0.001	57.32±12.28	p<0.001
Class year					
1st year	172 (26.7)	104.5 (68–125)		63.03±11.38	
2nd year	199 (30.9)	107 (60–125)		62.46±11.5	1>3, 2>3
3rd year	180 (28)	106 (54–125)	KW=3.060	59.26±10.53	F=4.014
4th year	93 (14.4)	103 (65–125)	p=0.382	61.19±11.27	p=0.008
Department					
Nursing	501 (77.8)	107 (60–125)		62.09±10.97	
Midwifery	27 (4.2)	102 (65–125)	1>4; 3>4	61.33±9.72	1>4; 3>4
Nutrition and Dietetics	16 (2.5)	115 (81–125)	KW=16.473	66.38±9.72	F=4.737
Social service	100 (15.5)	98 (54–125)	p=0.001	58.02±12.53	p=0.003
Income level					
Income less than expenses	162 (25.2)	104 (67–125)		61.75±10.84	
Income equal to expenses	407 (63.2)	106 (54–125)	KW=0.929	61.21±11.53	F=0.708
Income more than expenses	75 (11.6)	109 (70–125)	p=0.629	62.84±10.58	p=0.493
Family type					
Nuclear family	528 (82)	107 (54–125)	MU=24105.000	61.68±11.09	t=0.683
Extended family	116 (18)	101.5 (66–125)	p<0.001	60.89±11.96	p=0.495
Mother's education level					
Illiterate	73 (11.3)	97 (54–125)		60.4±11.04	
Literate	55 (8.5)	104 (60–125)		60.55±11.04	
Primary school graduate	244 (37.9)	108 (65–125)		62.05±10.71	
Secondary school graduate	108 (16.8)	106 (70–125)	3>1	61.14±11.9	
High school graduate	119 (18.5)	107 (68–125)	KW=17.314	61.7±11.97	F=0.408
University and above	45 (7)	111 (68–125)	p=0.004	62.29±11.48	p=0.843
Father's education level					
Illiterate	21 (3.3)	81 (65–118)		58.67±13.32	
Literate	23 (3.6)	92 (67–115)		58.57±10.5	
Primary school graduate	185 (28.7)	104 (66–125)	4>2.5>2	61.08±11.39	
Secondary school graduate	142 (22)	108 (54–125)	3>1.4>1.5 >1.6>1	62.23±11.4	
High school graduate	186 (28.9)	108 (66–125)	KW=25.846	62.08±10.62	F=0.851
University and above	87 (13.5)	106 (68–125)	p<0.001	61.7±11.7	p=0.514
Longest place of residence					
City	321 (49.8)	108 (60–125)	1>2	61.48±11.23	
Town	205 (31.8)	103 (66–125)	KW=7.305	61.22±11.46	F=0.300
Village	118 (18.3)	105 (54–125)	p=0.026	62.22±10.97	p=0.741
The geographical region of residence					
Eastern Anatolian Region	89 (13.8)	101 (54–125)		58.72±12.22	
Southeastern Anatolia region	134 (20.8)	103 (60–125)		61.76±10.2	
Marmara region	82 (12.7)	105.5 (66–125)		61.01±11.45	
Central Anatolian Region	114 (17.7)	112.5 (66–125)		63.11±10.74	
Black Sea region	139 (21.6)	108 (68–125)	5>1, 5>2	61.79±11.19	
Mediterranean Region	29 (4.5)	97 (70–125)	KW=27.846	58.1±13.57	F=2.332
Aegean region	57 (8.9)	108 (69–125)	p<0.001	64.12±10.77	p=0.031
Number of siblings					
0	21 (3.3)	108 (72–124)		63.1±10.74	
1	106 (16.5)	111 (66–125)	2>4, 3>4	62.79±11.7	3>4
2	160 (24.8)	110 (68–125)	KW=41.327	63.68±9.97	F=4.511
3 and more	357 (55.4)	102 (54–125)	p<0.001	60.11±11.51	p=0.004
Presence of a sister					
Yes	477 (74.1)	105 (54–125)	MU=35411.500	61.47±11.31	t=-0.238
No	167 (25.9)	108 (68–125)	p=0.033	61.71±11.08	p=0.812
Condition of parents					
Together	559 (86.8)	106 (54–125)		61.8±11.07	
Separate-divorced	43 (6.7)	104 (60–125)	KW=3.051	58.86±13.11	F=1.478
One of the parents passed away	42 (6.5)	101.5 (68–125)	p=0.218	60.74±11.44	p=0.229

* average age: 20.74±1.93, MU – Mann Whitney U test, t – Independent t-test, KW – Kruskal Wallis test, F – one way ANOVA test

have disabled friends than those who had; those who said yes to the education of disabled individuals together with individuals with no disabilities compared to those who said no; those who thought that a disabled woman should marry a man with no disabilities compared to those who thought that a disabled woman should marry a man with no disabilities and a disabled woman should not marry; those who thought that a disabled man should marry a woman with no disabilities compared to those who thought that a disabled man should marry a disabled woman and a disabled man should not marry.

Table 2. Relationship between some variables regarding disability and GPS and ATDWS*

Socio-demographic features	n (%)	GPS		ATDWS	
		Median (Min–Max)	Test and statistics	Mean±SD	Test and statistics
The presence of a disabled individual in the family					
Yes	116 (18)	106 (66–125)	MU=30056.500	59.74±11.42	t=-1.983
No	528 (82)	106 (54–125)	p=0.754	61.73±14.16	p=0.048
Kinship with the disabled person					
1st and 2nd degree kinship					
	54 (35.8)	103.5 (68–125)	MU=2545.000	58.22±13.17	t=-1.521
3rd-degree kinship					
	97 (64.2)	105 (68–125)	p=0.774	61.34±11.43	p=0.130
Presence of a disabled friend					
Yes	105 (16.3)	101 (60–125)	MU=24454.000	61.77±16.75	t=-0.817
No	539 (83.7)	107 (54–125)	p=0.028	59.84±10.74	p=0.415
Education of disabled individuals together with individuals with no disabilities					
Yes	495 (76.9)	108 (60–125)	MU=26888.000	62.03±11.76	t=4.839
No	149 (23.1)	97 (54–125)	p<0.001	54.68±11.7	p<0.001
Having received education on disability					
Yes	176 (27.3)	106 (65–125)	MU=40095.000	59.27±11.36	t=-3.159
No	468 (72.7)	106 (54–125)	p=0.605	62.39±11.1	p=0.002
Views about the marriage of disabled women					
A disabled woman should marry a disabled man					
	102 (15.8)	100 (69–122)	2>1.2>3	60.21±16.53	2>3.1>3
A disabled woman should marry a man with no disabilities					
	500 (77.6)	107 (68–125)	KW=29.282	61.07±10.86	W=32.213
A disabled woman should not marry					
	42 (6.5)	101.5 (75–125)	p<0.001	52.5±8.3	p<0.001
Views about the marriage of disabled men					
A disabled man should marry a disabled woman					
	110 (17.1)	99.5 (69–122)	2>1.2>3	58.72±16.7	2>3.1>3
A disabled man should marry a woman with no disabilities					
	489 (75.9)	107 (68–125)	KW=37.738	61.44±10.64	W=30.217
A disabled man should not marry					
	45 (7)	104 (75–125)	p<0.001	53.42±7.61	p<0.001

* MU – Mann Whitney U test, t – Independent t-test, KW – Kruskal Wallis test

Attitudes towards Disabled Women Scale mean score was found to be higher in those who did not have a disabled individual in the family compared to those

who had; those who said yes to the education of disabled individuals together with individuals with no disabilities compared to those who said no; those who did not receive education on disability compared to those who did; those who thought that a disabled woman should marry a disabled man or with no disabilities compared to those who thought that a disabled woman should not marry; those who thought that a disabled man should marry a disabled woman or with no disabilities compared to those who thought that a disabled man should not marry.

Table 3. Distribution of Students' GPS and ATDWS Total mean scores

Scales and sub-scales	Mean±SD	Median (Min–Max)
GPS total score	102.67±16.45	106 (54–125)
ATDWS total score	61.53±11.25	63 (35–85)
Disabled women and their private lives	24.08±4.96	24 (10–30)
Disabled women and disadvantages	13.77±3.85	14 (4–20)
Disabled women and social support	14.69±3.15	15 (6–20)
Disabled women and gender	8.98±2.88	9 (3–15)

Table 4. Relationship between Students' Attitudes towards Disabled Women Scale mean score and some variables*

	B (95% CI)	Beta	t	p	Zero-order	Partial
Constant	20.642 (8.598–32.686)		3.388	0.001		
Presence of a disabled individual in the family (reference=no)	-4.013 (-8.066–0.039)	-0.143	-1.958	0.052	-0.071	-0.161
Education of disabled individuals together with individuals with no disabilities (reference=no)	4.75 (0.527–8.972)	0.169	2.223	0.028	0.262	0.182
Views about the marriage of a disabled woman (reference=A disabled woman should not marry)	10.549 (3.414–17.683)	0.344	2.923	0.004	-0.001	0.237
A disabled woman should marry a disabled man						
A disabled woman should marry a man with no disabilities	7.062 (0.598–13.526)	0.260	2.159	0.032	0.115	0.177
GPS	0.323 (0.217–0.429)	0.445	6.043	<0.001	0.439	0.450
Kinship with the disabled relative (reference=3 rd degree relative)	-2.608 (-6.184–0.969)	-0.103	-1.441	0.152	-0.124	-0.119

* Multiple Linear Regression Analysis, B – unstandardized coefficient, Beta – standardized coefficient, F=9.905, p<0.001, R=0.541, R2=0.291, AdjR2=0.263, SE=10,415

An analysis of Table 3 shows that the GPS total mean score was 102.67±16.45 and the ATDWS total mean score was 61.53±11.25. ATDWS sub-scale scores were 24.08±4.96 for the disabled women and private life sub-scale; 13.77±3.85 for the disabled women and disadvantages sub-scale; 14.69±3.15 for the disabled wom-

en and social support sub-scale; and 8.98 ± 2.88 and for the disabled women and gender sub-scale.

Table 4 shows the multiple linear regression analysis performed with gender perception scale-related variables such as the presence of a disabled individual in the family; education of disabled individuals together with individuals with no disabilities; views about the marriage of disabled women; and kinship with the disabled relative. A positive and moderately significant correlation was found between gender perception and attitudes toward disabled women ($r=0.439$; $p<0.001$) ($r=0.439$; $p<0.001$) (Table 4).

The presence of a disabled individual in the family; education of disabled individuals together with individuals with no disabilities; views about the marriage of a disabled woman; kinship with the disabled relative; and the Gender Perception Scale score were found to explain 26.3% of the total variance for the attitudes towards disabled women ($R^2:0.291$, $AdjR^2:0.263$, $p<0.001$).

Discussion

This study investigates the effect of social gender equality perception among university students on attitudes towards disabled women.

Gender perception was found to be higher in women in this study ($p<0.001$). In their study conducted with university students, Roca-Hurtuna and Sanz-Ponce found that gender role perception was higher in women than men.²² Demirkoparan and Beydağ reported higher gender perception in women than men in their study conducted with nurses.²³ Ruiz et al. included female students in the departments of nursing and physiotherapy in their study and found no significant differences between gender role perception and gender.²⁴ Malkoc reported higher gender perception in men than women among participating social sciences students.²⁵ Our finding differs from the findings reported by Ruiz et al. and Malkoc.^{24,25} Hence, the literature reports different findings, which are considered to result from the differences in women's perception of the gender discrimination they are exposed to. This study found that the gender role perception of Nursing and Nutrition and Dietetics department students was significantly higher than that of social service department students ($p<0.001$). Yanikkerem et al. reported that students enrolled in the Departments of Nursing and Midwifery at the Faculty of Health Sciences had a higher perception of social equality than students in the Department of Social Service.²⁶ The finding in this study is similar to the one reported by Yanikkerem et al.; Kul Uçtu and Karahan found no relationship between gender perception and the department of study in health high school students.²⁷ Ünal et al. reported no relationship between predicting gender perception and the department of study.²⁸ This result is considered to result from being enrolled in departments that address women's

lives and social structure more closely and provide holistic care, which increases gender role perception. This study found that gender role perception was higher in nuclear families than in extended families ($p<0.001$). Coşkun et al. examined gender perception in students of the Faculty of Health Sciences and found that gender perception was higher in nuclear families.¹⁷ The study conducted by Korkmaz and Özbeşler found that gender perception was lower in university students who had extended families.²⁹ The findings in this study are similar to the findings reported by Korkmaz and Özbeşler and Coşkun et al. Besides studies indicating that gender perception is higher in nuclear families, some other studies indicate that it is higher in extended families and no relationship exists between family type and gender perception.^{30,31} This result is considered to be caused by the predominance of gender discrimination, social norms, and roles toward women in extended families.

Gender perception was found to be higher in participating individuals who had one or two siblings and who had a sister ($p<0.001$, $p<0.05$). Yanikkerem et al. found that the understanding indicating men's superiority and women's dependence on men was higher among university students who had three or more siblings compared to those who had no or two siblings.²⁶ Coşkun et al. determined that gender perception was higher in individuals who had sisters.¹⁷ In their study conducted with academics, Aydin et al. reported that the presence of a sister in the family had no relationship with gender perception. This condition is considered to increase the gender role perception of those who have a sister because it provides the opportunity to be closely involved in women's social, work, and education processes.

In this study, attitudes towards disabled individuals were found to be more positive in women than men ($p<0.001$). Roca-Hurtuna and Sanz-Ponce also found that women's attitudes toward disability were more positive than men among university students.²² In their study examining university students' attitudes towards disabled individuals and social likability, Gedik and Toker found that women's attitudes towards disability were more positive.³² The finding in this study is similar to the findings reported by Hurtuna and Ponce and Gedik and Toker. Ruiz et al. found that gender did not affect university students' attitudes toward disabled individuals. In their study including health personnel, Devkota et al. found that men had a more positive attitude towards disabled individuals than women.⁹ It is thought that the difference in the study results stems from the compassionate, gentle, understanding and maternal roles that society imposes on women.

This study found that the presence of a disabled individual increased attitudes toward disabled individuals ($p<0.05$). Roca-Hurtuna and Sanz-Ponce reported that university students who had disabled friends and

acquaintances in their family and business life had positive attitudes towards disabled individuals.²² Santilli et al. investigated the attitudes of coworkers toward their disabled colleagues and found that social relationships increased work performance.³³ Our findings are parallel to the literature. This finding is considered to result from having a disabled person in a close environment, which makes individuals avoid exhibiting wrong attitudes and behaviors towards disabled individuals. Having received education on disabled individuals was found to increase attitudes towards disabled individuals positively in this study ($p < 0.05$). Iwakuma et al. reported that the education on disabled individuals given to university students decreased students' perception of disability as a difficulty and made them feel closer to disabled individuals.¹⁸ Our finding is similar to the findings reported by Iwakuma et al. Devkota et al., who aimed to determine healthcare personnel's attitudes towards disabled people and found that receiving training on disability did not affect attitudes towards disabled individuals. Although our finding is different from the finding reported by Devkota et al., receiving education about disability increases attitudes toward disability because it decreases misconceptions and increases knowledge about disabled individuals.

Research findings indicate that students who hold the belief that disabled women should marry have more favorable sentiments towards disabled women. Bal investigated attitudes and gender perceptions towards women with orthopedic disabilities and found that the participants thought that disabled women should not marry or they should marry disabled individuals like them because disabled women reportedly cannot be good spouses and mothers.¹⁵ Our finding differs from the finding of Bal, which is related to the sample group of our study consisting of health sciences students, who receive training on disabled women, and their increased awareness on this issue.

In this study, gender perception was found to positively increase attitudes towards disabled individuals. Disabled individuals, who are in the disadvantaged group, were found to be exposed to a second obstacle due to their gender. Women are associated with gender roles such as being a good mother and good wife and having an ideal body. Disabled individuals cannot have these characteristics and therefore they face negative attitudes. Increased gender perception also increases attitudes towards disabled women positively.³⁴ The literature includes no studies that compared the gender perception scale with Attitudes toward Disabled Women Scale. This study is believed to be unique in this respect and thus contribute to the literature.

Study limitations

Data of this study were collected from students enrolled in a specific province, which is considered to be a limita-

tion. Additionally, it is not possible to draw causal conclusions based on this study because a cross-sectional design was used. Another limitation of the study is that it does not include open-ended questions about attitudes towards disabled women, and a mixed design was not utilized by adding qualitative analysis.

Conclusion

This study demonstrated that gender norms of students enrolled in the Faculty of Health Sciences at a public university had a notable impact on their views towards disabled women. Identifying the factors that influence the behaviors and attitudes of future health professionals towards disadvantaged populations throughout the provision of health services is crucial. To promote knowledge and understanding among students, it is crucial to arrange seminars focusing on the role of women in society, disability, and disabled women. These seminars should include informative case studies and involve volunteering efforts with individuals who have impairments. Engaging students in projects focused on disabled women might motivate them to develop educational strategies to address the prevailing challenges in this domain. This study is expected to provide a valuable contribution to experimental research in education. The findings of this study can serve as a reference for designing qualitative research.

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Declarations

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Author contributions

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

Conflicts of interest

The authors declare no conflict of interest.

Data availability

All data are available within the manuscript.

Ethics approval

A state scientific research and publication The Ethics committee decision (decision number 2022/10/12 dated 28.12.2022) was taken from the ethics committee.

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ORIGINAL PAPER

Tryptophan reduces the degree of brown adipose tissue whitening in rats with visceral obesity

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ABSTRACT

Introduction and aim. The relationship between brown adipose tissue (BAT) and visceral obesity (VO) is a topic of growing interest in scientific and medical research. The aim of this work was to investigate the effect of L-tryptophan on histomorphological abnormalities in BAT induced by a high calorie diet (HCD).

Material and methods. The study was performed on male Wistar rats 3 months of age. Control rats (group I) were fed a standard diet. VO in animals (groups II and III) was modelled by exposure to an HCD (45% fat and 31% carbohydrates) for 12 weeks. The rats in group III were also given L-tryptophan (80 mg/kg). Histological preparations were prepared from the interscapular bodies of the BAT. Indicators of lipid metabolism, oxygen consumption, subcutaneous oxygen tension and basal temperature were measured in the rats.

Results. It was found that the group of rats on HCD lead to the development of VO, and histomorphological changes occur in BAT indicating a decrease in its activity. Supplementation with L-tryptophan reduced the structural abnormalities in BAT, namely the accumulation of fat, the whitening of brown adipocytes, and prevented excessive loss of activity due to the deleterious effects of HCD.

Conclusion. Supplementation with L-tryptophan may have a potential benefit in preventing the development of excessive VO by preserving BAT activity.

Keywords. brown adipose tissue, obesity, tryptophan

Introduction

The global increase in obesity has become a serious problem with numerous consequences for the health of individuals and society as a whole.¹ Obesity often leads to the development of cardiovascular diseases, diabetes, fatty hepatosis, hormonal disorders, and even cancer.² At the same time, the effectiveness of existing methods of treatment and prevention of obesity is insufficient.

In recent years, researchers have turned their attention to brown adipose tissue (BAT) and its potential impact on obesity.³ Unlike white adipose tissue, which stores

energy in the form of triglycerides, BAT has a higher metabolic activity due to its thermogenic function, which allows it to burn calories and dissipate energy in the form of heat.⁴ Therefore, increasing the activity of BAT may be of great importance in caloric expenditure and contribute to the control of visceral obesity (VO).

At this time, the search for BAT-activating factors is relevant. These include: the influence of low temperatures; physical activity; the effect of some food additives, e.g. capsaicin in chilli peppers; the effect of hormones, especially irisin and natriuretic peptides, etc.⁵⁻⁸ BAT

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transplantation has also been shown to be a promising potential therapeutic approach in the fight against obesity.⁹ Although BAT activation has the potential to control obesity, the individual variability of its activity has certain complications. Not all people respond in the same way to stimuli that activate BAT, so individual approaches and the search for a universal factor that activates BAT are needed.

One such method is the use of the essential amino acid tryptophan. It is known that tryptophan is involved in the regulation of energy metabolism and food intake and has a direct effect on adipose tissue.¹⁰ Most studies on the effects of tryptophan on obesity have been conducted in animals. It has been shown that tryptophan reduces the weight of visceral fat in rats fed a high calorie diet (HCD).¹¹ Tryptophan metabolites (mainly serotonin and melatonin) have been studied for the activity of BAT and the development of obesity. It has been shown that the central and peripheral pools of serotonin act in opposite directions. For example, centrally acting serotonin, synthesized in the brain stem, inhibits food intake and reduces body weight, while peripheral serotonin, mainly from the gut, stimulates the development of obesity.¹² Melatonin has been found in most studies to inhibit the development of obesity by increasing BAT activity and energy expenditure.^{13,14} The effect of tryptophan on obesity in humans is poorly understood. For example, oral tryptophan (750 mg, twice daily for 3 months) was found to significantly increase weight loss in severely obese people.¹⁵ Other authors showed that administration of L-tryptophan (in a dose of 1-3g) reduced appetite and accelerated weight loss in patients.¹⁶

However, the effect of the influence of tryptophan on the histomorphometric changes in BAT that develop with VO has not been fully investigated. The mechanism by which tryptophan, according to most literature, is able to reduce the manifestations of VO and its negative consequences on the body is not known.^{17,18} Perhaps it does so by activating BAT. The question of the mechanisms of the relationship between the effect of tryptophan, the state of BAT and energy metabolism and their role in the pathogenesis of VO remains open and requires further study. Therefore, this study was conducted to investigate the efficacy of L-tryptophan supplementation in reducing the severity of obesity-related BAT structural disorders. And also to evaluate the possibility of using tryptophan in reducing the development of VO by preserving BAT activity.

Aim

The aim of this work was to investigate the effect of L-tryptophan on histomorphometric changes in brown adipose tissue induced by a high-calorie diet and to evaluate the possibility of its use in the prevention of visceral obesity.

Material and methods

Rats

Three month old Wistar rats (weight 360 ± 20 g) obtained from the vivarium of the Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine were used in the experiments. The rats were maintained at a temperature of 20°C and humidity of 40–60% with a 12-hour light/dark cycle. All protocols were approved by the Committee on Biomedical Ethics of Animal Care and Use of the Bogomoletz Institute of Physiology (Protocol No. 5, dated 31.11.19). Rats were sacrificed by decapitation under isoflurane anesthesia in accordance with the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986).

Modeling visceral obesity

The rats were randomly divided into 3 groups (12 animals in each group): I – control; II – animals that received HCD for 12 weeks; III – rats that received L-tryptophan (Ajinomoto Eurolysine S.A.S, France) at a dose of 80 mg/kg in addition to HCD.

Each rat of the control group received 20 g of standard feed daily with a fat content of 6%, protein content of 23%, and carbohydrate content of 55% (recipe K120-1 “Rezon-1”, Ukraine) and had free access to water. The daily caloric content per rat was 66 kcal. Experimental rats of II and III groups received HCD with a total daily caloric intake of 116 kcal per animal. The composition of such a diet, in addition to standard mixed feed, included pork lard, white breadcrumbs and sunflower seeds (fats – 45%, proteins – 9% and carbohydrates – 31%). It is this selected composition of HCD that leads to the development of pronounced signs of VO (the reproducibility of this pathology is 100%). The diet used ensures a high rate of food intake by the animals (97–100%).¹⁹ In addition, every second day the experimental rats received a 10% fructose solution instead of water, increasing the caloric content to 140 kcal per rat. This drinking regime does not reduce the animals' consumption of dry food and fluids and does not threaten to disrupt the body's water balance. The choice of fructose was based on the fact that its addition to the diet increases the negative effect of HCD on the development of obesity. As a high-fat, high-carbohydrate diet mimics the Western human diet, it is widely used to model obesity.

During the study period, the completeness of food intake was monitored daily in both control and experimental rats. Their general condition was assessed. Animals were weighed weekly. The presence of VO in the rats was diagnosed at the end of the experiment by determining the weight of visceral fat and the concentration of total lipids, triglycerides and cholesterol in the blood serum. These indicators can be used to determine the presence and degree of development of VO. Visceral fat was extracted mechanically from the peritoneal cav-

ity. BAT was removed from the interscapular area of the rat and its weight was determined.

Histomorphological analysis of the BAT

For histomorphometric studies, tissue samples were randomly selected from the interscapular bodies of the BAT, from which histological preparations were made according to the standard method.²⁰ Tissue samples were fixed in Bouin's fluid, dehydrated in alcohols of increasing concentration and embedded in paraffin. Paraffin sections of 6 μm thickness were prepared on a sliding microtome (MS-2, Reichert, Austria). The sections were stained with hematoxylin-eosin and by the Van Gieson method. Photomicrography was carried out using a light-optical microscope "Nikon Eslirse E100" (Japan) with a digital camera ("Levenhuk", USA). Morphometric analysis of photographs of serial sections was performed using the program "ImageJ 1.34".

For histomorphometric analysis, 5 sections of the BAT were taken from each rat. The relative areas of parenchyma, connective tissue and vessels were determined on the histological sections of the BAT. The stromal-parenchymal index (the ratio of the relative area of the connective tissue to the area of the parenchyma) and the trophic index (the ratio of the relative area of the vessels to the area of the parenchyma and connective tissue) were calculated.²¹ The mean diameter and cross-sectional area of adipocytes, their nuclei and cytoplasm were measured in 100 cells. The number of adipocytes was counted in 100 areas of 1000 μm^2 . The number of nucleoli was counted per 100 adipocyte nuclei. The number and area of lipid droplets in adipocytes were calculated. According to the number of lipid droplets, the adipocytes were divided into 3 types: A1 – contain 1 large lipid droplet; A2 – contain 1 large lipid droplet and several small ones; A3 – contain many small droplets. 100 adipocytes were counted on different sections. The number of adipocytes of each type was expressed as a percentage of the total number of cells counted. A morphometric grid was superimposed on the photomicrographs to facilitate cell counting and to determine the relative areas of the components of the BAT. Histomorphometric analysis of the BAT was performed on 10 photomicrographs from each rat at 800 \times magnification (23,000 μm^2).

Assessment of lipids in blood serum

The concentrations of total lipids, triglycerides, cholesterol, and high-density lipoproteins in rat blood serum were determined by a colorimetric-enzymatic method using standard reagent kits (Filisit-Diagnostics, Ukraine) and a biochemical analyzer (Sinnova, China). Standardized protocols were used to determine these indicators in blood serum.

Determination of oxygen consumption and basal body temperature

The determination of total oxygen consumption (VO_2) in rats was performed on an empty stomach in a closed gas exchange research system. The value of VO_2 was calculated in ml per 1 kg of body weight in 1 hour and brought to standard physical conditions (STPD): dry gas at a temperature of 0°C and a pressure of 760 mmHg. Rectal temperature was measured with an electronic medical thermometer "Beurer FT 09/1". Oxygen tension (PO_2) in the subcutaneous tissue of the rats' dorsal trunk was measured by *in situ* polarography using an open platinum electrode.

Statistical analysis

Statistical processing of the results was performed using the "SigmaPlot 14.5" software (Inpixon, CA). Data were tested for normality of distribution using the Shapiro-Wilk test. One-way analysis of variance was used to compare the samples. Homogeneity of variance was checked using Levene's test. Differences at $p < 0.05$ were considered significant. Quantitative data are presented as mean \pm standard deviation.

Results

In rats, signs of VO were found after 12 weeks on the HCD (group II). First of all, this indicated the increase in weight of visceral fat by 130.21% ($p < 0.05$). In the blood serum an increase in the concentration of total lipids, triglycerides and cholesterol by 52.38%, 67.76% and 26.44% ($p < 0.05$), respectively, compared with the control. In rats of group III receiving L-tryptophan in combination with HCD, indicators of lipid metabolism did not differ from control values (Table 1). The weight of visceral fat was 40.22% lower ($p < 0.05$) than in the rats of group II. The weight of interscapular brown fat was 78.51 and 50.33% ($p < 0.05$) higher in rats of groups II and III, respectively.

Table 1. Indicators of lipid metabolism in blood serum^a

Indicators	Control	High-calorie diet	High-calorie diet + L-tryptophan
Lipids, mmol/l	2.52 \pm 0.21	3.84 \pm 0.40*	2.60 \pm 0.24 [^]
Triglycerides, mg/dl	94.9 \pm 3.8	159.2 \pm 9.3*	97.6 \pm 3.8 [^]
Cholesterol, mmol/l	1.74 \pm 0.09	2.20 \pm 0.11*	1.74 \pm 0.09 [^]
High density lipoproteins, mmol/l	1.72 \pm 0.07	0.80 \pm 0.03*	1.68 \pm 0.08 [^]

^a* – $p < 0.05$ – significance of differences compared with the control, [^] – $p < 0.05$ – significance of differences compared with rats that received a high-calorie diet

Microscopic examination of sections from interscapular BAT of rats showed that they were composed of specialized adipocytes, which are different from white fat cells. The adipocytes contained a nucleus with several nucleoli, usually located in the center. The nucleus is

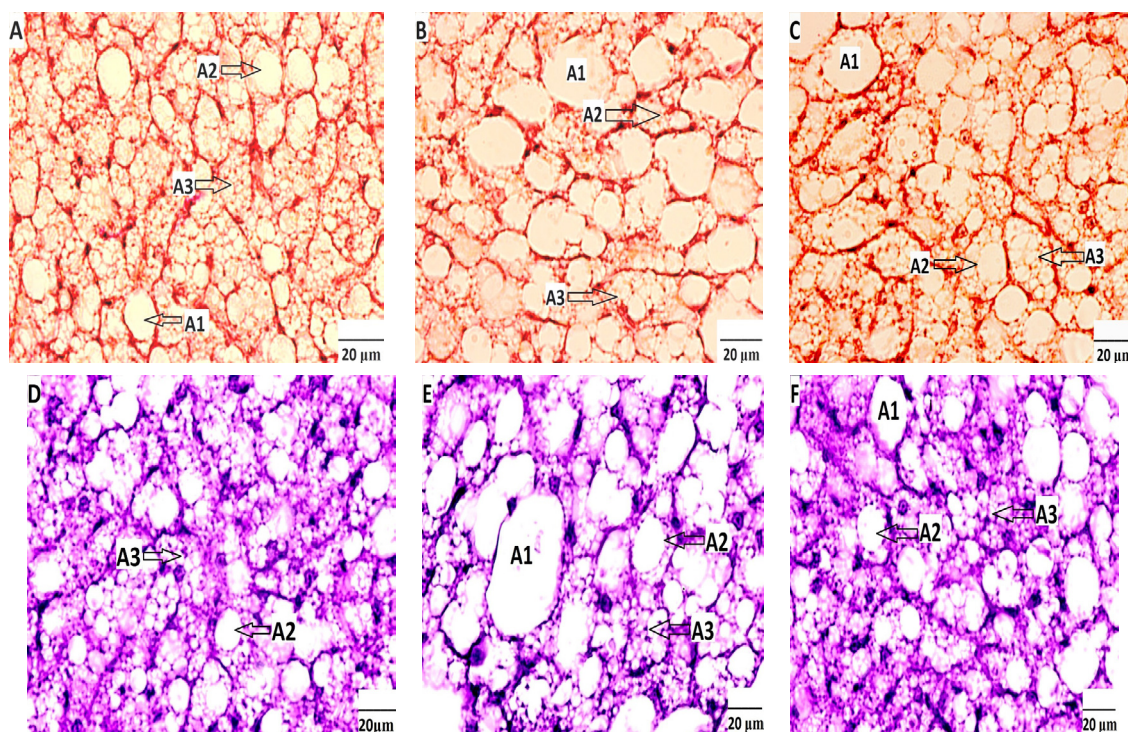


Fig. 1. Representative images of histological slides of brown adipose tissue (A, D – control rats, B, E – rats on high-calorie diet and C, F – rats treated with L-tryptophan in addition to high-calorie diet). A-C – Van Gieson stain, D-F – hematoxylin-eosin stain (800x), A1 – adipocyte with 1 large lipid droplet; A2 – adipocyte with one large and several small droplets; A3 – adipocyte with many small droplets

surrounded by many lipid droplets that are well separated from each other. This gave the cells a multi-chambered appearance. Such cells are actually functionally active brown adipocytes (type A3). In the HCD rats, the presence of adipocytes with a large lipid droplet and a nucleus displaced to the membrane was observed. We called this type of adipocyte A1. In terms of structure and function, these cells took on the characteristics of white adipocytes. The greater the degree of VO in the animal, the greater the number of white adipocytes in the BAT. In control animals, the white adipocytes in the BAT were small. Adipocytes were also found with a large lipid droplet surrounded by several small ones (type A2). These cells are a transitional type between white and brown adipocytes (Fig. 1).

As mentioned above, the number of white adipocytes was significantly increased in the BAT of rats on HCD, exceeding the control rate by 1800.12%. These rats had by 51.34% ($p < 0.05$) fewer brown adipocytes (A3) and by 110.52% ($p < 0.05$) more cells with one large lipid droplet and several small ones (A2) than the control (Fig. 2). The size of brown adipocytes was larger, namely: diameter by 18.72% ($p < 0.05$), area by 40.9% ($p < 0.05$) and their cytoplasmic area by 43.52% ($p < 0.05$). The nuclear-cytoplasmic ratio was less than by 22.89% ($p < 0.05$) compared with the control. The increase in adipocyte size is associated with an increase in lipid droplet area by 93.04% ($p < 0.05$). The number of lipid

droplets in the cells was less than by 45.51% ($p < 0.05$) compared with the control. The total number of adipocytes was less than by 31.91% ($p < 0.05$). The number of nucleoli in the nucleus of adipocytes in group II rats was less by 15.63% ($p < 0.05$), indicating a decrease in synthetic activity. There was also an increase in the relative area of connective tissue by 63.04% ($p < 0.05$) and a decrease in the area of vessels by 54.55% ($p < 0.05$) in the BAT of these rats (Table 2). This indicates the inhibition of oxygen transport to the parenchymal elements, the deterioration of conditions for the course of metabolism. In other words, the morphometric data obtained indicate a decrease in the functional activity of the BAT and its transformation into white fat.

Rats receiving HCD together with L-tryptophan showed less histomorphometric changes in BAT and significantly less evidence of brown to white adipocyte transformation. This was evidenced by a lower number of type A1 adipocytes (by 63.12%, $p < 0.05$), type A2 adipocytes (by 23.93%, $p < 0.05$) and a higher number of type A3 brown adipocytes (by 58.21%, $p < 0.05$) compared with group II rats (Fig. 2). When L-tryptophan was administered, the area of lipid droplets was smaller by 18.92% ($p < 0.05$) and their number was larger by 44.33% ($p < 0.05$). This resulted in a decrease in adipocyte area and cytoplasm by 15.92% and 16.91% ($p < 0.05$), respectively, and an increase in nuclear-cytoplasmic ratio by 18.75% ($p < 0.05$) compared with rats on

HCD alone. Administration of L-tryptophan also resulted in by 26.67% ($p < 0.05$) decrease in the relative area of connective tissue and by 40.00% ($p < 0.05$) increase in the area of blood vessels (Table 2). This indicates better perfusion and greater functional activity of the adipocytes than in the group II rats.

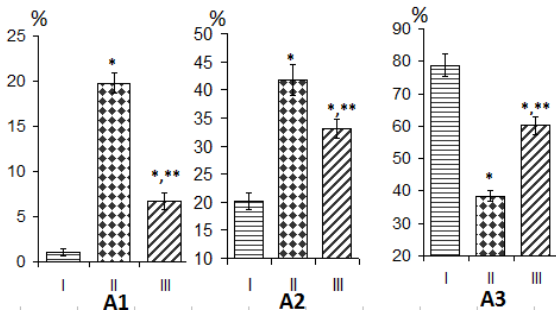


Fig. 2. The number of adipocytes of different types in BAT rats: A1 – contain 1 large lipid drop, A2 – contain 1 large lipid drop and several small, A3 – contain many small drops. The number of cells of each type is represented as a percentage of the total number of adipocytes. * $p < 0.05$ – significance of differences compared with the group I, ** $p < 0.05$ – significance of differences compared with the group II. I – control rats, II – rats on HCD and III – rats treated with L-tryptophan in addition to HCD

Table 2. Histomorphometric indicators of brown adipose tissue

Indicators	Control	High-calorie diet	High-calorie diet + L-tryptophan
Adipocyte diameter, μm	18.7 \pm 0.7	22.2 \pm 0.6*	21.1 \pm 0.4*
Area, μm^2			
adipocyte	300.0 \pm 15	422.7 \pm 18.6*	355.4 \pm 14* [^]
nucleus	23.1 \pm 0.9	25.3 \pm 1.1	25.2 \pm 0.9
cytoplasm	276.9 \pm 15.6	397.4 \pm 12.6*	330.2 \pm 15.1* [^]
nuclear-cytoplasmic ratio	0.083 \pm 0.008	0.064 \pm 0.004*	0.076 \pm 0.001 [^]
Number of adipocytes, pcs/1000 μm^2	2.57 \pm 0.11	1.75 \pm 0.09*	2.04 \pm 0.09* [^]
Number of nucleoli in the nucleus, pcs	1.60 \pm 0.07	1.35 \pm 0.05*	1.49 \pm 0.04
Number of lipid droplets in the adipocyte, pcs	17.8 \pm 0.6	9.7 \pm 0.4*	14.0 \pm 0.7* [^]
Area of lipid droplets, μm^2	11.5 \pm 0.6	22.2 \pm 1.2*	18.0 \pm 0.9* [^]
Relative area, %			
parenchyma	92.1 \pm 1.1	91 \pm 1.4	92.4 \pm 1.3
connective tissue	4.6 \pm 0.8	7.5 \pm 0.5*	5.5 \pm 0.2 [^]
vessels	3.3 \pm 0.4	1.5 \pm 0.2*	2.1 \pm 0.1* [^]
Stromal-parenchymal index	0.086 \pm 0.004	0.099 \pm 0.003*	0.082 \pm 0.005 [^]
Trophic index	0.036 \pm 0.005	0.016 \pm 0.003*	0.023 \pm 0.002* [^]

^a * – $p < 0.05$ – significance of differences compared with the control, [^] – $p < 0.05$ – significance of differences compared with rats that received a high-calorie diet

A significant change in oxygen metabolism and thermogenesis was observed in experimental rats receiving HCD. Rectal temperature increased the most in group II rats by 0.96°C ($p < 0.05$). In these rats there was a clear tendency to increase total oxygen consumption (+11.30%) and subcutaneous oxygen tension (+2.70

mmHg). Rectal temperature was 0.82°C ($p < 0.05$) higher in rats treated with HCD in combination with L-tryptophan than in control animals. Total oxygen consumption in this rats increased by 21.60% ($p < 0.05$) and subcutaneous oxygen tension by 9.31 mmHg ($p < 0.05$) compared with the control (Fig. 3). Thus, tryptophan activates oxygen metabolism, contributes to the utilization of excess dietary fats and increases heat production.

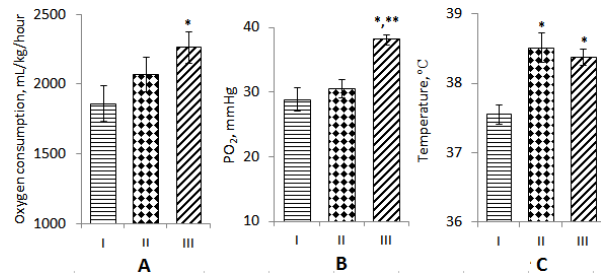


Fig. 3. Oxygen consumption (A), subcutaneous oxygen tension (B) and rectal temperature (C) of control rats (I), rats receiving high calorie diet (II) and rats receiving high calorie diet and L-tryptophan (III). * $p < 0.05$ – significance of differences compared with the group I, ** $p < 0.05$ – significance of differences compared with the group II

Discussion

The relationship between BAT and VO is a topic of growing interest in scientific and medical research. The unique ability of BAT to burn calories through thermogenesis has led to its investigation as a potential target for obesity reduction strategies.²² Although various methods of activating brown fat show promise, further research is needed to fully understand its role and how it can be used effectively and safely to prevent the development of excessive VO. Therefore, the search for new approaches to BAT activation may offer new opportunities in the fight against obesity and contribute to the improvement of global health care. The protective mechanisms against the excessive development of VO caused by tryptophan are complex and not yet fully understood. One possible mechanism is the activation of BAT by tryptophan or its derivatives.¹³

We found that a 12-week stay of rats in the HCD leads to the development of VO, as evidenced by a significant increase in the weight of visceral fat and indicators of lipid metabolism in blood serum. Histomorphological changes occur in BAT, indicating a decrease in its activity and intensification of brown adipocyte whitening processes. An increase in the size of lipid droplets and a change in the color of brown adipocytes made them more similar to cells of white adipose tissue, which are mainly involved in the accumulation of energy and not in its consumption, i.e. they do not contribute to an increase in body temperature. However, we did find an increase in body temperature in experimental rats. A high

level of total oxygen consumption and an increase in basal body temperature in the presence of VO indicates the possible presence of a certain degree of disruption of oxidation and phosphorylation processes, but does not exclude other mechanisms of temperature increase. For example, the number of beige adipocytes with thermogenic capacity may increase in white adipose tissue. The remaining brown adipocytes may increase their thermogenic activity to compensate for the decrease in their number. Improved mitochondrial function and increased expression of UCP1 in existing brown adipocytes can lead to increased heat production.²³ Activation of the sympathetic nervous system can significantly increase the thermogenic activity of brown and beige adipocytes.²⁴ Obesity is often associated with a chronic inflammatory process of low severity. It is characterized by an increase in levels of pro-inflammatory cytokines, which can affect the center of temperature regulation.²⁵ Activation of the immune system in obesity may also contribute to the development of hyperthermia.²⁶ Further studies are therefore needed to fully understand the complex interactions between obesity, inflammation and thermoregulation in laboratory rats.

Supplementation with L-tryptophan reduces structural abnormalities in the BAT, thereby reducing the accumulation of fat in her and preventing excessive loss of activity due to the deleterious effects of HCD. At the same time, there is a decrease in the number of white adipocytes and an increase in the number of active brown cells, which intensively produce heat.²⁷ Adipocytes not involved in thermogenesis acquire a structure more similar to white cells.²⁸ As a result, after exposure to L-tryptophan, indicators of lipid metabolism returned to control values and visceral fat weight decreased.

It is known that in obesity the activity of BAT decreases and the process of „whitening” occurs – the transformation of brown adipocytes into white ones.²⁹ Possible mechanisms include inhibition of vascular endothelial growth factor, redoxosome proliferator-activated receptor gamma coactivator 1 alpha and bone morphogenetic protein 7, which disrupts vascularization, mitochondrial biogenesis and differentiation of brown adipocytes. This can lead to the infiltration of T cells that secrete interferon-gamma, increase autophagy and disrupt metabolism. Whitening of BAT is thought to be under the control of genes such as b-AR, BMP and mitochondrial transcription factor A, as well as microRNAs, which regulate many processes including brown adipocyte differentiation.³⁰⁻³²

Thus, new data were obtained regarding the role of tryptophan in the mechanisms of obesity development and related metabolic disorders. Namely, it has been shown that tryptophan reduces the severity of structural damage of the BAT caused by HCD. This helps to increase the activity of the BAT. Important links in the

positive effect of tryptophan in VO are the activation of oxygen metabolism and the increase in the activity of thermogenesis, which contributes to the utilization of the excess amount of fats that come with food.

Study limitations

Although L-tryptophan at a dose of 80 mg/kg reduced the severity of BAT structural damage and the degree of adipocyte whitening induced by a HCD, the lack of a dose-dependent effect for the obesity model used limits the final conclusion.

Conclusion

Tryptophan supplementation may have a potential benefit for brown adipose tissue by reducing the severity of its structural damage and the extent of adipocyte whitening induced by a high calorie diet. Tryptophan also promotes increased basal metabolic rate and increased energy expenditure associated with the activation of thermogenesis. This may be of practical interest in the use of tryptophan and its derivatives in the clinic to prevent a decrease in brown adipose tissue activity and thus reduce the development of obesity. Enhancing the beneficial effects of tryptophan by combining dietary supplementation with other methods of brown adipose tissue activation will be the focus of our future research and may benefit obese patients.

Declarations

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Author contributions

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

Conflicts of interest

The authors declare no conflict of interests.

Data availability

The datasets 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 Ethics Committee on Biomedical Ethics of Animal

Care and Use of the Bogomoletz Institute of Physiology National Academy of Sciences of Ukraine (protocol No. 5 dated 11/31/19).

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ORIGINAL PAPER

Zn and Se supplementation abrogated metals-(metaloids) mixture-mediated ocular-thymus toxicity via modulation of oxido-inflammatory and antiapoptotic mechanisms in female Sprague Dawley rats

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ABSTRACT

Introduction and aim. This is an evaluation of the protective effects of Zn and Se in the eye and thymus of rats exposed to cocktail noxious metal mixtures (CNMM) (Al, Pb, Hg and Mn) in ameliorating ocular pathologies due to autoimmunity.

Material and methods. Female Sprague rats were grouped into eight (n=5) and orally exposed to various treatments for a period of 60 days: (1): the control group receive deionized water only; (2): the CNMM only group received lead acetate $Pb(C_2H_3O_2)_2$ (20 mg/kg), $AlCl_3$ (35 mg/kg), $HgCl_2$ (0.40 mg/kg) and $MnCl_2$ (0.56 mg/kg); (3) received CNMM+ZnCl₂, 0.80 mg/kg; (4) received CNMM+Na₂SeO₃, 1.50 mg/kg; (5) received CNMM+ZnCl₂, 0.80 mg/kg and Na₂SeO₃, 1.50 mg/kg combination. Oxidative stress markers, nuclear factor erythroid 2- related factor 2, nuclear factor kappa B, interleukin 6, tumor necrosis factor alpha and caspase-3 and histopathological changes were determined.

Results. CNMM decreased antioxidants levels but increased malondialdehyde and nitric oxide concentrations. CNMM increased levels of nuclear factor erythroid 2- related factor 2, and nuclear factor kappa B, interleukin 6 and tumor necrosis factor alpha and caspase-3. There was moderate retinal degeneration and total cell loss at the ganglionic cell layer in the eye; severe degenerative thymus, lymphocyte depletion and multifocal necrosis in CNMM only.

Conclusion. Supplementation with Zn and Se reduced the biochemical and histopathological changes in the eye and thymus in response to CNMM exposure.

Keywords. ameliorative effects of zinc and selenium, cocktail noxious metal mixture, eye, thymus

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Introduction

The eye is a sensory organ of vision located in the orbit and consists of three coats, three compartments and three fluids. The coat comprises the outer fibrous layer: (cornea, sclera, lamina cribrosa); the middle vascular layer or uveal tract (iris, ciliary body, choroids) and, the inner neuronal layer [pigment epithelium of the retina, retinal photoreceptors, and retinal neurons].¹ The eye transmits sensory impulses (clear image of objects) in the environment to the brain through the optic nerve and the posterior visual pathways to form visions.² The functions of all regions of the ocular surface system are closely integrated therefore, dysfunctions of or injury to one or more components of this system can lead to system-wide sequelae such as cicatrizing diseases and dry eye.³

Preclinical and clinical studies show the importance of heavy metals like cadmium and lead in the pathogenesis of eye diseases as their excessive exposure can lead to the development of age-related macular degeneration, cataracts, and glaucoma.⁴ Cadmium, lead and mercury cause oxidative stress, particularly in the cells of the retina and neurons possibly through reduced glutathione levels, which damage lipid membranes and DNA, thereby suggesting a possible route to increased risk of glaucoma.⁵ However, various trace elements such as iron, copper, zinc, selenium, calcium, magnesium, molybdenum, sodium, potassium and manganese help in maintaining the balance of prooxidative and antioxidative processes, regulation of fluid and ion flow through cell membranes of the ocular tissues.⁶

The immune system is one of the complex networks of mechanisms the eye has employed to maintain homeostasis and healthy ocular surface environment necessary to preserve visual function.⁷ Environmental toxins trigger the immunological events that shape the outcome of the diverse spectrum of autoimmune-based ocular surface disorders.⁷ The ocular surface contains its own local lymphoid tissues: conjunctiva-associated lymphoid tissue situated to sample antigens and maintain tolerance to commensal flora, with evolution of several mechanisms by the eye to modulate inflammation after environmental or microbial stress on the ocular surface.^{8,9} Therefore, abnormal activation of the immune system may result in autoimmunity to self-antigens localized to the ocular surface and associated tissues. Most challenges, physical and immunological, to the homeostasis of the eyeball usually arise due to defect in lubrication and/or host defense of the ocular surface.²

Ocular surface autoimmune diseases comprise various range of pathologies and manifest as ocular specific (dry eye, Mooren's ulcerative keratitis), systemic (Sjögren's syndrome, ocular cicatricial pemphigoid), or occur secondary to other common autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus).⁷ It is predicted that a combination of excessive

or atypical stimuli and/or immunoregulatory dysfunction, together with genetically predisposed factors and/or hormone imbalance provides an environment conducive to activation of autoreactive lymphocytes. The autoimmune response can be perpetuated by both T-cell-dependent and independent mechanisms.

Various subsets of regulatory T cells (Tregs) such as unconventional (CD8+, $\gamma\delta$ and NKT cells) and conventional Tregs (CD4+) which can be thymus-derived, naturally (nTreg) induced in response to specific antigens (iTreg) have been suggested to modulate the immune response within the ocular surface tissues and regional lymphoid organs. CD8+ T cells, $\gamma\delta$ T cells, and NKT cells present within the conjunctiva-associated lymphoid tissue of healthy subjects may provide protection against autoimmunity and likely contribute to both anti-microbial defense and suppression of autoreactive lymphocyte differentiation and/or function, the latter, by secreting TGF- β and/or IL-10.^{7,10} Tregs have been suggested to mediate autoimmune suppression by releasing soluble factors (TGF- β , IL-10), cell-cell contact that disables effector T cells and/or antigen presenting cells (APCs), and/or competing for soluble factors (IL-2 sequestration through high-level expression of the IL-2 receptor, CD25).

Environmental pollution with heavy metals (cadmium, lead, mercury) is of significance in the etiology of many eye diseases such as cataract, intraocular eye pressure and glaucoma.^{6,11} Reactive oxygen species (ROS)-NLRP3-IL-1 β signaling pathway axis was upregulated in environment-induced ocular surface diseases such as dry eye and corneal toxicity.^{12,13}

Certain metals like copper, zinc, and iron are utilized by the human cells to control significant metabolic and signaling functions making them essential for life while others such as the heavy metals: lead, cadmium, mercury, chromium, thallium, nickel, copper, zinc and bismuth; semi-metals like arsenic, tellurium and even non-metals-selenium are characterized by toxicity to humans or the environment.^{6,14} Zinc and iron respectively reduced the development of advanced age-related Macular degeneration (AMD) and oxidative stress implicated in the development of AMD.¹⁵

High concentration of mercury has been reported in cornea, iris, retina and lens, aqueous humor of humans with organomercury poisoning.¹⁶ Their toxic action is associated with their ability to accumulate in the body tissues and organs.

Oxidative stress and mitochondrial dysfunction are implicated in conditions affecting both the anterior segment (dry eye disease, keratoconus, cataract) and posterior segment (age-related macular degeneration, proliferative vitreoretinopathy, diabetic retinopathy, glaucoma) of the human eye, autoimmune uveitis.^{11,17-20} Increased susceptibility to disease may partially be at-

tributed to genetic and epigenetic changes that develop over time with continual environmental exposure and damage from endogenous and exogenous reactive oxygen species (ROS).¹⁷

The major chemical species associated with oxidative stress in the eye include Superoxide (O_2^-), Hydroxyl radical ($-OH$), Hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE).¹⁷ Thymus gland is a primary lymphoid organ of the adaptive immune system located in the upper anterior portion of the chest cavity, above the heart and behind the sternum.^{21,22} It is bi-lobed with two subcomponents: the cortex and the medulla made up of epithelial, dendritic, mesenchymal, and endothelial cells.²² The thymus gland is important in lymphoid cell homing and development, secretion of numerous cytokines, like IL-1 and -6, granulocyte colony stimulating factor (G-CSF), macrophage CSF (M-CSF) and GM-CSF essential during the various stages of thymocyte activation and differentiation and; the production and release of different hormones: thymosins, thymopoietin, thymulin.^{22,23} Thymus instructs T-cells preventing autoimmunity and maintain self-tolerance; mature T-cells (naïve T-cells) leave the thymus to peripheral lymphoid tissues like spleen and lymph nodes to establish the peripheral T-cell repertoire. Thymus also produces other alternative T-cell lineages, including regulatory T-cells (Treg cells), natural killer T (NKT) cells and $\gamma\delta$ -Tcells.²¹ Treg cells help to maintain self-tolerance by actively suppressing immune responses.²⁴

The thymus gland is a privileged site of T-cell generation, extremely vulnerable to toxic actions of chemicals.²⁵ The mechanisms of toxicity of thymus gland involve receptor binding (*Ah*, aryl hydrocarbon receptor); the Ca^{2+} -dependent activation of an endogenous endonuclease resulting in DNA fragmentation (programmed cell death or apoptosis) and interference with cell proliferation.²⁶ The consequences of thymus gland toxicity can be a decrease in output of newly generated T-lymphocytes (generation of a new T-cell repertoire), or induction of autoimmune symptoms by the creation of unwanted repertoire.

The physiological function of the thymus in mammals can be disturbed by introducing into the body increased doses of steroid hormones or by subjecting animals to stress. Salts of some heavy metals like cadmium, lead and mercury caused decreased DNA and vitamin C content *in-vitro*, reduced weight of thymus *in-vivo* with thymic involution, particularly visible in the cortical part of this gland.^{27,28} The thymolytic properties of the salts of these heavy metals are explained by their toxic effects on cell membranes, producing reactive oxygen species that result in lipid peroxidation, DNA damage, and depletion of cell antioxidant defense systems.^{29,30}

Zinc and selenium are vital for cellular metabolism, able to reverse damage produced by some heavy metals

such as cadmium on organs such as kidney and liver, possess anti-inflammatory, antioxidant, protective and immune properties.³¹⁻³⁴

Aim

The literature seems to be inundated with toxicity studies of individual metals and there is paucity of information on the toxicity of metal mixtures on some organs like the thymus and the eye. We, therefore, posited that zinc and selenium supplementation would down-regulate the inflammatory biomarkers and up regulate antioxidant transcription factors in the eye and thymus of cocktail noxious metal mixture (CNMM) exposed rats.

Material and methods

Chemicals

Lead acetate, mercury chloride, aluminum chloride, and manganese dichloride were bought from Sigma Chemical Co. (St. Louis, MO, USA). Rat ELISA kit of tumor necrosis factor alpha (TNF - α), interleukin 6 (IL - 6), caspase-3, nuclear factor erythroid 2- related factor 2 (Nrf2), nuclear factor kappa B (Nf-kB) and heme oxygenase - 1 (Hmox-1) bought from Elab Science Biotechnology Company, (Beijing, China) were used.

Animals and treatments

In vivo study

Female (weight-matched 100-200 g and aged 8-10 weeks, n=25) Sprague Dawley rats from the Department of Pharmacology, Animal House, University of Port Harcourt, Nigeria were kept under standardized conditions, with water and food *ad libitum*, in accordance with the ethical principles on animal research adopted by the University of Port Harcourt institutional Centre for Research Management and Development Animal Care and Use Research Ethics Committee (UPH/CEREMAD/REC/18). Animals were housed in standard polypropylene cages under room temperature $25\pm 2^\circ C$ with a 12-h light/dark cycles throughout the duration of the experiment. The animals were acclimatized for two weeks before the commencement of the study. The animals received standard feed and deionized water *ad libitum*. All protocols were approved by the University of Port Harcourt institutional Centre for Research Management and Development Animal Care and Use Research Ethics Committee (number: UPH/CEREMAD/REC/18).

Experiment was conducted in accordance with the "Guide for the Care of Laboratory Animals" approved by the National Academy of Science.

Experimental design

Animals were weight matched and divided into 5 groups (n=5) and various treatments were administered by oral gavage. The animals' treatment protocol lasted for 60 days as follows:

Group 1 received deionized water only and served as the control.

Group 2: Rats were given Cocktail Noxious Metal Mixture (CNMM) only Pb, (20mgkg⁻¹), Hg (0.40mgkg⁻¹), Mn (0.560mgkg⁻¹) and Al (35mgkg⁻¹).

Group 3 rats received CNMM+ZnCl₂, 0.80 mg/kg.³⁵

Group 4 were administered CNMM+Na₂SeO₃, 1.50 mgkg⁻¹.³⁶

Group 5 received CNMM+ZnCl₂, 0.80 mgkg⁻¹ and Na₂SeO₃, 1.50 mgkg⁻¹ combined.

Body, eye and thymus weights, feed and fluid consumption

The body weight of rats was measured weekly and at the end of the 60th day of experiment; the eye and thymus weight was recorded directly after the sacrifice on day 60. The absolute weight of the eye and thymus (g)=mean of eye and thymus weight for each group taken.

The relative weight of eye and thymus

$$\begin{aligned} & (\text{g}/100 \text{ g body weight})= \\ & = \frac{\text{eye and thymus weight for each group}}{\text{final body weight}} \times 100. \end{aligned}$$

Feed (g) and fluid consumption (ml) were recorded daily.

Harvesting and necropsy of eye and thymus

At the end of 60 days of treatment, animals in each group were euthanized using pentobarbital (50mg/kg) intra-peritoneally. The eye and thymus of each rat were harvested, rinsed in cold saline water, weighed and used for both biochemical parameters and heavy metal analyses.

Analysis of heavy metals

The eye and thymus tissues (20 mg) were digested separately using 2 ml of perchloric acid and 6 ml of nitric acid. The samples were subsequently kept for 30 min before heating at 105°C until digestion was completed and the solutions made up to 15 mL (final volume) with deionized water. The Solar thermo elemental flame Atomic Absorption Spectrometer (Model SG 71906) was used to determine Lead (Pb), Aluminum (Al), Mercury (Hg) and Manganese (Mn) concentrations.³⁷ Limits of detection (LoD) were 0.001 mgkg⁻¹ for Aluminum (Al), Mercury (Hg) and Manganese (Mn) and 0.01 mgkg⁻¹ for Pb, whereas the limits of quantification (LoQ) were 0.0033 mgkg⁻¹ for Al, Hg and Mn and, 0.033 mgkg⁻¹ for Pb.

Assay of antioxidant enzymes

GPx activity was measured using a well-established method of Rotruck, et al.³⁸ A known amount of enzyme preparation was allowed to react with hydrogen peroxide and GSH for a specified time period. The GSH content remaining after the reaction was measured by Ellman's reaction.³⁹ This method is based on the development of yellow color when dithionitrobenzoic acid

(DTNB) is added to compounds containing sulfhydryl groups.

Catalase (CAT) activity was measured using slight modification of the technique by Claiborne.⁴⁰ This technique is based on the principle that catalase in the sample will split hydrogen peroxide which can be estimated at 240 nm using a spectrophotometer.⁴⁰ Superoxide dismutase (SOD) activity was estimated with the technique previously illustrated by Misra, et al.⁴¹ This method is based on the principle that at pH 10.2, SOD has the capacity to inhibit the autoxidation of epinephrine.

Assessment of oxidative stress markers

The lipid peroxidation marker, malondialdehyde (MDA) level was assayed using the standard method.⁴² MDA reacts with the chromogenic reagent, 2-thiobarbituric acid (TBA) under acidic medium to produce a pink colored complex with 532 nm absorbance.

Nitric oxide (NO): this assay adapted the Griess reaction technique.⁴³⁻⁴⁴ One microliter 100 µl of heart and lungs (separately) supernatant was added to 100 µl acidic Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid). The absorbance was read at 540 nm against blank.

Enzyme-linked immunosorbent assay

The inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), heme oxygenase and Hmox-1, apoptotic marker caspase 3, and transcription factors Nf-kB, Nrf2 in the homogenized eye and thymus cell supernatant was detected using commercially available ELISA kits following the manufacturer's instructions. All experiments were conducted in triplicate.

Assessment of inflammatory markers and transcription factor markers

IL-6, TNF-α, Nr2, Hmox-1, Nf-kB and Caspase 3 activities were measured with IL-6, TNF-α, Nrf2, Hmox-1, Nf-kB and Caspase 3 Activity Assay Kit (Beyotime Institute of Biotechnology, Jiangsu, China) according to the manufacturer's directions. Eye and thymus samples were lysed for 15 min on ice. The ovarian and thyroid homogenate were centrifuged at 16,000g for 10 min at 4°C. Standards or samples were added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for rat IL-6 and avidin-horseradish peroxidase (HRP) conjugate were added successively to each micro plate well and incubated. Free components were washed away. The substrate solution was added to each well; only those wells that contain rat IL-6, biotinylated detection antibody and avidin-HRP conjugate appeared blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turned yellow. The

optical density was measured spectrophotometrically at a wavelength of $450 \text{ nm} \pm 2 \text{ nm}$. The optical density value was proportional to the concentration of rat IL-6. Concentrations of rat IL-6 in the samples were calculated by comparing the optical density of the samples to the standard curve. This procedure was repeated for TNF- α , Nrf2, Hmox-1, Nf-kB and caspase 3.

Histopathological examinations

After fixing with 10% neutral buffered formalin for 72 hours, eye and thymus were separately embedded in paraffin by standard histological method. The tissues were sectioned coronally in $5 \mu\text{m}$ thickness, and then slices were dewaxed followed by hydration procedures. The sections were stained with hematoxylin-eosin stain kit (Vector Laboratories, USA) according to the manufacturer's instructions. The sections of the eye and thymus were evaluated under a light microscope and photographed with image acquisition parameters settings at $100\times$ and $400\times$.

Statistical analysis

All the results were expressed as Mean \pm Standard deviation (std). Microsoft Xlstat 2014 was used in performing analysis of variance and Tukey multiple comparison pairwise tests to check if the concentration of the biomarkers were significantly ($p < 0.05$) different between groups. Pandas was used in obtaining the descriptive statistical parameters (biomarkers and heavy metals mean conc.). Graph Pad Prism 5[®] was used in plotting all graphs (GraphPad Software, California, USA).

Results

Effect of zinc and selenium on body weight, absolute and relative weight of eye and thymus in CNMM

The absolute weight of the eye was significantly reduced ($p < 0.05$) in the various metal mixtures compared to the control group (deionized water); in the thymus, significant weight reduction was observed only in CNMM+Zn and CNMM+Se groups compared to the control (Table 1). However, there was no difference in the relative weights of the eye and thymus (Table 1). A significant reduction in body weight was observed in the metal mixture only and the different combinations with trace elements; feed intake was non-significantly reduced compared to the control group with Table 1). The percentage body weight of the rats treated with essential trace elements is shown in Figure 1. The weight of the rat in the control group ranged from 126–200 g with an increase of 7.4%, the CNMM group ranged from 117–181 g with an increase of 6.35%, the group treated with CNMM+Zn ranged from 60–163 g with an increase of 10.25%, the rat treated with CNMM+Se ranged from 76–125 g with a weight increase of 7.95% and rats ex-

posed to CNMM+Zn +Se had a range of 40–127 g with an increase of 11.5%.

Table 1. Effect of zinc and selenium on the body weight, absolute and relative weight of eyes and thymus of female albino rats exposed to CNMM*

Treatment	Absolute weight eyes (g)	Relative weight eyes (%)	Absolute weight thymus (g)	Relative weight thymus (%)	Feed intake	Fluid intake
Deionized H ₂ O (only)	0.80 \pm 0.71a	0.40 \pm 5.02	0.30 \pm 0.0 ^a	0.15 \pm 0.00	154.10 \pm 23.42	242.98 \pm 35.35
CNMM (only)	0.55 \pm 0.97a	0.30 \pm 9.90	0.30 \pm 0.14 ^a	0.17 \pm 19.8	148.27 \pm 16.42	210.07 \pm 33.46
CNMM+Zn	0.53 \pm 0.0 ^a	0.33 \pm 0.33	0.23 \pm 0.1 ^a	0.14 \pm 0.83	147.94 \pm 22.13	195.62 \pm 43.20
CNMM+Se	0.46 \pm 0.0 ^a	0.30 \pm 8.45	0.21 \pm 0.0 ^a	0.14 \pm 5.63	127.63 \pm 35.47	209.74 \pm 51.27
CNMM+Zn+Se	0.51 \pm 0.1 ^a	0.33 \pm 1.98	0.34 \pm 0.0 ^a	0.22 \pm 1.13	127.82 \pm 29.85	203.85 \pm 53.26

* values expressed as mean \pm standard deviation, n=5, different superscripts (a, b, c) are significantly different from each other at $p < 0.05$

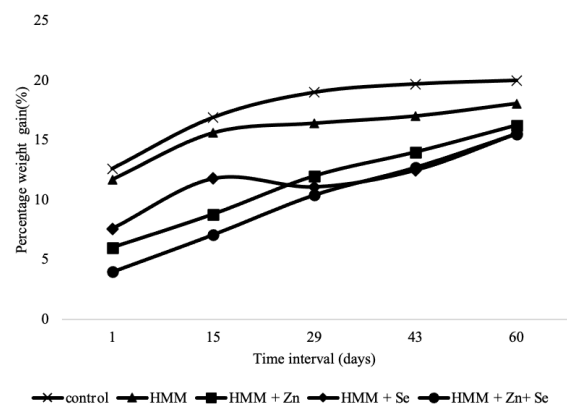


Fig. 1. Effect of Zn and Se on the percentage body weight gain

Antioxidants profile in the eyes of rats treated with zinc and selenium extract after CNMM exposure

SOD in the eyes was significantly reduced ($p < 0.05$) in CNMM only group when compared to deionized water and increased in the CNMM+Zn, CNMM+Se and CNMM+Se+Zn groups treatment groups compared to deionized water and HMM only. There was a significant SOD increase in compared to CNMM+Zn only (Fig. 2A); GPx was reduced in CNMM only and CNMM+Zn only groups but increased in CNMM+Zn+Se group; a significant increase was observed in CNMM+Se only and CNMM+Zn+Se groups compared to CNMM +Zn only group (Fig. 2B).

GSH significantly decreased in CNMM and CNMM+Zn only groups compared to deionized water group; significantly increased in CNMM +Se and CNMM+Se+Zn groups compared to CNMM+Zn only but

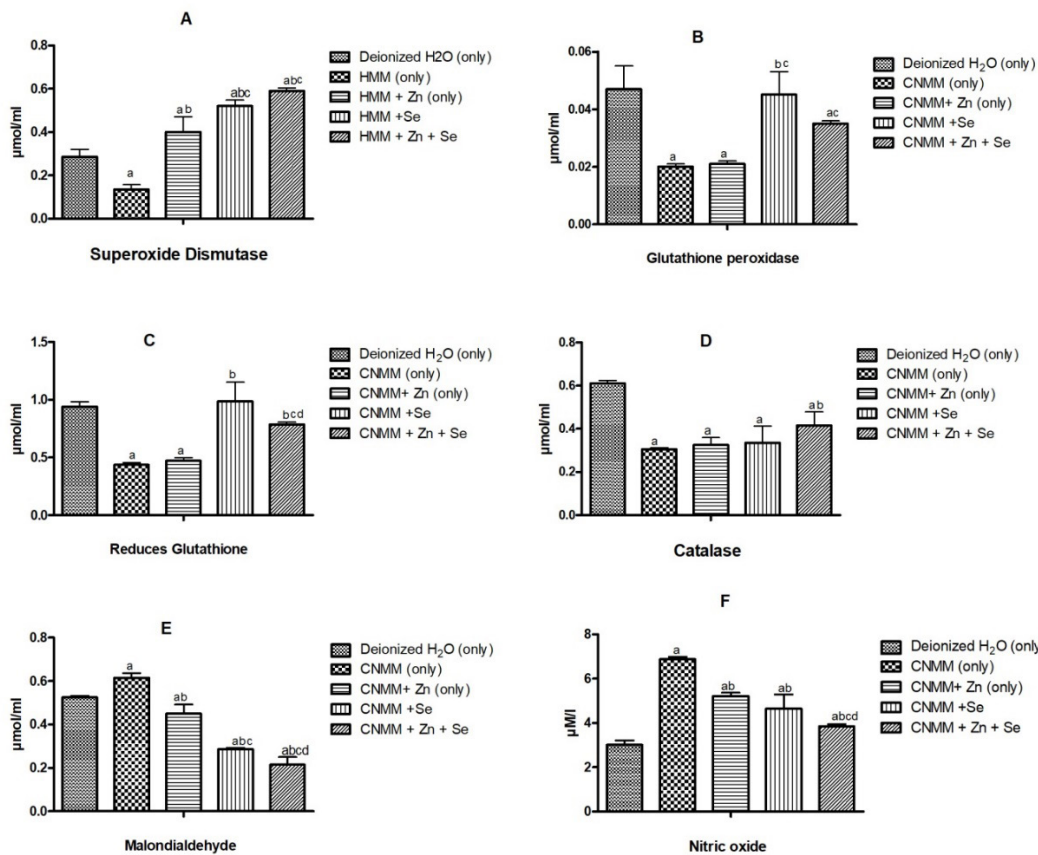


Fig. 2. Essential elements (Zn and Se) on antioxidants (SOD, GPx, CAT, GSH) MDA and NO levels in eye of female albino rats after exposure to CNMM, a – p<0.05 compared to deionized H₂O, b – p<0.05 compared to CNMM, c – p<0.05 compared to CNMM+Zn, d – p<0.05 compared to CNMM+Se

decreased in CNMM+Se+Zn group compared to CNMM+Se (Fig. 2C).

A significant reduction in CAT was seen in all the various metal treatment groups compared to deionized water but an increase in CNMM+Se+Zn compared to CNMM only (Fig. 2D). Similarly, significant reduction of MDA in all metal treated groups except CNMM only group compared to deionized water was observed; MDA decreased in the various metal treated groups compared to CNMM only and also in CNMM +Se and CNMM+Se+Zn groups compared to CNMM+Zn only (Fig. 2E).

However, NO increased significantly in all the metal treated groups compared to the control but decreased in the other metal treatment groups compared to CNMM only; a reduction was observed in CNMM +Zn+Se compared to CNMM+Zn and CNMM +Se (Fig. 2F).

Antioxidants profile in the thymus of rats treated with zinc and selenium after CNMM exposure

In the thymus, the level of the SOD significantly increased in CNMM+Se and CNMM+Se+Zn groups compared to the control and in all the other metal treated groups compared to CNMM only; CAT also in-

creased in CNMM+Zn+Se compared to CNMM+Se (Fig. 3A).

A significant decrease of GPx was seen in all the metal treated groups compared to the control and an increase in CNMM+Zn+Se compared to CNMM only group (Fig. 3C). GSH level was reduced and increased respectively compared to CNMM only group and CNMM+Se (Fig. 3D). MDA level was significantly reduced in CNMM+Se and CNMM+Se+Zn compared to control and CNMM only groups (Fig. 3E).

NO significantly increased in all the heavy metal treated groups compared to control but decreased in the other metal treated groups compared to CNMM only group (Fig. 3F).

Pro-inflammatory markers (IL-6, TNF-α and Casp 3) and transcription factors (NF-κB and Nrf2) in the eyes of rats treated with zinc and selenium following CNMM exposure

In the eyes, pro-inflammatory cytokines IL-6, TNF-α and Casp 3 were significantly increased in CNMM only compared to the control but decreased in the other metal treated groups compared to CNMM only (Fig. 4A, 4B and 4E). Similarly, Nf-kβ was significantly increased in CNMM only compared to the control but decreased

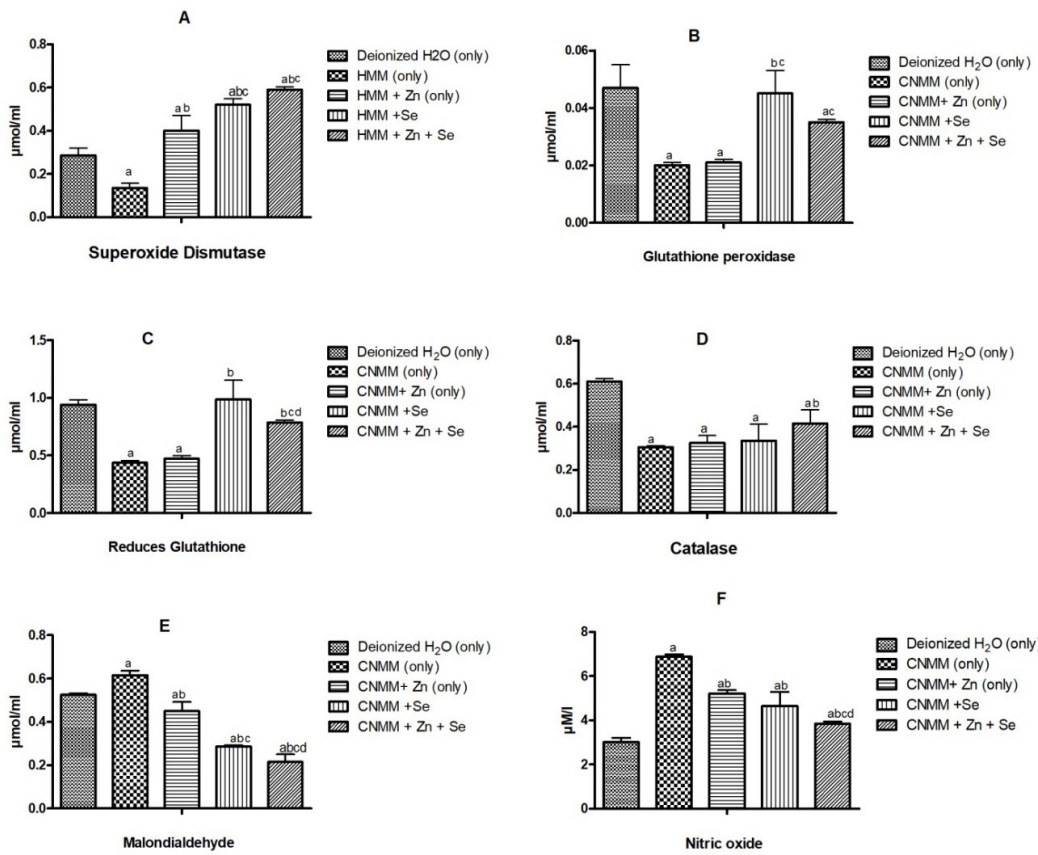


Fig. 3. Essential elements (Zn and Se) on antioxidants (SOD, GPx, CAT, GSH) MDA and NO levels in thymus of female albino rats after exposure to CNMM, a – $p < 0.05$ compared to deionized H₂O, b – $p < 0.05$ compared to CNMM, c – $p < 0.05$ compared to CNMM+Zn, d – $p < 0.05$ compared to CNMM+Se

only in CNMM+Zn and CNMM+Se groups compared to CNMM only (Fig. 4D).

Nrf2 significantly increased in the various heavy metal treatment groups compared to control but decreased significantly in the other metal treated groups compared to CNMM only; reduced in CNMM+Se compared to CNMM+Zn (Fig. 4C).

Pro-inflammatory markers (IL-6, TNF- α and Casp 3) and transcription factors (NF- κ B and Nrf2) in the thymus of rats treated with zinc and selenium following CNMM exposure

A significant increase in thymic IL-6, TNF- α and Casp 3 was observed in CNMM only compared to the control but decreased in the other metal treated groups compared to CNMM only; also, significant reduction of TNF- α in CNMM+Zn+Se compared to CNMM+Zn and CNMM+Se groups was observed (Fig. 5A, 5B and 5E).

Nrf2 and Nf- κ B were increased in the CNMM only and CNMM +Zn+Se groups compared to the control but decreased in the other metal treated groups compared to HMM only, however, Nf- κ B was increased in CNMM +Se and CNMM+Zn+Se groups compared to

CNMM+Zn group (Fig. 5C and 5D).

Histology of the eye of rats treated with zinc and selenium following CNMM exposure and treatment

The eye of the rats that received deionized water only showed normal architecture of the retina layers: retina pigmented epithelium (RPE), lamina of the rods and cones (LRC), external limiting membrane (ELM), outer nuclear layer (ONM), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GCL), optic nerve fiber layer (OFL) and vitreous chambers (VC) (Fig. 6A). The CNMM only treated group showed moderate retinal degeneration seen as less distinct lamination of the retinal layers; considerably cell loss at the nuclear layer (NL) with no demarcation of the outer and inner nuclear layers; the IPL is enlarged and: there is total cell loss at the ganglionic cell layer GCL (Figure 6b).

The eye of CNMM+Zn treated rats showed mild retinal degeneration with decrease in thickness and/or density of cells at the nuclear and GCL. The lamination of the retinal layers was still well preserved and the RPE showed slight disruption (Fig. 6C) while the CNMM+Se group showed normal retinal layer (R) and VC (Fig. 6D).

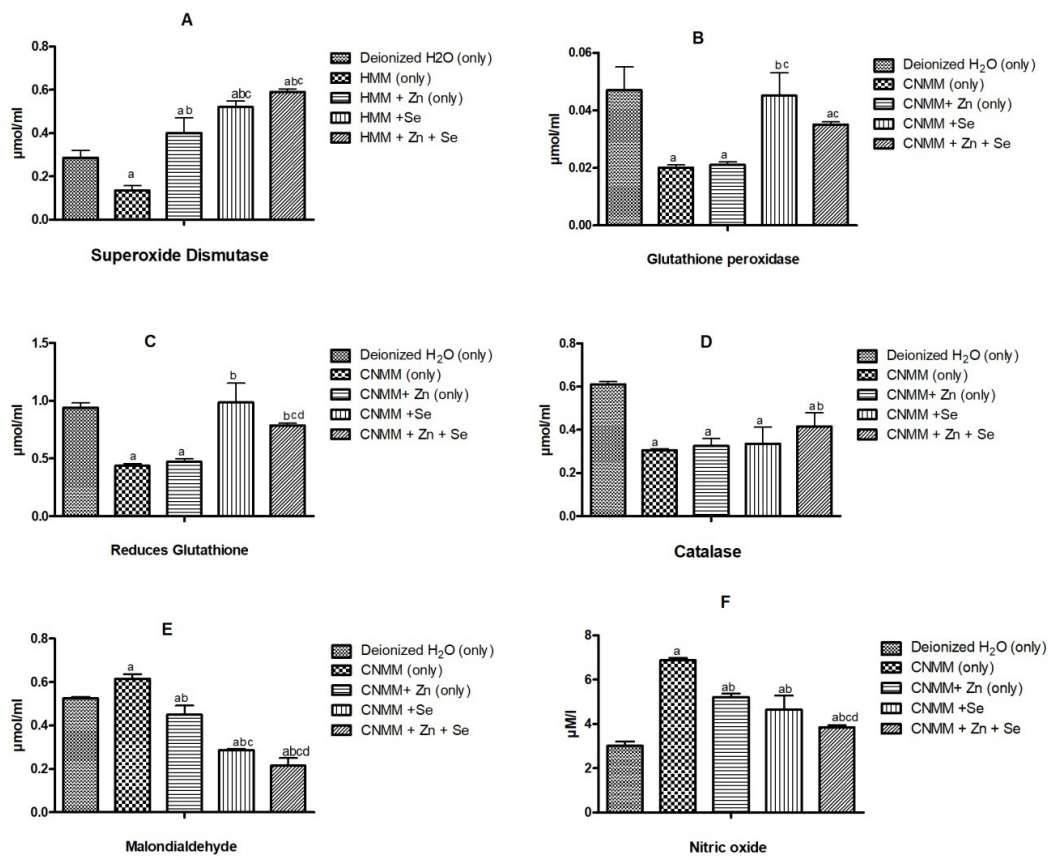


Fig. 4. Effect of essential elements on the pro-inflammatory cytokines (IL-6, TNF-a, transcription factors (Nrf2, Nfkb), and caspase 3 in eye of female albino rats after CNMM (Pb, Hg, Mn and Al) exposure, a – p<0.05 compared to deionized H₂O, b – p<0.05 compared to CNMM, c – p<0.05 compared to CNMM+Zn

The group exposed to CNMM and combination of essential trace elements (Zn+Se) showed moderate retinal degeneration (Fig. 6E). The lamination of the retinal layers is less distinct at the outer segment (OS), the outer photoreceptor segments were absent; the thickness of the outer nuclear layer (ONL) was considerably reduced; at the inner segment (IS) the ganglion cells were reduced and there is associated retinal wall disruption (RWD) and retinal detachment (RD).

Histology of the thymus of rats treated with zinc and selenium following CNMM exposure and treatment

The photomicrograph of the thymus of rat in the control group showed moderate atrophy with lymphocyte depletion accompanied by multifocal areas of necrosis (arrows), thickened interlobular septum (S) and prominent blood vessel (BV) is noticed (Fig. 7A). The group that received CNMM only showed severe degenerative thymus, lymphocyte depletion, multifocal necrosis (arrows) and thickened interlobular septum with associated fatty change (S) (Fig. 7B).

CNMM+Zn treated group showed mild atrophy with lymphocyte depletion and multi-focal necrosis (arrows) (Fig. 7C). Similarly, photomicrograph of thymus of rats

that received CNMM+Se showed mild atrophy with lymphocyte depletion and diffuse necrosis (Fig. 7D).

The group treated with CNMM and combination of essential trace elements (Zn+Se) showed moderate atrophy, lymphocyte depletion and diffuses necrosis (Fig. 7e).

Discussion

Heavy metals share common mechanisms of toxicities such as oxidative stress and inflammation.⁴⁵ ROS production is regulated by enzymatic antioxidants, including GPx, CAT, and SOD, and other non-enzymatic antioxidants like reduced glutathione, ascorbic acid and tocopherol.⁴⁶ SOD is a major part of the antioxidant system while GSH sustains cellular redox balance. Reduction in GSH and SOD levels leads to excessive utilization of superoxide and hydrogen peroxide causing lipid peroxidation by hydroxyl radicals and increased cellular content of MDA.⁴⁶

This study showed that treatment with CNMM (Al, Pb, Mn and Hg) resulted in decreased CAT, SOD, GPx and GSH levels with increased MDA and nitric oxide (NO) levels in the eye and thymus of rats. HMM co-treatment with essential elements (zinc and selenium) singly or in combination, increased CAT, GPx and

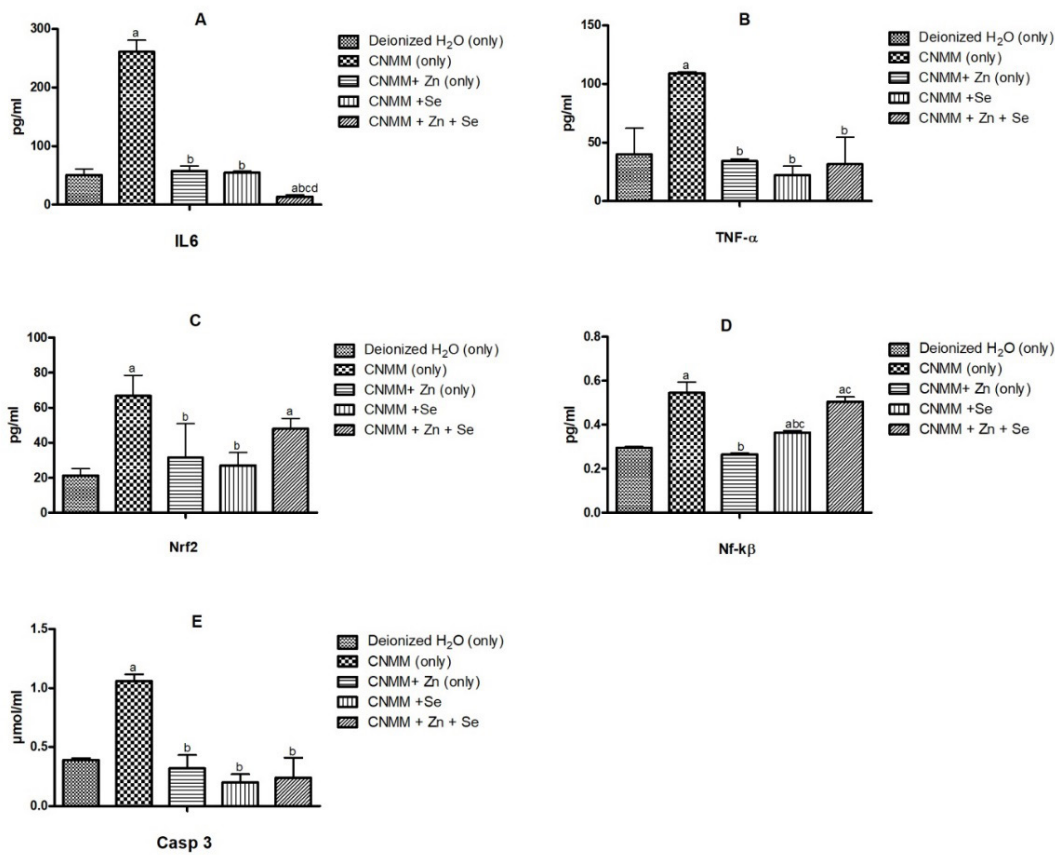


Fig. 5. Effect of essential elements on the pro-inflammatory cytokines (IL-6, TNF- α , transcription factors (Nrf2, Nfkb), and caspase 3 in thymus of female albino rats after CNMM (Pb, Hg, Mn and Al) exposure, a – $p < 0.05$ compared to deionized H₂O, b – $p < 0.05$ compared to CNMM, c – $p < 0.05$ compared to CNMM+Zn, d – $p < 0.05$ compared to CNMM+Se

GSH levels with decreased levels MDA and nitric oxide (NO) in these organs but not as with the control.⁴⁵ noted that essential elements like selenium (Se), cobalt (Co), copper (Cu), and zinc (Zn) are cofactors or structural components of some antioxidant enzymes. Superoxide dismutase is an isoenzyme that contains Cu and Zn (CuZn-SOD) and so, Zn supplementation might increase CuZn-SOD activity. Zn also reduces the toxicity of cadmium and lead by displacing them from the bonds to important enzymes.⁴⁷ GSH is an essential component of the antioxidant system and serves as a cofactor for GSH transferase (GST), which helps remove certain drugs and chemicals as well as other reactive molecules from the cells.⁴⁸ GPx is an important selenium-containing enzyme that protects many cells from oxidative damage caused by hydrogen peroxide and other reactive oxygen intermediates.

GPx, is also capable of neutralizing lipid hydroperoxides and this possibly explains the significant reduction of MDA and NO levels in groups co administered with the Se or its combination in our work.⁴⁹ Formation of endogenous aldehydes and their derivatives, such as MDA due to lipid peroxidation causes high reactivity and toxicity for cell components and so, causes oxida-

tive stress and tissue damage.⁴⁹ The increased nitric oxide production can be attributed to increased nitrate/nitrite level upregulated by iNOS.⁵⁰ Inhibitors of ROS and NOX generation, modification of cellular signaling pathways that regulate ROS production and antioxidant defense may reduce ROS levels.⁵¹ Our study showed that Zn and Se have potent antioxidant activity against metal mixture induced oxidative stress and also, provided cellular protection through scavenging hydroxyl radicals and inhibiting lipid peroxidation evidenced in reduced MDA and NO levels.

In this study, rats exposed to CNMM showed increased levels of pro inflammatory cytokines (IL-6, TNF- α), Caspase 3 proteins and transcription factors: nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor kappa B (NF- κ B) in the eye and thymus. This is in line with the work of with significantly increased serum levels of IL-1, IL-6 and TNF- α in the group of workers chronically exposed to lead compared to control values.⁵² However, Nrf2 expression was decreased in the testicular tissue of mice exposed to Cd and in cerebellum and cerebral cortex of rats challenged with heavy metal mixture.^{30,49} Several cytokines, transcription factors like NF- κ B and Nrf2 are regulated by

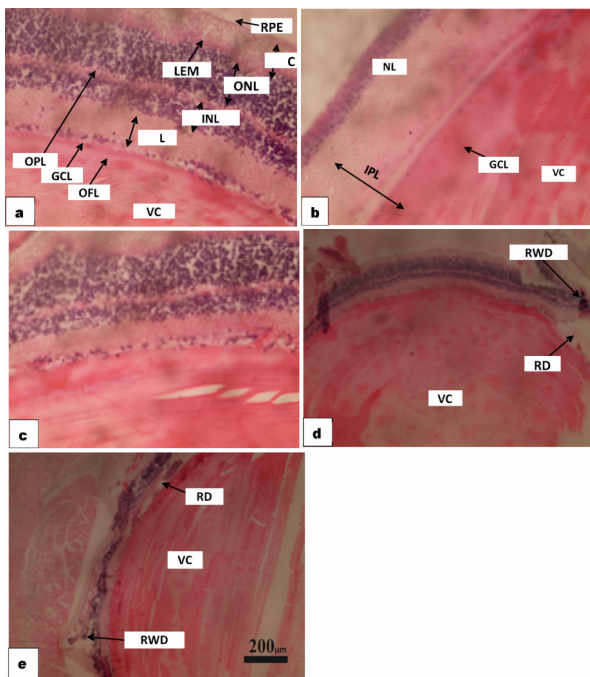


Fig. 6. Photomicrographs of light microscope of rat thymus of various treatment groups, cross sections of eyes were stained with hematoxylin and eosin (400X). A: rat eye treated with iodized water only showing normal architecture of the retina layers, B: rat eye treated with CNMM only exhibited moderate retinal degeneration seen as less distinct lamination of the retinal layers, C: rat eye treated with CNMM+Zn showed mild retinal degeneration with decrease in thickness and/or density of cells at the nuclear and ganglion cell layers, D: rat eye treated with CNMM+Se showed RPE with slight disruption, E: rat eye treated with CNMM+Zn+Se showed moderate retinal degeneration

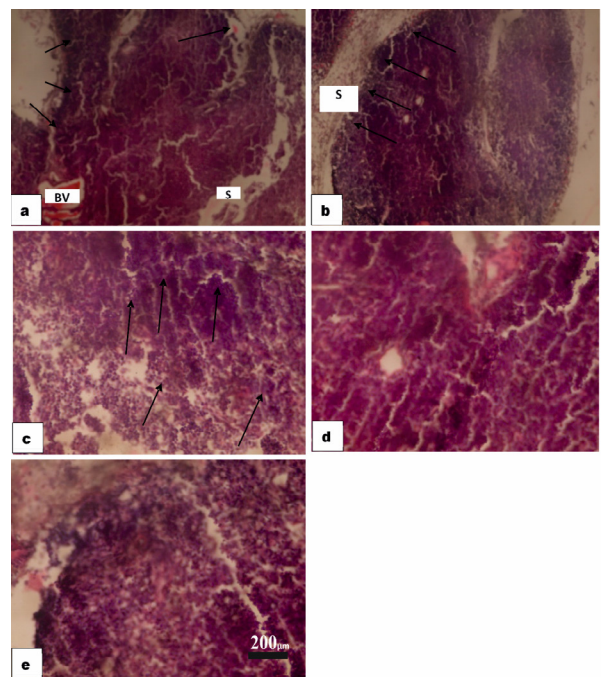


Fig. 7. Photomicrographs of light microscope of rat thymus of various treatment groups, cross sections of eyes were stained with hematoxylin and eosin (400x). A: rat thymus treated with iodized water only showed moderate atrophy with lymphocyte depletion, B: rat thymus treated with CNMM only showed severe degenerative thymus, lymphocyte depletion, C: rat thymus treated with CNMM+Zn showed mild atrophy with lymphocyte depletion, D: rat thymus treated with CNMM+Se showed mild atrophy with lymphocyte depletion, E: rat eye treated with CNMM+Zn+Se showed moderate atrophy, lymphocyte depletion

ROS and their up regulation could be attributed to increased lipid peroxidation observed in our work.^{31,49,52} As an adaptive mechanism, Nrf2 is quickly up-regulated in cells and tissues in response to oxidative stress at early stage.⁵³

Heavy metal mixtures can affect the Nrf2 pathway which is important in cellular defense against oxidative stress.⁵⁴ A luciferase assay also indicated that the levels of activator protein-1 and Nf-κB transcription factors were upregulated in metal mixture *in vitro*.⁵⁵ Heavy metals mediate germ cell apoptosis with oxidative stress and CNMM in our study potentiated apoptotic effect in the eye and thymus by upregulating the pro-apoptotic proteins, caspase-3.⁵⁰

Oxidative stress is known to induce the release of proinflammatory cytokines that in turn, triggers signaling cascades of the inflammatory processes.⁵² This study demonstrated the ability of CNMM to increase the levels of potent pro-inflammatory cytokines such as TNF-α, IL-6 and transcription factors (NF-κB and Nrf2) con-

sistent with *in vitro* and animal studies which showed that various heavy metals induced high level expression of cytokines.⁵⁶ The up regulation of TNF-α, IL-6, NF-κB and Nrf2 by the treatment with heavy metal mixture in our study increased MDA and NO levels with down regulation of the antioxidant system in both eye and thymus. The activation of Nrf2 in the CNMM treated rats could be an initial compensatory mechanism in response to the toxic effect of the heavy metals evidenced by increased inflammatory cytokines.

However, co-treatment with Zn and Se reduced the levels of IL-6, TNF-α, Caspase 3 proteins and NF-κB but Nrf2 was activated.⁵⁷ reported that zinc decreased the expression of inflammatory cytokines and molecules by inhibition of NF-κB activation via A20 and PPAR-α pathways. Nrf2, an important cellular defense factor against oxidative stress regulates intracellular antioxidants and other proteins to neutralize reactive oxygen and/or nitrogen species (RNS) and also acts as target gene for NAD(P)H quinone oxidoreductase-1 (NQO-1), heme

oxygenase-1 (Hmox-1), superoxide dismutase (SOD) and glutathione S-transferase.⁵³ Activation of Nrf2 can trigger antioxidant enzyme expression and diminish oxidative stress.⁵⁸ Nrf2 has the ability to inhibit the expression of pro-inflammatory cytokines, like TNF- α and IL-6, as well as inducible nitric oxide synthase (iNOS) as Nrf2 knockout in mice significantly aggravated acute inflammation.⁵⁹ Activation of Nrf2 can trigger antioxidant enzyme expression and diminish oxidative stress.⁵⁸ Our work demonstrated the up regulation of antioxidants, reduced lipid peroxidation and down regulation of inflammatory cytokines with the activation of Nrf2 with Zn and Se supplementation. Nrf2 activation also induces cytoprotective gene expression to counteract the toxic effect of ROS and so, an important regulator of processes leading to toxicity and inflammatory diseases.⁵⁹ Zn stimulates the Nrf2 expression and transcription, likely by activation of Akt-dependent inhibition of Fyn nuclear translocation. The anti-inflammatory effect of Nrf2 can be due to the ability to inhibit the expression of pro-inflammatory cytokines, like TNF- α and IL-6, as well as inducible nitric oxide synthase.⁵⁹

This study also showed that heavy metal mixture exposure potentiated apoptotic effect in the eye and thymus by upregulating the pro-apoptotic protein (caspase-3). Histological findings in this work showed moderate retinal degeneration and total cell loss at the ganglionic cell layer in the eye; severe degenerative thymus, lymphocyte depletion and multifocal necrosis in rats exposed to CNMM only. It has been proposed that heavy metals may mediate germ cell apoptosis with oxidative stress.⁶⁰ Oxidative stress impairs calcium ion channels and alters the mitochondrial membrane potential, leading to cytochrome C release, which enhances caspase cascade and fragmentation of DNA. Co-treatment with Zn and Se in this study counteracted the apoptotic cascade produced by metal mixture exposure therefore; the anti-apoptotic effect of Zn and Se might be due to their antioxidant capacity.⁶¹

Excessive ROS formation is closely related to inflammation and contributes to the pathogenesis of numerous diseases.⁶² Oxidative stress triggers the pathophysiology of various ocular disorders, like dry eye disease, uveitis, pterygium, keratoconus, Fuchs endothelial corneal dystrophy, diabetic keratopathy.⁶¹⁻⁶⁵ The visual system is very vulnerable to oxidative stress due to its composition of several susceptible tissues with high metabolic activities.

Heavy metals exposure affects innate and adaptive immune systems through activation of inflammatory events such as release of cytokines and chemokines that results in pathologic conditions like autoimmune diseases.⁶⁶ Lymphatic organs are an important part of the body's immune system and play roles in preventing many diseases.⁴⁶ TNF- α is important in inflammation, innate and adaptive immune responses and has been

implicated in a wide variety of human diseases.^{45,56} Lead is related to the induction of oxidative stress, and alteration of immune response that could influence on suppression of T-helper type 1 cells and enhancement of T helper type 2 (Th2) cells which are significantly related to IL-6 production.⁶⁷

Zinc and selenium have the ability to modulate immune response through the production of antibodies or anti-inflammatory cytokines. Selenium is vital in the functioning of enzymes involved in antioxidant system of the immune cells.⁶⁸

Heavy metals affect immune response by altering the relative distribution of different T cell subpopulation such as CD4⁺, CD8⁺, Th1, Th2, Th17, Treg.⁶⁹ Treg cells help to maintain self-tolerance by actively suppressing immune responses.²⁴ The immune privilege of the ocular surface is actively maintained through a variety of immunoregulatory mechanisms that prevent the disruption of immune homeostasis.⁷⁰ Suppression of effector T cells in the local lymphoid compartment is an essential mechanism maintaining the integrity of the ocular surface; Treg in the lymph nodes draining the ocular surface have been shown to potently suppress sensitization of naive T cells and function of activated T cells, thus preventing the loss of ocular surface immune quiescence.⁷⁰⁻⁷²

Reception of signal from proinflammatory cytokines (e.g., IL-6) by the immune cells inhibits the function of Foxp3 with the induction of Th17 differentiation.⁷³ Mature antigen presenting cells bearing self-antigen migrate from the thymus to regional lymph nodes through afferent lymphatic vessels where they prime naive T cells, which then differentiate into the CD4⁺ T helper cell subsets, T_H1 and T_H17. The effector T_H1 and T_H17 cells migrate through efferent blood vessels to the ocular surface, where they are thought to induce epithelial damage and tear dysfunction via proinflammatory cytokine release. IL-6 inhibits Treg differentiation and induces the development of Th17 cells.⁷⁴ The conversion of immunosuppressive Foxp3⁺ Tregs to pro-inflammatory Th17 cells has been identified as a critical factor in the pathogenesis of autoimmune diseases. T_H1 cells secrete the proinflammatory cytokines TNF- α , interferon- γ (IFN- γ), and IL-2, which activate macrophages. T_H17 cells secrete the cytokine IL-17, which stimulates the production of other proinflammatory molecules, recruits neutrophils, and has been shown to promote corneal epithelial barrier disruption.⁴⁹ There is also a correlation between IL-6 levels, disease severity and ocular surface parameters. In this study, the up regulation of these inflammatory cytokines in CNMM exposure would cause lack of tolerance in the eye which could be mitigated by Zn and Se supplementation as evidenced by reduced levels of these cytokines.

Zinc is required for thymic development, production of naive T lymphocytes, clonal expansion, Th1/Th2,

differentiation and normal T lymphocyte function. Zn modulates the activity of several kinases and phosphatases that enhance Nrf2 activity and selenium enhances Nrf2 target gene expression.⁷⁵ The cross-talk between Nrf2 and other transcription factors, including the aryl hydrocarbon receptor, NF- κ B, tumor suppressor protein p53, and Notch making Nrf2 is important in regulating immune defense, differentiation, and tissue regeneration, as well as cell death. NF- κ B is one of the major immune response transcription factors.^{57,64} RelB, an NF- κ B transcription factor subunit is essential for the development and differentiation of medullary thymic epithelial cells and changes in thymic function may decrease peripheral tolerance and hasten autoimmune disease.⁷⁶ TNF- α is one of the most potent physiologic inducers of NF- κ B in human lens epithelial cells and this contributes to the amplification of inflammation.⁶⁴

One limitation of the present which will be addressed by immunoblotting in future study is demonstration of increased Nrf2 nuclear trafficking from the cytosol following co-treatment with essential elements.

Conclusion

Taken together the present study has demonstrated that CNMM only exposed rats showed increased levels of MDA and NO, IL-6 and TNF- α , attenuated antioxidants and caspase 3 in the eye and thymus. Zn and Se attenuated adverse effects of HMM exposure. Zinc and selenium supplementation ameliorated CNMM ocular-thymus intoxication in female rats. Our study demonstrated the role of zinc and selenium as anti-oxidant and anti-inflammatory agents and can mediate in immunosuppression and ocular dysfunctions in rats. Zinc and selenium are involved in regulation of redox reactions and several molecular signaling pathways such as Caspase 3, NF- κ B and Nrf2 in heavy metal cocktail toxicity in rats.

Declarations

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Author contributions

Conceptualization, O.E.O and C.N.O.; Methodology, O.E.O, A.N.E.; Software, X.X.; Validation, O.E.O, A.N.E. and C.N.O.; Formal Analysis, M.A, O.E.O, A.N.E; Investigation, M.A, A.N.E. and C.N.O.; Resources, X.X.; Data Curation, M.A, A.N.E. and C.N.O; Writing – Original Draft Preparation, T.U, O.E.O, AC, D.N.A.; Writing – Review & Editing, T.U, AC, AC, D.N.A, OEO; Visualization, O.E.O, A.N.E. and C.N.O.; Supervision, O.E.O, A.N.E; Project Administration, O.E.O.

Conflicts of interest

Authors confirm that there was no conflict of interest.

Data availability

All data have been provided.

Ethics approval

All the procedures involving the animals and the experimental protocol followed guidelines for the safe use of animals in research and were approved by the University of Port Harcourt animal research committee (UPH/CEREMAD/REC/18).

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REVIEW PAPER

Forensic aspects of salbutamol overdose – doping, abuse, and suicide

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ABSTRACT

Introduction and aim. Salbutamol is a popular drug used in respiratory diseases. With the increasing prevalence of the use of this substance for therapeutic purposes and its availability on the market, the frequency of its use for other purposes has also risen due to its effects outside the respiratory system. The aim of the study was to investigate the medico-legal aspects of salbutamol.

Material and methods. Medical literature databases such as PubMed, Scopus, Web of Science and Google Scholar were searched. The search was carried out in accordance with the specified purpose of the keyword research using Boolean operators.

Analysis of the literature. In sports, the use of salbutamol is strictly regulated by anti-doping regulations. Recreational substance abuse and accidental overdoses, mainly among children and the elderly, are also important. Rare cases of suicide attempts associated with the use of salbutamol have also been reported.

Conclusion. Salbutamol overdoses are usually not life threatening. However, one should remember about the possibility of accidental overdose, especially among the elderly and children taking the drug chronically. Currently, the use of salbutamol for recreational purposes is rare. In sports, the status of salbutamol use, especially among athletes who do not require its use for therapeutic reasons, is still a controversial issue.

Keywords. abuse, doping, forensic toxicology, overdose, salbutamol, suicide

Introduction

Salbutamol was first synthesized in 1968. It is a substance worth careful analysis from various perspectives because, despite more than half a century passing, it remains one of the primary drugs used in the treatment of respiratory diseases such as asthma and chronic obstructive pulmonary diseases (COPD).^{1,2} Despite the fact that this drug has been used for a long time, there are few available publications focusing on the risks associated with its use and on the diagnostics in cases of overdose, which can even result in the death of the patient. Salbutamol is a selective and short-acting agonist of β_2 adrenergic receptors that can be found, for example, in bronchi, blood vessels and the uterus, par-

ticularly on the membranes of smooth muscle cells.³ When ingested, nebulized or taken via an inhaler, salbutamol binds to β_2 adrenergic receptors on the surface of smooth muscle cells. This interaction in the respiratory system triggers a series of events that lead to the relaxation of the muscles surrounding the airways. This bronchodilation effect helps to alleviate symptoms like wheezing, shortness of breath, and chest tightness.⁴ In patients with asthma, it is used to stop an asthma attack and as a preventive measure against exercise-induced bronchoconstriction in people with exercise-induced asthma.⁵ While, in COPD it is used to treat periods of exacerbation of the disease.⁶ Due to the β receptors being located in areas other than the smooth muscles, sal-

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butamol can be associated with certain undesirable side effects. In the heart, there are 4 times more β_1 adrenergic receptors than β_2 adrenergic receptors, so impact of salbutamol on the heart should be minor. Based on an *in vitro* study that compared the effects of a non-selective agonists of β adrenergic receptors with salbutamol, it can be concluded that β_2 receptor stimulation alone is not sufficient for the early induction of diastolic dysfunction. However, salbutamol in higher doses also acts on β_1 adrenergic receptors.^{7–9} After administration of salbutamol, the most common cardiovascular symptom is tachycardia. Cases of arrhythmia and angina pectoris have also been reported.^{4,5} Effects on the musculoskeletal system may manifest themselves as tremors, however studies also suggest that high doses may cause myopathy.⁵ Salbutamol can have metabolic effects: hypokalemia, as well as an increase in glucose, pyruvate and free fatty acids. There is also an observed elevation in insulin levels, which is a consequence of hyperglycemia. For this reason, it should be used with extreme caution in people with diabetes.^{10–18} Also, through research conducted on humans, the influence of this substance on adipose tissue has been demonstrated, where stimulation of β_2 adrenergic receptors leads to increased glucose uptake by brown adipose tissue (BAT).¹⁹ According to research conducted on rodents, it appears that β_3 adrenergic receptors are responsible for the activation of BAT, but the presence of the same mechanism in humans has not been confirmed.^{20–22} In terms of the nervous system, salbutamol can cause hallucinations and trigger or prevent anxiety depending on the dose taken. However, it's important to emphasize that salbutamol's impact on the nervous system is generally mild. The central nervous system effects of salbutamol, such as increased alertness, are usually more noticeable when the medication is taken in higher doses. However, due to its bronchodilatory effect, it may help manage stress during an asthma attack. On the other hand, patients with epilepsy who received salbutamol showed a reduction in the frequency of seizures. Some studies have explored the idea that salbutamol's effects on these receptors might influence neuronal excitability and neurotransmitter release, potentially contributing to a decrease in seizure activity.⁵ Although salbutamol is a bronchodilator, rare cases of paradoxical bronchospasm have been reported, the mechanism of which is not fully understood.²³ After inhalation, salbutamol is quickly absorbed and transported through the bloodstream. The primary route of metabolism occurs in the liver, where cytochrome P450 enzymes facilitate its transformation into inactive metabolites. These metabolites are then eliminated primarily through the kidneys.²⁴ Due to the prevalence and high availability of salbutamol, it is also necessary to take into account its medico-legal aspects. The effects caused in the body resulting from its prop-

erties and mechanism of action may be used for purposes that are not in line with the accepted principles, for example to improve sport performance. In addition, salbutamol overdose, intentional or accidental, is associated with life-threatening consequences, especially among children and elders.

Aim

The aim of the study was to investigate the medico-legal aspects of salbutamol, with particular emphasis on overdoses and illegal or criminal uses, including doping and poisoning.

Material and methods

This narrative review was developed on the basis of a literature reviews available in recognized medical literature databases and libraries such as PubMed, Scopus, Web of Science and Google Scholar. The search was carried out using Boolean operators and keywords reflecting the purpose of the work e.g. “salbutamol”, “abuse”, “doping”, “poisoning”, “overdose”, “intoxication”, “suicide”, “diagnostics” and “post-mortem.” Then, on the basis of the selected literature, an analysis of the state of knowledge was made, specifying the most important aspects defined by the purpose of the work. Due to the limited access to full versions of scientific papers, “gray literature” containing only abstracts of conference presentations was excluded from the study. In order to properly ensure the legal context, the applicable legal provisions, in particular regarding doping in sport, were additionally analyzed.

Analysis of the literature

Substances affecting adrenergic receptors were already used 5000 years ago in China. During that time, *Ephedra sinica* was utilized. It contains ephedrine, which, among other effects, non-selectively stimulates α and β adrenergic receptors. In Chinese medicine, it was used to alleviate respiratory system diseases and as a stimulant.^{3,25}

However, earliest publications on adrenergic receptors appeared much later in 1948. Raymond P. Ahlquist proposed the classification of these receptors into α and β types, and the final, currently known classification and structure of receptors were described in 1993.^{26,27} The next step in popularizing substances affecting adrenergic receptors was the introduction of isoprenaline in 1947. It has a non-selective impact on β adrenergic receptors. Initially, isoprenaline gained significant popularity among asthmatic patients. However, cases of fatal outcomes were recorded due to the use of this medication in high doses. This led to a decline in sales.^{28,29} In response to the demand for a bronchodilator with fewer side effects, the synthesis of salbutamol in 1968 by a team led by David Jack proved significant. It was the world's first selective β_2 receptor agonist and was in-

troduced under the trade name Ventolin. Compared to its predecessor, isoprenaline, salbutamol exhibits fewer side effects and has a longer duration of action after administration.^{1,2} Following the synthesis of salbutamol, the next step was to obtain a substance with a longer duration of action. The first drug in the class of long-acting beta-2 receptor agonists obtained was salmeterol (trade name Serevent).

Dosage and route of administration

The most popular form of administering salbutamol is through inhalation. The oldest among the currently available inhalation methods is the use of a nebulizer. Several types of these devices are available on the market. Their common feature is turning liquid medicine into a mist. The aerosol is inhaled by the patient using a face mask or mouthpiece. Nebulizers can be pneumatic (jet nebulizers), meaning they use compressed air or oxygen. They can be divided into two subcategories: breath-enhanced and breath-actuated. These devices have large sizes and generate significant noise. A newer generation includes nebulizers with a mesh containing multiple holes (vibrating mesh technology). Aerosol is produced through vibration. During use, they generate less noise, are more efficient, and more portable. Currently, they are more expensive than jet nebulizers.^{30–33} In the case of adults, the nebulization solution is prepared by diluting 2.5 to 5 mg of salbutamol in 2–5 ml of 0.9% sodium chloride, while for children aged 5–12 years, the dose of salbutamol is half as much.¹⁰ A Metered-dose inhaler (MDI) is a device that delivers a specific amount of medication to the lungs in the form of an aerosol. The aerosol is released from the inhaler by pressing the top of the canister containing the drug. MDIs can be used with or without a spacer. Taking medications with an attached spacer to the MDI yields better results and reduces errors in drug administration. This is a very convenient way to take salbutamol because the inhaler itself is portable, the administration time is short, and it can be used in any conditions. However, patient training is required, especially regarding the correct coordination of inhalation.^{34–36} MDIs are available, delivering 100 µg of salbutamol in a single inhalation dose. The dosage for adults is 100–200 µg for the relief of acute episodes of bronchospasm and for the prevention of bronchospasm. The prevention of exercise-induced bronchospasm requires a dosage of 200 µg. The maximum daily dose is 800 µg. The dosage for children aged 6–12 is similar, but the maximum dose is 400 µg.¹⁶ Another method of inhaling salbutamol is using a dry-powder inhaler (DPI). Similar to MDIs, the medication is delivered directly to the lungs, but in the form of powder rather than an aerosol. The initiation of medication release from the inhaler is also different. The powder is drawn into the lungs during the patient's inhalation, requiring at least a

minimum inspiratory effort. This method does not require synchronizing inhalation with the pressing of the top of the canister, as in MDIs. However, with higher doses, dry powder may cause coughing. Studies have shown that the use of spacers with MDIs decreases the risk of incorrect application by patients.^{35,37–39} The DPI is available in doses of 100 or 200 µg per inhalation, and the dosage is the same as in the case of MDI.^{14,15} Salbutamol is less commonly administered orally. It is available in the form of tablets or syrup. It can also be used in this form for treating asthma, but it is less effective compared to the inhalation form. Its onset of action is slower, and it has more side effects.⁴⁰ For adults, the recommended dose of salbutamol is 2–4 mg three to four times a day, with a maximum daily dose ranging from 16 to 32 mg depending on the manufacturer. In children aged 6–12, the suggested dose is 2 mg three to four times a day, with a maximum daily dose of 8 or 24 mg, depending on the manufacturer.^{12,13} The syrup contains 2 mg of salbutamol in 5 mL and it is administered 3–4 times a day. The recommended dose is 4 mg for adults, 2 mg for children aged 6–12, and 1–2 mg for children aged 2–6.¹¹ Salbutamol is also available in the form of an injectable solution. This allows the administration of the medication subcutaneously, intramuscularly and intravenously. Indications for administering salbutamol in this form include asthmatic conditions and severe bronchospasm. Intravenous administration of salbutamol may be less effective and cause more side effects than its inhaled counterpart. For subcutaneous or intramuscular administration, the dosage is 8 µg/kg. On the other hand, intravenous administration requires dilution in sodium chloride with or without dextrose and administration at a rate of 3–20 µg per minute (dosage varies depending on the manufacturer).^{17,18}

Pharmacokinetics and metabolism

The pharmacokinetics of salbutamol depends primarily on the route of administration. The most common method of administering salbutamol is through inhalation using an MDI with a spacer. Following this method, the onset of action is observed approximately 5 minutes after ingestion. The bioavailability of salbutamol is low, mainly due to local administration, limiting the amount of the substance entering the bloodstream. The bronchodilatory effect of salbutamol does not correlate with its serum concentration. The highest serum concentration is observed after 3–4 hours, and the average half-life of plasma activity is 4–6 hours. Clinically, it is observed that the average duration of action of salbutamol is around 6 hours.^{3,41–44} Clinically used preparations containing salbutamol are a racemic mixture of the R- and S-enantiomers. The metabolism of salbutamol primarily occurs in the liver and gastrointestinal tract, where it undergoes conjugation with sulfate through sulfo-

transferase, leading to the formation of the metabolite – salbutamol – 4³-O-sulphate. Salbutamol partially unchanged and its metabolites are then excreted from the body primarily through urine.^{45,46}

Table 1. Characteristics of salbutamol administration routes^{10–18,31,40}

	Nebulizer	Metered-dose inhaler (MDI)	Dry-powder inhaler (DPI)	Salbutamol p.o.	Salbutamol i.v, i.m, s.c.
Drug form	Solution for oral inhalation	Liquefied gas propellant	Dry powder	Tablets or syrup	Solution
Method of use	Inhalation via face mask or mouthpiece, breath-enhanced or breath-actuated	Inhalation by pressing the top of the canister containing drug	Inhalation the powder into the lungs	Orally	Subcutaneously, intramuscularly, intravenously
Dosage	Adults: 2.5-5 mg dissolved in 0.9% sodium chloride Children 5-12 years old: 1.25-2.5 mg dissolved in 0.9% sodium chloride	Adults: 100 or 200 µg, daily max dose 800 µg Children 5-12 years old: 100 or 200 µg, daily max dose 400 µg	Adults: 100 or 200 µg, daily max dose 800 µg Children 5-12 years old: 100 or 200 µg, daily max dose 400 µg	Adults: 2 to 4 mg 3–4 times a day Children 5-12 years old: 2 mg 3-4 times a day	s.c and i.m – 8 µg/kg i.v – 3–20 µg per minute
Advantages	No coordination of inhalation required	Portable	Portable, no coordination of inhalation required	Portable, no coordination of inhalation required	No patient cooperation required
Disadvantages	Non-portable, noisy, long drug administration time	Most MDIs do not have dose counter (difficult to determine how many doses left), correct coordination of inhalation required	At least minimum inspiratory effort required, cough in case of higher doses	Later effect than after inhalation, more side effects	Risks associated with administration subcutaneously, intramuscularly, intravenously

Interactions

Interactions between salbutamol and other drugs are a significant aspect that requires attention when treating patients with respiratory conditions alongside other medical conditions. Salbutamol, as a bronchodilator, can interact with certain substances, potentially affecting treatment efficacy or increasing the risk of side effects. If salbutamol is used concurrently with other drugs affecting adrenergic receptors and exerting similar effects, there may be a potential intensification of side effects, such as increased heart rate, elevated blood pressure, and the risk of cardiac arrhythmias.¹⁶ Additionally, the use of formoterol (a long-acting agonist of β_2 adrenergic receptors) may lead to an increased tolerance to the bronchodilation effect of salbutamol.⁴⁷ Salbutamol should not be combined with non-cardioselective beta-blockers like propranolol as they block its

effects on beta2-receptors and hence its bronchodilatory effects.^{48–50} Interactions also occur between salbutamol and methylxanthine derivatives such as theophylline. There are studies showing improvement in spirometric parameters with the use of a combination of salbutamol and theophylline.^{51,52} On the other hand, it has been demonstrated that the combination of these two drugs can lead to more pronounced tachycardia and supraventricular extrasystoles. Additionally, the effectiveness of theophylline was found to be higher when not used in conjunction with salbutamol.^{53,54} Studies have shown that salbutamol binds to monoamine oxidases (MAO) and inhibits their activity. Monoamine oxidases are enzymes that catalyze the oxidation reaction of monoamines. Salbutamol exhibits a greater preference for binding to MAO-B than to MAO-A^{55,56}. In a situation where a patient is taking both salbutamol and drugs from the group of MAO inhibitors, there may be a synergistic effect. The result of such a combination could be an intensification of adverse effects, particularly related to the cardiovascular system (increase in blood pressure).^{16,57} Also, tricyclic antidepressants (TCAs) are used in the treatment of depression, and they too can intensify adverse effects on the cardiovascular system such as prolonged the QTc interval.⁵⁸ One of the adverse effects of salbutamol can be hypokalemia. Therefore, it is risky to use it with other drugs that cause a decrease in blood potassium levels. Examples of such drugs include non-potassium sparing diuretics, which encompass carbonic anhydrase inhibitors, loop diuretics, and thiazides.³ Caution should also be exercised when using salbutamol and digoxin. A study conducted on healthy volunteers indicates that salbutamol lowers the serum concentration of digoxin. However, the concentration in skeletal muscles remains unchanged.⁵⁹ Using desflurane in patients taking salbutamol is also risky, as it is associated with the potential for hypotension.⁵⁸

Detection and interpretation

In recent years, there has been a significant development in methods for detecting salbutamol in body fluids. The methods used allow to find substances in concentrations at the level of femtograms per milliliter.⁶⁰ In general, methods of detection and determination of concentrations can be divided into physico-chemical and immunoassays.^{61,62} Physico-chemical detection methods include, for example, mass spectrometry with gas or liquid chromatography. They are characterized by high hardware requirements, are time-consuming and have higher cost.^{62,63} Their advantage, however, is that they can be used for micro-volume probes for analysis.⁶⁴ Immunoassays are faster, less expensive and more accessible.⁶¹ They use specific antibodies labelled with enzymes, chemiluminescent molecules, metal particles or DNA molecules to detect substances.^{60,62,65} When using

methods based on immunoassays, it is also necessary to take into account the possibility of cross-reactions, especially with other low molecular weight molecules with a similar structure, such as clenbuterol.^{60,65,66} Blood or urine may be used as biological material to detect salbutamol in the body and to determine its concentrations.^{67–70} Some of the dose taken is also metabolized in the liver.⁶⁷ However, when inhaled, the drug has a predominantly local effect and is partially absorbed into the bloodstream, bypassing the liver and the first pass effect. A portion of the inhaled dose can also be swallowed and absorbed into the digestive system.⁷⁰ In the blood, salbutamol may be measured both in serum and plasma.⁶¹ The concentration of salbutamol in urine may vary depending on the urine density, which is influenced by hydration levels during exercise. In order to reduce the number of false negative results, it is postulated to adjust the urine specific gravity to a standardized level.⁷¹ When interpreting the results, especially trace concentrations, the possibility of environmental contamination should be taken into account.⁶⁰ Salbutamol is sometimes used to increase muscle mass gain in farm animals such as pigs.^{62,72} Moreover, ultra-sensitive methods detect the presence of trace amounts of salbutamol in water.⁶⁶ In addition, the metabolism of the medicine in the human body differs between people who use the medicine chronically (e.g. asthma) and those who use it occasionally (e.g. to improve performance) or individuals not using the drug.⁷³

Doping

Doping in sports is an unethical practice involving the use of substances or methods that enhance an athlete's physical performance beyond their natural abilities. The primary goal of doping is to achieve better sports results by increasing strength, endurance, speed, or the body's ability to recover. Sports organizations such as the World Anti-Doping Agency (WADA) undertake comprehensive efforts to detect and eliminate doping from sports, ensuring the integrity of competition and safeguarding athletes' health.^{74,75} Salbutamol has also found application in sports, as athletes are at higher risk experiencing of exercise-induced asthma (EIA) and exercise-induced bronchospasm (EIB) compared to the general population. EIA refers to the narrowing of airways triggered by exercise, leading to symptoms such as wheezing, coughing, shortness of breath, and chest tightness in individuals with a history of asthma. EIB, on the other hand, is a similar condition that can also occur in individuals without a previous asthma diagnosis. Both EIA and EIB are caused by the exposure of airways to cold, dry air during exercise, leading to inflammation and constriction. For athletes, these conditions can be challenging to manage, as they can significantly impact performance and overall well-being. However, it's important to note

that athletes with EIA or EIB should not be discouraged from participating in sports. Proper management, including pre-exercise use of bronchodilators like salbutamol (with adherence to anti-doping regulations), warm-up routines, and appropriate medical guidance, can help individuals with these conditions continue to engage in physical activities safely and effectively.^{74,76,77} The second aspect is taking the drug by non-asthmatic athletes to improve results. Striking a balance between legitimate medical needs and preventing its misuse for a doping purpose poses a challenge in the ongoing fight against doping in sports. As it is a controversial subject, the position of the WADA on such use of salbutamol has been changing over the years. Currently, all selective and non-selective substances acting on β_2 adrenergic receptors are only allowed in strictly defined cases. The use of inhaled salbutamol is permitted only at a maximum dose of 1600 μg over 24 hours in divided doses not to exceed 600 μg over 8 hours starting from any dose. The acceptable concentration in urine (1000 ng/mL) was also established, the exceeding of which indicates taking the drug in unacceptable doses (Table 1). In the event of a positive doping control result, the athlete may undergo a pharmacokinetic test in order to prove that they have been taking the drug in doses complying with the anti-doping rules. This is aimed at ruling out the possibility that the athlete's body metabolizes the drug in an atypical manner.⁷⁸ Despite the relationship between the dose taken and the concentration achieved in urine, studies suggest that it is not possible to determine the exact dose of the drug taken based on the concentration in a urine sample.^{79–82} Literature on this subject shows discrepancies regarding the desired effects of salbutamol in terms of doping. An *in vitro* study suggests that this substance may have androgenic activity and exhibit an anabolic effect.⁸³ There are studies conducted on people suggesting that taking salbutamol increases muscle strength and contractility. The effect varies depending on the muscle group and better results were achieved when the drug was administered orally rather than inhaled.^{84–86} One meta-analysis ruled out positive effects of salbutamol on aerobic and anaerobic performance and strength in healthy athletes. Additionally, it has been suggested that due to the lack of strong evidence that taking salbutamol can lead to improved athletic performance, salbutamol should be removed from WADA's list of banned substances.⁸⁷ According to another meta-analysis, the intake of salbutamol by healthy athletes improves their anaerobic capacity, however, it is not clear whether such effects are achievable at therapeutic doses approved by WADA.⁸⁶ Due to the above unclear or even contradictory reports, determining the objectives of anti-doping regulation regarding salbutamol is impossible.

Table 2. Concentrations of salbutamol related to effect^{75,82,88,89}

Maximal level approved by WADA (urine)	Therapeutic level (blood)	Rescue level (blood)	Toxic level (blood)	Lethal level (blood)
1000 ng/mL	4–20 ng/mL	20–40 ng/mL	>30 ng/mL	>160 ng/mL
4.18 nmol/L	0.0167–0.0836 nmol/L	0.0836–0.1672 nmol/L	>0.1254 nmol/L	>0.6696 nmol/L

Abuse and accidents

The form of salbutamol abuse or overdose varies greatly. The misuse of salbutamol is a concerning issue that can have significant health implications. The therapeutic concentration of the drug and the toxic concentration in the serum differ by only 10 ng/mL. Meanwhile, the concentration considered lethal is approximately 8 times higher than the therapeutic dose (Table 2). In the literature, already in the 1980s, authors drew attention to the risk of salbutamol abuse among children. Lack of proper education of the patient or their caregiver on the dosage of the drug can lead to accidental or intentional intake of doses higher than recommended.⁹⁰ Children who do not fully comprehend the potential risks associated with misusing medication might engage in such behavior without realizing the potential harm. Especially in a stressful situation during an acute asthma attack when they administer large doses of medication to control the symptoms. Cases of symptomatic overdose of salbutamol in children have also been reported.^{91–93} Determining poisoning in such cases due to the non-specific set of symptoms will pose significant diagnostic problems, therefore it is crucial to collect a thorough interview regarding the possibility of drug overdose. The main symptoms in such cases included cardiac arrhythmias in the form of tachycardia with QT prolongation, hypokalemia, glycemic disturbances and metabolic acidosis.^{93,94} In cases of suspected overdoses, it is crucial to quickly collect blood for analysis due to the short half-life of salbutamol, which is 3–6 hours.⁸⁹ In addition, young children who are left unattended may accidentally take too much medicine by taking and ingesting a medicine that belongs to someone else.⁹⁵ In such cases, overdoses leading to life-threatening cardiac disorders have been reported.⁹⁶ Moreover the abuse of salbutamol by children can stem from different factors and take various forms. There have been reports of pediatric patients misusing salbutamol to relieve anxiety or induce euphoria.⁹⁷ The ease of access to inhalers, if other family members are using prescribed medication, can contribute to this phenomenon. When the drug is used for recreational purposes, it usually involves the inhaled form. Then, the gases used as a carrier of the substance in the inhaler also contribute to the intoxication effect combined with the action of salbutamol. There are also publications on adult patients addicted to this drug. In some

cases, in addition to the anxiolytic effect, even hallucinations are said to have occurred. The off-label use of salbutamol as a means to alleviate anxiety is a topic that has gained attention. The mechanisms underlying this reported effect are not fully understood, and the potential risks and side effects of using salbutamol for anxiety relief have not been comprehensively studied.⁹⁸ Chronic abuse of salbutamol can result in a range of complications, including hypokalemia and acute overdose leads to sinus tachycardia, ventricular and supraventricular tachycardia and myocardial ischemia. Additionally, a case of diagnostic difficulties associated with recurrent supraventricular tachycardia resulting from chronic salbutamol abuse was also described.⁹⁹ Additionally, cases of chronic abuse have been reported in association with Munchausen syndrome.¹⁰⁰ Furthermore, relying on salbutamol inappropriately can lead to a diminished effectiveness of the medication over time. This means that when it is genuinely needed to manage asthma symptoms, it might be less effective due to tolerance development. Chronic misuse might also exacerbate underlying respiratory issues and increase the risk of respiratory infections, given that prolonged bronchodilation.¹⁰¹ Furthermore the geriatric population is also important as it is particularly prone to overdose. In such cases, overdosing may be associated with progressive dementia changes resulting in an additional dose being taken due to memory problems or difficulties using the inhaler and the wrong way of drug administration. This is especially dangerous due to the widespread occurrence of polypharmacy and the possible interaction between the drugs used by the patient, reduced cognitive abilities, visual impairment, deterioration of liver, kidney and heart function in this population. The possibility of overlapping with pre-existing arrhythmias and those caused by drug overdose is also particularly important. An unusual example of misuse of inhaled salbutamol is multiple applications of the drug directly to the skin for self-injury and burns.^{102,103} In the analysis of the abuse of this drug, it is important to also consider non-medicinal ingredients present in for example MDI. For instance, in the most well-known product containing salbutamol - Ventolin, besides the medication, there is 1,1,1,2-tetrafluoroethane (HFA-134a). Studies conducted on animals and healthy volunteers have shown that this is a relatively safe gas. This means that HFA-134a should not pose a threat even if a large dose of the medication is ingested. However, rapid emptying of containers containing this gas lowers their temperature, which in extreme cases can lead to frostbite.^{104–107} In the case of a salbutamol overdose, substances with antagonistic effects, such as those that block β_2 adrenergic receptors (e.g., propranolol), should be administered, and electrolyte imbalances such as hypokalemia should be corrected.^{108,109}

Suicides and poisonings

Possibility of poisoning and death caused by intentional overdose or criminal administration of xenobiotics is one of the challenges in post-mortem diagnostics. A multi-threaded analysis is then required using post-mortem diagnostic tools, information obtained during the investigation conducted by the authorities and the medical data of the deceased. In such cases, the key issues are also deciding whether there was a homicide or a deliberate overdose of the drug with the intention of suicide. There have been cases of the use of salbutamol for suicidal purposes described in literature.¹¹⁰ The dosage of salbutamol is usually related to its route of administration, but the lethal dose of the substance still requires clarification. Although in most cases the patients are saved without damage to their health, there have been cases of poisoning that resulted in death.^{111,112} A particularly vulnerable group are patients with chronic respiratory diseases (e.g. asthma) due to easy access to the drug; moreover, the presence of a chronic disease increases the overall risk of suicide, it also applies to the pediatric population.¹¹³ In addition, due to the scant symptoms resulting from small excess doses, many suicide attempts may go unnoticed and the symptoms ignored, which makes it difficult to provide the patient with appropriate psychological care. In addition, chronically ill patients often choose drugs they take on a daily basis for suicide attempts. The most common form of medication used in self-poisoning are oral form of salbutamol.^{114,115} The key procedure in such cases is the analysis of the victim's environment, as in the case of suicide, multiple empty packages of the substance consumed can usually be found. In cases of salbutamol overdose, death is caused by cardiac arrhythmias (mainly ventricular and supraventricular arrhythmia) caused by sympathetic receptors stimulation and hypokalaemia.^{93,111} This creates significant diagnostic problems during post-mortem examination, due to the possible lack of detectable changes during autopsy, which may suggest sudden cardiac death in a functional mechanism.^{116,117} Under such circumstances, particularly in cases where there are no witnesses or if the individual is found deceased without any attempts at rescue, authorities may discontinue their investigation. Then toxicological and histopathological tests become crucial to confirm or exclude poisoning.^{118,119} In similar cases, important information is provided by post-mortem examination of salbutamol concentrations in blood and urine, however, due to the frequent use of the drug in the population, its result should be interpreted in consideration of the patient's medical documentation regarding the drugs taken.^{120–124} The differential diagnosis should also take into ac-

count a severe asthma attack as the cause of death in which salbutamol overdose occurred during life-saving procedures.^{125–127} An attack may occur as a result of a stressful situation, e.g. during a robbery. Death can be a result of a bronchospasm, but also the bronchodilator overdose.¹²⁵ In such cases life-threatening overdose occurs as a result of unknowingly and unintentionally taking too much of the drug by the victim as a rescue in emergency situation. An important issue is also determining the criminal administration of salbutamol in cases of poisoning or the Munchausen by proxy syndrome, where the victim is often a child and the perpetrator is their parent.¹¹⁵

Conclusion

The growing popularity of salbutamol results in an increase in its use for non-therapeutic purposes. The uncommon substance overdoses typically end up with mild complications and a full recovery of patient. However, one should remember about abuse of substances for recreational and sports performance purposes, inconsistent with anti-doping regulations. In addition, special care should be taken of people regularly taking salbutamol for health purposes, especially children and elderly, due to possibility of accidental overdose, e.g. by taking an additional dose. Further research is needed to better define suicidal and criminal use, particularly in chronically ill population, including post-mortem procedures for suspected overdose. Additionally, future research should investigate the post-mortem concentrations of salbutamol to establish clearer thresholds that differentiate between therapeutic use and accidental or intentional misuse.

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Author contributions

Conceptualization, M.K.; Methodology, M.K. and S.R.; Validation, M.K., S.R. and A.T.; Resources, M.K. and S.R.; Writing – Original Draft Preparation, M.K. and S.R.; Writing – Review & Editing, A.T. and C.Ž.; Supervision, C.Ž.; Project Administration, M.K.;

Conflicts of interest

All authors declare that they have no conflicts of interest.

Data availability

No datasets were generated or analyzed during the current study

Ethics approval

Not applicable.

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REVIEW PAPER

Semaglutide – a long – awaited cure for obesity or an elusive treatment?

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ABSTRACT

Introduction and aim. Obesity is a challenging disease that affects various organs and cannot be easily treated. Therefore, the discovery that taking semaglutide results in weight loss caused great excitement amongst patients and medical professionals. The aim of this literature review is to explore the benefits and dangers of semaglutide and its effectiveness in treating obesity.

Material and methods. Review of articles published in PubMed since 2017 until first quarter of 2024.

Analysis of literature. Obesity is an increasing problem of the world population. There are different approaches in obesity treatment. Semaglutide seems effective in treating diabetes, as well as conditions that emerge from long-lasting obesity. It showed positive influence regarding diabetic neuropathy, cardiovascular risk and more. It is important to remember about possible adverse events. The most prominent are gastrointestinal symptoms, such as nausea, diarrhea, vomiting, and abdominal pain. New reports show complications with simultaneous use of anesthesia or coexisting depression. Larger studies on semaglutide-related side effects will be published in 2025 and in 2026.

Conclusion. Semaglutide is an anti-diabetic drug showing promising effects in treating diabetes and in alleviating conditions caused by obesity. It is worth remembering that its side effects have not yet been fully investigated.

Keywords. semaglutide, treatment, weight loss

Introduction

Semaglutide is a glucagon-like peptide-1 receptor agonist (GLP-1 RA). In its structure, it is similar to incretin glucagon-like peptide-1 (GLP-1), which is secreted in response to the food intake. GLP-1 has a short half-life in circulation (1–2 minutes) due to degeneration by the dipeptidyl peptidase-4 (DPP-4) and neutral endopeptidase (NEP). Due to modification of the chemical structure, semaglu-

tide has a much longer half-life in the blood of about seven days (184 hours).¹⁻² According to this, semaglutide can be administered either orally (Rybelsus) or by subcutaneous injection (Ozempic, Wegovy).

Semaglutide causes stimulation of pancreatic beta cell GLP-1 receptors, which leads to insulin secretion and at the same time inhibits glucagon release from alpha-pancreatic cells.¹⁻³ Semaglutide reduces food intake by lower-

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ing appetite, slowing down digestion in the stomach and gut mobility, improving glycemic control and activation of mechano-receptors to produce satiation signals. It also it decreases brain food reward anticipation, thus helping reduce body weight.^{1,3,4,5} Semaglutide is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes (T2D), for long-term weight management in patients with overweight or obesity, and for decreasing the incidence of cardiovascular events and physical functioning.^{1,3,4,5} Possible side effects include nausea, diarrhea, vomiting, constipation, abdominal pain, fatigue, hypoglycemia, gastroesophageal reflux disease (GERD), and increased risk of cholelithiasis. The U.S. Food and Drug Administration (FDA) label warns of potential association with pancreatic cancer and thyroid cancer.⁵ Semaglutide can minimize urges to binge eat so it can be used to treat compulsive dysfunctional eating behaviours.⁶

Semaglutide was approved by the U.S. FDA in 2017 for the treatment of T2D; in 2021 it was the 90th most commonly prescribed medication in the US, with 8 million prescriptions.^{3,7} In this article we will try to answer the question of why semaglutide is such a popular drug, what are the benefits and the potential dangers of its use.

Aim

The aim of this review is to highlight the importance of semaglutide's role in treating obesity along with its potential benefits in other areas, and, on the other hand, consider possible side effects that may impact the decision of including this medication in patient treatment.

Material and methods

A review of literature was performed to discover the latest findings on semaglutide. The scientific articles were reviewed by using PubMed. We have considered reviews and original papers from 2017 until first quarter of 2024.

Analysis of the literature

Obesity as a challenge in medicine

Obesity is one of the most common diseases in the world. The main criteria are the BMI (body mass index) value and excessive accumulation of body fat. The limit for obesity is considered BMI above 30 kg/m², first degree obesity is defined as that in the range of 30–34.99 kg/m², second degree 35–39.99 kg/m², third degree obesity extreme obesity above 40 kg/m².^{8–11}

Currently, there is an increase in the number of patients suffering from obesity, as well as an increasing number of patients with complications of the disease.⁸ Obesity in 2022 affected about 16% of the adult population worldwide.¹⁰ In the 1990s, the disease affected only 8% of adults worldwide.¹⁰

The occurrence of obesity can be due to many factors. Main factors that may lead to obesity are monogen-

ic mutations in *PCSK1*, *POMS*, *LEP*, and *LEPR* genes. In addition, obesity can be inherited by multiple genes, and epigenetics plays a major role in the onset of the disease.¹² The *PCSK1* gene is responsible for the processing of hormones, including POMC. Mutations in the *POMC* gene lead to hyperphagia and early-onset obesity. The *LEP* and *LEPR* genes are responsible for fat metabolism and calorie intake.¹² Other reason is gender. Women have a greater desire for sweet meals compared to men, and women are more likely to experience binge-eating disorders conducive to the development of obesity.^{9,13} It also depends a lot on the environment in which the individual is raised, introducing a proper diet and cultivating healthy activity in preschool age may not lead to the occurrence of obesity.^{9,14}

There are four main heterogeneous groups in patients with obesity:

- metabolically abnormal obese – this is the most common obesity phenotype. This group includes patients with a BMI >30 kg/m² with metabolic disorders such as diabetes, hyperlipidemia, elevated rates of inflammation, as well as fatty liver.^{15–17}
- metabolically healthy obese – these include patients with a BMI >30 kg/m² who are metabolically healthy, that is, without insulin and lipid disorders. They have good physical endurance and less fat around the abdomen. They have a lower risk of heart disease compared to metabolically abnormal obese.^{15–17}
- metabolically obese normal weight – this group includes patients with a BMI below 25 kg/m² who have lipid disorders, and insulin resistance and are inactive physically. They have an increased risk of developing T2D and cardiovascular disease.^{15–16}
- sarcopenic obesity – is associated with a decrease in muscle mass and an increase in the amount of body fat in elderly patients who are chronically ill, and have reduced physical activity. To diagnose sarcopenic obesity, the muscle mass in the body is estimated and compared to the body weight of the patient. Patients have increased blood pressure.^{15,16,18}

One of the main complications is non-alcoholic fatty liver. It occurs, depending on the population, in about 40–70% of obese people. Lifestyle changes and weight reduction are recommended as treatment, as well as pharmacological treatment including GLP-1 agonists and currently promising sodium/glucose cotransporter 2 (SGLT-2) inhibitors not yet registered for non-alcoholic fatty liver disease (NAFLD).^{19,20} Teheri et al. showed in a cohort study that the use of Empagliflozin in patients with Liver Steatosis and Fibrosis without T2D reduced the number of changes measured by elastography controlled attenuation parameter and liver stiffness measurement.²¹

Another complication affecting a patient's quality of life is the impact of obesity on bone tissue. Obese people are less likely to develop osteoporosis, but the risk

of falls is high, and they have increased bone fragility, which results in a high risk of fractures.²²

Obesity also disrupts lipid management. This is associated with decreased levels of adiponectin, and increased levels of inflammatory cytokines. This promotes metabolic disorders and thus leads to insulin resistance, which may result in T2D.²³⁻²⁴

In the course of obesity, complications from the cardiovascular system may occur. Obese people have a higher risk of heart attack, stroke, hypertension, heart failure, and atrial fibrillation.²⁵

Methods of treating obesity include:

- diet, physical activity, and lifestyle change – all three components of treatment are based on changing the patient's habits. The diet should be based on the caloric deficit, as well as based on the patient's preferences.²⁶⁻²⁷ The physical activity of the patient should be about 300 minutes of moderate to vigorous physical activity per week.²⁷ Cognitive-behavioral psychotherapy can help change a patient's lifestyle. The patient should be under the care of a multidisciplinary team.²⁸
- bariatric surgeries – are a method of treatment for patients with a BMI above 40 kg/m² and over 35 kg/m² with diseases resulting from obesity. The use of the method brings good results in patients: decreased BMI, improved health-related quality of life and improved lipid management.^{9,29,30}
- pharmacological treatment - these include:
 - orlistat – reduces the absorption of fat from the gastrointestinal tract, thereby reducing the number of calories taken,
 - liraglutide – a GLP-1 receptor agonist,
 - the combination of bupropion and naltrexone – its probable mechanism of action is an effect on the hypothalamic melanocortin system and the mesolimbic reward system.
 - semaglutide – a GLP-1 receptor agonist.³¹⁻³²

In the following two paragraphs we will discuss semaglutide's effects. We gathered some of the information described below in Table 1.

Table 1. Comparison of semaglutide's effects

Effects of semaglutide	Benefits	Disadvantages
Metabolic	Reduction of HbA1c Decreased fasting insulin to normal levels	Hypoglycemia (if combined with sulfonylurea and/or insulin therapy)
Gastrointestinal	Decreased liver fat content Higher reduction of liver enzymes	Nausea, diarrhea, vomiting, abdominal pain, constipation, etc.
Surgical	Decrease in odds of developing sepsis and readmission	Higher risk of aspiration due to delayed gastric emptying
Body image	Weight loss	"Ozempic face"
Synergy with other diseases	Decreased disorders in estrous cycle in polycystic ovary syndrome (PCOS)	First occurrence of depression or relapse Rhabdomyolysis Bullous pemphigoid

Positive aspects of semaglutide

Oral treatment with semaglutide leads to a reduction of HbA1c and weight in patients. A Spanish multicenter longitudinal study on 1018 people with T2D showed that $\geq 5\%$ weight loss and HbA1c reduction $> 1\%$ was achieved in 19.1% of patients at 6 months and 37.5% of patients at 12 months.³³ Similar findings regarding HbA1c were obtained in the IGNITE, PIONEER REAL, GLIMPLES and Japanese population studies.³⁴⁻³⁷

HIV positive patients

The data on semaglutide effectivity among people with HIV (PWH) is scarce. The antiretroviral therapy may be one of the causes for gaining weight, especially integrase strand transfer inhibitors (INSTI) such as dolutegravir and bictegravir are associated with weight gain.³⁸ As PWH are already at higher risk of cardiovascular complications and T2D, the untreated obesity further increases their risk. Haidar et al. performed a cohort observation of PWH that received semaglutide treatment. They reported a significant loss of body weight after 1 year and significant HbA1c reduction.³⁸ There was no difference between people receiving and not receiving INSTI proving that patients treated with INSTI also lost weight.

Diabetic neuropathy

Semaglutide seems to play a protective role in the development of diabetic peripheral neuropathy. An Australian study on 22 T2D patients treated with dulaglutide or semaglutide showed reduction of nerve cross-sectional area 1 month and 3 months post-treatment. Additionally, improvement in sural nerve conduction was reported.³⁹ However, due to the sample size, those findings should be confirmed by other studies.

Surgical implications

Diabetes mellitus (DM) without appropriate management increases the risk of postoperative complications. A study on knee arthroplasty in patients treated with semaglutide showed a large decrease in odds of developing sepsis and readmission.⁴⁰ Similar findings were obtained in the study on total hip arthroplasty.⁴¹

Semaglutide also seems to be effective in the treatment of weight recurrence after bariatric surgery. In a Kuwait study of 115 patients who were treated with semaglutide after sleeve gastrectomy 62.9% achieved $\geq 5\%$ weight loss and 11.4% had $\geq 10\%$. At 6 months of treatment, 80% of patients had $\geq 5\%$ total body weight loss, 48.6% had $\geq 10\%$ and 26% achieved $\geq 15\%$ total body weight loss.⁴² Those findings are consistent with the results of a Japanese study on a group of 29 patients treated with semaglutide after laparoscopic sleeve gastrectomy.⁴³ The findings of both studies are confirmation of the earlier results presented by Murvelashvili et al. in which semaglutide was effective regardless of surgery type.⁴⁴

Cardiovascular risk

Obesity is a known risk factor for cardiovascular risk factors such as dyslipidemia, T2D, hypertension and sleep disorders. It is also an independent risk factor for the development of cardiovascular disease and cardiovascular disease mortality.⁴⁵ The analysis of patients from SUSTAIN 6 and PIONEER 6 trials showed that in the group of patients, the overall hazard ratio for major cardiovascular events (MACE) and also for its components such as cardiovascular deaths (CV), myocardial infarction (MI) and strokes was lower for the patients who received semaglutide in comparison with the placebo group.⁴⁶ The post-hoc analysis of SUSTAIN 6 participants showed that the presence of polyvascular and single vascular disease was associated with a greater risk of MACE compared with no vascular disease. In those who were administered with semaglutide, the risk of MACE was lower in comparison to the placebo group thus patients with T2D might have greater absolute benefits from the treatment.⁴⁷ Semaglutide seems to reduce MACE also in non-diabetic patients. The SELECT trial analyzed a group of 17604 patients of age 45 years or older with a BMI of 27 or greater and had established cardiovascular disease. The treatment of a subcutaneous semaglutide dose of 2.4 mg once a week for a mean duration of 33 months reduced the risk of MACE by 20%.⁴⁸ Semaglutide was associated with changes of multiple biomarkers of cardiovascular risk such as blood pressure, waist circumference, glycemic control, nephropathy, levels of lipids and C-reactive protein.⁴⁸

The cross-national study showed that in non-diabetic patients with heart failure with preserved ejection fraction (HFpEF), semaglutide not only decreased body weight but also improved patient outcomes in the 6-minute walk test in comparison to the placebo. Additionally, the patients were asked to complete a Kansas City Cardiomyopathy Questionnaire clinical summary score (KCCQ-CSS) which is a standardized instrument that quantifies heart failure-related symptoms. The score reflects health status. After 52 weeks of semaglutide treatment patients achieved a significantly higher score in KCCQ-CSS than in the placebo group.⁴⁹

Polycystic ovary syndrome (PCOS)

In rodent studies, semaglutide seems to have a positive impact on PCOS outcomes. Xiong et al. reported that in PCOS mice the semaglutide treatment restored imbalance in gut microbiota induced by PCOS resulting in metabolic parameters improvement.⁵⁰ Another study showed that semaglutide reduced increase of body weight caused by dehydroepiandrosterone-induced PCOS. Additionally, semaglutide decreased fasting insulin to normal levels and showed a trend toward ameliorating glucose tolerance.⁵¹ Histological analysis showed that semaglutide decreased disorders in estrous

cycle. In addition, semaglutide increased the quantity of corpus luteum and decreased the quantity of cystic follicles.⁵¹ Those studies show promising results of semaglutide in PCOS and were partially confirmed by Carmina et al. In a group of 27 obese patients with PCOS who were unresponsive to a lifestyle modification after three months treatment BMI, body weight, fasting glucose and insulin significantly decreased.⁵² In 21 of the patients, menstrual disorders improved and 15 of women achieved normal menses.⁵²

Non-alcoholic liver diseases

A multinational randomized study treatment of non-alcoholic steatohepatitis (NASH) related cirrhosis with semaglutide for 48 weeks showed that there is no significant improvement in fibrosis compared with placebo.⁵³ However in patients without cirrhosis, semaglutide led to significantly greater number of patients with NASH resolution with no worsening of fibrosis compared to placebo.⁵⁴ In Flint et al. study of NAFLD semaglutide did not shown difference in comparison to placebo in change of liver stiffness assessed by magnetic resonance elastography. However, semaglutide significantly decreased liver fat content at each timepoint. Compared to the placebo semaglutide group achieved a higher reduction in liver enzymes.⁵⁵

Negative aspects of semaglutide

The recent rise of Ozempic and Wegovy intake by patients without a diagnosis of diabetes or pre-diabetic state is raising concerns within the medical community. While its efficacy in treating diabetes was demonstrated by many researchers and potential adverse effects do not outweigh the positive results of that specific therapy, there is a demand for putting in order reported adverse events so that the medical professionals who are prescribing semaglutide would be more aware of the safety of mentioned drug.⁵⁶

Gastrointestinal symptoms

To begin with, the most common adverse effects are associated with the gastrointestinal system.⁵⁷ They are also often the reason for the discontinuation of the drug.⁵⁸ The most common are: nausea, diarrhea, vomiting, abdominal pain and constipation, however, it must be noted that their intensity is connected to the dose escalation (during the period of escalation or shortly after) and a few days later they tend to resolve.⁵⁷⁻⁶⁰ There have been reports suggesting an increased occurrence of pancreatitis and pancreatic carcinoma, although further research on that subject is necessary, as some researches challenge the link between those diseases and described drug.⁵⁶ Other examples of gastrointestinal problems after taking semaglutide include: impaired gastric emptying, gastritis, oropharyngeal discomfort,

dry mouth, hiccups, abnormal feces and more.⁵⁷ Studies showed that a patient's age and body weight have a connection with a higher occurrence of the events mentioned above. On the other hand, patient's sex does not seem to have an impact in that subject, Prevalence of the gastrointestinal adverse effects is significantly higher in the middle- aged group than in the elderly group (>65 years).⁵⁷ Moreover, in defiance of a theory that gastrointestinal adverse events enhance the weight loss effect, it was proved that they do not have a great input in that matter.⁶⁰

Anaesthesiological dangers

Another danger resulting from taking semaglutide is a delayed gastric emptying. The report about a 31-year-old female undergoing the esophagogastroduodenoscopy (EGD) procedure showed that this procedure had been stopped due to a large amount of food found in the stomach during the procedure. That finding was associated with a high risk of aspiration, therefore the endoscopic examination had to be discontinued. It was stated that the patient took prescribed Ozempic and fasted for over 10 hours prior to the EGD. Next examination was scheduled a month after and the patient's last dose of Ozempic was taken a week before the EGD – that time there was no food in the stomach and the procedure was successful.⁶¹ There is also a case report about a 66-year-old man who was qualified for an elective treatment of a renal nodule. He fasted for 9 hours and his last dose of semaglutide had been taken 6 days prior the operation, however, the doctors were not informed about the patient taking that medication. The outcome was similar to the previous example: solid food was found in the stomach; the doctors did not continue the procedure and the pulmonary aspiration was avoided due to the anaesthesiologist's choice of a rapid sequence induction. However, had there been a pulmonary aspiration, the patient could have been permanently disabled or he might have even died.⁶² As it was shown in the examples above, the usual 8-10 hours fasting period before an operation is not enough to ensure proper patient preparation, thus there was a call for specific guidelines in anaesthesiology concerning patients on Ozempic. Those were announced by the American Society of Anaesthesiologists and according to them, patients who take a daily dose should skip a dose one day prior to the operation and those with a weekly dose should discontinue Ozempic a week prior to the operation.⁵⁹

Aesthetic controversies

After anaesthesiologists, the next group of doctors who raise concerns about Ozempic, are plastic surgeons. Many of them reported a new phenomenon that is a much older look of patients who took Ozempic. It is caused by a rapid facial fat loss. In usual circumstances,

facial fat provides a volume that makes face look young. Sudden fat loss observed in Ozempic intake but also in diseases that contribute to malnourishment, attributes to changes in key face areas which are responsible for youthful look. For example, wrinkles get deeper, skin loses its turgor in cheeks, nasolabial folds, tear troughs and more. The size of lips and cheeks may change and that impacts the primary face structure. It is worth noting that in case of regaining weight, fat will not be redistributed to the face, so the aging effect is permanent and a very prominent adverse event of taking Ozempic.⁵⁹

Diabetic retinopathy

Furthermore, there are researches that make medical society aware of possible danger of diabetic retinopathy complications (DRC) that might be enhanced by Ozempic. Some reports say it may be even the main cause for retinopathy in up to 5% of patients.⁵⁸ So far, there have been published results of the SUSTAIN 6 research with 3297 patients being involved, divided into semaglutide or placebo group. In that trial there was no exclusion associated with diabetic retinopathy (DR). It was shown that DRC were significantly higher in the semaglutide group; more patients developed vitreous hemorrhage; more of participants were in need of retinal photocoagulation in comparison to placebo group.⁶³⁻⁶⁴ Further investigations of that subject – the FOCUS trial – will be released in 2025 or in 2026.^{58,64}

Another group of adverse events, less frequent, but worth noticing, are psychiatric related issues such as first occurrence of depression or deteriorated mood in a patient with a long psychiatric treatment history. In both cases symptoms of fatigue, sleep relapse, disturbed concentration and decision making showed up after about a month of semaglutide treatment. Improvement of patients' state was observed after discontinuation of semaglutide. This effect is suspected to be caused by semaglutide interfering with dopaminergic neuron activity.⁶⁵ Moreover, there are singular reports of the following problems that appeared after patients started semaglutide therapy: bullous pemphigoid, rhabdomyolysis and hypoglycemia. In the first two examples, interference from other drugs was excluded and symptoms were relieved after stopping semaglutide intake, whereas hypoglycemia occurred only when accumulated with sulfonylurea and/or insulin therapy.^{56,66,67}

Conclusion

Semaglutide is a very promising medication that could be used not only to reduce glucose level but also for other various ailments. It gives hope especially to patients that suffer from obesity complications. For years, there were many attempts to treat obesity, however, with different results. Semaglutide stands out because of its pleiotropic effects, of which the most desired are decreased glu-

coase level, reduction of HbA1c and weight loss. It is worth noting that this medication comes with some side effects. The most well-known are those from gastrointestinal system, such as nausea, vomiting, constipation. There is a need of further investigation on possible negative outcome of semaglutide treatment. Many researches on that topic are announced to be released in 2025, 2026 or later.

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Author contributions

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

Conflicts of interest

The authors declare no competing interests.

Data availability

Not applicable.

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REVIEW PAPER

Preiser disease in pediatric population – a review

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ABSTRACT

Introduction and aim. Preiser disease is an avascular necrosis of the scaphoid. It is a rare disease, and even less common among the pediatric population. Disease is idiopathic, occurring without trauma or scaphoid non-union. Diagnostic methods include classic radiology, CT, MRI and bone scan. Treatment may be both surgical and conservative, and main aim is pain relief and widening range of motion of the wrist. The main aim of this paper is to summarize available knowledge about Preiser disease in the pediatric population.

Material and methods. The paper is based on scientific publications available in PubMed and NCBI databases. After evaluation of abstracts, papers were selected and analyzed, considering the references cited.

Analysis of the literature. Preiser disease is rare in the pediatric population. Children tend to present better results after conservative treatment than adults. Currently there are no unified treatment recommendations in Preiser disease, as knowledge about this condition is still limited. Wider research is vital to unify management recommendations.

Conclusion. Preiser disease can affect patients of both sexes at any age, but it is exceedingly rare in children. There is a need for controlled randomized studies to establish the treatment standards, which is challenging due to the rarity of the disease. By now, non-surgical treatment tends to be the most common among the pediatric population. Surgical methods implemented in this condition include vascularized bone grafts and proximal row carpectomy.

Keywords. avascular necrosis, orthopedics, pediatric orthopedics, Preiser disease

Introduction

Preiser's disease (PD) is an idiopathic avascular necrosis (AVN) of the scaphoid. It is not connected with any trauma, acute scaphoid fracture or delayed or absent bone union.¹ The exact etiology is still undefined.² This disease was firstly reported by Preiser in 1910.^{3,4} It is significantly less common than scaphoid fracture and Kienbock's disease, which is avascular necrosis of the lunate.^{3,5} It is worth mentioning that there are reports in literature describing patients with avascular necrosis of both lunate and scaphoid, but concomitant AVN of more than one carpal bone is uncommon.^{6–8} Dominant extremity is affected twice more commonly than

non-dominant.^{1,9} Bilateral location is extremely rare, as only a few cases were described.² PD has a predisposition towards females, but can affect every age group and both sexes.^{4,5,10} Although, occurrence in pediatric population is exceedingly rare.^{1,11} Average age of presentation is 42 years.¹²

Aim

The main aim of this review is to summarize the knowledge about Preiser disease in pediatric patients. The data is very limited due to the rarity of the disease. Review was conducted to sum up diagnostic and treatment methods in PD, but according to the literature some of

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the therapies were only implemented in adults and there is no data regarding its use in patients under 18 years.

Material and methods

To conduct this review, the search of PubMed and Google Scholar databases was conducted for articles raising the problem of the Preiser's disease in patients younger than 18 years. Key words such as 'preiser disease', 'idiopathic avascular necrosis scaphoid' were used. The papers were assessed based on their titles, abstracts and full texts, with the main inclusion criteria being that they described idiopathic avascular necrosis of the scaphoid in pediatric population. The most important criteria of exclusion were if the paper did not raise the problem of Preiser disease, was focused on avascular necrosis of other bones or other conditions, or pediatric cases were not mentioned. After final evaluation, 6 papers were included to the review. The review we conducted was not a systematic review.

Analysis of the literature

Typically Preiser disease occurs without prior trauma, and symptoms are not specific and lasting for months, what may lead to misdiagnosis. Manifestations include:^{1,3,4,7,9,13}

- wrist pain on its dorso-radial side, including chronic pain,
- limited range of motion,
- tenderness of scaphoid area,
- swelling of dorsal side of the hand
- grip strength loss
- stiffness of the wrist.

In the literature, one can find two classifications of Preiser disease. The most popular one was proposed by Herbert and Lanzetta in 1994, consisting of 4 stages, according to radiographic scans. In 2001, Kalainov et al. suggested dividing the disease into 2 stages based on magnetic resonance images. The Kalainov classification suggest that type 1 is more likely to be connected with steroid use and worse prognosis, while type 2 tends to be linked with prior wrist trauma and prognosis is more positive.^{4,9} Classifications are presented in Table 1.³⁻⁵

Table 1. Classifications of Preiser disease

Herbert and Lanzetta	Kalainov et al.
Type 1 – normal X ray, positive bone scan	Type 1 – involving the entire scaphoid
Type 2 – increased density of proximal pole	
Type 3 – proximal pole fragmentation	Type 2 – involving proximal part of scaphoid
Type 4 – carpal collapse	

According to a study conducted by Tomori and co-workers, most cases of PD concerned patients with systemic use of steroids, which is type 1 in the Kalainov classification.⁷ It is connected with reducing blood

circulation and bone microcirculation, caused by multiple systemic glucocorticoid intake, as they may lead to local hypertension and vascular constriction. Steroids may be also responsible for necrosis of osteocytes and osteoblasts and for increased number of bone marrow adipocytes and their hypertrophic transformation.¹⁴ Systemic diseases reported by them that may be connected with development of post-steroid PD were renal transplantation, systemic lupus erythematosus and autoimmune hemolytic anaemia.^{7,14} Although, Tomori et al. narrated a case where Preiser disease was induced by repeated local glucocorticoid injection.^{14,15} Demiroglu and colleagues described a case of PD in a five-fingered hand – in this extremely rare condition scaphoid is usually absent or hypoplastic. In their female patient bone was present, but avascular, which resulted in development of the disease.¹⁶ In the literature one can find a case of Preiser disease development in a patient with Holt-Oram syndrome, which is a condition characterized by anomalies in the morphology of the upper limbs and cardiac abnormalities.^{17,18} PD may also occur in patients with hematological diseases, such as leukemias.¹⁹ Other factors leading to PD are presented in Table 2.^{1,2,10,14,20,21} According to Konarski and co-workers, in atraumatic AVN, the use of alcohol and steroids may be responsible for osteonecrosis development in up to 80% of the cases.²¹

Table 2. Risk factors of Preiser disease

Risk factors of Preiser disease
– alcohol
– nicotine
– scleroderma
– diabetes
– chemotherapy
– hemoglobinopathies
– infection
– vasculitis
– thumb hypoplasia
– cardiovascular diseases
– collagen diseases

Preiser disease in pediatric population

PD is even more rare in pediatric population than among adults. This leads to the fact that treatment algorithms are unclear and not standardized.¹¹ In literature, 6 cases of scaphoid avascular necrosis were reported, and the youngest patient was 9 years old. Even though Preiser disease shows predisposition towards females, all pediatric cases described in literature regarded males. Risk factors were not distinguished in every case, but trauma was reported in three cases.^{10,11} Three patients were treated with immobilization and the results were positive.¹¹ Two patients were treated surgically – one with vascularized bone graft (VBG) and proximal row carpectomy (PRC), and second one with VBG.^{10,11} Summary of the cases is presented in Table 3.^{10,11,22-26}

Avascular necrosis can be described as necrotic changes in the bone caused by interrupted blood supply.²¹ The most common location is femoral head, but it may be present in other locations, including carpal bones.²¹ Scaphoid is located on the radial side of wrist in its proximal row, and takes part in flexion, extension and radial or ulnar deflexion.³ Morsy et al. conducted a cadaver study in 2019 which revealed that scaphoids may be divided into two types. Type I – “slender” scaphoid, tends to have wider intraosseous vascular supply. Type II – “full” scaphoid, as the vascularity is less solid, are connected with higher risk of non-union, avascular necrosis or Preiser disease.²⁷ Vascularization of scaphoid can be described as poor, although its AVN occurs rarely.¹ Blood supply for proximal part of scaphoid is assured by radial artery via dorsal scaphoid artery. This variant is the most common, but there are vascular variations in which blood supply is ensured by branches of radial artery and intercarpal artery. Distal scaphoid vascularization comes from radial artery or from its superficial palmar branch. Dorsal supply covers 70 to 80% of the vascularity of the scaphoid, while palmar supply covers the rest.^{3,10,28}

Table 3. Pediatric cases of Preiser disease reported in literature

Study	Age/ sex	Risk factor	Treatment	Comment
Amundsen et al. ¹⁰	17 M	Trauma	Pisiform vascularized bone graft and proximal row carpectomy	
Fujibuchi et al. ¹¹	10 M	Trauma	Immobilization for 2 months	– reported left wrist pain in pre-ossified scaphoid – ossific nucleus with fragmentation and collapse – positive results after 5 years follow-up
Jensen and Leicht ²³	10 M		Immobilization	– good clinical result was obtained
Gallie et al. ²⁴	12 M	Trauma	Immobilization	– no fracture was reported – MRI and bone scintigraphy revealed total ischemia of scaphoid – MRI after 6 months showed incomplete revascularization
Zheng et al. ²⁵	9 M		Not mentioned	– bone scintigraphy was implemented – disease did not show progression
Barthel et al. ²⁶	13 M	No risk factors	Vascularized bone graft	– clinical and radiological improvement was obtained

Repetitive trauma, stress and overloading of the joint may lead to its swelling what may negatively affect intraosseous and extraosseous blood supply.¹⁴ Interrupted blood circulation may be also caused by thrombosis, thromboembolism or fat embolus, but the last factor is not common in carpal AVN.²⁹ Vascular disruption leads to ischemia, necrosis of cellular bone constituents, such as osteocytes and bone marrow, followed by bone collapse.^{28,29} This process leads to painful sensations and limited range of motion and function of the wrist.²⁸ Among

potential pathogenesis patterns of PD one can distinguish diffuse ischemic necrosis of the bone (type 1) and type 2, where avascular necrosis concerns only a part of scaphoid.⁵ Although AVN are more common within carpal bones in pediatric population, one can find a case describing avascular necrosis of distal radius.¹³

When looking for diagnosis of Preiser disease one can implement classic radiology, computed tomography (CT), bone scan, arthroscopy and magnetic resonance imaging (MRI), which is considered to be a method of choice.^{3,30} X-ray and CT scans are used to divide cases according to the Herbert and Lanzetta classification, which is the most widely used rating.^{1,3} This classification included bone scan results as well, but nowadays this examination is replaced by MRI.¹⁰ Bone scan with isotope typically presents enhanced ingestion in affected bone area.³¹

Classic radiology, even though is considered as initial diagnostic method, may not reveal pathological changes on early stages.^{28,32,33} So when PD is suspected, magnetic resonance is suggested method, as its sensitivity is higher.^{28,34} Although, bone sclerosis or fracture presence may be evaluated when analyzing X-rays. Posteroanterior and lateral views are required to make a complex diagnosis.⁹ Late stages of PD may present as cystic structures, bone fragmentation or collapse on classic radiology scans.³⁵

CT let physicians to visualize potential fractures and bone fragmentation or cystic structures and makes possible to assess exact morphology of the scaphoid.^{9,31}

MRI is helpful when dividing cases into two types according to Kalainov classification. When osteonecrosis occurs, MRI images present signal loss in both T₁ and T₂ weighted scans. Ischemia leads to decreased signal in T₁ images, and hyperintensive images in T₂ scans. Bone marrow hyperintensity enhanced by gadolinium administration can suggest preserved vascularity of scaphoid.^{1,3,34} MRI can reveal bone edema and necrosis areas and it is helpful when assessing vascularization sufficiency of scaphoid after intravenous administration of contrast agent.^{14,33,34,36,37} Type 2 in Kalainov classification – AVN concerning proximal part of scaphoid, is linked with potentially better prognosis.³⁰ Post-operatively, MRI scan is used to evaluate revascularization.¹⁰

Arthroscopy is another method that can be implemented in diagnostic process, but there is no wide report or study in this topic, and evidences are limited to case studies.⁹ It allows to assess chondral surfaces and carpal ligaments, or to perform synovectomy, if needed. Arthroscopy provides a possibility not only for diagnosis, but also to treat affected wrist. In cases of fracture or bone fragmentation, curettage can be implemented.⁹

Histopathological image of PD may reveal necrotic areas with necrotic debris presence, with empty lacunae, without functional cells.¹⁴

There are no unified recommendations for PD treatment.^{7,38} Treating AVN in pediatric patients is challenging, as it is important to avoid destruction of open physis, which may lead to limb dysfunctions.¹³ Main goal in therapy is pain relief, widening range of motion and function and delaying arthritis development.²⁸ Early stages may be treated by immobilization, which tends to be more effective than physiotherapy, activity pattern modification or NSAIDs.^{1,9,13} Hyaluronic acid injections were reported in literature as a method with good but temporary outcome.¹ It is worth remembering that these methods do not lead to slowing further PD development.^{5,9,22} Surgical methods used in managing more advanced stages of PD are vascularized bone graft, proximal row carpectomy, closing radial wedge osteotomy, curettage or partial arthrodesis (capitolunate or four-corner) and 3D modelled prosthesis.^{3,5,38–44} Surgical methods should be reserved for advanced cases of PD as the bone growth is not completed in children, in contrast to adults. Vascularized bone grafts may be acquired from distal radius, iliac crest, rib or pronator quadratus pedicle.^{45–49} In literature methods like scaphoid excision or its arthroscopic proximal pole resection, silicone prosthesis, denervation, radial osteotomy, but according to reports, this techniques are not in a frequent use, mostly due to unsatisfactory outcomes.^{3,20} Vascularized bone grafts can result in pain relief, wider range of motion and improvement of vascularization, which is assessed in MRI scan post-operatively. This method can be implemented to preserve carpal anatomy.²⁰

Agarwala et al. suggested that bisphosphonate therapy may lead to the improvement in cases of avascular necrosis other than femoral head.⁵⁰ Therapies may be with alendronate only or combined – alendronate taken orally with intravenous injections of zoledronic acid. This treatment leads to collapse prevention, clinical image improvement and potential avoidance of arthroplasty. Researchers suggested that combined therapy revealed better outcomes than single alendronate intake.⁵⁰ Although, study held by Agarwala and colleagues did not include any pediatric patient to their study – youngest patients were 18 years old.

According to recent reports, the use of mesenchymal stromal cells in the treatment of osteonecrosis appears to have promising prospects, as a safe method with a rare occurrence of any complications.⁵¹

Although exact management recommendations for both children and adults are not established, in literature, it is suggested to initially treat young patients conservatively, especially in early stages.¹ According to Lenoir and co-workers, pediatric patients, unlike adults, may present complete cure or stop of disease progression.²² Children affected with PD can also obtain spontaneous revascularization, in contrast to adult patients.^{10,22} Researches described that both conservative

and surgical approaches led to positive clinical outcomes.^{23,24,26} It is vital to remember that AVN may occur in pre-ossified bone – case of not yet ossified scaphoid affected was described by Fujibuchi et al. Their experience show that conservative treatment with immobilization resulted in good clinical outcome.¹¹ Main limitation of the study was the rarity of the disease, which is even more rare in pediatric population. This leads to limited data availability. The same limitations lead to the lack of unified recommendations regarding treatment of the PD in young patients. Further studies evaluating larger groups of patients are needed to expand the knowledge about the disease, diagnostic process and to create universal recommendations and guidelines for both surgical and conservative management methods of Preiser disease.

Conclusion

Preiser disease is a rare condition, and even less frequent in pediatric population, as literature is limited to 6 reports of patients younger than 18 years. As it is an idiopathic avascular necrosis of the scaphoid, the main symptoms include pain in dorso-radial part of the wrist and limited range of motion. Diagnostic method of choice is MRI, as classic radiology tends to appear as negative. Treatment standards are not stated, both regarding children and adults. In the pediatric population there is a predisposition to treat patients non-surgically, especially in the early stages. There is a need for more complex studies and wider research concerning this disease is needed among the pediatric population.

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Conflicts of interest

Authors do not declare any conflict of interest.

Data availability

The data that support the findings of this study are available on request from the corresponding author.

Ethics approval

Not applicable.

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REVIEW PAPER

Valentino's syndrome – a systematic review of a life-threatening mimic of acute appendicitis

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ABSTRACT

Introduction and aim. Valentino's syndrome is an atypical but potentially lethal presentation of a perforated peptic ulcer. It closely mimics appendicitis. Literature about this disorder is sparse due to its rarity. Hence, this article was compiled to gain an understanding of this disorder. This article was composed to review the profiles of patients with Valentino's syndrome reported in the recent literature.

Material and methods. A systematic literature search was conducted through electronic databases, including PubMed, ResearchGate, and Scopus, using the key words and terms "Valentino's syndrome," "perforated duodenal ulcer simulating acute appendicitis," and "perforated peptic ulcer mimicking acute appendicitis." Only English literature was considered for inclusion in this study, and the time frame was fixed between 2012 and 2023.

Analysis of the literature. Twenty-seven cases from both genders, ranging in age from 7 to 76 years, were included in the review. In 20 cases, the diagnosis was made at laparotomy for a presumed diagnosis of acute appendicitis. Six cases had undergone appendectomy as their first surgical operation, but their condition had worsened postoperatively and had undergone re-exploration at a delay of 3 to 10 days (mean 6 ± 2.86 days; mode 3 days). One patient required a third abdominal exploration.

Conclusion. Valentino's syndrome has a very high rate of misdiagnosis. There is a need to increase awareness of this disorder among healthcare professionals so that it might be considered a differential diagnosis while a patient is being evaluated for acute abdomen

Keywords. appendicitis, Graham's patch, peptic ulcer, perforation, peritonitis, Valentino's syndrome

Introduction

One of the most common causes of right lower quadrant (RLQ) abdominal pain is acute appendicitis.¹ Typically beginning in the periumbilical region, the pain advances to the RLQ and is connected to anorexia, vomiting, and nausea.¹ However, a variety of common and uncommon disorders may have identical clinical manifestations. Valentino's syndrome [syn: Valentino appendix] is one such rare condition wherein a perforated peptic ulcer very closely mimics acute appendicitis.²⁻⁴ As depicted in Figure 1, fluids leaking out of the perforated gastric or duodenal ulcer stream along the right paracolic gutter

reach the RLQ, causing peritoneal and periappendiceal inflammation.

Although there are certain distinctions, the clinical signs and symptoms often follow a three-phase pattern that is comparable to a typical perforation of the peptic ulcer as follows:⁵

First Phase: Severe upper abdominal pain like a bolt from the blue, due to perforation of the ulcer and leakage of acidic contents for 0–2 hours after onset.

Second Phase: Abdominal pain shifting to the right lower quadrant within 2–12 hours after onset, as a result of tracking of the leaking fluid from the perforated ulcer

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along the right paracolic gutter, causing peritoneal and periappendiceal inflammation as depicted in Figure 1.

Third Phase: If not diagnosed and managed within 12 hours, generalized peritonitis sets in and manifests as diffuse abdominal pain and abdominal distension with acute circulatory collapse.

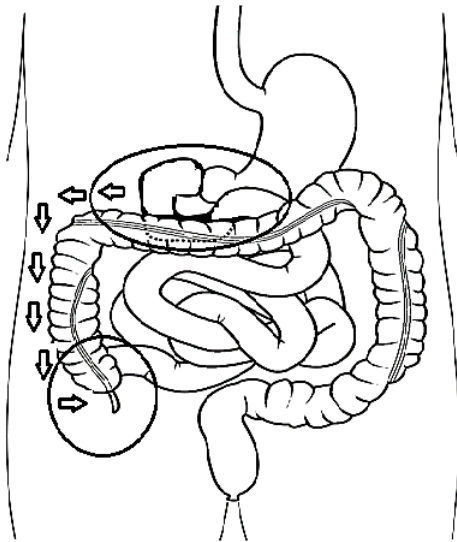


Fig. 1. Etiopathogenesis of Valentino's syndrome (Image credits: Sajad Ahmad Salati – author)

Historically, Valentino's syndrome (VS) is named after Rudolph Valentino, a well-known American actor who was born in Italy. He underwent an appendectomy in 1926, at the age of 31, after exhibiting symptoms and signs of appendicitis. He didn't get better, though, and after a week, he passed away due to peritonitis. The autopsy revealed that he actually had a perforated peptic ulcer.² This condition, if misdiagnosed and ignored, can have ramifications similar to those that Rudolph Valentino suffered a century ago.

Peptic ulcer perforations occur in around 2–15% of patients and have a mortality rate ranging from 1.3% to 25%. They are among the most uncommon complications related to the disease.⁶ More than half of the cases are female; compared to males, they are older and have more comorbidities.^{6–7} Numerous etiologic factors, such as the use of steroids, non-steroidal anti-inflammatory medicines (NSAIDs), smoking, *Helicobacter pylori*, and a diet high in salt, have been found to operate by influencing acid secretion in the stomach mucosa. In most patients, there are multiple factors present that tend to increase the ulcerogenic impact.⁸

Aim

Because VS is considered a rare manifestation of a perforated peptic ulcer and the majority of the relevant literature consists of solitary case reports, its exact epidemiology is still unknown, and healthcare professionals are not well-informed about it. Considering this, any analysis

was undertaken to examine the recently published case reports of VS in order to gain a deeper comprehension of the characteristics of this rare disease that places patients at a significant risk of complications and death.

Material and methods

Methods

A systematic literature search was conducted through electronic databases, including PubMed, ResearchGate, Scopus, and Google Scholar, using the key words and terms “Valentino's syndrome”, “perforated duodenal ulcer simulating acute appendicitis” and “perforated peptic ulcer mimicking acute appendicitis”. The search was carried out by using individual keywords with a combination of Boolean logic (AND). Only English literature was considered for inclusion in this study. The time frame fixed was 2012–2023.

Criteria for considering studies

Articles, including case series and case reports, that provided a precise and comprehensive account of the variables were included in the review process.

Participants and outcome measures

Only those cases were included where the diagnosis of Valentino's syndrome had been established through surgical exploration. The ten variables (Table 1) reported and analyzed include: [i] age of the patient; [ii] gender; [iii] duration of symptoms; [iv] history of NSAID intake; [v] clinical features; [vi] provisional diagnosis on clinical assessment; [vii] findings on imaging; [viii] management; [ix] operative findings; and [x] H-pylori eradication status.

Exclusion

Excluded were the original studies, systematic reviews, or meta-analyses that offered condensed data without a comprehensive analysis of the variables. Additionally, non-English language articles were not included.

Risk of bias

The study analyzed articles that were obtained via Open Access, made available by Qassim University and Saudi Digital Library through institutional subscriptions, or else obtained through requests to the authors through the ResearchGate platform; consequently, it is possible that some articles that were not accessible through these sources may have got overlooked.

Methodological quality checking

For comparison with the checklist that was self-drafted for this study, checklist items from Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) and previously published peer-reviewed literature were used.

Data synthesis (extraction and analysis)

Data related to the nine variables was extracted and arranged in form of Tables 1 and 2. The collected data was then analyzed with Statistical Package for Social Sciences (IBM-SPSS version 27.0, Armonk, NY, USA) and Microsoft Excel (Office Version 16, Microsoft, Redmond, Washington, USA).

The characteristics of participants were described with descriptive statistical analyses such as simple frequencies, measures of central tendency, and measures of variability. Then, the information was presented using frequencies, summary measures, tables, and figures as shown in the results. $p < 0.05$ and 95% confidence level were used as a difference of statistical significance. Moreover, the details of the included cases were provided in form of Table 1 and 2.

Analysis of the literature

Study selection

The electronic database search resulted in a total of 66 articles; 24 were identified in PubMed, 16 in ResearchGate, and 13 in Scopus. After excluding 18 duplicated articles, 35 were used to screen titles and abstracts, after which 31 potentially relevant articles in English were assessed for the eligibility criteria. Finally, 24 articles were included in the review after the inability to retrieve 4 articles and the detection of deficient data in 3 articles, as shown in Figure 2. No automation tools were used, and all the exclusion and inclusion of articles was undertaken by the authors manually.

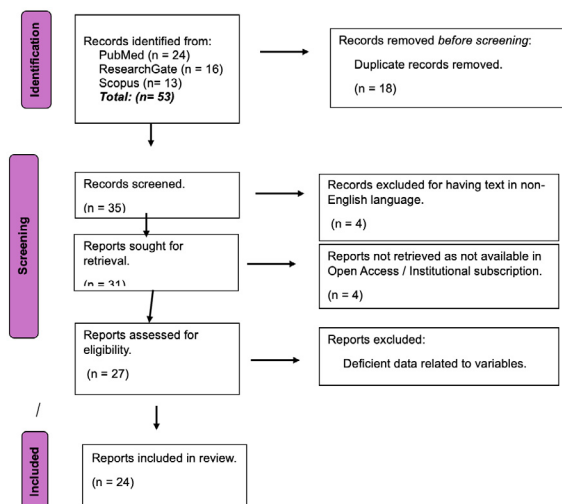


Fig. 2. Flowchart of the reviewed articles

There were 24 articles, including 22 case reports (each with a single patient) and 2 case series (with 2 and 3 patients). There were 27 cases ($n=22$; 81.5% males and $n=5$; 18.5% females), ranging in age from 7 to 76 years (mean 33 ± 16.96 years). 4 (14.8%) cases had a positive history of prolonged NSAID intake, and 1 (3.7%) case

had consumed a methylprednisolone course a week before this complication. For convenience in description, the included cases can be categorized into two types: type A ($n=21$, 77.8%) who had reported symptoms without any history of intervention, and type B ($n=6$, 22.2%) who had undergone appendectomy as their first surgical operation and got diagnosed when instead of recovery, their condition had worsened, leading to re-exploration. The duration of symptoms at presentation for cases of type A ranged from 4 hours to 7 days, and the majority of them ($n=13$; 48.1%) had reported within 24 hours. Six cases (type B) did not show expected improvement after appendectomy and deteriorated. Re-exploration was undertaken from 3 to 10 days (mean 6 ± 2.86 days; mode 3 days) post-appendectomy. 2 cases were detected during prolonged index admission, and 4 cases were readmitted to the same or some other facility after being discharged home.

The clinical features cases included right lower abdominal pain/tenderness ($n=21$; 100%), pain in the upper abdomen with shift to the right lower quadrant ($n=8$; 35%), nausea/vomiting ($n=14$; 75%), and fever ($n=6$; 30%) in 21 type A. Type B cases had presenting features of peritonitis and sepsis in their second admission, whereas in the first admission, they had been managed as acute appendicitis on the basis of clinical presentation. The provisional diagnosis on the basis of clinical features was acute appendicitis (with or without perforation or lump formation) in 26 (96.3%) cases and peritonitis with viscus perforation in 1 (3.7%); in the second admission, all six Type B cases were diagnosed as peritonitis. In 3 female patients, ovarian pathology (Mittelschmerz, ovarian cyst, ovarian torsion) was considered a second differential diagnosis, whereas in 1 pediatric case, Meckel's diverticulitis was considered a differential diagnosis. The final diagnosis was perforated peptic ulcer in 26 (96.3%) cases and acute pancreatitis in 1 (3.7%) case.

As far as imaging is concerned, three modalities were used (alone or in combination) during the assessment, including a plain X-ray abdomen (with or without an X-ray chest), an ultrasound abdomen, and a CT scan of the abdomen. Plain X-rays were conducted in 11 cases (40.7%) and in 5 (18.5%); the images were within normal limits; pneumoperitoneum was detected in 5 (18.5%); and a sentinel loop in RLQ was seen in 1 (3.7%). Ultrasound Abdomen was conducted in 15 (55.6%), and intraperitoneal free fluid was found in 10 (37%), pneumoperitoneum in 5 (18.5%), features of acute appendicitis in 5 (18.5%), and ovarian cyst in 1 (3.7%) case. A CT scan of the abdomen had been advised in 11 cases (40.7%), and intraperitoneal free fluid was revealed in 10 (37%), intraperitoneal air pockets/pneumoperitoneum in 6 (22.2%), oral contrast leakage through the duodenum in 1 (3.7%), features of acute

Table 1. Study characteristics of the patients (demographics and preoperative assessments)*

Serial number	Series	Year of publication	Number of cases	Age (years)/ Gender (M-F)	Duration of symptoms (hours-h; days)-d	Presenting complaints	H/o NSAIDs intake	Provisional Diagnosis	Findings on imaging
1	Wijegoonewardene, et al. ²	2012	1	30/F	4h	RLQ abdomen pain /tenderness * Got symptoms 4 hours after removal of intrauterine contraceptive device	-	Appendicitis	USG: Minimal free fluid in pelvis
2	Mahajan et al. ³	2014	1	21/M	8h	-RLQ abdomen pain /tenderness -Nausea/vomiting * Epigastric pain to start with and then shift to RLQ	Yes	Appendicitis	-
3	Mbarushimana et al. ⁴	2014	1	12/M	4h	RLQ abdomen pain /tenderness -Nausea/vomiting	-	Perforated appendix/Meckel diverticulum	Plain X-ray: Pneumoperitoneum
4	Sultan and Pal ⁹	2015	3	48/M 28/M 30/M	7 d PA (Type B) 10 d PA (Type-B) 3d PA (Type-B)	Abdominal distension and pain; fever, bile discharge through appendectomy wound, peritonitis - Nausea/vomiting Abdominal distension and pain; septic shock, peritonitis. Nausea/vomiting * Type B; Pain abdomen, High grade fever, dehydration, features of peritonitis	- - -	Post appendectomy, Peritonitis Post appendectomy, Peritonitis Post appendectomy, Peritonitis	- - CT: Oral contrast spillage in the duodenal area
5	Blundell et al. ¹⁰	2015	1	14/M	12h	RLQ abdomen pain /tenderness -Nausea/vomiting - Pain epigastrium shifting to RLQ; Fever, positive family history of PUD.	-	Appendicitis, peritonitis	X-Ray: Pneumoperitoneum; Football sign
6	Hussain et al. ¹¹	2016	1	7/M	1d	RLQ abdomen pain /tenderness * Pain in epigastrium and umbilical areas	-	Appendicitis	-
7	Ramirez-Ramírez et al. ¹²	2016	1	29/M	2d	RLQ abdomen pain /tenderness	-	Perforated Appendicitis	-X-Ray: Pneumoperitoneum
8	Chávez et al. ¹³	2017	2	26/M 76/M	8h PA (Type-B)	RLQ abdomen pain /tenderness -Nausea/vomiting Fever RLQ abdomen pain /tenderness -Nausea/vomiting Periumbilical pain that shifted to RLQ; fatigue, anorexia	Yes -	Peritonitis 1-Appendicitis; 2. Post appendectomy; Peritonitis	-X ray: Sentinel loop in RLQ USG: Free fluid in pelvis CT: Pneumoperitoneum, free fluid, X-Ray: 8 days post-appendectomy, dilated bowel loops in RLQ and pneumoperitoneum USG: Pericaecal free fluid before appendectomy
9	Amann et al. ¹⁴	2017	1	18/F	1d	RLQ abdomen pain /tenderness -Nausea/vomiting * Pain upper abdomen; similar pain 12 days prior treated with H2-blockers	Yes	Appendicitis; Ovarian torsion	CT: Pneumoperitoneum within the greater sac and free
10	Sgro et al. ¹⁵	2017	1	32/M	4h	RLQ abdomen pain /tenderness	-	Appendicitis;	-
11	Iloh et al. ¹⁶	2018	1	45/M	3d PA (Type-B)	RLQ abdomen pain /tenderness -Nausea/vomiting - At onset, sudden epigastric pain shifting to RLQ; appendectomy undertaken followed by generalized abdomen pain after 3 days.	-	1-Appendicitis; 2. Post appendectomy, Peritonitis	USG: Free fluid in peritoneal cavity
12	Yildiz et al. ¹⁷	2018	1	17/M	-	RLQ abdomen pain /tenderness -Nausea/vomiting	-	Appendicitis	X-ray: Normal Study USG: Free fluid in RLQ

13	Mohan et al. ¹⁸	2018	1	17/M	1d	RLQ abdomen pain /tenderness -Nausea/vomiting -Fever	-	Appendicitis	X-ray: Pneumoperitoneum USG: Free fluid in RLQ and pelvis CT: Free fluid in peritoneal cavity; no clear perforation
14	Brandon et al. ¹⁹	2019	1	24/F	-	RLQ abdomen pain /tenderness	-	Appendicitis; mittelschmerz; ovarian cysts	USG: Transvaginal US showed fluid in cul-de-sac CT: Inconclusive; appendix not visualized
15	Phadnis et al. ²⁰	2019	1	42/M	4h	RLQ abdomen pain /tenderness -Nausea/vomiting	Yes	Appendicitis	X-ray: Normal USG: Free fluid with thick appendix (6mm)
16	Nousios et al. ²¹	2020	1	51/M	6h	RLQ abdomen pain /tenderness -Nausea/vomiting * Pain abdomen with positive signs of acute appendicitis; history of duodenal ulcer for 10 years with <i>H. pylori</i> eradication	Yes	Appendicitis	X-ray: Normal chest and abdomen Xray
17	Karthik et al. ²²	2020	1	60/M	3d	RLQ abdomen pain /tenderness * Fever, pain in right groin, restlessness; H/O PPIs intake PUD for 10 years.	-	Appendicitis	X-ray: Normal USG: "Probe tenderness" and fluid collection in the RLQ CT: Free air in the RLQ and paracolic gutter
18	Lopez-Casillas et al. ²³	2020	1	52/M	1d	RLQ abdomen pain /tenderness -Nausea/vomiting * Fever, dehydration,	-	Appendicitis	USG: Free fluid, increased fat echogenicity, a retrocecal 10 mm swollen appendix
19	Rodrigo et al. ²⁴	2022	1	31/F	5d PA (Type B)	RLQ abdomen pain /tenderness -Nausea/vomiting * Appendectomy followed by clinical deterioration and bilious discharge through surgical site; re-exploration undertaken on 5th day. H/O steroid intake	-	Appendicitis	USG: Swollen appendix; a right-sided 4 cm × 4.5 cm ovarian cyst CT: Post-appendectomy. CT showed multiple fluid collections, no pneumoperitoneum
20	Devri et al. ²⁵	2022	1	22/M	2d	RLQ abdomen pain /tenderness - Pain in right groin, fever	-	Pain in right groin, fever	USG: "Probe tenderness" and fluid collection in RLQ. CT: Air pockets over right kidney and the right paracolic gutter; fluid in Morison's pouch
21	Arumugam et al. ²⁶	2022	1	28/M	4d	RLQ abdomen pain /tenderness -Nausea/vomiting	-	Appendicular mass	USG: "Probe tenderness" in the RIF CT: Acute edematous pancreas with peripancreatic fluid collection suggestive of acute pancreatitis
22	Machaku et al. ²⁷	2023	1	23/M	7d	RLQ abdomen pain /tenderness -Nausea/vomiting -Fever, guarded lower abdomen	-	Perforated appendix, perforated peptic ulcer	USG: Suggestive of perforated appendicitis
23	Villamil-Angulo et al. ²⁸	2023	1	59/M	3d	RLQ abdomen pain /tenderness -Nausea/vomiting Generalized abdomen pain shifted to RLQ, diarrhea	-	Appendicitis	-
24	Komatreddy et al. ²⁹	2023	1	50-/F	4d	RLQ abdomen pain /tenderness -Nausea/vomiting Pain started in epigastrium, then shifted to RLQ	-	Peritonitis due to Perforated Appendix	X-ray : Normal USG: Fluid collection in pelvis CT: Multiple localized right subhepatic fluid collections with inflamed appendix

* type B – patient diagnosed only after appendectomy

Table 2. Study characteristics (management, operative findings and *H. pylori* status)

Serial number	Series	Number of cases	Management	Operative findings	Postoperative <i>H. pylori</i> eradication
1	Wijegonewardene et al. ²	1	Exploratory laparoscopy; ligation; repair of prepyloric ulcer with falciform ligament patch	0.5 cm anterior prepyloric ulcer; turbid fluid in peritoneum; appendix normal	Yes
2	Mahajan et al. ³	1	Exploratory laparoscopy, ligation and repair of DU perforation	Perforated ulcer in the first portion of duodenum with bile leak	-
3	Mbarushimana et al. ⁴	1	Exploratory laparoscopy, ligation and repair of DU perforation	A large volume of turbid fluid tracking to the pelvis and a 0.5 cm perforation in the anterior wall of the first part of the duodenum	Yes
4	Sultan and Pal ⁸	3	Exploratory laparotomy; ligation; Graham patch repair of duodenal ulcer; postoperative active ventilation for 4 days, ICU care, hospitalization for 13 days.	Perforated duodenal ulcer (DU), anterior D1	-
			Exploratory laparotomy; ligation; Graham patch repair of duodenal ulcer; postoperative active ventilation for 5 days, ICU care, abdominal dehiscence management	Perforated duodenal ulcer (DU)	-
			Exploratory laparotomy; ligation; Graham patch repair of duodenal ulcer; postoperative active ventilation for 4 days, ICU care	Perforated duodenal ulcer (DU)	-
5	Blundell et al. ¹⁰	1	Exploratory laparoscopy; ligation; modified Graham repair of duodenal ulcer	Duodenal ulcer perforation	-
6	Hussain et al. ¹¹	1	Laparotomy started with grid iron incision for appendectomy and then extended by Rutherford Morrison incision; repair of duodenal ulcer by Graham patch; ligation; appendectomy.	Lot of brownish-green fluid; normal appendix; perforation in the anterior wall of first part of duodenum	-
7	Ramirez-Ramirez et al. ¹²	1	Exploratory laparoscopy; ligation; repair of duodenal ulcer with Graham patch; appendectomy	A 5 mm perforated duodenal ulcer on the anterior surface of the first part of the duodenum; hyperemic caeca appendix with fibrin and peri-appendicular fluid.	-
8	Chávez et al. ¹³	2	Exploratory laparoscopy; ligation; modified Graham repair of antral perforation 1 st operation: laparoscopic appendectomy; 2 nd operation: exploratory laparotomy Repair of duodenal perforation by Graham patch; ligation	Free fluid with gastric contents; 1 cm gastric antrum, 2 inches from the pylorus 1 st operation: Purulent fluid along paracolic gutter and pelvis; inflamed mesoappendix 2 nd operation: 5 mm perforation in anterior wall of 2 nd part of duodenum.	-
9	Amann et al. ¹⁴	1	Exploratory laparoscopy and Graham patch repair of DU perforation	Small, perforated ulcer in the first portion of duodenum	Yes
10	Sgro et al. ¹⁵	1	Exploratory laparoscopy and Graham patch repair of DU perforation	Perforated ulcer anteriorly in the first portion of duodenum	-
11	Iloh et al. ¹⁶	1	Exploratory laparoscopy; ligation; primary repair of pre-pyloric ulcer with biopsy;	5 liters of purulent fluid; 3 mm anterior prepyloric ulcer	Yes
12	Yildiz et al. ¹⁷	1	Exploratory laparoscopy and repair of anterior gastric ulcer with pedicled omentum	Normal appendix, pus flowing from upper abdomen, perforated anterior gastric ulcer	-
13	Mohan et al. ¹⁸	1	Exploratory laparoscopy, ligation, Graham patch repair of perforated duodenal ulcer	Sero-purulent fluid collection, appendix normal, sealed anterior perforation in first part of duodenum	-
14	Brandon et al. ¹⁹	1	Exploratory laparoscopy converted to minilaparotomy; ligation, repair of duodenal ulcer with Graham patch	Cloudy fluid in peritoneal cavity; normal appendix, pinhole perforation in the first part of the duodenum.	-
15	Phadnis et al. ²⁰	1	Exploratory laparoscopy converted to laparotomy; ligation; Graham patch repair of DU perforation	500 ml of bile-stained fluid, perforated ulcer in the first portion of duodenum	-
16	Nousios et al. ²¹	1	Exploratory laparotomy; repair of perforated duodenal ulcer with omentoplasty	Retropitoneal perforation of a duodenal ulcer	-
17	Karthik et al. ²²	1	Exploratory laparoscopy; ligation, Graham patch repair of DU perforation	Bile-stained fluid and food particles in retroperitoneum; no intraperitoneal fluid; appendix normal; a retroperitoneal perforation (approximately 3 mm × 4 mm) of posterior wall of duodenum	-
18	Lopez-Casillas et al. ²³	1	Exploratory laparoscopy, ligation, modified Graham patch repair (and biopsy) of perforated duodenal ulcer	150 cc seropurulent fluid collection, appendix edematous, anterior perforation in first part of duodenum	-
19	Rodrigo et al. ²⁴	1	1 st : Open appendectomy and ovarian cystectomy done - 2 nd : Exploratory laparotomy; ligation and repair of duodenal ulcer; patient got complicated with drain induced colonic perforation requiring re-laparotomy and intensive care.	Perforation at the posterior aspect of the junction of first and second part of the duodenum.	-
20	Devi et al. ²⁵	1	Exploratory laparotomy; ligation, Graham patch repair of DU perforation	Intraperitoneal fluid; 5 mm × 5 mm perforation of anterior wall of second part of duodenum	-
21	Arumugam et al. ²⁶	1	-	-	-
22	Machaku et al. ²⁷	1	Exploratory laparoscopy, ligation, appendectomy and modified Graham patch repair of pyloric perforation	Free fluid in peritoneal cavity, gastric contents around appendix and cecum, fibrinous exudates, 0.5×0.2-cm perforation on the anterior pyloric antrum.	-
23	William-Jungulo et al. ²⁸	1	Exploratory laparoscopy; ligation; repair of pyloric antrum ulcer with Cellan Jones patch; appendectomy	Purulent fluid, appendiceal inflammation features; fibrinous exudate with 5mm ulcer over anterior pyloric antrum	-
24	Konatreddy et al. ²⁹	1	Exploratory laparoscopy, ligation, modified Graham patch repair of perforated duodenal ulcer; appendectomy	Free intraperitoneal fluid and pus flakes; perforation in anterior wall of first part of duodenum	-

edematous pancreatitis in 1 (3.7%), and 1 study (3.7%) was inconclusive.

Surgical exploration was undertaken in 26 (96.3%) cases, and the patient with acute pancreatitis was managed conservatively. The surgical approach was open laparotomy in 19 (66.7%) and laparoscopic in 7 (24.9%). In 2 cases (7.4%), the approach was laparoscopic to start with but was converted into open due to difficulties during the operation, and in 5 cases (18.5%), laparotomy followed the index appendectomy as the second procedure.

The surgical steps in 26 cases that underwent surgical management involved thorough peritoneal lavage/toilet repair of ulcers in all and reinforcement with greater omentum utilizing techniques including Graham patch in 14 (53.8%), modified Graham patch in 7 (26.9%), and Cellan Jones in 1 (3.8%). In 1 case (3.8%) of pyloric ulcer, a falciform ligament patch was used. Appendectomy was undertaken in 6 (38.5%) of cases during surgical exploration, including the 4 cases (15.4%) that had appendectomy as the primary procedure. A biopsy of the ulcer was retrieved in 1 (3.8%) case, and 1 (3.8%) case required further re-exploration to treat colonic perforation caused by surgical drain-induced pressure necrosis. The majority of the perforated ulcers were located on the anterior surface of the first part of the duodenum (n=15; 57.7%), followed by the anterior surface of the stomach over the prepyloric region or antrum (n=6; 23.1%), the posterior surface (retroperitoneal) of the first part of the duodenum (n=3; 11.5%), and the anterior surface of the second part of the duodenum (n=2; 7.7%), as shown in Figure 3. *Helicobacter pylori* was tested and eradicated in only 4 (15.4%) cases.

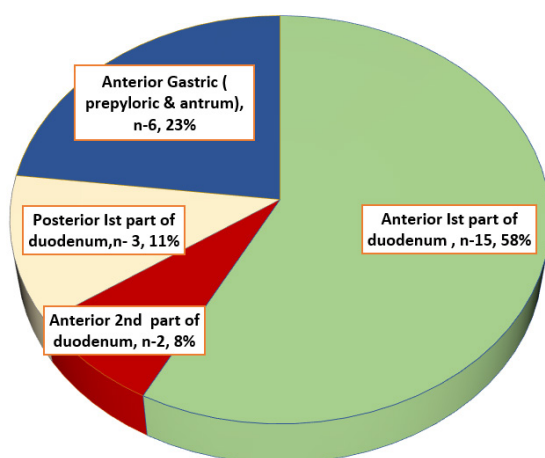


Fig. 3. Distribution of perforated peptic ulcers

Discussion

Despite improvements in diagnostic technology over the past century, cases of Valentino syndrome continue to be published, and in our review, all of the patients had acute appendicitis as a tentative diagnosis. Due to chemical peritonitis in RLQ, patients frequently expe-

rience abdominal tenderness and discomfort that is remarkably similar to peritonism induced by acute appendicitis.²⁷ Proper assessment and imaging can aid in diagnosis, when there is awareness about this possibility and the condition is suspected preoperatively. In this review, we found that, three modalities were used (alone/combination) during assessment including plain X-ray abdomen (with or without X-ray chest), ultrasound abdomen and CT scan of abdomen.

Plain X-rays were taken in 11 cases (40.7%), and in half of those, the pictures were considered normal; pneumoperitoneum was only found in 5 cases. Ultrasound abdomen was performed in 15 (55.6%), intraperitoneal free fluid was found in 10 (37%), pneumoperitoneum in 5 (18.5%), features of acute appendicitis in 5 (18.5%), and ovarian cyst in 1 (3.7%) case. An abdominal CT scan had been done in 11 cases (40.7%), and intraperitoneal free fluid was revealed in 10 (37%), intraperitoneal air pockets/pneumoperitoneum in 6 (22.2%), oral contrast leakage through duodenum in 1 (3.7%), features of acute edematous pancreatitis in 1 (3.7%), and 1 study (3.7%) was inconclusive. This data shows that a CT scan with contrast is most helpful in the detection of peptic ulcer perforation, but it has been ordered in only 40.7% of cases as the condition has not been suspected. Ansari et al. found that a CT scan with intravenous and oral contrast is the most valuable imaging technique to identify peptic ulcer perforation, and only in rare circumstances may surgical exploration be necessary for reaching the diagnosis.³⁰

3 (11.1%) of the patients in this review had perforations in the duodenum's posterior wall, which resulted in retroperitoneal collections. Several manifestations that might be seen on a CT scan and indicate retroperitoneal perforation have been noted in the literature. These include retroperitoneal free air, mainly around the right kidney, known as the "veiled right kidney sign" a thickened duodenal wall, and free air around the duodenum.^{3,21} Plain chest and abdominal x-rays may illustrate free intraperitoneal air under the right diaphragm or around the right kidney, pointing towards the possibility of perforation of the duodenum.²¹ In a recent study, Wang et al. examined the correlation between CT scan results and the locations of perforated peptic ulcers and discovered that this modality gives gastrointestinal surgeons greater information.³¹ According to Ishiguro et al., CT scans can help diagnose perforated peptic ulcers as well as predict the amount of accumulated intraperitoneal fluid in patients. This knowledge may be used to inform treatment choices and gauge the seriousness of postoperative complications.³²

In this review, there are 6 cases in which an appendectomy served as the index procedure. The majority of these patients were sent home after their index operations but were readmitted after returning back in deteriorated

condition. Despite the fact that the patients' symptoms continued even after the appendectomy, the delay in re-exploration was 6 ± 2.6 days. These startling statistics highlight the need to raise awareness of this ailment so that it can be suspected when patients behave in an unusual way.

Based on clinical signs and imaging, an appendectomy was performed in the case described by Chavez et al.¹³ During the procedure, purulent fluid was discovered in the right paracolic gutter and cul-de-sac in addition to inflammation of the mesoappendix. After the surgery, the patient stayed in the hospital for an additional three days while experiencing severe epigastric pain (8/10 on the Visual Analogue Scale). Ketorolac was administered to control the pain. The patient reported back with features of generalized peritonitis, and a plain X-ray showed massive pneumoperitoneum. The patient underwent re-exploration, wherein a perforated ulcer was detected. The patient had a prolonged hospital stay. The appendix's histology report ruled out appendicitis. Similarly, the case reported by Rodrigo et al. also suffered due to misdiagnosis.²⁴ She underwent appendectomy for provisionally diagnosed acute appendicitis and, on the 4th postoperative day, reported back with fever, generalized abdominal pain, and purulent bile-stained discharge from the surgical site. A CT scan revealed multiple intraperitoneal fluid collections in the right lower quadrant and pelvis, and exploratory laparotomy was undertaken for thorough peritoneal lavage and repair of the duodenal ulcer. She got further complicated when the drain caused colonic perforation, requiring another laparotomy and prolonged intensive care. Similarly, the other four cases also had a stormy post-appendectomy course, but there was no reported mortality. Limiting surgical delay in patients with perforated peptic ulcers is of paramount importance, and every hour of delay from admission to surgery was found by Buck et al. to be associated with increased morbidity and an adjusted 2–4 percent increased probability of death.³³

Only 4 (15.4%) of the cases had *Helicobacter pylori* testing, and this tendency is inconsistent with the suggestions made in the literature. In order to compare the long-term outcomes in terms of ulcer recurrence, Tomtitchong et al. searched the Cochrane Controlled Trials Register, Medline, and Embase for controlled trials of duodenal ulcer perforation patients using the simple closure method plus postoperative *H. pylori* eradication therapy versus simple closure plus antisecretory non-eradication therapy.³⁴ They found that *H. pylori* eradication after simple closure of duodenal ulcer perforation was better for the prevention of ulcer recurrence as compared to the operation plus antisecretory non-eradication therapy. They recommended testing for *H. pylori* infection in all patients with duodenal ulcer perforation and starting eradication therapy in all infected individuals.³⁴ In a randomized trial with comparable goals, Ng et al. discovered that in patients with perforated duodenal ulcers caused by *H. py-*

lori, eradicating *H. pylori* prevents ulcer recurrence and that in cases of generalized peritonitis, no immediate acid-reduction surgery is required.³⁵

Gisbert and Pajares investigated *H. pylori* prevalence in perforated peptic ulcers and the effects of antibiotic therapy.³⁶ They discovered that whereas the percentage rises to 90–100% in cases of noncomplicated ulcers, the mean prevalence of *H. pylori* infection in patients with perforated peptic ulcers is approximately 65–70%. Patients with *H. pylori* infection is more likely to experience recurrent ulcer disease after peptic ulcer perforation, proving the importance of the bacteria in the condition. All patients with perforated ulcers caused by *H. pylori* should begin *H. pylori* eradication medication as soon as possible in the postoperative phase in order to avoid or at least lessen ulcer recurrence and ulcer perforation.³⁷ Regardless of prior NSAID use, Metzger et al.'s research revealed that 73.3% of patients with perforated peptic ulcers tested positive for *H. pylori*.³⁸ A timely and effective *H. pylori* eradication therapy for perforated peptic ulcers was also discovered to lower the relapse rate following straightforward closure without any acid-reduction surgery. They also discovered that a triple eradication procedure had a great response rate.

In this review, 1 (3.7%) case had taken a course of methylprednisolone a week prior to this problem, and 4 (14.8%) cases had a history of extended NSAID use. Due to their anti-inflammatory and analgesic effects, NSAIDs make up around 8% of all prescriptions worldwide and are widely accessible over-the-counter for a variety of reasons.³⁹ NSAIDs use, together with exposure to the *H. pylori* infection, is a significant risk factor for peptic ulcer perforation, along with smoking, chronic stress, alcoholism, old age, and cocaine use.^{39,40}

No patient underwent any acid-reduction surgery, as is the norm currently, and management in 26 cases that underwent surgical management involved a thorough peritoneal toilet to remove collections and simple repair of the ulcer, reinforced with greater omentum utilizing techniques including Graham patch in 14 (53.8%), modified Graham patch in 7 (26.9%), and Cellan-Jones repair in 1 (3.8%). In 1 case (3.8%) of pyloric ulcer, a falciform ligament patch was used. These are standard procedures mentioned in the literature, and the methods adopted generally depend on the preference of the operating surgeon.⁴¹ Depending on the patient's health and the surgeon's expertise, either an open or a laparoscopic method can be used.^{42,43} In the review, open laparotomies were used in 19 (66.7%) and laparoscopic procedures in 7 (24.9%) patients.

There is one case in this review by Arumugam et al. that is likely the only one of its kind to have been recorded in the literature, where the patient reported having clinical signs indicative of appendicitis, but a CT scan revealed acute edematous and bulky inferior part of the head and

an uncinata process of the pancreas, with peripancreatic fluid collection noted in the infrapancreatic region, pancreaticoduodenal groove, and precaval region.²⁶ Blood work further confirmed acute pancreatitis, and the patient was managed conservatively. The imaging had been crucial in making the treatment decision; otherwise, he might have possibly ended up with surgical treatment for the suspicion of the appendicular abscess.

Conclusion

Valentino's syndrome is an atypical presentation of a perforated peptic ulcer that closely mimics appendicitis. The misdiagnosis rate for this condition is very high. Focused assessment and imaging studies can help in reaching the correct diagnosis. If the diagnosis is missed and an appendectomy is started, then the presence of bile-stained or turbid peritoneal fluid and the lack of an obviously inflamed appendix should trigger a search for a potential perforated peptic ulcer. After an appendectomy, if the patient doesn't get better or worsens, appropriate imaging should be undertaken to rule out Valentino's syndrome. There is a need to enhance the awareness level of this disorder among healthcare professionals so that it might be considered a potential differential diagnosis while a patient is being evaluated for pain in the right lower abdomen.

Declarations

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Author contributions

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

Conflicts of interest

No conflict of interest is declared by the author.

Data availability

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

Ethics approval

Not applicable.

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REVIEW PAPER

Growth differentiation factor 15 – a review of current literature on biological roles and clinical significance

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ABSTRACT

Introduction and aim. Growth differentiation factor 15 (GDF15), a member of the TGF- β superfamily, plays crucial roles in various physiological and pathological processes including inflammation, apoptosis, angiogenesis, cell repair, growth, metabolic regulation, and immune response. This review aims to discuss the biological roles and clinical significance of GDF15 and to analyze its impact across different medical fields such as cardiology, oncology, neurology, gynecology, and areas related to aging and metabolic disorders.

Material and methods. A review was constructed through a literature search on PubMed and Google Scholar databases, focusing on studies from 2014 to 2024, using relevant keywords.

Analysis of the literature. Recent research highlights GDF15's potential as a biomarker in cardiovascular diseases, its role in cancer progression and resistance to therapies, and its significance in metabolic regulation affecting conditions like obesity, diabetes, and cachexia. Emerging research also points to its role in aging, mitochondrial diseases, and systemic conditions such as sepsis, liver, and lung disorders.

Conclusion. GDF15's involvement in multiple pathological states and its broad impact across various medical disciplines underline its potential for future clinical applications. Understanding GDF15's complex roles could lead to novel therapeutic strategies and enhance prognostic assessments in diverse medical fields.

Keywords. biomarker, GDF15, GFRAL

Introduction

Growth differentiation factor 15 (GDF15), an approximately 25 kDa protein encoded by the GDF15 gene in humans, is a member of the transforming growth factor beta (TGF- β) superfamily.^{1,2} Initially identified as macrophage inhibitory cytokine-1 (MIC-1), GDF15 is also known by several other names, including nonsteroidal anti-inflammatory drug-activated gene-1 (NAG-1), prostate differentiation factor (PDF), placental bone morphogenetic protein (PLAB), placental transforming growth factor-beta (PTGF-beta/PTGFB).

GDF15 is expressed at low concentrations in various organs under normal conditions and is upregulated in response to cellular damage and organ injuries, including those affecting the liver, kidney, heart, and lungs.^{1,3,4,5} Its levels in the blood increase with age, inflammation and in response to cellular stress.^{6,7}

The effects of GDF15, including cell proliferation and differentiation, are mediated through binding to transmembrane receptor with kinase activity, GFRAL (glial-cell-derived neurotrophic factor family receptor α -like).^{1,3} GFRAL is an orphan receptor suggested to

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play a role in neuroprotection and brain development, and it is a distant homolog of the glial-cell-line-derived neurotrophic factor (GDNF) family of receptors. GDF15 was found not to interact with other, GFRAL related, neurotrophic factor receptors GDNF family receptor α (GFRA) 1–4.⁸ Experimental data indicate that the interaction between GDF15 and GFRAL leads to the internalization of the ligand-receptor complex within the cell. Further studies have shown that GFRAL signaling via GDF15 depends on the co-receptor RET, forming a GFRAL-RET-dependent complex.⁸

While the precise functions of GDF15 are still being elucidated, current understanding suggests its involvement in regulating inflammatory pathways and influencing processes such as apoptosis, angiogenesis, cell repair, and growth. Elevated levels of GDF15 have been associated with cardiovascular and neoplastic disorders as well as neurodegeneration, highlighting its broad impact on health and disease.^{1,4,6,9,10,11} The discovery of the GFRAL receptor offers new therapeutic avenues, particularly in improving prognosis in cancer patients.¹² Additionally, GDF15 plays a crucial role in processes such as cancer drug persistence and metastasis.^{13,14} It was also linked to poor prognosis in cancer patients post-radiation therapy, with radiation-resistant cancer cells exhibiting higher GDF15 expression levels.¹⁵

In terms of metabolic regulation, GDF15 influences body mass by reducing food intake, improving insulin resistance, and promoting fat breakdown. This positions GDF15 as a promising target in addressing the global obesity epidemic.^{16,17} On the other hand, elevated GDF15 levels are associated with anorexia/cachexia syndromes in various diseases, such as cancer, renal failure, heart failure, and chronic obstructive pulmonary disease. Thus, targeting GDF15-GFRAL interaction could be a therapeutic goal for anorexia/cachexia syndrome.¹⁸

GDF15 is emerging as a pivotal biomarker and therapeutic target in various pathological states. This review aims to comprehensively examine the biological functions of GDF15 and its potential clinical applications across various medical disciplines. The rationale behind this review is to consolidate the emerging evidence of GDF15's multifaceted roles in disease pathology and therapy, providing a cohesive understanding that can facilitate future research and clinical strategies.

The novelty of this review lies in its interdisciplinary approach, synthesizing data from cardiology, oncology, neurology, gynecology, metabolic disorders, and aging (Table 1). By highlighting GDF15's widespread impact, the review underscores its potential as a universal biomarker and therapeutic target, thereby opening new avenues for treatment and management across diverse medical fields.

Table 1. Roles and clinical implications of GDF15 across various medical disciplines^{1,6,7,13,15-17,19-86}

Medical field	Key findings	Potential therapeutic applications
Cardiology	<ul style="list-style-type: none"> - Elevated levels indicate cardiovascular risk and correlate with disease severity and mortality. - Protective effects include antioxidative, anti-inflammatory, and anti-apoptotic actions. 	<ul style="list-style-type: none"> - Biomarker for stratification and management of heart disease risk. - Therapeutic target for modulating metabolic activity and aiding cardiac regenerative processes.
Oncology	<ul style="list-style-type: none"> - Elevated levels in various cancers correlate with poor prognosis and survival. - Involvement in drug resistance and metastasis. - Interaction with immune responses. 	<ul style="list-style-type: none"> - Prognostic biomarker. - Target for therapeutic intervention to counteract drug resistance and metastasis.
Metabolic disorders	<ul style="list-style-type: none"> - Reduces food intake and promotes fat breakdown. - Improves insulin resistance. - Elevated levels linked to anorexia/cachexia syndromes. 	<ul style="list-style-type: none"> - Potential therapy for the management of obesity and diabetes. - Therapeutic target for anorexia/cachexia syndrome.
Aging and mitochondrial diseases	<ul style="list-style-type: none"> - Unclear correlation with age. - Marker for mitochondrial disease severity. - Involvement in cellular stress responses. 	<ul style="list-style-type: none"> - Diagnostic marker for aging and mitochondrial diseases. - Therapeutic target to modulate aging processes and mitochondrial function.
Gynecology	<ul style="list-style-type: none"> - Increased levels during pregnancy. - Associated with nausea and vomiting in pregnancy, hyperemesis gravidarum, and gestational diabetes mellitus. - Lower plasma levels linked to miscarriages. 	<ul style="list-style-type: none"> - Target for managing nausea and vomiting in pregnancy and hyperemesis gravidarum. - Early detection marker for gestational diabetes mellitus. - Potential therapy for reducing miscarriage risk.
Neurology	<ul style="list-style-type: none"> - Elevated levels in neurodegenerative diseases and stroke outcomes. 	<ul style="list-style-type: none"> - Diagnostic biomarker for neurodegenerative diseases. - Prognostic marker for stroke outcomes.
Liver diseases	<ul style="list-style-type: none"> - Reduced expression in liver fibrosis. - Protective role in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. 	<ul style="list-style-type: none"> - Therapeutic target for treating liver fibrosis and non-alcoholic fatty liver disease.
Sepsis	<ul style="list-style-type: none"> - Elevated levels in sepsis correlate with inflammatory markers and organ damage. - Modulates macrophage inflammation and polarization. 	<ul style="list-style-type: none"> - Diagnostic marker for sepsis. - Therapeutic target to modulate immune response and improve sepsis outcomes.
Pulmonology	<ul style="list-style-type: none"> - Protective role in acute lung injury. - Potentially protecting cells from fatty acid overload. 	<ul style="list-style-type: none"> - Biomarker for bronchopulmonary dysplasia, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, pulmonary hypertension, and coronavirus disease 2019.
Thrombosis	<ul style="list-style-type: none"> - Elevated levels in deep vein thrombosis patients and mouse models. - Potential role in endothelial cell function and thrombosis. 	<ul style="list-style-type: none"> - Therapeutic target to improve anticoagulant and fibrinolytic functions in thrombosis.
Systemic autoimmune diseases	<ul style="list-style-type: none"> - Linked to acute and chronic inflammation. - Potential role in systemic autoimmune diseases like type I diabetes and rheumatoid arthritis. - Increased plasma levels in lupus erythematosus, rheumatoid arthritis, and idiopathic inflammatory myopathies. 	<ul style="list-style-type: none"> - Target for modulating immune responses in autoimmune diseases. - Diagnostic and prognostic biomarker in lupus erythematosus, rheumatoid arthritis, and idiopathic inflammatory myopathies.
Dermatology	<ul style="list-style-type: none"> - Reduced levels in psoriasis. - Role in age-related pigmentation and melanogenesis. - Influences skin inflammation and keratinocyte proliferation. 	<ul style="list-style-type: none"> - Therapeutic target for treating psoriasis and age-related pigmentation alterations.

Aim

This comprehensive overview seeks to bridge existing knowledge gaps and propose new directions for research and clinical practice, emphasizing the significance of GDF15 in improving patient outcomes.

Material and methods

A review was conducted based on a literature search of the PubMed and Google Scholar databases, concentrating on studies published between 2014 and 2024. The search utilized relevant keywords, including “GDF15”, “GDF-15”, “growth differentiation factor 15”, “MIC-1”, “MIC1”, “NAG-1”, “PDF”, “PLAB”, and “PTGFB”.

Analysis of the literature

Role of GDF15 in cardiovascular disorders

In the cardiovascular context, GDF15 exhibits protective actions, including antioxidative, anti-inflammatory, and anti-apoptotic effects.⁶ GDF15 expression is markedly increased in cardiomyocytes after ischemia/reperfusion. It inhibits leukocyte activation and recruitment by directly interfering with chemokine signaling and integrin activation. Additionally, GDF15 activates the nitric oxide pathway (NOS/NO/nuclear factor κ B or NF κ B) and the forkhead box (FOX) transcription factor Foxo3. This leads to antioxidant effects and promotes neovascularization.⁶ Its potential as a therapeutic target in modulating metabolic activity and aiding cardiac regenerative processes in cardiovascular diseases presents an exciting avenue for research. However, *in vitro* studies indicate that the expression of GDF15 mRNA (messenger ribonucleic acid) and its protein levels tend to be increased in the vessel walls affected by atherosclerosis.⁷ Furthermore, the absence of GDF15 may result in a significant reduction of aortic arch lumen stenosis, decrease in fluorodeoxyglucose uptake, reduced presence of inflammatory CD11b(+) (cluster of differentiation 11b) and IL-6(+) (interleukin 6) leukocytes, as well as apoptotic cells.⁷ The varying research findings on GDF15 highlight the complexity of its function and emphasize the need for further investigation to fully understand its implications in cardiovascular diseases. Future studies should aim to elucidate the precise mechanisms by which GDF15 influences cardiovascular pathology and identify potential therapeutic applications.

GDF15's role as a predictive biomarker for adverse cardiac events further underscores its clinical relevance.^{6,19,20} GDF15 appears in the blood in many pathological conditions, making it a nonspecific marker. The conditions where increased levels of GDF15 are detected include: vascular diseases (e.g., atherosclerosis, acute coronary syndrome, stroke), heart failure, arterial hypertension, acute inflammatory states, atrial fibrillation, chronic kidney disease, anemia, bleeding, metabolic syndromes, diabetes, tobacco smoking, cancers, and advanced age.^{1,20}

In the general population, cardiovascular diseases are the main cause of increased GDF15 levels in the blood.¹ Due to its relatively simple method of precise measurement, GDF15 represents a promising element in the prevention of both primary and secondary cardiovascular events. Studies indicate three cardiovascular risk ranges based on GDF15 concentrations: concentrations <1200 ng/l correspond to low cardiovascular risk (the upper limit of reference values for healthy individuals), 1200–1800 ng/l indicate moderate (average) risk, and >1800 ng/l suggest high risk.^{21,22} The strong association between GDF15 concentration and cardiovascular disease risk factors, susceptibility to these diseases, their severity, and overall mortality (including due to cancer) designates GDF15 as a ‘predictor of death’.^{1,21} The large randomized controlled trial conducted by Hagström et al. demonstrated that higher concentrations of GDF15 are associated with an increased risk of various cardiovascular and non-cardiovascular events in patients with stable coronary heart disease.²⁰ GDF15 was independently linked to mortality from cardiovascular causes and hospitalization for myocardial infarction, heart failure and stroke. These findings underscore the significance of GDF15 as a biomarker in assessing the risk of adverse outcomes in patients with stable coronary heart disease.²⁰

In the realm of chronic heart failure, GDF15 distinguishes itself as an independent prognostic marker, adding incremental value to NT-proBNP in the prediction of long-term mortality.^{22,23} Notably, there is emerging evidence suggesting that GDF15 might even surpass NT-proBNP as a predictive marker, thereby highlighting its potential as a significant tool in the risk assessment and management of heart failure.²³ GDF15 has been observed to increase over time in heart failure patients.²⁴

While GDF15 shows promise as a biomarker and therapeutic target, several limitations exist, including its nonspecificity due to elevated levels in various pathological conditions, unclear mechanisms of its influence on cardiovascular pathology, and the need for robust, large-scale studies to transition from research to clinical practice. Future research should focus on clarifying the detailed mechanisms of GDF15 in cardiovascular diseases, conducting large-scale, longitudinal studies to validate its predictive and prognostic utility, and exploring therapeutic interventions to modulate its effects. Addressing these limitations will enhance the potential of GDF15 in improving cardiovascular health and patient outcomes.

GDF15 in oncology

GDF15 appears to be a multifaceted player in cancer biology, with its role ranging from participating in various cellular processes that influence cancer development

and progression to serving as a potential biomarker for diagnosis and prognosis.²⁵ Further research is needed to unravel the intricacies of GDF15's involvement in different cancer types and to explore its therapeutic implications.

Increased expression of GDF15 is observed in tumors of various organs, particularly in the gastrointestinal system, prostate gland, breast, skin, and brain.^{25,26} Elevated levels of secreted GDF15 are associated with poorer prognosis and survival in certain types of cancer. GDF15 is regulated by tumor suppressor pathways such as p53 (tumor protein 53), GSK-3 β (glycogen synthase kinase-3 beta), and EGR-1 (early growth response protein 1), and can be induced by drugs and dietary substances exhibiting anti-cancer properties.²⁵

GDF15 is implicated in colorectal cancer (CRC), exhibiting higher expression in CRC tissues compared to adjacent normal tissues. Excessive GDF15 expression significantly promotes cell viability, invasion, and migration, along with inducing changes favoring epithelial-to-mesenchymal transition (EMT), including increased N-cadherin, vimentin, and Twist1 (Twist-related protein 1) expression, and reduced E-cadherin expression. Utilizing GDF15-shRNA (short hairpin RNA) strategies demonstrated that reducing GDF15 inhibited cell viability, invasion, and migration in colorectal cancer cells.²⁷

Elevated levels of GDF15 were observed in various malignant phenotypes of glioma. It is associated with the progression of malignancy through the NF- κ B (Nuclear factor kappa-light-chain-enhancer of activated B cells) pathway. GDF15 is closely linked to inflammatory responses, immune cell infiltration, and immune system regulatory components, particularly in low-grade glioma (LGG). High expression levels of GDF15 predict poor survival in LGG. Thus, GDF15 emerges as a promising prognostic biomarker for LGG.²⁸

It has been discovered that GDF15 is increased during the carcinogenesis of cervical cancer. The introduction of additional GDF15 stimulated the growth of cervical cancer cells, accelerating the cell cycle transition from G0/G1 (gap 0/gap 1) to S (synthesis) phase. GDF15, by transactivating receptor tyrosine-protein kinase ErbB2, promotes the proliferation of cervical cancer cells by increasing the expression of cyclin D1 and cyclin E1, and reducing the expression of p21 (protein 21), through the activation of PI3K/AKT (phosphoinositide 3-kinase/protein kinase B) and MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) signaling pathways.²⁶

In a study on hepatocellular carcinoma (HCC), it was discovered that GDF15 is associated with an increase in regulatory T cells (Tregs), which suppress the immune response.²⁹ Deletion of the GDF15 gene in HCC transforms the tumor microenvironment from

immunosuppressive to inflammatory. GDF15 supports the generation of peripherally induced regulatory T cells (iTregs) and enhances the inhibitory function of naturally occurring regulatory T cells (nTregs). GDF15 achieves this through interaction with the CD48 (cluster of differentiation 48) receptor on T cells, regulating the activity of STUB1 (stabilin 1), an E3 (ubiquitin) ligase responsible for the degradation of forkhead box P3 (FOXP3) protein. Neutralizing antibodies against GDF15 effectively eliminate HCC in mice, suggesting the potential use of GDF15 blockade in the treatment of this cancer.²⁹

Fatigue associated with cancer is a debilitating sense of exhaustion related to the disease or its treatment. Blocking the GFRAL receptor, involved in the action of GDF15, counteracted cisplatin-induced fatigue. These findings suggest that the GDF15/GFRAL axis may be a promising therapeutic target for treating cancer-related fatigue.³⁰ Furthermore, GDF15 contributes to weight loss due to anorexia and cachexia. Blocking GDF15 and GFRAL can reverse weight loss in tumor-bearing mice, suggesting a potential therapeutic strategy. Neutralizing antibodies against GDF15 and GFRAL, such as NGM120, are currently being clinically tested for the treatment of cancer and cancer-related anorexia-cachexia syndrome.³¹

GDF15 plays a significant role in cancer cell resistance to therapy, particularly evident in breast cancer studies. GDF15, along with the GFRAL receptor, was found to be involved in inducing the formation of DTP (drug-tolerant persister) cells following eribulin therapy. GDF15 is excessively secreted after eribulin treatment. The direct involvement of GDF15 and its GFRAL receptor in inducing DTP by eribulin was confirmed by increased killing of DTP cells by eribulin in loss-of-function studies of GDF15 and GFRAL. Combined therapy of eribulin and anti-GDF15 antibody kills breast cancer DTP cells. These findings suggest that targeting GDF15 may help eliminate DTP cells and prevent the development of acquired resistance.¹³ Moreover, GDF15 was identified as a gene associated with an unfavorable prognosis in breast cancer patients after radiotherapy. Breast cancer cells resistant to radiotherapy exhibited elevated GDF15 expression compared to radiation-sensitive cells. These resistant cells not only showed increased EMT features, including enhanced migration and invasion, but also displayed stem cell-like characteristics such as mammosphere-forming ability, a high percentage of stem cells, and expression of stem cell markers. Silencing GDF15 increased the sensitivity of radioresistant cells to radiation while inhibiting EMT and stem cell-like features. These findings indicate that GDF15 contributes to the resistance of breast cancer cells to radiotherapy by promoting EMT and stem cell properties. The potential use of GDF15 as a novel prognostic bio-

marker for breast cancer patients after radiotherapy and its consideration as a therapeutic target to enhance radiotherapy effectiveness in breast cancer treatment are noteworthy.¹⁵

In conclusion, GDF15 plays a crucial role in cancer biology, offering significant potential as a biomarker and therapeutic target. However, its complex functions and the variability of its effects across different cancer types necessitate a comprehensive and critical approach to fully harness its clinical potential.

Role of GDF15 in food intake regulation, anorexia/cachexia, diabetes and obesity

GDF15 has been recognized for its intriguing connection with food intake, regulation of appetite and energy balance.¹⁶ GDF15 exerts its effects on food intake through its interaction with GFRAL receptor. GFRAL is expressed in the hindbrain, particularly in the area postrema and nucleus of the solitary tract, which are regions involved in the regulation of appetite and energy homeostasis. It has been discovered that GFRAL signals through the co-receptor of the tyrosine kinase receptor (RET).¹⁶

The data from preclinical animal studies suggest that endogenous GDF15 plays a protective role in the development of obesity, and its absence exacerbates the progression of obesity.³² Using various methods, including GFRAL null mice, viral-mediated knockdown of GFRAL in the hindbrain, and anti-GFRAL blocking antibodies, researchers demonstrated that GDF15 causes a significant and dose-dependent reduction in food intake in rodents through the GFRAL receptor, which is specifically expressed in the hindbrain's area postrema and nucleus of the solitary tract, and this effect is inhibited by GFRAL blockade, indicating that the interaction between GDF15 and GFRAL is essential for achieving the reduction in food intake.^{8,33,36} Furthermore, when challenged with a high-fat diet, GFRAL null mice gained substantially more weight compared to wild-type mice.^{8,33,36}

Administration of GDF15 analogs consistently led to weight loss by reducing food intake. Therefore, GDF15 represents an attractive option in combating the current global obesity epidemic.¹⁶ Furthermore, GDF15 influences body weight by altering dietary preferences, delaying gastric emptying, improving insulin resistance, promoting fat breakdown, and protecting β cells from apoptosis.^{17,32} Therefore, GDF15 is a promising therapeutic target in the treatment of not only obesity, but also conditions such as diabetes.¹⁷ Male mice without GDF15 exhibited poorer glucose tolerance, lower locomotor activity, and reduced metabolism compared to wild-type mice.³²

In another study, a fusion protein of GLP-1 (glucagon-like peptide-1) and GDF15 labeled as QL1005 was developed, examining its potential for reducing body weight in animals. QL1005 demonstrated higher effica-

cy than semaglutide in *in vitro* studies. In obese mice, it led to a reduction in body weight, food intake, levels of insulin, fasting glucose, and triglycerides. These effects were attributed to the balanced impact of GLP-1 and GDF15 on metabolic pathways. The long-acting, dual protein GLP-1/GDF15 appears promising in the development of new metabolic drugs.³⁷

Studies indicate that metformin increases the levels of GDF15 in the blood by stimulating its synthesis in the kidneys. Focusing on the kidneys as the site of metformin action and its regulation of GDF15 may be crucial for understanding the drug's impact on energy homeostasis. Silencing the expression of GDF15 in the kidneys or GFRAL in specific areas of the medulla abolished metformin's ability to reduce food intake and weight gain.³⁸

The mechanisms by which the body responds to the availability of fatty acids are less understood than those controlling glucose levels. GDF15 inhibits the consumption of high-fat diets, but paradoxically, its levels increase in obese mice on such a diet. Studies have shown that fatty acids, especially linolenic acid, increase GDF15 levels in a dose-dependent manner. GDF15 mRNA expression was significantly higher in the kidneys than in the gastrointestinal tract. Linolenic acid reduced food intake and body mass in wild-type mice, but not in mice lacking the GFRAL receptor. These findings suggest that GDF15 acts as a fatty acid sensor, protecting cells from fatty acid overload.³⁹

The flip side of harnessing the influence of GDF15 on nutrition and metabolism is also revealed in the topic of cachexia/anorexia. The nutritional deficiencies associated with diseases such as cancer, are common and often fatal. It occurs most frequently in the advanced stages of cancer and is likely a result of molecules released by cancer cells. The GDF15, produced in large quantities by cancer cells, plays a significant role by influencing feeding centers in the brain, leading to anorexia and weight loss.⁴⁰ The associations between GDF15 levels and nutritional indicators confirm the potential of this cytokine as a target for cancer-associated cachexia therapy.⁴⁰ Neutralizing antibodies targeting GDF15 and GFRAL, such as NGM120, are currently being clinically tested in the treatment of cancer and cancer-related anorexia-cachexia syndrome.³¹

GDF15 in aging and mitochondrial diseases

GDF15 is emerging as a significant participant in the field of aging research, as it is associated with various physiological processes and age-related conditions.

A cross-sectional study involving a group of 120 healthy individuals of different ages revealed a positive correlation between GDF15 levels and age, while also noting a negative correlation with telomere-related parameters. The results suggest that GDF15 may serve as

a potential biomarker for the aging process, influencing the risk of age-related disorders.^{41,42} However, another study on the impact of GDF15 on the proliferation of old chondrocytes found higher levels of this factor in young adults compared to older individuals. The addition of GDF15 increased chondrocyte proliferation, indicating a role for GDF15 in processes related to cartilage aging, possibly through the activation of the TGF- β pathway.⁴³ Both studies shed light on the complex role of GDF15 in aging processes, serving as both a biomarker and an activator of cellular processes, which may have implications for understanding and potentially modifying aging processes and related conditions. However, the results inconsistency highlights the need for more comprehensive longitudinal studies to determine whether GDF15 is a reliable aging biomarker.

GDF15 plays a significant role in healthy aging, influencing mitochondrial function, cellular vitality, and preventing chronic diseases. Maintaining mitokine homeostasis through lifestyle can impact life extension.⁴⁴ The influence of GDF15 on mitochondrial function has led to its recognition as a diagnostic marker in mitochondrial diseases. A moderate positive correlation was found between GDF15 levels and the severity of mitochondrial disease in carriers of the m.3243A>G mutation. Higher GDF15 levels were associated with the presence of diabetes, cardiomyopathy, and kidney disorders.⁴⁵ Cell lines with a mutation causing MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes), treated with lactic acid or pyruvate, showed increased expression and secretion of GDF15. Serum GDF15 levels were also significantly higher in patients with mitochondrial diseases than in those with other conditions. These results suggest that GDF15 may be a promising biomarker for the diagnosis and evaluation of the therapeutic effectiveness of pyruvate in mitochondrial diseases.⁴⁶ The results of another study suggest that measuring GDF15 is the most useful first-line test for identifying patients with mitochondrial respiratory chain deficiency. In a comparative study of biomarker levels in patients with mitochondrial disease and healthy control individuals, it was found that GDF15 was significantly higher in mitochondrial disease patients (on average six times higher). Additionally, GDF15 measurement showed greater effectiveness in diagnosing mitochondrial disease than other studied biomarkers.⁴⁷

GDF15 is one of the most active proteins associated with aging and various age-related diseases. Although GDF15 plays a protective role in many tissues during stress and aging processes, there are reports that its chronic elevation in old age may lead to harmful effects.⁴⁸

GDF15 in gynecology

Research has shown a significant increase in GDF15 levels in the placenta and circulation during pregnan-

cy.^{49,50} The protein, often secreted by the placenta and abdominal organs, plays a role in nutritional stress.⁵⁰⁻⁵³ The human trophoblast appears to produce a significant amount of GDF15 already in early pregnancy.⁵¹

The increase in GDF15 levels in the blood is associated with vomiting, weight loss, and cachexia, underscoring the potential therapeutic use of GDF15 as a target in the context of nausea and vomiting in pregnancy (NVP) and hyperemesis gravidarum (HG).^{50,54,55} The discovery of GFRAL receptor in the brain sheds light on mechanisms related to nausea and confirms the role of GDF15 in NVP and HG.^{50,56} Furthermore, genetic studies, particularly genome-wide association studies (GWAS), have demonstrated that genes within the GDF15-GFRAL axis are associated with the risk of NVP and HG.^{50,57-59}

Decreased levels of GDF15 in the serum and trophoblast tissue of pregnant women have been strongly associated with miscarriages. Immunohistochemistry and enzyme-linked immunosorbent assay (ELISA) studies show that women who miscarried had significantly lower levels of GDF15 compared to those who had live births.⁶⁰ In mice, GDF15 knockout was related to increased embryo resorption and reduced migration and invasion abilities of extravillous trophoblast cells, facilitated by the upregulation of the TGF- β /Smad1/5 pathway and by inhibition of the Jagged-1 (JAG1)/NOTCH3/HES1 pathway.^{60,61} The embryonic resorption could be reversed with the administration of recombinant GDF15. These findings suggest that sufficient levels of GDF15 are crucial for trophoblast invasion and proper placental development, indicating that GDF15 supplementation could potentially help maintain early pregnancy and reduce the risk of miscarriage.⁶¹

Increased levels of GDF15 are significantly associated with gestational diabetes mellitus (GDM) during the third trimester of pregnancy. However, GDF15 levels did not show a clear correlation with GDM-related adverse perinatal outcomes. These findings indicate that GDF15 could serve as a biomarker for GDM, though further research is needed to confirm its clinical utility and significance.⁶²

GDF15 in neurology

A meta-analysis comprising eight studies revealed significantly higher levels of GDF15 in patients with neurodegenerative diseases compared to healthy control individuals.⁶³ This is further supported by another study that aimed to investigate the association between GDF15 levels and cognitive impairment without dementia (CIND) or Alzheimer's disease (AD), taking into account the presence of cerebrovascular disease (CeVD). Elevated GDF15 levels were significantly associated with disease groups, especially in patients with CeVD.⁶⁴ These findings suggest that serum GDF15 lev-

els may serve as a promising diagnostic biomarker for neurodegenerative diseases.

Relationships between GDF15 and ischemic stroke and its consequences were also demonstrated. In a study focused on the Chinese population, an analysis of single nucleotide polymorphisms (SNPs) in the GDF15 gene explored the association with susceptibility to ischemic stroke. The study suggests that the rs1804826G/T polymorphism of the GDF15 gene and sGDF-5 levels are associated with the development of ischemic stroke in the Chinese population, highlighting the role of the GDF15 gene in this context.⁶⁵ In another investigation, the association between GDF15 levels and three-month mortality in patients treated with revascularization therapy following ischemic stroke was assessed. GDF15 levels in serum were measured at admission and subsequent time points. Deceased patients had higher GDF15 levels at the beginning of the study. The analysis indicated that GDF15 levels at admission were independently associated with mortality after 3 months. This suggests that GDF15 concentration at admission may serve as a potential prognostic marker for patients with ischemic stroke treated with revascularization therapy.⁶⁶ To integrate GDF15 as a diagnostic or prognostic biomarker in clinical practice, algorithms incorporating GDF15 levels with clinical parameters and imaging findings need to be developed and validated. The cost-effectiveness of incorporating GDF15 measurement into routine clinical practice should be evaluated to ensure it provides a tangible benefit to patient management and outcomes.

Impact on liver and metabolic disorders

A correlation has been demonstrated between GDF15 concentrations and the development of liver diseases and metabolic disorders. One study aimed to investigate the role of GDF15 in liver fibrosis progression. It was found that GDF15 expression is reduced in patients with liver fibrosis. GDF15 deficiency exacerbated fibrosis, while GDF15 overexpression alleviated the process. GDF15 acts by reprogramming macrophage metabolic pathways, resulting in an anti-inflammatory effect. Transplanting GDF15-treated macrophages mitigated inflammation and liver fibrosis progression. The results suggest that GDF15 could be a potential therapeutic target for treating liver fibrosis by modulating liver macrophages.⁶⁷

Another study suggests that GDF15 also plays a significant role in non-alcoholic fatty liver disease (NAFLD).⁶⁸ In the pathogenesis of NAFLD, mitochondrial dysfunction and inflammasome activation are crucial. GDF15 is essential for regulating this process. The study showed that GDF15 expression is decreased in the liver tissues of NAFLD patients and cell models of steatosis. GDF15 overexpression in transgenic mice alleviated obesity, liver steatosis, and AIM2 (absent in melanoma 2) inflammasome activation. Additionally, GDF15

limited oxidative stress, mitochondrial damage, and dsDNA (double-stranded deoxyribonucleic acid) accumulation, potentially contributing to weakening AIM2 inflammasome activation. These results suggest that GDF15 could be a potential therapeutic target for NAFLD by suppressing oxidative stress and inflammation.⁶⁸

In another study, it was found that in non-alcoholic steatohepatitis (NASH), the expression of ARRB1 (Arrestin beta 1) is decreased. ARRB1 was found to interact with GDF15 and facilitate the transport of GDF15 precursor (pro-GDF15) to the Golgi apparatus for processing and maturation.⁶⁹ Recombinant GDF15 treatment reduced lipid accumulation in the absence of ARRB1 both in vitro and in vivo. Re-expression of ARRB1 in NASH models improved liver disease, and this effect was enhanced in the presence of excessive pro-GDF15 expression. Conversely, the standalone effect of excessive pro-GDF15 expression was weakened in models lacking ARRB1.⁶⁹

The role of GDF15 is, however, more complex, as indicated by the inconsistencies among sources. GDF15 can exert both protective and harmful systemic effects, playing a role in the mitochondrial unfolded protein response (UPRmt) and may even be elevated in metabolic diseases such as obesity and NASH.⁷⁰

Sepsis

Mitochondria are crucial organelles regulating various cellular processes, and mitochondrial stress, associated with electron transport chain defects and proteostasis disruptions, is linked to numerous diseases. Sepsis, a life-threatening condition, involves metabolic disturbances and impaired immune response. Mitochondrial stress is anticipated to play a role in sepsis pathogenesis. Cells activate homeostasis maintenance systems in response to mitochondrial stress, potentially leading to the activation of genes associated with cell survival and death. GDF15 has been identified as a key secreted protein in response to mitochondrial dysfunction.⁷¹ GDF15 shows elevated levels in sepsis, correlating with inflammatory markers and organ damage indicators involved in SOFA (sequential organ failure assessment) score. It has high diagnostic value in sepsis, especially when combined with other markers. In vitro studies indicate that GDF15 may regulate macrophage inflammation, inhibit M1 (classically activated macrophages) polarization, support M2 (alternatively activated macrophages) polarization, enhance macrophage phagocytic and bactericidal functions. Additionally, GDF15 exhibits protective effects by inhibiting the JAK1/STAT3 (Janus Kinase 1/signal transducer and activator of transcription 3) pathway activation and NF- κ B p65 subunit nuclear translocation. Dynamic monitoring of GDF15 levels may be crucial in sepsis assessment and prognosis.⁷²

GDF15 in pulmonology

GDF15, secreted by tracheal epithelial cells, shows therapeutic potential by inhibiting excessive fibroblast activity and promoting epithelial repair. In a rat model with tracheal stenosis, the addition of GDF15 alleviated the degree of narrowing, suggesting its potential efficacy as a therapy for benign tracheal and bronchial stenosis.⁷³ Furthermore, research on mice lacking the GDF15 gene and exposed to ricin toxin demonstrated that the absence of GDF15 intensified lung injury, resulting in weight loss, decreased survival, and elevated levels of inflammation-related cytokines. These findings highlight a critical role for GDF15 in modulating the immune response in acute lung injury, suggesting potential avenues for new research and anti-inflammatory therapies in lung diseases.⁷⁴

GDF15 is proposed as a potential biomarker in lung diseases such as bronchopulmonary dysplasia in newborns, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), and pulmonary hypertension, aiding in diagnosis and prognosis.⁷⁵

Study by Husebo et al. explored GDF15's role in COPD. High plasma GDF15 levels correlated with higher exacerbation rates, increased mortality, and greater declines in FEV1 and FVC. Increased GDF15 levels also predicted more frequent exacerbations and higher mortality independently. The study indicated that GDF15 acts as a fatty acid sensor, potentially protecting cells from fatty acid overload.⁷⁶ Elevated levels of GDF15 were also found in the sputum and lung tissue of smokers and COPD patients compared to non-smokers. Experiments showed that cigarette smoke exposure increases GDF15 expression in human bronchial epithelial cells and mouse lungs. Importantly, GDF15 deficiency reduced cigarette smoke-induced pulmonary inflammation in mice. These findings suggest that GDF15 contributes significantly to the inflammatory processes in the lungs caused by cigarette smoke, implicating its potential role in the pathogenesis and progression of COPD.⁷⁷

Elevated GDF15 levels are associated with increased subclinical coronary atherosclerosis in COPD patients who are free of clinical cardiovascular disease. This relationship holds true even after adjusting for baseline cardiovascular disease risk, co-morbidities, severity and impact of COPD, markers of cardiac stress, and inflammation. These findings suggest that GDF15 independently contributes to subclinical coronary atherosclerosis in ever-smokers with COPD, highlighting its potential importance in the cardiovascular health of these patients.⁷⁸

Infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), has led to a pandemic affecting the respiratory and cardiovascular systems. Cytokine storms in COVID-19 patients contribute to vascular dysfunction, leading to severe complications

such as acute respiratory distress syndrome (ARDS). GDF15 serves as a strong predictor of poor prognosis in critically ill patients, playing a central role as a tissue tolerance mediator in response to inflammation.⁷⁹

Thrombosis

The relationship between GDF15 and thrombosis is a topic of interest in medical research. The study explores the interplay between GDF15 and deep vein thrombosis (VTE). Elevated GDF15 levels in VTE patients and mouse models of thrombosis prompt an investigation into how GDF15 influences the development of deep vein thrombosis. The study suggests that inhibiting GDF15 could offer therapeutic implications for improving the anticoagulant and fibrinolytic functions of endothelial cells in the context of venous thrombosis.⁸⁰

Systemic autoimmune diseases

GDF15 is linked to acute and chronic inflammation, particularly in autoimmune diseases like Type I diabetes and rheumatoid arthritis. However, its role in systemic autoimmune diseases is not well understood. Studies on GDF15-deficient mice show that its absence contributes to lymphoproliferation, T, B, and plasma cell expansion, and elevated anti-DNA antibodies, leading to accelerated kidney inflammation. GDF15 deficiency also enhances *in vitro* lymphoproliferative responses and induces type I interferon in spleen cells exposed to toll-like receptor (TLR) stimuli. In individuals with lupus nephritis (LN), reduced GDF15 expression and increased type I interferon in the kidneys suggest GDF15's regulatory role in suppressing lymphocyte activation and proliferation, while negatively influencing Toll-like receptor-driven type I interferon signaling.⁸¹

A study by Xu et al. demonstrated the involvement of GDF15 in the pathogenesis of systemic lupus erythematosus (SLE). The results indicated that GDF15 levels are higher in SLE patients compared to the healthy population. To further investigate this finding, recombinant GDF15 was administered to pristane-induced lupus mice, which resulted in reduced histological scores and decreased percentages of CD8+, CD11b+, CD19+, and CD11C+ cells, as well as TH2 cells and pro-inflammatory cytokines (IL-1 β , IL-2, IL-4, IL-21, and IL-22). These findings suggest that GDF15 is associated with the pathogenesis of lupus and may help reduce the activity of the disease.⁸²

The activity of GDF15 has also been studied in patients with rheumatoid arthritis (RA) and idiopathic inflammatory myopathies (IIM). It has been shown that the serum concentration of GDF15 in patients with RA or IIM is higher compared to a healthy population.^{83,84} Moreover, in RA patients, significantly higher levels of this factor were observed in individuals with active disease. Serum GDF15 levels showed a positive correlation

with erythrocyte sedimentation rate (ESR), morning stiffness, disease activity score-28 (DAS28), tender joint count, and carotid intima-media thickness (CIMT).⁸³ In IIM, elevated levels of GDF15 were also detected in diseased skeletal muscle. GDF15 predominantly localizes to small regenerating or denervated muscle fibers. In patients with sporadic inclusion body myositis, GDF15 co-localizes with the characteristic protein aggregates found within affected muscle fibers.⁸⁴

While current studies provide valuable insights into GDF15's potential roles, the precise molecular mechanisms through which GDF15 modulates immune responses are not fully elucidated. Further research is needed to explore these pathways in detail. The dual role of GDF15 in promoting and inhibiting inflammation in different contexts complicates its use as a biomarker or a therapeutic target.

GDF15 in dermatology

The multifaceted role of GDF15 underscores its significance in unraveling the complexities of skin conditions such as psoriasis and age-related pigmentation alterations, offering potential therapeutic avenues for these dermatological concerns.

Psoriasis, marked by excessive keratinocyte proliferation and inflammation, exhibits reduced GDF15 levels. In psoriasis, GDF15 is inhibited by TNF- α (tumor necrosis factor), exacerbating psoriasis symptoms. GDF15 deficiency intensifies psoriatic changes and increases skin inflammation. GDF15 regulates cytokine and chemokine synthesis, inhibits the TAK1/NF- κ B (transforming growth factor- β activated kinase 1/NF- κ B) pathway in keratinocytes, and directly influences neutrophil adhesion and migration by inhibiting Rap1 (Ras-related protein 1) activation. Recombinant mouse GDF15 administration alleviates psoriasis symptoms in a mouse model, suggesting therapeutic potential for treating this skin condition.⁸⁵

In a study on aging skin pigmentation, increased levels of GDF15 expression were observed in aging fibroblasts exposed to UV (ultraviolet) radiation and in photoaged skin with hyperpigmentation. Experiments using human melanocytes showed that GDF15 stimulates the melanogenesis process by activating the β -catenin signaling pathway and increasing the expression of MITF/tyrosinase (Microphthalmia-associated transcription factor/tyrosinase). Studies on ex vivo skin culture and a reconstructed model of human skin confirmed the stimulatory effect of GDF15 on skin pigmentation, suggesting its potential role in age-related pigmentation.⁸⁶

Conclusion

The review underscores GDF15's pivotal role across various biological processes and medical disciplines. GDF15 emerges not only as a crucial player in normal

physiological regulation but also as a key factor in the pathophysiology of numerous diseases. Its involvement in inflammatory pathways, apoptosis, angiogenesis, cell repair, and growth highlights a multifunctional role, affecting a wide spectrum of organ systems and disease processes.

In the realm of cardiovascular disorders, GDF15 serves as both a protective agent and a biomarker for disease severity and prognosis. Its elevated levels are indicative of cardiovascular risk and correlate with the severity and mortality of heart diseases, positioning it as a promising tool for risk stratification and management in cardiology.

GDF15's role in oncology is equally significant. Its varied expression in different cancer types and its influence on tumor progression, metastasis, and resistance to therapies mark it as a potential biomarker for cancer prognosis and a target for therapeutic intervention. The GDF15-GFRAL axis, in particular, offers new insights into the management of cancer-related anorexia and cachexia, as well as cancer cell resistance to treatment.

In metabolic disorders, GDF15 has shown a profound impact on body weight regulation, appetite control, and energy homeostasis. Its role in obesity, diabetes, and cachexia underscores its potential therapeutic utilization in these prevalent global health challenges.

The review also highlights GDF15's emerging role in aging and mitochondrial diseases, where it could act as a biomarker for aging processes and mitochondrial dysfunction. Its regulatory role in autoimmune diseases, sepsis, and liver diseases further emphasizes its systemic impact.

GDF15's influence extends to gynecology, neurology, and dermatology, indicating its broad relevance in medical science. In these fields, it serves as a biomarker and potential therapeutic target, offering novel insights into disease mechanisms and treatment strategies.

Overall, GDF15 represents a potential biomarker and therapeutic target with wide-ranging implications in health and disease. The concise overview of the diverse roles and implications of GDF15 in various medical disciplines is presented in Table 1.

GDF15's complex role in various physiological and pathological processes demands further research to fully exploit its potential in clinical practice. Understanding the multifaceted nature of GDF15 will be crucial in developing novel diagnostic and therapeutic approaches for a multitude of diseases, potentially improving patient outcomes across various medical specialties.

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Conflicts of interest

The authors declare no competing interests

Data availability

No datasets were generated or analyzed during the current study.

Ethics approval

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agreed with the content of the manuscript as written. The paper complies with the Helsinki Declaration, EU Directives and harmonized requirements for biomedical journals.

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REVIEW PAPER

Composite index of anthropometric failure and conventional anthropometric indices of undernutrition among children in India – a systematic review and meta-analysis

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ABSTRACT

Introduction and aim. The high prevalence of childhood undernutrition continues to be a major public health issue in India. This systematic and meta-analysis study employed both the composite index of anthropometric failures (CIAF) and conventional to determine the magnitude of undernutrition in Indian children.

Material and methods. A comprehensive literature search was conducted from 2005 to June 2023 using electronic databases, including Google Scholar, Web of Science, SCOPUS, PubMed, J-Gate, and ScienceDirect. A random-effects model for pooled prevalence rates was generated and heterogeneity was assessed using the I^2 index and Q statistics.

Analysis of the literature. CIAF revealed a higher prevalence of undernutrition than conventional anthropometric indices in children aged 0 to 72 months. The combined prevalence of stunting and underweight was 37% (95%CI: 0.32-0.41), and wasting was 22% (95%CI: 0.18-0.25) ($p < 0.01$). However, according to CIAF categorization, the pooled prevalence of undernourishment was reported to be 55% (95% CI: 0.50-0.60; $p < 0.01$). CIAF's higher prevalence highlights its effectiveness in capturing childhood undernutrition, accounting for children with multiple concurrent nutritional deficiencies in population.

Conclusion. This systematic review and meta-analysis investigated the assessment of undernutrition using conventional anthropometric indices compared with CIAF, which aggregate different aspects of undernutrition, especially when children have multiple undernutrition issues. Appropriate, target-specific interventions are necessary to improve the overall nutritional status of Indian children.

Keywords. child undernutrition, composite index of anthropometric failure, meta-analysis, nutritional status, public health, systematic review

Introduction

India has the world's largest population and fastest-growing economy yet continues to struggle with access to nutrition and healthcare services.¹⁻³ Undernutrition is a condition that occurs when an individual's nutritional requirements are out of coherence with their intake.⁴ The prevalence of undernutrition becoming a severe public health issue concerns Indian children as well, classified as one of the causes of child mortality,

and increasing the chances of illness burden. Despite significant economic growth, the magnitude of child mortality remains high due to undernutrition.⁵ However, undernutrition is closely linked to factors such as poverty, socio-economic disparities, and demographic conditions.⁶⁻¹² The Comprehensive National Nutrition Survey (2016-2018) found that 38% of children (<5 years) are stunted and that 35% are underweight among Indian children.^{13,14} The second major target of

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the Sustainable Development Goal, is to alleviate famished factors and improve nutritional status.^{16,17} The conventional anthropometric indices of stunting (low height-for-age), underweight (low weight-for-age), and wasting (low weight-for height) are widely used around the world to assess the severity of childhood undernutrition.^{17,18} Children who are undernourished in various undernutrition indices are not accurately represented by these conventional anthropometric indices due to the overlapping nature of assessment.^{9,19-22} The Composite Index of Anthropometric Failure (CIAF) offers the overall magnitude of undernutrition as an aggregate single measure over conventional anthropometric indices, contributing to the identification of single, double or multiple anthropometric failures in children. Further, the CIAF, a typical anthropometric measure consisting of seven categories, evaluates the prevalence of stunting, wasting, and underweight in children in several combination measures and presents an additional and reliable measure to ascertain the actual degree of undernutrition.^{18,23,24}

This systematic review and meta-analysis examined undernutrition status using both conventional anthropometric indices and CIAF among children in India. When a child's measurements deviated from the mean by more than minus two standard deviations (-2SD), this may indicate that they might be lacking in important nutrients and food intake in their first phase of life.^{18,25-29} The included studies have examined different groups of children using both nutritional assessment approaches (i.e., conventionally used anthropometric measures and CIAF) to observe how many of them had nutritional deficiencies or undernutrition. It is interesting to note that using the CIAF method frequently showed that there were significantly more undernourished children than when using only conventional measurements.^{3,9,20-21,30-42} The present study focuses on the use of both conventional anthropometric indices and CIAF as a measure of child undernutrition in India. The main objective of this study is to determine whether the CIAF provides a more accurate estimate than conventional anthropometric indices of child undernutrition. Moreover, by improving the accuracy of our assessments, target-specific interventions and policies can be made that are more effective and directly aimed at reducing child mortality, nutritional and disease burden. Nandy et al. reported that in order to address undernutrition in India, a study should ascertain the extent of the issue, which has been grossly underestimated.⁵

A newly proposed composite measure of CIAF proposed by Nandy et al. assigns a single combined value to the undernourished children in a population, which has identified that the CIAF reveals a more alarming prevalence of undernutrition after studying data from 24,396 Indian children.⁵ In comparison to commonly utilized

measures of stunting (45%), underweight (47%), and wasting (16%), the CIAF estimates that 60% of children show anthropometric failures in different categories of undernourishment in children. This novel method enables us to gain a deeper understanding of and recognize more children who are found to be susceptible and who require nutritional assistance.^{5-9,19-22,25,34,42-49} In order to provide a more thorough understanding of the prevalence of undernutrition in India, the present study was designed around this premise. In addition, despite notable economic growth, the country continues to struggle with this significant health issue, with a high prevalence (>40.0%) of children under the age of five suffering from undernutrition in India.^{3,9,20-22,24,33,35,37,42,46,48,50-58} According to the National Family Health Survey (NFHS)-4, stunting was 38.4% and decreased to 35.5% in NFHS-5, wasting was 21% in NFHS-4 and reduced to 19.3% in NFHS-5, and underweight was 35.8% in NFHS-4 and 32.1% in NFHS-5. While all three indicators of undernutrition have decreased, but the overall prevalence remains high, indicating that both chronic and acute undernourishment affect a considerable proportion of children under the age of five in India.⁵⁹

Aim

It is imperative to investigate the relationship between undernutrition and its concomitant factors, particularly how these factors vary across nutritionally vulnerable populations. Given the foregoing, the present systematic review and meta-analysis employed both conventional anthropometric measures (i.e., stunting, underweight, and wasting) and CIAF in children to determine the magnitude of undernutrition across various geographical regions in India. Furthermore, this study compares the outcomes of conventional anthropometric indices, and the CIAF approach to determine which method is more accurate to determine the actual magnitude of undernutrition to implement any appropriate nutritional intervention in the target population. Further, this systematic review and meta-analysis also aims to contribute to the development of focused interventions that can lower morbidity and disease burden by providing a thorough understanding of undernutrition and assessments among children in India.

Material and methods

Search strategy

Between 2005 and June 2023, a thorough literature search was carried out using international search engines or databases, including Google Scholar, Web of Science, Scopus, PubMed, J-Gate, and ScienceDirect. The Medical Subject Headings (MeSH) terms CIAF, OR "Childhood Anthropometric Indices of Failure", OR "Anthropometric Failure in Children", OR "Childhood Growth Failure", OR "Malnutrition in Childhood",

“Under Five Composite Index of anthropometric failure AND undernutrition” OR “Childhood undernutrition Composite Index” OR “Anthropometric Failure in Under-Five Children” OR “Under-Five Growth Failure” OR “Under-Five undernutrition”, “0-72 months” OR CIAF AND “Composite Index of anthropometric failure AND undernutrition” OR “Anthropometric Failure in Children aged 0 to 72 months” OR “Growth Failure in Infants and Young Children” OR “CIAF and Nutritional Deficiency” OR “Undernutrition with Anthropometric Failure” OR “Malnutrition with Anthropometric Failure”, “Childhood Undernutrition” OR “Undernutrition in Children” OR “Nutritional Deficiency in Childhood” OR “Child undernutrition” OR “Severe undernutrition in Childhood” OR “Weight-for-height deficit in Children” was combined within the search strategy along with “India.” In order to find additional studies, the present systematic review and meta-analysis study applied the snowball technique to search the list of pertinent references that were cited in the published manuscripts selected for the analysis. To ensure the rationale of the studies, this systematic review excluded un-reviewed articles, conference abstracts, and dissertations and theses. The entire set of search results is then transferred, and duplicates are removed using the reference management program Endnote 21.0.1.⁶⁰ The eligibility of the remaining references was further examined by following the protocols of inclusion and exclusion.

Protocols of inclusion and exclusion

Study design

Studies included in this analysis had to offer primary information on anthropometric indices and child health parameters. Both cross-sectional and longitudinal research investigations had been taken into consideration.^{42,49,57} Review articles, editorials, pieces of opinion, and studies that lacked primary data were all disqualified for the meta-analysis.

Population

The studies focused on children aged 0–72 months during the study period; priority for cross-sectional studies was considered. Studies focusing on higher age-groups or non-Indian populations with specific health conditions unrelated to the parameters of interest were excluded.

Time period

The present systematic review and meta-analysis study has included the studies published from 2005 to 2023. Search results declared zero results, which indicated that no study was found that applied both conventional and CIAF among Indian children (0–72 months) before 2005.

Anthropometric variables

In research articles that examined stunting, underweight, wasting, and CIAF, these parameters were required to be clearly defined and measured in the studies (Table 1). Studies that were not examined or examined only one of the parameters were excluded.

Data availability

The studies reported the total number of cases and provided specific values for each anthropometric measure along with the population distribution based on the age parameter (0–72 months). The research articles that did not report the total number of cases or participants or did not provide specific values for the indices were also excluded.

Eligibility criteria

Research studies that take into account the age range of 0 to 6 years and household surveys in India were included if they demonstrated the distribution of CIAF along with the conventional anthropometric measures of nutritional status (e.g., stunting, underweight, and wasting). The NFHS data have been used for selected studies that followed the above-mentioned study criteria. Articles reflecting upon only the CIAF or other single anthropometric indices were removed.

Table 1. Classification of Anthropometric failure assessed by CIAF (classification based on Nandy et al.⁵)

Group name	Description	Wasting	Stunting	Underweight
A	No failure: children whose height and weight are above the age-specific norm (i.e., above – 2 z-scores) and do not suffer from any anthropometric failure.	No	No	No
B	Wasting only: children with acceptable weight and height for their age but who have subnormal weight for height.	Yes	No	No
C	Wasting and underweight: children with above-normal heights but whose weight for age and weight for height are too low.	Yes	No	Yes
D	Wasting, stunting, and underweight: children who suffer from anthropometric failure on all three measures.	Yes	Yes	Yes
E	Stunting and underweight: children with low weight for age and low height for age but who have an acceptable weight for their height.	No	Yes	Yes
F	Stunting only: children with low height for their age but who have acceptable weight, both for their age and for their short height.	No	Yes	No
Y	Underweight only: children who are only underweight.	No	No	Yes

Study selection

Two authors independently reviewed the published titles and abstracts. Both authors independently determined whether or not to include the possibly eligible research articles after acquiring their full-texts. Any disagree-

ments among the authors were considered at each level of screening, and their consensus was used to establish the eligibility and inclusion of all listed publications. The authors, on the other hand, used risk-of-bias methodologies to meticulously analyze the quality assessments of published studies. The studies were limited to those published in English for the sake of research simplicity.

Initial screening

Following the literature search, the titles and abstracts of the retrieved references are reviewed to eliminate any unnecessary research and reduce the chance of bias and inaccuracies in the studies. The research studies that look at both conventional anthropometric indicators and CIAF are used to determine qualifying study levels. This could include studies on undernutrition in the target population. Relevant primary studies with diverse study designs, such as randomized controlled trials, cohort studies, case-control studies, or cross-sectional studies, were examined for inclusion in the study design. Studies that meet the inclusion criteria or demonstrate potential relevance go on to the next stage of the evaluation process. Full-text publications from these chosen studies are retrieved and given a more thorough evaluation before being included in the systematic review.

Full-text screening

The full-text of any potentially eligible studies is then obtained from electronic databases. Each study was thoroughly reviewed to ensure that it met the inclusion criteria. During these stages, the research articles were meticulously checked to ensure they met the requirements. The present study specifically examined whether the chosen studies took into account children aged 0-6 years, carried out household surveys, and displayed the distribution of the CIAF along with stunting, underweight, and wasting. Furthermore, researchers made certain that the studies were written in English for ease of comprehension and further analysis. The number of excluded studies and the reasons for exclusion were reported using the standard Preferred Reporting Items for systematic reviews and meta-analyses (PRISMA) guidelines (Fig. 1).⁶¹ This stringent full-text screening process ensured that only the most relevant and high-quality studies were included in the final analysis, improving the validity and reliability of our research findings.

Data extraction

Data for this analysis were precisely and conscientiously collected from 49 studies and research articles that presented the findings of CIAF and conventional anthropometric measures focused on child undernutrition assessment and were done between 2005 and 2023. Google Scholar, J-Gate, PubMed, Science Direct, SCOPUS, and Web of Science were among the electronic da-

tabases searched; however, the search was confined to the keywords following the MeSH terms. The present investigation identified similar networking terms and built the search strategy around them. The following information was gathered from each study that followed the STROBE guidelines and basic study details are included in the Excel sheet, including the initial author, year of publication, study location, total cases, and conventional anthropometric indices, including CIAF.

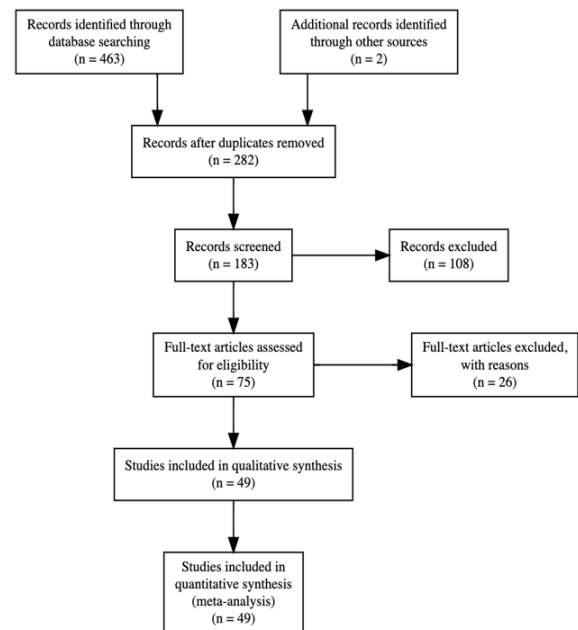


Fig. 1. The PRISMA flowchart illustrates the step-by-step process of study selection based on predefined exclusion and inclusion criteria for the systematic review

Data analysis

The R-statistical programming language was used to analyze the data.^{62,63} This systematic review utilized the R-packages 'metadata' and 'meta', which provide a variety of functions for conducting meta-analysis, by importing the prepared Excel file and calculating the pooled prevalence rates of stunting, underweight, wasting and CIAF using a random effect model because the random effect model considers both within-study and between-study variability. A significant amount of statistical heterogeneity was also estimated. The I^2 index and Q statistics were calculated for all of the studies to quantify their heterogeneity. Stunting, underweight, wasting, and CIAF had I^2 values of 99% and 98%, respectively, indicating the degree of heterogeneity between signifying anthropometric indices across studies. This systematic review further examined potential publication bias using funnel plots. The goal of this test is to detect the small study effect, which can indicate publication bias. Finally, the present study used forest plots to visualize the results of the heterogeneity between the studies. The size of each square box in

the plot corresponds to the study's sample size, and the lines extending from each side of the square boxes represent the 95% confidence interval (CI), percent weight, pooled estimate with 95% CI, significance of Chi-square statistics, and I^2 statistics. The square boxes also represent each study's 95% CI and 95% weight. The goal of the meta-analysis used in this study was to compile and produce the information contained in the CIAF and other conventional anthropometric indices.

Analysis of the literature

The results are based on research conducted on the prevalence of undernutrition in Indian children. This present study initially located 463 records in this systematic review using various electronic databases. Finally, 52 population or study groups (49 total studies) in total, including cross-sectional studies, were selected for this systematic review and meta-analysis study. The present study has identified that, of the 49 studies, 37 reported undernutrition assessments in accordance with WHO child growth standards (1995, 2006), and 12 articles reported using the National Centre for Health Statistics (1977) growth standard. This was after carefully examining the research articles in accordance with the inclusion criteria. There were 3,56,957 participants in total. There were 2,48,055 children between the ages of 0 and 72 months in the largest study.⁶⁴ The present study divided the studies into three sub-groups based on the search period, which was from 2005 to 2023. Sub-group 1 was the initial stage for conducting research on and publishing the CIAF, with studies ranging from 2005 to 2010. Sub-group 2 includes studies from 2011 to 2017 and represents the middle of publications in which researchers recognize CIAF as a valuable indicator of childhood undernutrition. The most recent stage of the investigation is Sub-group 3, which includes research from 2018 to 2023. By dividing the timeline into three groups, the present study can estimate the trend, development, and shifts in anthropometric indices over time. The present study adhered to the guidelines provided by PRISMA and the Meta-Analysis of Observational Studies in Epidemiology.⁶⁵

According to this systematic review analysis, the prevalence of stunting, underweight, and wasting is depicted in Figures 2–4 and Table 2, respectively. The pooled prevalence of stunting was 36.31% (95% CI: 0.3191–0.4097, $I^2=98.3$, $H=7.59$, $p<0.001$). In the case of underweight, it was 36.93% (95% CI: 0.3312–0.4091, $I^2=98.4$, $H=7.86$, $p<0.001$), while wasting was 20.52% (95% CI: 0.1765–0.2372, $I^2=97.5$, $H=6.36$, $p<0.001$). However, the magnitude of CIAF (Fig. 6) was observed to be significantly higher (i.e., 54.80%, 95% CI: 0.4965–0.5985, $I^2=97$, $p<0.001$) than the child undernutrition assessed utilizing conventional anthropometric measures. The test of heterogeneity analysis using Wald and the Likelihood Ratio Test (LRT) both yielded highly sig-

nificant values, indicating substantial variability across studies in both conventional anthropometric measures and CIAF ($p<0.01$).

Based on a random-effects model, the analysis between six geographical regions revealed significant heterogeneity, with $I^2=99.6\%$ (τ^2 values: 0.0272 and 0.0007). The Q-test yielded a significant value of 809.59, indicating significant variation across regions. The Q-value for the between-group analysis was 21.04 (d.f., 6; $p<0.01$), indicating significant regional variability. The overall findings revealed 59% (95% CI: 0.56–0.62, $I^2=100$), indicating a significant difference among the reported studies ($p<0.01$) (Table 3). However, region-specific analysis revealed that the northern region had a 60% (95% CI: 0.48–0.72, $I^2=96$, $p<0.01$) distribution, the central region had a 47% (95% CI: 0.26–0.68, $I^2=100$, $p<0.01$) distribution, the eastern region had a 51% (95% CI: 0.44–0.57, $I^2=96$, $p<0.01$) distribution, and the western region had a 60% (95% CI: 0.32–0.68, $I^2=97$, $p<0.01$).

The results showed significant variations in prevalence rates of CIAF across the regions of India. The large variation in most countries highlights the importance of taking regional variables into account when managing childhood undernutrition and single, double, or multiple anthropometric failures. This systematic review and meta-analysis has derived that four of the forty nine studies were designated as “India,” with four studies conducted in the Northern region of India (Region 1),^{38,40,66,67} five in the Central region (Region 2)^{20,45,47,51,68} nineteen in the Eastern region (Region 3),^{19,31–34,42,43,49,50,53–56,58,69–73} two in the North Eastern region (Region 4)^{9,22} nine in the Western region (Region 5)^{35–37,39,41,46,74–76} and six in the Southern region (Region 6).^{2,23,59,77–79}

Table 2. This table presents the results of quantifying heterogeneity and conducting tests of heterogeneity for four anthropometric indices (stunting, underweight, wasting, and CIAF) in a systematic review with 52 included studies and a total of 356,957 observations. The proportion is represented with 95% CI for each index

Anthropometric indices	(a) No of studies 'K'=52, (b) No of Observation 'o'=356957, (c) Proportion=95% CI						Test of Heterogeneity			
	No of Event (e)	Random	tau ²	tau	I ²	H	Q	d.f.	p	
Stunting	142834.5	0.3631	0.4969	0.7049	98.3%	7.59	Wald	2941.44	51	0.001
							LRT	3162.60	51	0.001
Underweight	134893.2	0.3693	0.3693	0.5985	98.4%	7.86	Wald	3154.01	51	0.001
							LRT	3226.58	51	0.001
Wasting	73206.73	0.2052	0.4388	0.6624	97.5%	6.36	Wald	2059.81	51	0.001
							LRT	2084.60	51	0.001
CIAF	201887	0.5480	0.5573	0.7465	97.4%	6.24	Wald	1982.66	51	0.001
							LRT	2452.17	51	0.001

Table 3. This table presents the results of the meta-analysis conducted in different regions, analyzing the prevalence of the CIAF index. The table includes information on the number of studies (K) in each region, the estimated proportion value, the 95% CI, and measures of heterogeneity (τ^2 and τ)

Regions	K proportion	95% CI	τ^2	τ	Q	I^2	Between groups
India	4	0.5889	0.0007	0.0272	809.59	99.6	
Region 1 (North)	4	0.6014	0.0131	0.1146	72.15	95.8	
Region 2 (Central)	5	0.4721	0.0559	0.2365	951.90	99.6	Q=21.04
Region 3 (East)	21	0.5058	0.0218	0.1476	558.93	96.4	d.f.,=6
Region 4 (N. East)	2	0.4987	0	0	0.43	0.0	p=0.0018
Region 5 (West)	9	0.6014	0.0131	0.1143	309.04	97.4	
Region 6 (South)	7	0.6034	0.0382	0.1954	596.45	99.0	

Table 4 shows that sub-group 1 (2005–2010) included a total of seven investigations. The CIAF was observed to have a proportionate value of 0.5613 (95% CI: 0.0517 to 0.2275). The τ^2 and τ values were 0.2275 and 0.0517, respectively, indicating the level of variation or heterogeneity among the investigations. The heterogeneity test yielded a statistically significant Q-value of 1993.88 and an I^2 score of 99.7. These results indicate that there was a lot of variation among the trials in this sub-group. The present study examined 18 studies in the second sub-group (2011–2017). The calculated proportion value for the CIAF was 0.5839, with a 95% CI ranging from 0.0121 to 0.1098. τ^2 and τ were both 0.1098 and 0.0121, respectively. The heterogeneity test revealed a Q value of 328.68 and an I^2 value of 94.8. These findings indicate that there was significant diversity among the studies in this sub-group as well. Sub-group 3 (2018–2023) had 27 studies and the calculated proportion value for the CIAF index was 0.5163, with a 95% CI of 0.0231 to 0.1519. τ^2 and τ were both 0.1519 and 0.0231, respectively. The heterogeneity test yielded a Q-value of 1787.24 and an I^2 value of 98.5. The sub-group results show variable proportion values for the CIAF throughout time periods. For between-group heterogeneity, the Q-value was 2.82 (d.f., 2; $p>0.05$). As a result, while each sub-group exhibits high heterogeneity within itself, there is an insignificant difference in heterogeneity between sub-groups, showing that the variability reported in the research is unaffected by time periods (Fig. 5–7).

Eggers' test was used for each anthropometric measure to quantify publication bias, as shown in (Table 5). The publication bias indicates a nominal result that was statistically insignificant for all outcomes ($p>0.05$). Each anthropometric index's p-value is greater than the sig-

nificance level of 0.05. To clarify further, the present study has calculated and plotted a funnel plot (Fig. 8), which highlights any potential publication bias or small study impacts. The funnel plot displays the effect size or bias, on the horizontal axis and the standard error of the effect size on the vertical axis.

Table 4. The results of the meta-analysis conducted on three sub-groups based on studies conducted in different time periods (2005–2023), which were majorly categorized as initial, mid, and advanced, referring to the research and publication phase, sub-group 1 (2005–2010), sub-group 2 (2011–2017), and sub-group 3 (2018–2023). Each sub-group consists of a specific number of studies (K) and a proportion value with a 95% CI for the CIAF.

Sub-groups	K proportion	95% CI	τ^2	τ	Q	I^2	Between groups
Sub-group 1 (2005–2010)	7	0.5613	0.0517	0.2275	1993.88	99.7	Q=2.82,
Sub-group 2 (2011–2017)	18	0.5839	0.0121	0.1098	328.68	94.8	d.f.,=2,
Sub-group 3 (2018–2023)	27	0.5163	0.0231	0.1519	1787.24	98.5	p=0.2437

Table 5. Anthropometric indices and publication bias assessment using Egger's test

Anthropometric Indices	Bias (95% CI)	p
Stunting	-0.1601 (1.1823–0.0293)	0.8928
Underweight	1.2230 (1.2127–0.0303)	0.3181
Wasting	0.2911 (0.9870–0.0296)	0.7693
CIAF	0.1327 (0.9681–0.0236)	0.8916

Discussion

Child undernutrition remains a serious public health issue in low-to middle-income countries, which has a significant adverse impact on health status by increasing morbidity, mortality and disease burden.^{23,75,80} According to the findings, children utilizing CIAF showed a significantly greater prevalence of undernourishment than children using conventional anthropometric indicators such as stunting, underweight, and wasting in India (Fig. 9). The present study also found that the proportion of children facing undernutrition was notably higher in various Indian regions (Fig. 10): among urban, rural, slum and tribal children less than 6 years old, 59.8%, 5.9%, 58.64%, 61.8%, and 55.32% respectively,^{24,30,44,48,51} under 6 years old slum children in Tamil Nadu 68.6%²³, Gujrat, 60.5% and 73.4%,^{35,74} Jammu and Kashmir 73.2%,⁶⁶ Maharashtra 47.8%,³⁶ West Bengal 41.2%,⁵⁵ 43.5%,⁵² and 24.16%,⁴¹ Telangana 39.6%⁴: under 6 years of age, tribal children of Assam is 28.6%, 51.0%,^{9,22} Kerala 41.1% and 66.9%,⁵⁷ Maharashtra, 38% and 66%,^{75,76} Odisha 54.4%,⁷³ West Bengal 66.3% and 69.6%^{33,49}: among the urban children's younger than 6 years, the CIAF proportions in Andhra Pradesh 56%,⁷⁸ Chhattisgarh 54.16%,⁴⁷ Delhi 62% and 60.5%,^{38,40} Kar-

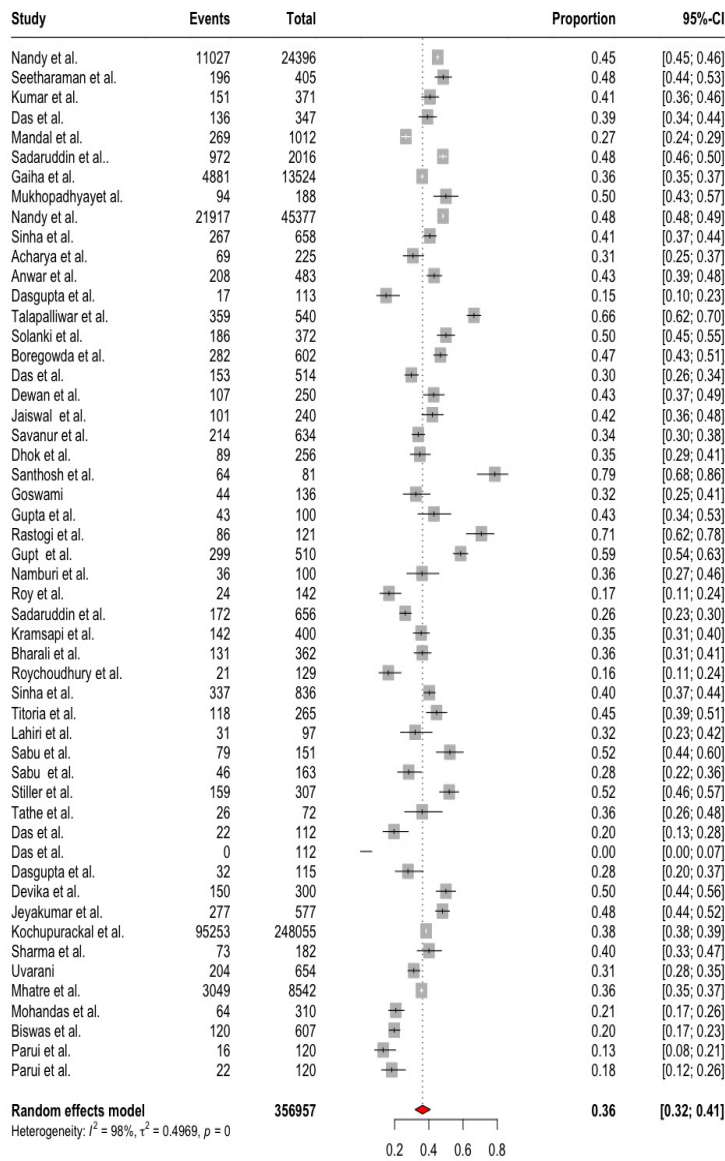


Fig. 2. The forest plot presents the results of the meta-analysis conducted on the variable stunting using a random-effect model. The plot displays individual study estimates (represented by squares) of the effect size, along with their corresponding 95%CI represented by horizontal lines.

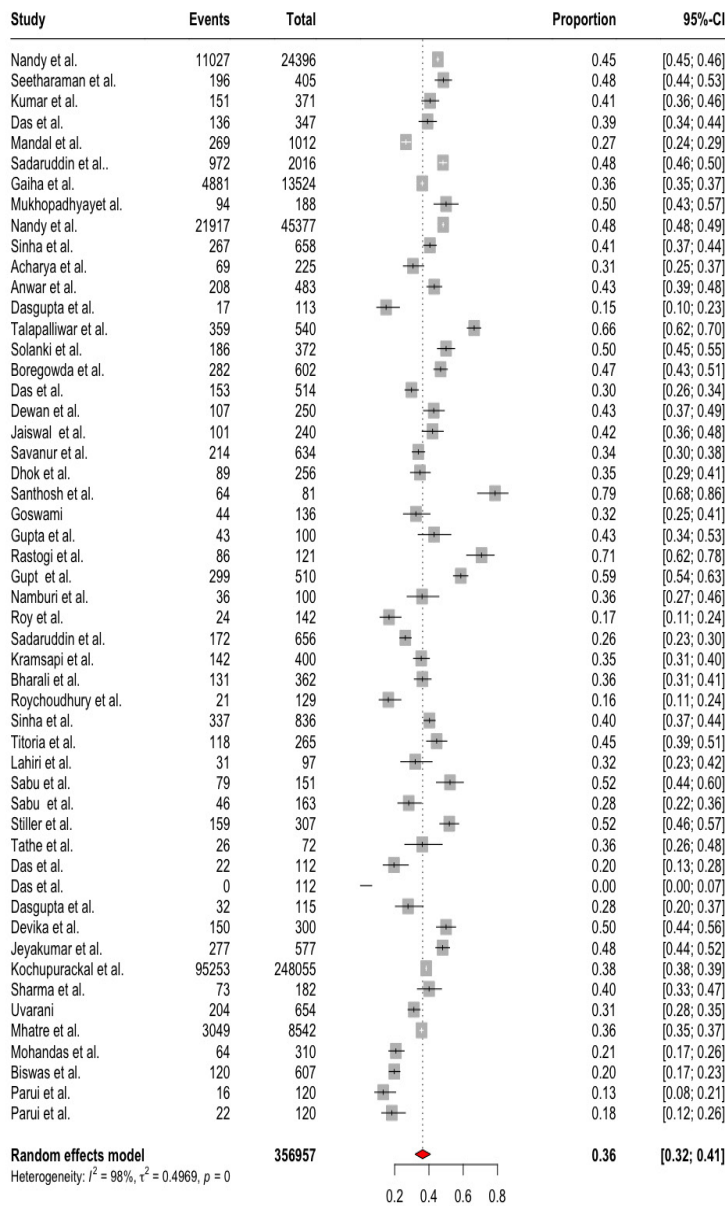


Fig. 3. The forest plot presents the results of the meta-analysis conducted on the variable underweight using a random-effect model. The plot displays individual study estimates (represented by squares) of the effect size, along with their corresponding 95%CI represented by horizontal lines

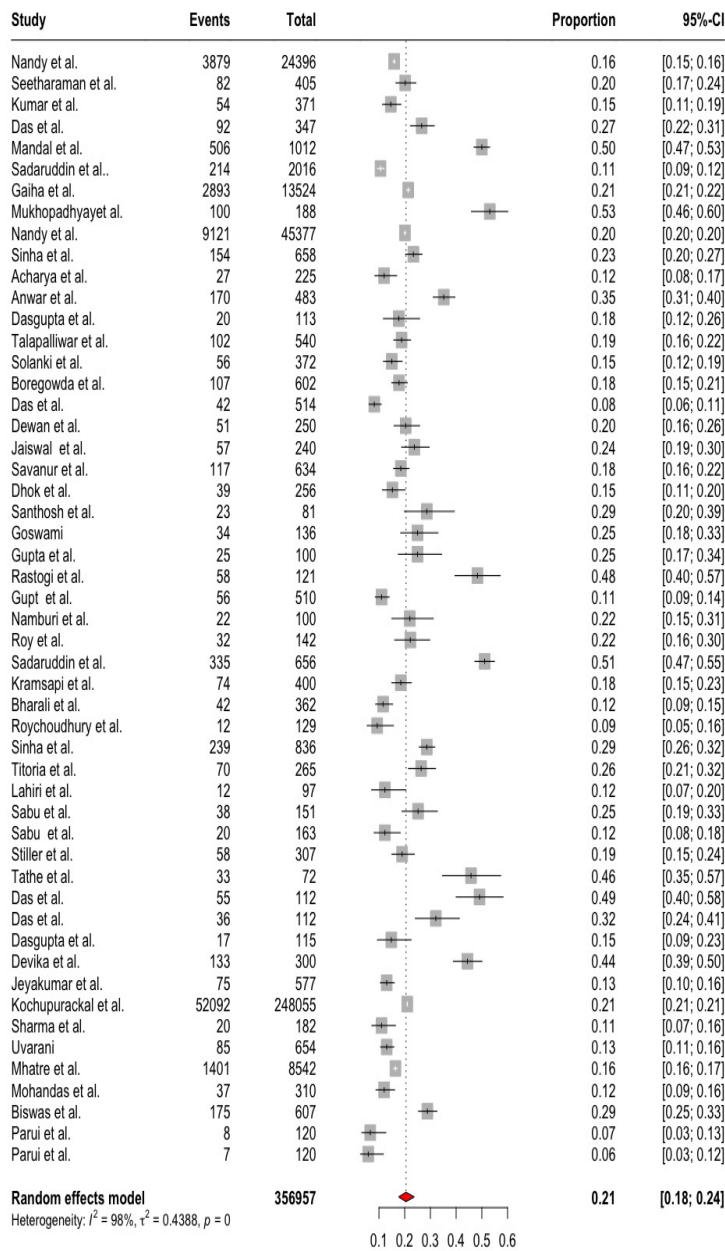


Fig. 4. The forest plot presents the results of the meta-analysis conducted on the variable wasting using a random-effect model. The plot displays individual study estimates (represented by squares) of the effect size, along with their corresponding 95%CI represented by horizontal lines

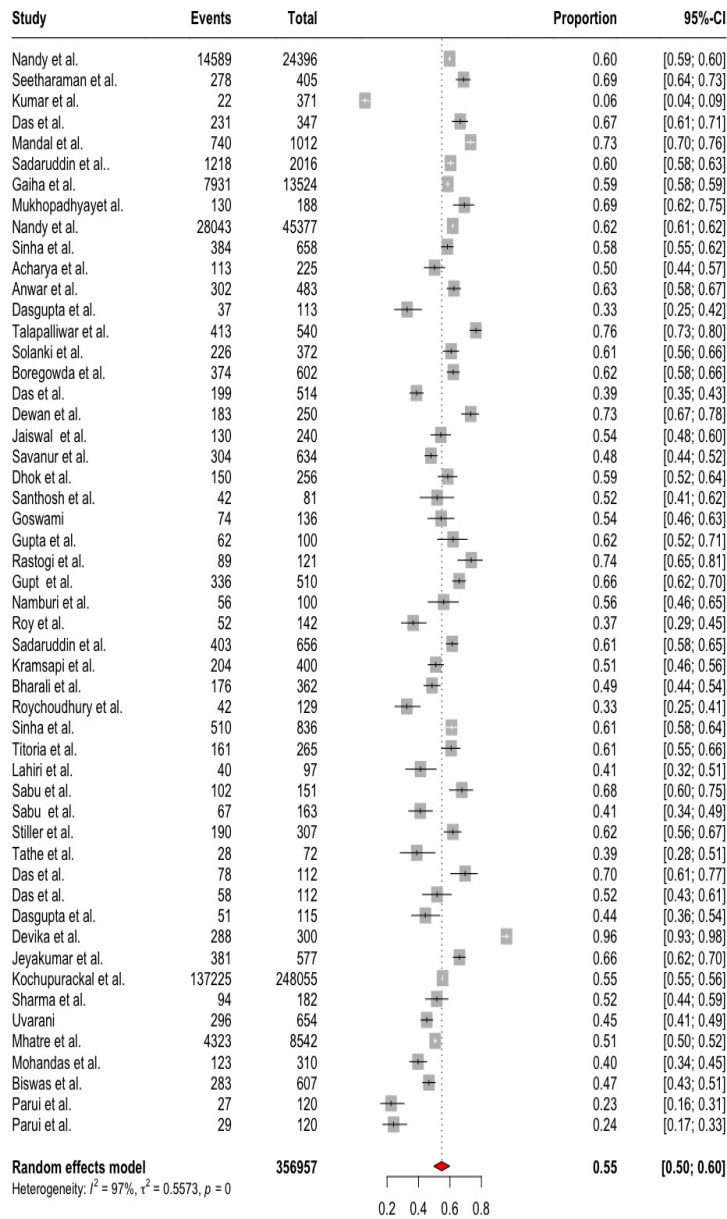


Fig. 5. The forest plot illustrates the results of the meta-analysis conducted on the CIAF using a random-effect model. The plot provides a visual representation of the effect sizes and their 95% CI from individual studies included in the systematic review

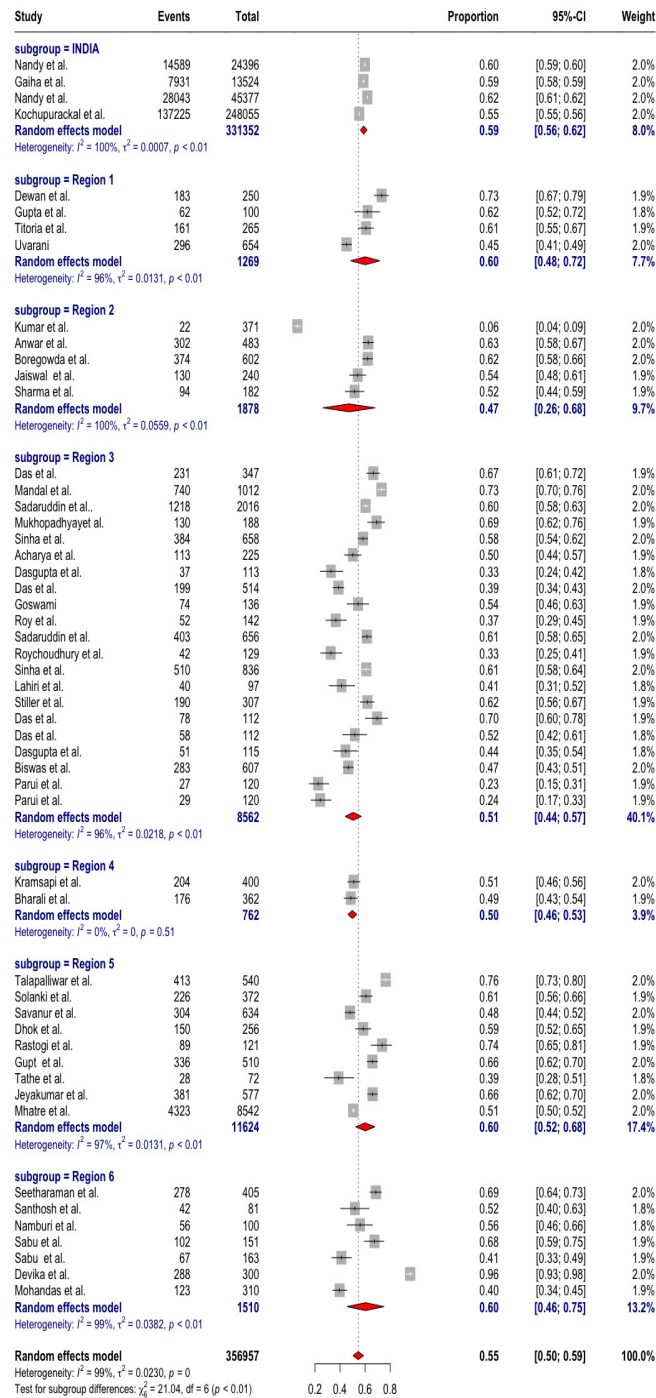


Fig. 6. This forest plot presents the results of the meta-analysis conducted on different regions to assess the proportion of CIAF. Each row in the plot represents a separate region, and the size of the squares corresponds to the weight of the individual studies within the region (Region: North=1, Central=2, East=3, Northeast=4, West=5, South=6)

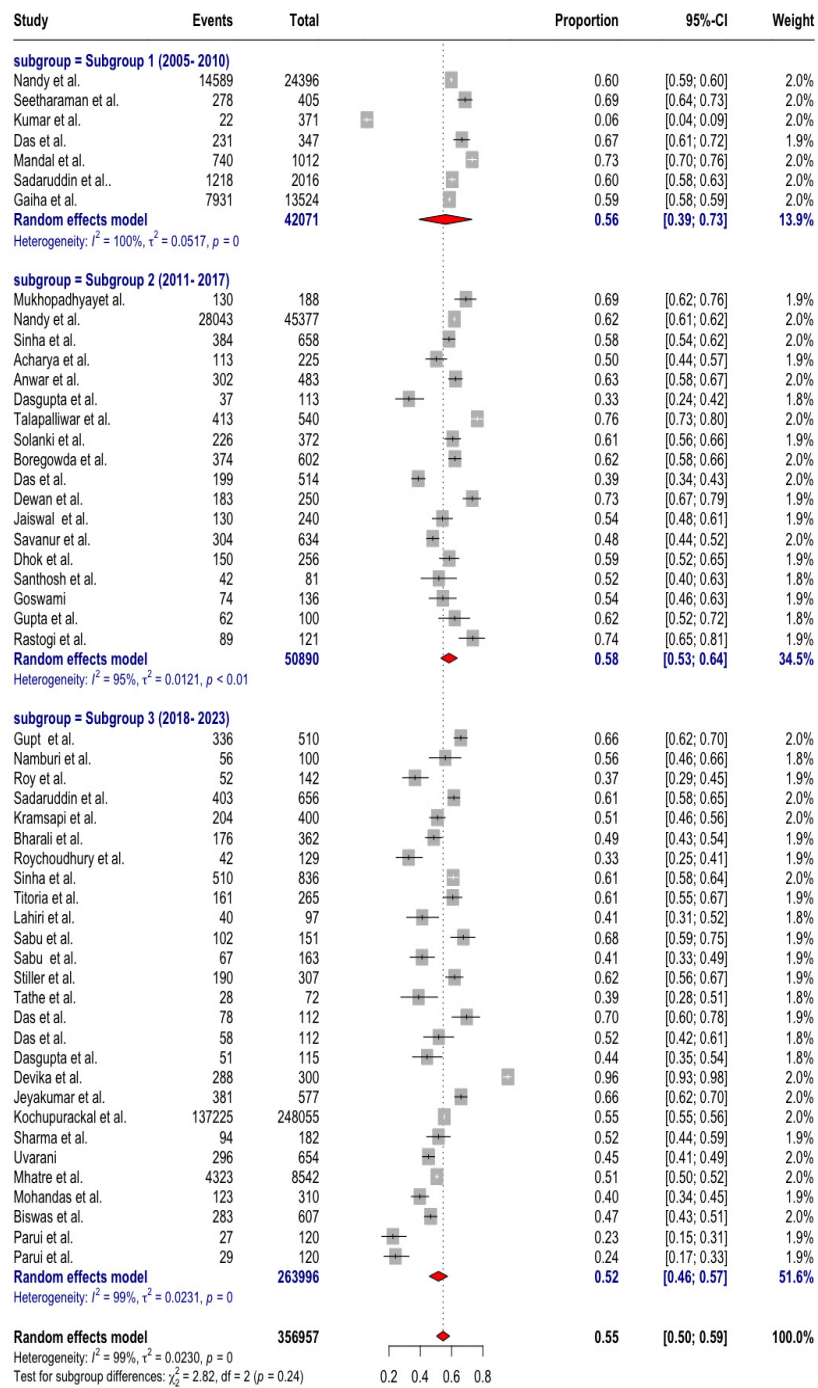


Fig. 7. This forest plot presents the results of the meta-analysis conducted on three sub-groups, representing different time periods (2005–2010, 2011–2017, and 2018–2023), to assess the proportion of CIAF. Each row in the plot represents a separate sub-group, and the size of the squares corresponds to the weight of the individual studies within the sub-group

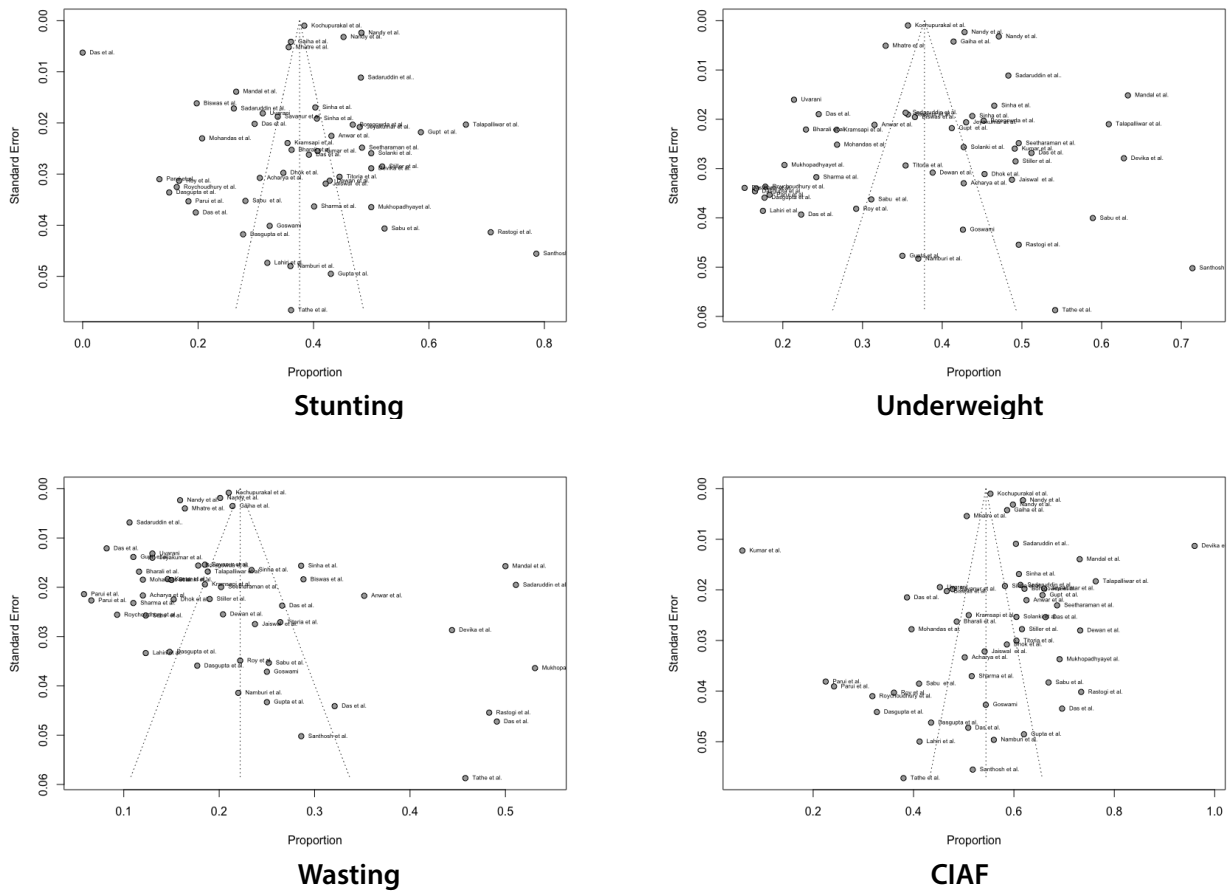


Fig. 8. Funnel plot illustrating the distribution of selected research articles examining publication bias in measured anthropometric indices (stunting, underweight, wasting, and CIAF), the analysis was conducted using generalized linear mixed models methodology

nataka 51.8%,⁷⁷ Maharashtra 58.59% and 65.7%,^{37,39} Tamil Nadu 96%,⁷⁹ Uttar Pradesh 62.5%,⁴⁵ West Bengal 31.8% and 22.5%.^{42,70} Under 6 years, rural children reflect a proportion in Chhattisgarh 62.1% and 51.6%,^{20,68} Haryana 76.3%,⁶⁷ Maharashtra 76.3% and 50.6%,^{41,46} West Bengal 73.1%, 60.4%, 69.1%, 58.21%, 50.2%, 32.7%, 38.7%, 36.1%, 61.3%, 61%, 61.6%, 50.9% and 46.62%.^{19,31,32,34,43,49,50,53-55,69,71,72} Several populations based investigations have suggested that the CIAF may accurately determine the cause-specific mortality and co-morbidity in health records.^{19,22,24,31,33,41,42,44,45,48,49,68,69} This specialized ability is beyond the reach of conventional anthropometric indicators, which struggle to distinguish groups of populations facing multiple anthropometric failures related to health and nutritional challenges. The CIAF along with its detailed sub-categories (i.e., B-Y), provides a clear and precise understanding of undernutrition, where conventional indices are unable to determine the actual magnitude due to their overlapping outcomes.^{24,31,39,42,66,71,76} However, many children from less privileged backgrounds tend to experience multiple health challenges in their

respective measurements.^{19,20,30,32,51,70} These children are likely to be susceptible to health risks due to their poor socio-economic conditions, belonging to underprivileged segments, inadequate resource allocation at intra-household level and being unable to utilize appropriate healthcare facilities.^{52,54,72,75,78,81} To find out the effects of various determinant variables, including income, education, occupation, demographics and lifestyle, on CIAF, in-depth analysis becomes necessary at population levels.^{9,54,72,75} Several studies have reported the connections between different types of anthropometric failures or nutritional deficiency related issues and socio-economic, demographic, and lifestyle factors in the population.^{31,36,49,42,52,72,73,75} This valuable information can be helpful to guide government agencies and policymakers in constructing effective policies and/or appropriate nutritional intervention programmes.^{26,82-84} The present investigation has carried out a systematic review and meta-analysis utilizing standard anthropometric measures and CIAF to describe the magnitude of undernutrition among Indian children (below 6 years old) during an 18-year period (2005–2023). The preva-

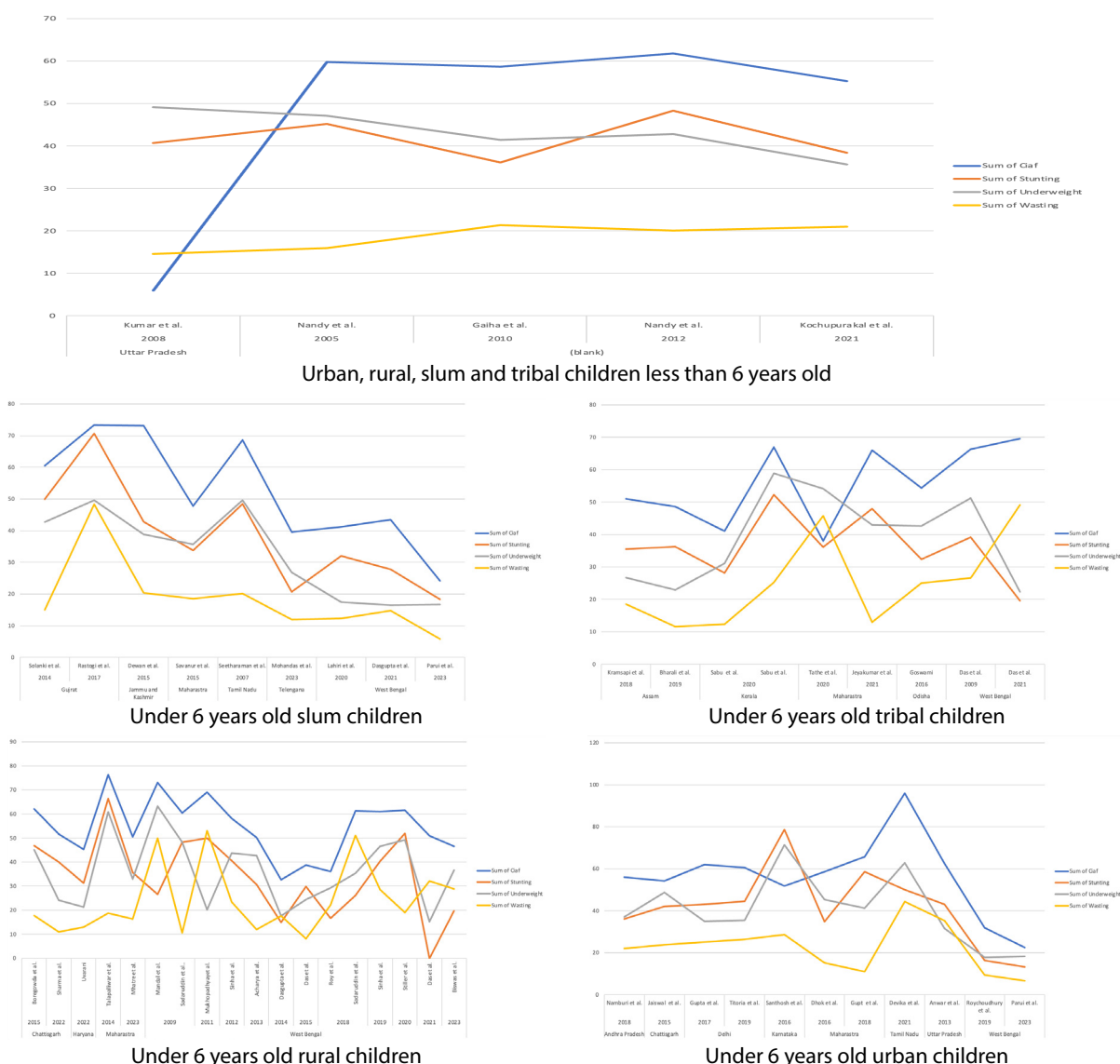


Fig. 9. Graphs depict trends among urban, rural, tribal, and slum children from 2005 to 2023 for conventional anthropometric indices and CIAF reporting, providing insights into the evolving undernutrition trend

lence of wasting was found to be 22% (95% CI: 0.18–0.25, $p < 0.001$), and the combined prevalence of stunting and underweight was 37% (95% CI: 0.32–0.41, $p < 0.01$). According to CIAF categorization, the pooled prevalence of undernourishment was reported to be 55% (95% CI: 0.50–0.60, $p < 0.001$). The majority of children scored a higher magnitude of undernutrition on the CIAF, and stunted and underweight children were more common, which suggested the existence of long-term chronic rather than acute undernutrition.^{4,9,17,52} Stunting, underweight, wasting, and CIAF are among the more severe types of undernutrition in Indian children under the age of six, according to several studies.^{21,25,30,52,83,85–88} The high burden of infectious diseases, recent food insecurity, socio-economic status, and/or the high prevalence of childhood undernutrition all point to a scenario of both chronic and acute undernutrition.^{21,24,35,36,43,45,54,71,73,75} It is believed that intra-household food or resource alloca-

tion, cultural customs, social influences, environmental factors, and restricted access to healthcare services remain the main contributors to such severe nutritional manifestations in the population.^{10,72,85}

In addition to affecting dietary intake, socio-economic status, food preferences or feeding practices, these factors are considered to be crucial for maintaining adequate nutritional status throughout physical growth and development.^{35,36,75} Further, food taboos, low hemoglobin levels, and literature was located using electronic databases like PubMed, PMC, Google Scholar, and Scopus. The present investigation did not include any grey literature, theses, dissertations, or research manuscripts from other print or online databases. The time frame for the literature search was limited to June 2005 through June 2023. Only reports which studied Indian children were included in this analysis, and the magnitude of undernutrition was evaluated using both conventional an-

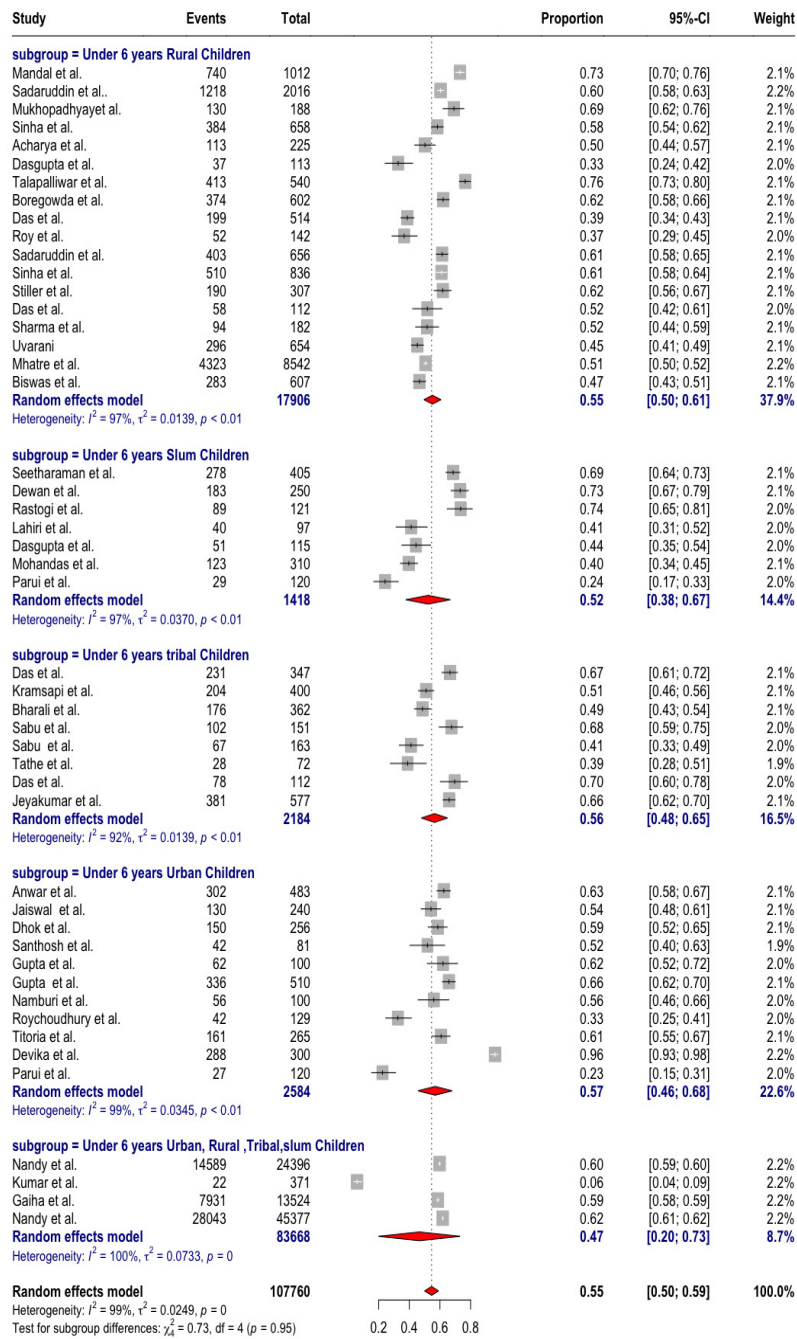


Fig. 10. This forest plot presents the results of the meta-analysis conducted on five sub-groups, representing different study groups that consist of urban, rural, slum, and tribal children, to assess the proportion of CIAF. Each row in the plot represents a separate sub-group, and the size of the squares corresponds to the weight of the individual studies within the sub-group

thropometric measurements and the CIAF. Since only a significant portion of studies were presented in the analysis's use of only English-language published articles, publications on non-Indian children published during the study period and children not found within the considered age groups (e.g., 0–6 years) were not considered for this study. The study's time frame was only between June 1 and July 20, 2023, when the published manuscripts were searched.

Conclusion

India is not an exception to the fact that undernutrition is one of the most serious public health problems in children. Due to demographic disparities, inadequate nutritional status, educational attainment, and food security, children suffer disproportionately from undernutrition in India. The present investigation has utilized both conventional anthropometric measurements and the CIAF to highlight the magnitude of undernutrition in children. Undernutrition was significantly higher in children who received CIAF. The findings shed light on the prevalence and relationships of stunting, underweight, wasting, and CIAF in children. This information can be used to develop evidence-based interventions and policies to combat undernutrition and anthropometric assessment related investigations. It is impossible to overstate the importance of adequate healthcare facilities and maternal education on optimal nutrition for pregnancy and breastfeeding, marriage age, and family planning

Declarations

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Author contributions

Conceptualization, S.S.R. and N.M.; Methodology, S.S.R.; Software, S.S.R.; Validation, S.S.R. and N.M.; Formal Analysis, S.S.R. and N.M.; Resources, S.S.R.; Writing – Original Draft Preparation, S.S.R.; Writing – Review & Editing, N.M.; Visualization, S.S.R. and N.M.; Supervision, N.M.; Funding Acquisition, S.S.R.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Data availability

Data supporting the results of this study shall, upon appropriate request, be available from the corresponding author.

Ethics approval

This systematic review and meta-analysis did not involve direct human participation or interaction; hence,

ethics approval and consent to participate were not required. However, research ethical guidelines and best practices were adhered to in the selection, review, and reporting of the studies included in this analysis.

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REVIEW PAPER

The use of topical metronidazole in the management of seborrheic dermatitis – a systematic review and meta-analysis of randomized controlled trials

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ABSTRACT

Introduction and aim. Topical metronidazole, with its well-known anti-inflammatory and antibacterial properties, could be beneficial for managing seborrheic dermatitis (SD), but studies report conflicting results. The aim was to evaluate the efficacy and safety of topical metronidazole in the treatment of SD.

Material and methods. A systematic search of Medline, Embase, and CENTRAL was conducted from inception to April 2024. Randomized controlled trials (RCTs) comparing metronidazole to any comparator for SD were included in this study. Data were pooled using random-effects models.

Analysis of the literature. Seven RCTs were included. Overall, topical metronidazole did not significantly reduce SD symptom severity when compared to any comparator/treatment. However, it significantly reduced symptom severity compared to placebo after 4 to 8 weeks (standardized mean difference (SMD) -3.00, 95% CI, -5.21 to -0.78). Specifically for facial SD, metronidazole showed significant symptom reduction (SMD -0.85, 95% CI, -1.41 to -0.29). No significant differences were found in the proportion of patients with clinical improvement or side effect frequency. Most studies had a high risk of bias and lacked information on missing data and assessor masking.

Conclusion. Topical metronidazole demonstrates potential for managing SD, but current trials lack quality. Larger, high-quality trials are needed to confirm its efficacy and compare it with other treatments for SD.

Keywords. dermatitis, meta-analysis, metronidazole, seborrheic

Introduction

Seborrheic dermatitis (SD) is a common chronic inflammatory skin condition characterized by erythema, scaling, and pruritus, mainly affecting areas rich in se-

baceous glands such as the scalp, face, and trunk. While the pathogenesis is not fully understood, fungal overgrowth, particularly of *Malassezia* species, and the dysregulation of immune responses play pivotal roles in its

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development.¹ Treatment typically involves topical antifungal and anti-inflammatory agents, and also phototherapy. Thus, corticosteroids and calcineurin inhibitors aim to reduce inflammation, while topical antifungals such as azoles, ciclopirox olamine, and zinc pyrithione attempt to combat *Malassezia* overgrowth. Additionally, keratolytic agents like salicylic acid, tar, selenium sulfide, and zinc pyrithione are used to soften and remove dermal crusts. Many of these treatments appear to have multiple mechanisms of action, with the nature of some mechanisms being quite obscure.^{2,3}

Metronidazole, a synthetic nitroimidazole derivative, is primarily known for its antimicrobial activity against anaerobic bacteria but also for its potentially useful anti-inflammatory properties. It has been shown to be effective in treating various dermatological conditions and was the original topical therapy approved exclusively for rosacea, where it still has a central role in management.⁴ Although the possible mechanisms of action in the management of SD are not fully understood, its observed anti-inflammatory effects alone make it an intriguing option for therapy. Several randomized controlled trials (RCTs) assessing the effectiveness of topical metronidazole in treating SD have been conducted, but they have yielded conflicting findings. For example, Parsad et al. randomized 44 patients with SD to receive either 1% topical metronidazole gel or a placebo, and subsequently observed a statistically significant reduction in the mean severity score at 8 weeks.⁵ Conversely, Koca et al. conducted an RCT comparing 0.75% metronidazole gel to a placebo for 8 weeks (n=84) and found no statistically significant difference in the change in mean severity score between the two groups by the end of the study.⁶

Despite the availability of various treatments for SD, there remains a critical need for effective therapies with minimal side effects. The inconsistent findings from previous randomized controlled trials (RCTs) underscore the necessity for further investigation into the efficacy of topical metronidazole. As such, a comprehensive synthesis of existing data via a systematic review and subsequent meta-analysis is required to provide a clearer understanding of topical metronidazole's potential role in managing SD. To our knowledge, this study represents the first systematic review and meta-analysis on this topic. By consolidating evidence from multiple RCTs, it aims to provide valuable insights that could inform clinical practice and shape the direction of future research on the topic.

Aim

This systematic review and meta-analysis aimed to assess the efficacy and safety of topical metronidazole in the treatment of SD.

Material and methods

The protocol for this study was registered with Open Science Framework (<https://doi.org/10.17605/OSF.IO/9X48M>). This review employed the 2020 Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guideline.⁷

We identified relevant studies through a systematic search of Medline, Embase and the Cochrane CENTRAL Register of Controlled Trials from inception to April 13, 2024. We also manually checked the reference lists of included studies to further identify any relevant studies. There were no language restrictions. The search strategy is provided in Appendix S1. Two reviewers (E.L. and S.S.) independently performed screening of titles and abstracts for relevance and selected studies after examining the full text of potentially eligible articles. Any discrepancies were resolved through discussion with the third reviewer (S.V.).

Studies included were the RCTs identified as above, and which also met the following criteria: a) participants were adults or adolescents diagnosed by a healthcare practitioner (explicitly stated or implied within context), with SD of the scalp, face or both, based on clinical case definition, with or without laboratory confirmation and b) studies comparing metronidazole at any strength and dose, and any topical formulations, to placebo, no treatment, or any active interventions (e.g., topical antifungal drugs). Primary outcomes were, a) symptom severity scores for erythema, pruritus and scaling, etc., measured using any type of systematic symptom severity assessment and, b) the proportion of individuals who experienced clinical resolution and/or global improvement of symptoms (defined as $\geq 50\%$ symptom improvement) based on physician assessment. A very important secondary outcome in this study was the frequency of side-effects.

Data extraction

Requisite data were extracted independently and in duplicate by two reviewers (E.L. and S.K.) and entered into a custom-designed data extraction form. For all outcomes, we used the initial number of participants randomized to each trial arm and performed the analyses irrespective of how the authors of the original trials had analyzed the data (intention-to-treat principle).⁸ Two reviewers (E.L. and S.S.) independently assessed the risk of bias within each study by using the Cochrane Risk of Bias (ROB 2.0) tool.⁹ Each domain was assessed and categorized into low risk, high risk, or unclear risk of bias. Discrepancies were resolved by consensus.

Statistical analysis

Data were pooled using the DerSimonian and Laird random-effects model.¹⁰ For continuous outcomes such as improvement in symptom severity, we used

the standardized mean difference (SMD) with its 95% confidence interval (CI) in summarizing results. For dichotomous outcomes such as the proportion of persons who had clinical resolution/global improvement of symptoms, we expressed the estimate of effect size as a risk ratio (RR) with 95% CI. Thus, RR >1 reveals a beneficial effect of the treatment. Adverse events data were also summarized with RRs. In each meta-analysis, we evaluated heterogeneity by using the I² statistic.¹¹ We assessed publication bias using funnel plot asymmetry and small-study effects using Egger's regression test.¹² A p-value of less than 0.10 was taken as statistical evidence of the presence of small-study significant effects. We performed subgroup analyses based on the comparison group as available. Statistical analyses were conducted using STATA version 16.0 (StataCorp, College Station, TX, USA).

The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach was used to rate the quality of evidence (high, moderate, low, and very low) of estimates derived from meta-analyses using the GRADEpro program version 3.6.1 (McMaster University, 2014).¹³

Analysis of the literature

The database search yielded 2480 records. The removal of 736 duplicates, left 1744 titles and abstracts, which were screened according to the predetermined eligibility criteria. A total of 271 studies were retrieved for further screening, of which 263 were excluded as they were not investigating topical metronidazole in the treatment of SD. Overall, 7 studies were included in this systematic review (Fig. 1).

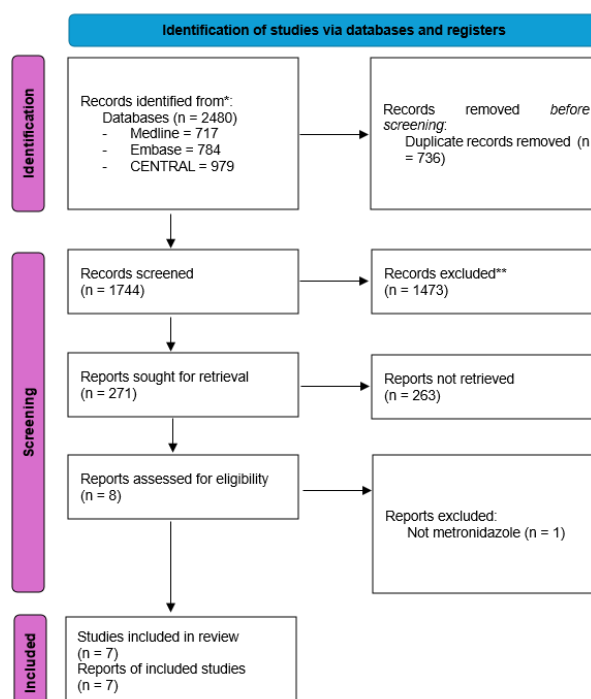


Fig. 1. PRISMA flow diagram

The characteristics of the 7 included studies are summarized in Table 1.^{5,6,14–18} The studies were published from 2001 to 2013. Two different strengths of topical metronidazole (0.75% or 1%) had been examined against control or a comparator (topical antifungal or calcineurin inhibitor).

Figures 2 and 3 depicts the risk of bias assessment for each included study. The majority of the studies were rated at overall high risk of bias; most of these studies did not provide sufficient information on missing outcome data and whether outcome assessors were masked.

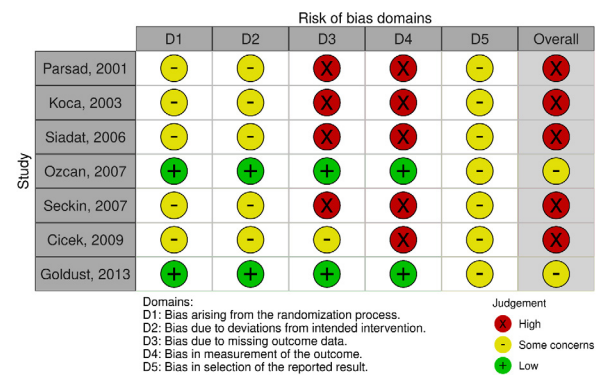


Fig. 2. Risk of bias assessment for each included study

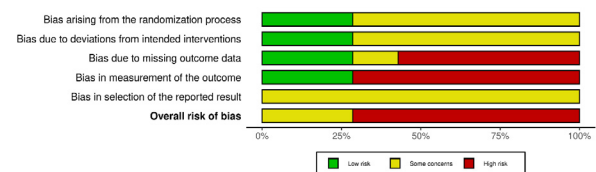


Fig. 3. Summary of the risk of bias assessment for included studies

Overall, there was no statistically significant difference in reduction of SD symptom severity observed between topical metronidazole and any comparator (including control, ketoconazole and pimecrolimus) (Fig. 4). In comparison to a placebo only, the use of topical metronidazole for 4 to 8 weeks shows statistically significant effectiveness in reducing symptom severity (standardized mean difference (SMD), -3.00, 95% confidence interval (CI), -5.21 to -0.78, three RCTs). However, there is a high level of heterogeneity (I²=96.7%), because the studies differed in the site of the SD (overall or face only), as well as the potency of metronidazole used, and the duration of follow-up. The funnel plot did not reveal a strong indication of publication bias (Fig. 5), and Egger's regression test did not indicate presence of small study effects (p=0.167). In a subgroup analysis that included only studies assessing facial SD, topical metronidazole showed a statistically significant reduction in symptom severity compared to control, with a moderate level of heterogeneity (SMD, -0.85, 95% CI, -1.41 to -0.29, two RCTs, I²=59.4%) (Fig. 6).

Table 1. Characteristics of included studies*

Author, year	Country	Participants	Participants age Mean±SD (years)	Sample size	Intervention (I)	Control/ comparator (C)	Duration of treatment
Parsad, 2001 ⁵	NR	SD, diagnosed according to clinical criteria	I: 23.8 C: 24.2	Metronidazole (n=22), vehicle (n=22)	Metronidazole 1% gel twice daily	Vehicle gel twice daily	8 weeks
Koca, 2003 ⁶	Turkey	Mild to moderate SD of the face, diagnosed according to clinical criteria	I: 35.9±5.87 C: 32±6.47	Metronidazole (n=50), vehicle (n=34)	Metronidazole 0.75% gel twice daily	Vehicle gel twice daily	8 weeks
Cicek, 2009 ¹⁴	Turkey	Face SD, diagnosed clinically	I: 30.7±7.35 C: 31.6±8.27	Metronidazole (n=21), pimecrolimus (n=21)	Metronidazole 0.75% gel twice daily	Pimecrolimus 1% cream twice daily	8 weeks
Siadat, 2006 ¹⁵	Iran	Face SD, confirmed by a dermatologist	I: 27.4 C: 25.5	Metronidazole (n=28), vehicle (n=28)	Metronidazole 1% gel	Vehicle gel	8 weeks
Ozcan, 2007 ¹⁶	Turkey	SD, diagnosed according to clinical criteria	I: 26±11.5 C: 26±8.7	Metronidazole (n=33), vehicle (n=34)	Metronidazole 0.75% gel twice daily	Vehicle gel twice daily	4 weeks
Seckin, 2007 ¹⁷	Turkey	Face SD	I: 42.1 C: 34.7	Metronidazole (n=30), vehicle (n=30)	Metronidazole 0.75% gel once in the morning + ketoconazole cream as vehicle once in the evening	Ketoconazole 2% cream once in the evening + metronidazole gel as vehicle once in the morning	4 weeks
Goldust, 2013 ¹⁸	Iran	SD	I: 30.18±12.64 C: 34.14±10.68	Metronidazole (n=78), sertaconazole (n=78)	Metronidazole 1% gel twice daily	Sertaconazole 2% cream twice daily	4 weeks

* NR – not reported

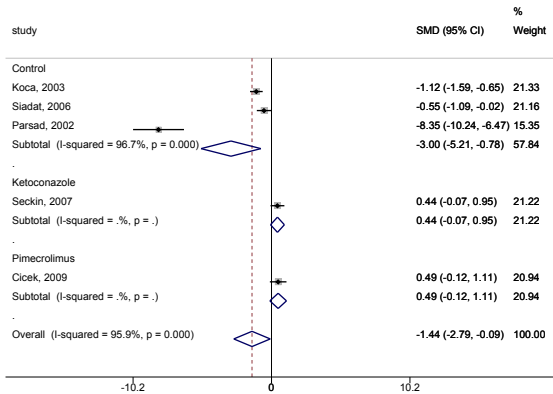


Fig. 4. Forest plot showing meta-analysis of comparison: Metronidazole vs any comparator, outcome: mean reduction in severity of symptoms

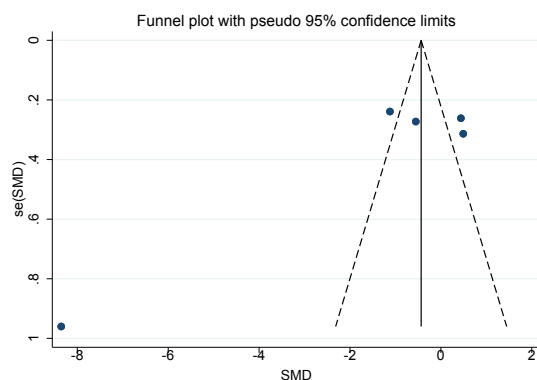


Fig. 5. Funnel plot of comparison: Metronidazole vs any comparator, outcome: mean reduction in severity of symptoms

In a meta-analysis of data from four RCTs, no statistically significant difference was observed for the proportion of patients who had clinical resolution and/or global improvement of symptoms (risk ratio (RR), 2.06,

95% CI, 0.97 to 4.37, $I^2=68%$) (Fig. 7). Conversely, in a subgroup analysis that included only studies assessing overall SD, use of topical metronidazole showed a statistically significant improvement (RR, 3.86, 95% CI, 1.65 to 9.07, two RCTs, $I^2 = 0%$) (Fig. 7).

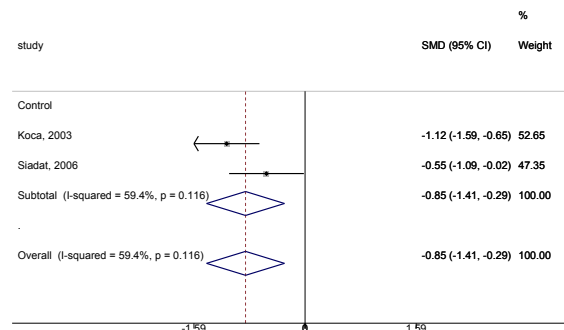


Fig. 6. Forest plot showing meta-analysis of comparison: Metronidazole vs control, outcome: mean reduction in severity of symptoms (facial SD only)

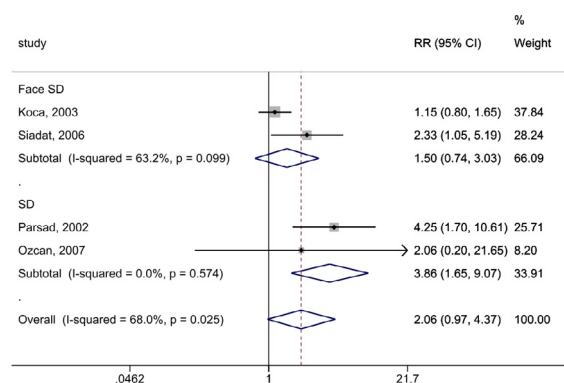


Fig. 7. Forest plot showing meta-analysis of comparison: Metronidazole vs control, outcome: proportion of persons who had clinical resolution/global improvement of symptoms based on physician assessment

There was no statistically significant difference detected in the occurrence of side effects between topical metronidazole and control (SMD, 2.07, 95% CI, 0.66 to 6.47, three RCTs, $I^2=20.2\%$) (Fig. 8).

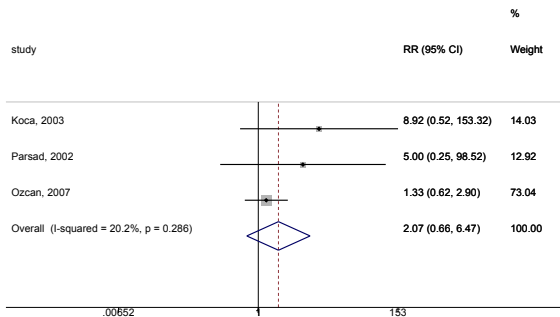


Fig. 8. Forest plot showing meta-analysis of comparison: Metronidazole vs control, outcome: side effects

Overall, the quality of evidence based on applying GRADE criteria to findings from the meta-analysis was generally rated as very low to low (Fig. 9).

Discussion

SD is a common chronic dermatological disorder that affects persons of all ages. There is no definitive treatment for SD, but topical steroids and antifungal agents are commonly used to control the condition. It is suggested that topical metronidazole could be useful in treating SD because of its anti-inflammatory effects. However, the efficacy of metronidazole for that purpose remains controversial. Several small-sized RCTs have been conducted to assess the efficacy of metronidazole in treating SD but the findings were inconsistent. Because studies with small sample sizes are typically associated with inflated effects, we performed a meta-analysis of these trials to provide a more precise quantitative summary of efficacy. This systematic review and meta-analysis represents the first comprehensive evaluation of topical metronidazole for the treatment of SD.

Our findings suggest that topical metronidazole may be effective for the treatment of SD compared to no treatment; however, the quality of the trials included in our review was generally poor. To establish a more robust understanding of its efficacy, there is a clear need for higher quality trials with larger sample sizes. Furthermore, none of the trials included in our analysis provided solid evidence regarding the comparative effectiveness of topical metronidazole against other treatment modalities for SD. Therefore, further research is warranted to clarify the comparative efficacy and safety profile of topical metronidazole in the management of SD.

The clinical effectiveness of topical antifungals and anti-inflammatories is well-established in treating SD but their prolonged usage may be associated with local adverse effects, such as antifungal-related contact dermatitis.¹⁹ Although the frequency cannot be reliably estimated, post-marketing surveillance of ketoconazole shampoo has reported incidents of hair discoloration, abnormal hair texture, itching, skin burning sensation, contact dermatitis, hypersensitivity reactions including angioedema, alopecia, rash, urticaria, skin irritation, dry skin, and application site reactions.²⁰ The prolonged use of another approach to treatment, the use of topical corticosteroids, is contra-indicated due to the potential risks of skin atrophy, striae, telangiectasias, hypopigmentation, and tachyphylaxis.²¹ Another concern is corticosteroid withdrawal can occur upon cessation after prolonged use.²² Because SD is a chronic condition, ongoing maintenance therapy is often necessary, necessitating prolonged use over several weeks to months of treatment.²³ In view of the above information, it can be seen that, a variety of other treatment options are needed to effectively manage SD.

For patients who require frequent use of topical corticosteroids, alternative treatments such as topical

Metronidazole compared to Control for SD

Certainty assessment							Summary of findings				
Participants (studies) Follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of evidence	Study event rates (%)		Relative effect (95% CI)	Anticipated absolute effects	
							With Control	With Metronidazole		Risk with Control	Risk difference with Metronidazole
Symptom severity score (Metronidazole vs Control)											
(5 RCTs)	very serious	serious	serious	very serious	none	⊕○○○ Very low	NA	NA	-	The mean symptom severity score (Metronidazole vs Control) was 0	0 (0 to 0)
Symptom severity score (Metronidazole vs Placebo)											
(3 RCTs)	very serious	not serious	serious	not serious	none	⊕○○○ Very low	NA	NA	not estimable	0 per 1,000	
Symptom improvement (Metronidazole vs Control)											
(4 RCTs)	very serious	serious	serious	serious	none	⊕○○○ Very low	NA	NA	not estimable	0 per 1,000	
Symptom severity score: Face only (Metronidazole vs Placebo)											
(2 RCTs)	serious	serious	not serious	not serious	none	⊕⊕○○ Low	NA	NA	not estimable	0 per 1,000	

Fig. 9. Summary of findings

calcineurin inhibitors (tacrolimus 0.1% ointment and pimecrolimus 1% cream), crisaborole 2% cream, or roflumilast 0.3% foam may be considered. These alternatives do not produce the adverse effects associated with topical corticosteroids, such as skin atrophy and telangiectasias.²⁴ However, topical calcineurin inhibitors received a boxed warning from the U.S. Food and Drug Administration due to concerns about a potential association with lymphoma and skin cancer.^{25,26} Consequently, the American Academy of Dermatology advises against continuous long-term use of topical calcineurin inhibitors and recommends limiting their application.²⁷ Furthermore, the high cost of topical calcineurin inhibitors could limit their general use.²³

The use of systemic antifungals is hindered by various limitations, including the risk of systemic side effects such as abnormal liver function or gastrointestinal disorders, and potential interactions with other concurrent systemic medications.²⁸ Another commonly used treatment, zinc pyrithione has recently been classified as carcinogenic, mutagenic or reproductive toxicity in cosmetics by EU commission regulation 2021/1902.²⁹ Hence, considering these constraints, there is a clear need to explore and consider alternative therapeutic options for the management of SD. The long-term safety record and very low costs of topical metronidazole would merit it being a treatment of choice even if it were only equally effective as existing treatments.

SD often occurs concurrently with rosacea, making it one of the most common skin conditions to coexist with rosacea. Although these two disorders are unrelated, a clinical study revealed that 26% of patients with rosacea also had facial SD, and 28% had SD of the scalp.³⁰ In cases of coexistent facial SD and rosacea, effectively treating both conditions can be challenging. Prolonged use of even mild topical corticosteroids, prescribed for SD, may worsen rosacea and should ideally be avoided or used sparingly and intermittently. Conversely, topical metronidazole 1% gel or cream, commonly used for rosacea, may also alleviate mild SD.³¹

Given the recurrent nature of SD, periodic treatments are necessary to maintain the disease in remission. While treatment with topical antifungals and anti-inflammatory agents are typically effective, some patients may not respond adequately.⁴ Therefore, topical metronidazole potentially provides a viable therapeutic alternative to the reliance on topical steroids in this subset of patients. Additionally, it is important to note that there are only very limited studies comparing the efficacy of metronidazole to other treatments such as topical corticosteroids, antifungals, and calcineurin inhibitors. Further research, particularly high-quality RCTs, is needed to better understand the comparative effectiveness of these treatment options in managing SD.

Study limitations

The current systematic review has several limitations that need to be acknowledged. Firstly, the search strategy was limited to English language publications only, thereby potentially excluding relevant studies published in other languages. Additionally, the findings of this review are based on RCTs with unclear or high risk of bias. Consequently, our results should be interpreted cautiously, and further research is necessary to validate the findings of this review. Sensitivity analysis was not performed based on risk of bias as assessed by the ROB 2.0 tool. This is because the included studies were either of unclear or high risk of bias. Moreover, high heterogeneity was observed among the included studies, which may impact the robustness and generalizability of our findings.

Conclusion

In conclusion, while topical metronidazole shows potential for treating SD, the overall quality of trials reviewed fall short of the level needed to recommend it as a treatment option. Larger, higher-quality trials are needed to fully understand its efficacy. Additionally, further very carefully designed clinical research is required to determine how metronidazole compares to other treatment options for SD. Cost and safety considerations could well tip the balance towards metronidazole even if it was found only to be equally efficacious as existing treatments.

Declarations

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Author contributions

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Conflicts of interests

The authors have declared no competing interests.

Data availability

All data generated or analyzed during this study are included in this published article.

Ethics approval

Not applicable.

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CASUISTIC PAPER

Neonatal lupus in an infant with maternal history of Jessner's lymphocytic infiltrate

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ABSTRACT

Introduction and aim. Neonatal lupus erythematosus (NLE) is a rare autoimmune condition occurring in infants born to mothers with circulating antinuclear autoantibodies, particularly anti-Ro and anti-La. It presents with a characteristic cutaneous rash, which may be accompanied by systemic manifestations. The condition is mostly self-limited, although congenital heart block, if present, is irreversible. We report a rare case of cutaneous NLE in an infant of a mother with a prior history of Jessner's lymphocytic infiltration of skin (JLIS), which is arguably a variant of cutaneous lupus erythematosus (LE).

Description of the case. This report concerns an infant presenting with cutaneous manifestations of NLE, without any systemic involvement. The mother was asymptomatic, but had been previously diagnosed with JLIS. The diagnosis of NLE was made based on serological results from both the infantile and maternal blood. Conservative management was sufficient to achieve complete resolution.

Conclusion. The case underscores the importance of considering NLE in infants presenting with annular rash, even in asymptomatic mothers with no known rheumatic disease. It also suggests that JLIS may belong to the same spectrum as LE, rather than being a separate entity. This warrants careful prenatal monitoring in mothers with a concurrent or prior diagnosis of JLIS.

Keywords. cutaneous lupus erythematosus, Jessner's lymphocytic infiltrate, neonatal lupus

Introduction

Neonatal lupus erythematosus (NLE) is a spectrum of cutaneous and systemic manifestations occurring in infants due to transplacental transfer of maternal antinuclear autoantibodies. The most common manifestations include cutaneous rash, hepatitis, cytopenias and congenital heart block. While most of these are transient and resolve within a few months with clearance of maternal antibodies, congenital heart block (CHB) is irreversible and requires urgent intervention.¹⁻⁴ The mothers of affected infants often have an autoimmune

connective tissue disorder, although they may remain asymptomatic and undiagnosed at the time of delivery.⁵

Jessner's lymphocytic infiltration of the skin (JLIS) is an entity that has been debatably considered as a dermal variant of lupus erythematosus (LE).⁶ It is a benign disease characterized by asymptomatic erythematous papules, or annular plaques, usually located on the face or back, with histological evidence of perivascular or periadnexal lymphocyte infiltration under a normal epidermis.⁷

NLE is a rare condition with only a few reported cases in current literature. Furthermore, a case of NLE,

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where the mother had a prior diagnosis of JLIS, has not been previously reported and ours is the first such case.

Aim

We present the case of a two-month old male infant with cutaneous NLE, born to a mother with no known history of collagen vascular disease, but previously diagnosed with JLIS.

Description of the case

A two-month-old male baby presented with reddish-brown patches on the face and body. The lesions first appeared about one week after birth, as a red rash around both the eyes. This was followed after two weeks by multiple raised patches on the face and body, which seemed to aggravate on sun exposure. On consultation with a primary physician, desonide cream was applied for a week, after which the redness decreased. There was no history of any fever, irritability or systemic complaints. The baby was born by Caesarean delivery (elective) without any complications during the antenatal and perinatal period. The parents were non-consanguineous and did not have any previous issue. The mother complained of having developed a reddish elevated skin lesion on the right cheek with aggravation on sun exposure, four years back. A skin biopsy from the lesion was suggestive of Jessner's lymphocytic infiltrate. The lesion had resolved after two months of treatment with topical steroids, with no subsequent recurrence. She had no history of recurrent fever, arthralgia, photosensitivity or any other cutaneous or systemic complaints.

Examination of the infant revealed multiple erythematous to hyperpigmented macules and annular plaques distributed bilaterally on the face, trunk and extremities (Fig. 1). The facial lesions involved the forehead and periorbital areas. Mucosal involvement was absent and no abnormality was detected on systemic evaluation.



Fig. 1. Erythematous macules and annular plaques distributed on the face, trunk and extremities

Laboratory investigations of the baby demonstrated negative results on potassium hydroxide (KOH) mount for fungal elements and qualitative serum Venereal Disease Research Laboratory (VDRL) test, but a strongly positive antinuclear antibody (ANA) titer of 1:1000 speckled pattern, along with positive anti-Ro and anti-La antibodies. Blood counts revealed anemia (hemoglobin 8.5 g/dL, erythrocyte count 2.9 million cells/cmm) and normal leucocyte and platelet counts. Peripheral smear revealed normocytic normochromic anemia. Liver enzyme levels were within normal limit. An electrocardiogram and a two-dimensional echocardiogram did not reveal any cardiac abnormality. Maternal serum was found to be positive for ANA (131.8 IU/mL; reference range <20 IU/mL), as well as, anti-Ro and anti-La. Thus, a diagnosis of cutaneous NLE was made based on the characteristic skin lesions and laboratory findings. Photo-protection was advised along with iron, vitamin C and D₃ supplementation. The lesions showed gradual resolution on subsequent monthly follow-up and the hemoglobin level normalized by three months. The ANA titer repeated every four months showed a gradual fall and was negative at 14 months of age. Anti-Ro and anti-La, repeated at 18 months, were found to be negative. The mother remained clinically asymptomatic throughout follow-up.

Discussion

NLE is a rare condition with an incidence of 1 in 20,000 live births, preferentially affecting females.^{2,3} The maternal autoantibodies implicated in 98% cases are anti-Ro/SS-A and anti-La/SS-B, in addition to anti-U1-ribonucleoprotein (anti-U1-RNP).⁴ These antibodies bind to their corresponding antigens in neonatal tissue causing inflammation, which may sometimes progress to fibrosis and scarring, especially in the cardiac conduction system.^{1,4} Mothers often have known rheumatic diseases such as systemic lupus erythematosus (SLE), Sjogren syndrome, rheumatoid arthritis, mixed connective tissue disease (MCTD) or undifferentiated autoimmune conditions.¹⁻³ In up to 40-60% cases, seropositive mothers may also be asymptomatic.¹ Our patient is the first reported case of NLE, where the mother had a prior diagnosis of JLIS.

JLIS is a benign condition, first described by Max Jessner and Norman Kanof, characterized clinically by firm erythematous papules or annular plaques on photo-exposed areas, especially the face.⁸ The nomenclature is based on the histological feature of a dense dermal perivascular or periadnexal lymphocytic infiltrate. This entity has arguably been linked to LE by several authors, including Jessner himself.⁶⁻⁸ A comparative study between 32 patients with JLIS and 14 with tumid LE showed remarkable similarities between the two in terms of clinical and histopathological characteristics.⁷

Clinically, common features shared by the two entities included predilection for middle-aged females, facial lesions, photosensitivity, lesional morphology, lack of systemic involvement and good response to antimalarials. Histologically, both conditions showed absent or minimal epidermal changes and dermal lymphocytic infiltrate. The only significant pathological differences were the relatively lower frequency of epidermal atrophy and a comparatively denser sleeve-like perivascular lymphocytic infiltrate in JLIS as compared to LE tumidus.⁷

Although several authors have described certain immunohistochemical features such as polyclonal CD8 infiltrate and plasmacytoid monocytes in JLIS as distinctive features differentiating it from LE, most of these studies were inadequately designed and excluded patients with features suggestive of LE from the study population giving rise to biased results.^{9,10} Similarly, a longitudinal study of 100 patients with JLIS reported no evolution to LE during follow-up arguing in favor of the two being separate entities, but this study had excluded two patients with typical JLIS since they were already diagnosed with discoid LE.⁹ On the contrary, Lipsker et al. in their analysis of 210 patients of JLIS, found 7.6% patients with typical features of LE, occurring either previously, concomitantly or later in life. This led them to propose the classification of JLIS as a dermal variant of LE.⁶ The occurrence of NLE in our patient in the context of maternal history of JLIS further substantiates this hypothesis.

The cutaneous manifestations noted in our case were representative of a typical presentation of NLE. In severe cases, cardiac conduction abnormalities may occur ranging from prolonged PR interval to complete heart block. The risk of CHB is lower in infants of mothers with SLE, as compared to those with Sjogren syndrome or undifferentiated autoimmune syndrome.¹ Other manifestations of NLE include cytopenias, hepatitis, neurological abnormalities (hydrocephalus, macrocephaly, aseptic meningitis) and pulmonary involvement (pneumonitis).^{1,2} Lipsker et al. mentioned that among patients with cutaneous LE, dermal variants, like tumid LE and possibly JLIS, have the best prognosis in terms of systemic involvement and cutaneous sequelae.⁶ In accordance with this, the absence of extracutaneous manifestations in our case further indicates a low risk of NLE complications in cases with maternal JLIS.

The diagnosis of NLE is based on characteristic clinical features and presence of specific antibodies in the infantile and maternal sera. It should be differentiated from seborrheic dermatitis, atopic dermatitis, tinea corporis, early congenital syphilis, annular erythemas, and Langerhans cell histiocytosis.^{1,3} Cutaneous lesions usually improve with photoprotection and topical steroids. In severe cases, systemic steroids or antimalarials may be necessary. A multidisciplinary approach is imperative for early detection and management of extra-cutaneous manifes-

tations, especially in case of CHB, where a pacemaker is invariably required. Affected infants are also more prone to develop autoimmune diseases in later life.³

Parents should be counselled regarding the two-to-three-fold higher risk of recurrence of NLE in subsequent pregnancies, particularly if the infant has CHB.¹ In such cases, prenatal monitoring by fetal echocardiography starting from the 16th gestational week is recommended.² Furthermore, our case underscores the importance of monitoring pregnant mothers with concurrent or previous history of JLIS. Frequent ANA titer estimation and, if required, anti-Ro, anti-La and fetal echocardiography should be carried out to rule out the development of NLE in such cases.

Our study was limited by resource constraints, as a result of which, we could not conduct serial titers of anti-Ro and anti-La antibodies, which are more specific for NLE, during follow-up. A skin biopsy from the lesions would also have added diagnostic value to our case. Additionally, the diagnosis of JLIS in the mother was based solely on the histopathologist's record as neither the clinical nor the histological images were available to us.

Conclusion

Our case highlights the importance of suspecting NLE in infants presenting with typical cutaneous lesions, even in the absence of maternal history of autoimmune connective tissue diseases. The development of NLE in the scenario of maternal history of Jessner's lymphocytic infiltrate emphasizes the possibility of JLIS belonging to the spectrum of LE, rather than a separate entity. Consequently, pregnant mothers who have had JLIS at any point in their lives, should be carefully followed up during pregnancy for signs of NLE developing in the fetus.

Declarations

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Author contributions

Conceptualization, B.K.K., G.N.R.N. and S.C.; Methodology, A.D.; Software, B.K.K. and A.D.; Resources, B.K.K. and S.C.; Data Curation, B.K.K. and A.D.; Writing – Original Draft Preparation, A.D. and B.K.K.; Writing – Review & Editing, G.N.R.N. and S.C.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Data availability

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

Ethics approval

Informed consent for inclusion was obtained from the legal guardian of the study participant, who was a minor.

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CASUISTIC PAPER

Congenital epulis in a newborn – a case report in Benin City, Nigeria

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ABSTRACT

Introduction and aim. Congenital epulis is a rare benign tumor that affects the oral cavity of newborns which typically presents as a solitary mass on the maxillary alveolar bridge at birth, with a predilection for the female gender. The rarity of congenital epulis is underscored by the limited number of reported cases in the literature, as evidenced by the infrequent occurrence of this condition in newborns.

Description of the case. This case report presents a rare case of congenital epulis, a benign gingival tumor in a newborn. The report includes a brief literature review to provide insights into the clinical characteristics, diagnostic approaches, and management of congenital epulis.

Conclusion. This case contributes to the current knowledge regarding rarity of occurrence and emphasizes the need for reporting, early intervention and multidisciplinary collaboration in effective management.

Keywords. alveolar ridge, congenital epulis, gingiva, granular cell tumor, newborn

Introduction

Neonatal congenital epulis (CE) is an uncommon tumor. It is referred to as granular cell tumor or congenital gingival granular cell tumor due to its unique histologic characteristics. The term congenital granular cell epulis is the one used by the WHO.¹ Newmann originally reported it in 1871, and its 9–10:1 ratio indicates a preference for females.^{2–4} The anterior maxillary alveolar ridge is where epulis is mostly seen.⁵ It typically manifests as a single mass, while 10% of cases involve multiple lesions. It can originate from the tongue or mandibular gingiva as well.^{2,6} It typically stops growing after birth.⁷

Clinically, congenital epulis presents as a pedunculated protuberant lump that might obstruct breathing or food intake. One still does not know the precise histogenesis. Prompt surgical resection is the advised course of action. There have been no instances of malignant

transformation, tumor recurrences, or harm to the dentition in the future.⁶ A small number of instances with congenital epulis have been observed to spontaneously resolve.⁸

Aim

We summarize the pertinent literature and report on a female neonate who has congenital epulis, a solitary tumor in the anterior mandibular alveolar ridge.

Description of the case

A 6-hour old female neonate was brought into the out born section of the Neonatal intensive care unit (of a medical facility in Benin City), via Accident and Emergency accompanied by the father with complaints of a ‘big’ growth noticed on the mandibular alveolar ridge immediately after birth.

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Mother was a 28-year-old P3 + 0 with secondary level of education. Her pregnancy was not scheduled at any hospital. However, the mother went to a traditional birth attendant at intervals for 'checkups.' No history suggestive of preeclampsia or bleeding per vagina in the antenatal period. There is history suggestive of malaria in the third trimester of pregnancy for which some herbal concoctions (contents unknown) were given to her by the trained afro-traditional birth attendant. The child was born at term via spontaneous vaginal delivery at the traditional birth attendant maternity home after about 12 hours of labor. No history of premature rupture of membrane or peripartum pyrexia.

The neonate had an APGAR score of 8/1, 9/5 and the birth weight was 2.7 kg. After birth, no bleeding or discharge was noticed from the growth which was large enough to be apparent. Growth was single and no other similar lesions were observed on other parts of the body.

On presentation, child was afebrile, anicteric, acyanosed, not dehydrated, not pale, and not in any painful or respiratory distress. Random blood sugar was 48 mg/dL as the baby had not been fed after birth. Hypoglycemia was promptly corrected as per protocol. Vital signs were as follows: temperature: 36.4°C, heart rate: 128 bpm, respiratory rate: 44 cpm, SpO₂ in room air: 99%. On examination, a 3×2 cm ovoid pedunculated mass was noticed on the left aspect of the mandibular alveolar ridge (Fig. 1). The mass was non-tender, pink in color, fluctuant, and vascular. No spinal or bony deformities were observed. The systemic examination done was insignificant. A clinical diagnosis of congenital epulis was made. Differential diagnosis of granular cell tumor, hemangioma, rhabdomyosarcoma, dermoid cyst, fibroma and teratoma were made.

The neonate was placed on appropriate intravenous fluid and samples were also taken for full blood count and electrolyte, urea and creatinine which were within normal values. The pediatric surgeon and the maxillofacial surgeon were promptly informed to review.

On the first day of life, fluid was changed from 10% dextrose water to 8% dextrose saline. Upon review of the neonate by the pediatric surgeon, a nasogastric tube was passed for feeding with infant formula starting at 15 mL two hourly. No feeding difficulties or regurgitation was noticed. It was also agreed that the tumor was resectable and that it will be carried out by a maxillofacial surgeon. Daily cleaning of the growth two hourly with normal saline after yellowish slough was noticed was also commenced.

Due to hospital administrative issues and without affecting the patient's life, the surgery was delayed. During the successive days, vital signs were stable, and volume of feeds were increased by 5–10 mL every day which was well tolerated by the neonate. On the 8th day of life, the decision for surgery was made after payment and all neces-

sary logistics had been carried out. At about 12 midnight, feeding was stopped, and baby was placed on nil per oral. Surgery was done the next day under general anesthesia. An incision was made to the peduncle and minimal bleeding was controlled with diathermy. The growth was excised and sent to the laboratory for histology.



A



B



C

Fig. 1. A and B: Congenital epulis with a lengthy base linked to the lower bridge of the gingiva, C: Post-operation wound

Feeding was recommenced at 11 PM that night. Baby was placed on intravenous ceftazidime and gentamicin for five days duration, paracetamol for three days and was also breastfed from two days post operation till discharge on the 12th day of life. The neonate was dis-

charged in a stable condition and was scheduled for a follow up visit in a week.

Histopathological examination showed a polypoidal fragment of tissue lined by stratified squamous epithelium with a thin layer of parakeratosis. The sub-epithelial stroma is composed of nodules of large polygonal cells with eosinophilic granular cytoplasm and small central nuclei. There is no focus of atypia. Features were consistent with that of congenital granular cell tumor (Fig. 2). The tumor was also reckoned to be benign and capable of spontaneous regression.

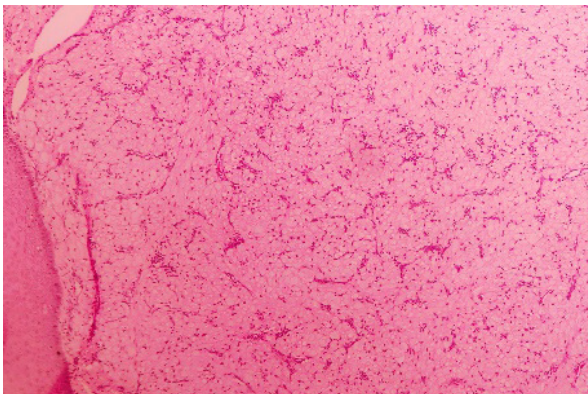


Fig. 2. Hematoxylin and eosin stain $\times 100$ showing stratified squamous epithelium with a thin layer of parakeratosis (H&E, 100 \times)

Discussion

The etiology of congenital gingival granular cell tumor is unknown. It is believed to emanate from primitive neural crest mesenchymal cells. Histopathologic examination is required for definitive diagnosis.⁹

Prenatal diagnosis has made it possible to institute a multidisciplinary approach to its management involving the neonatologist, ear, nose, and throat surgeon, pediatric surgeon, and pediatric anesthetist. A magnetic resonance imaging (MRI) of the fetal brain could prove useful as it will outline the characteristics of the mass.¹⁰ It would show a mixed mass with fibrofatty, mucosal and cartilaginous components.¹¹

This neonate had no prenatal investigations done since the parents registered with a traditional birth attendant instead of registering at a hospital with proper antenatal care. Ultrasound has been shown to be effective in visualizing this lesion as early as 26 weeks gestation.¹²

Prenatal ultrasound or MRI may show a very large mass that would make vaginal delivery difficult, and a caesarian section can be planned for. Prenatal diagnosis can therefore be useful in instituting counselling and discussing the modality of intervention with parents and caregivers.^{1,13,14}

Due to its appearance, its occurrence can be frightening to the parents and caregivers of the neonate hence a good knowledge of this rare but benign tumor is nec-

essary. Very few cases are reported in literature and to the knowledge of the authors this rare condition has only been reported once in Benin City by Edetanlen and Ogboh, 2019.¹⁵

The tumor commonly occurs at the maxillary alveolar ridge but in this present patient, it was found in the mandible.¹⁶ However, surgical excision is generally indicated due to interference with feeding or respiration.¹⁷

The differential diagnosis of congenital epulis include granular cell tumor. Granular cell tumor though similar histologically to congenital epulis unlike it rarely affects neonates, is rarely located at the gingiva, and shows reactivity to S-100 protein and laminin.¹⁸

Historically, the anomaly has traditionally been treated with either broad or specific anesthesia and total removal via surgery. A case study including the use of a CO₂ laser with general anesthesia has also been published.¹⁹ Congenital epulis has not been known to develop into cancer or grow after delivery. There have been no reports of recurrence, even with partial excision. Incomplete resection has not been shown to cause a recurrence and most lesions have a penchant to involute.⁸ There have been a few documented cases of complete regression without surgical intervention.^{1,16}

It is a rare tumor with few case reports. A review of cases from 2000 to 2017 by Sohal et al. reported 156 cases globally.²⁰ This report was intended to add to the body of knowledge of the few cases reported so far from Nigeria and other African countries.²¹⁻²⁵

Conclusion

Congenital epulis is a very rare benign lesion that affects the oral cavities of neonates. The small number of instances that have been described in the literature clearly indicates how uncommon this ailment is. It can sometimes cause problems with breathing and eating due to its large size, so it's important to make a diagnosis as soon as possible and the right care instituted. Early surgical intervention is important because deglutition and respiratory difficulty can be severe though spontaneous regression without intervention has been documented in the literature. An analysis of clinical as well as histopathological data is key to making an accurate diagnosis of this rare lesion.

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Author contributions

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K.A.; Investigation, S.O., O.A.E. and K.A.; Resources, S.O., O.A.E. and K.A.; Data Curation, K.A.; Writing – Original Draft Preparation, S.O., and O.A.E.; Writing – Review & Editing, S.O. and K.A.; Supervision, K.A

Conflicts of interest

The authors declare no competing interests.

Data availability

No datasets were generated or analyzed during the current study.

Ethics approval

Before obtaining the report, the child's parents gave their informed permission.







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CASUISTIC PAPER

A rare case of hypertrophic olivary degeneration in a patient with pontine hemorrhage

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ABSTRACT

Introduction and aim. Hypertrophic olivary degeneration (HOD) is an extremely rare disease that affects the inferior olivary nucleus (ION) in the medulla oblongata and is also referred to as hypertrophic degeneration of the inferior olives. This type of degeneration is characterized by trans-synaptic degeneration resulting from the blocking of afferent impulses in the ION, which is distinct from HOD. In this report, we present a clinical case of bilateral HOD that was identified and confirmed by neuroimaging two months after pontine hemorrhage.

Description of the case. A 52-year-old male was admitted to a university hospital because of gait imbalance, difficulty in swallowing and speaking, psychomotor agitation, visual hallucinations, and full vertical and horizontal gaze paralysis. He had a history of hypertension, hyperlipidemia, and obesity. The patient received symptomatic supportive treatment, without surgery. Ten weeks after the vascular incident, neurological examination revealed six cases of right cranial nerve palsy, vertical pendular nystagmus, dysarthria, decreased throat reflexes, and lingual and pharyngeal paresthesia. Repeated magnetic resonance imaging revealed bilateral hyperintense foci of 15×6 mm in the medulla oblongata, as detected by T2-weighted and fluid-attenuated inversion recovery. Two months of treatment with clonazepam, carbamazepine, and vestibular exercise resulted in no positive changes. Rehabilitation and psychotherapy were then continued.

Conclusion. Contrast MRI is required to confirm HOD, whereas non-contrast magnetic resonance imaging is the only method used for imaging and is not always reliable because it can cause neoplasia, infarction, demyelinating disorders, infections, and other similar problems.

Keywords. dentato-rubral-olivary pathway, hypertrophic olivary degeneration, magnetic resonance imaging, pendular vertical nystagmus, pontine hemorrhage

Introduction

Hypertrophic olivary degeneration (HOD) is an extremely rare disease that affects the inferior olivary nucleus

(ION) in the medulla oblongata and is also referred to as hypertrophic degeneration of the inferior olives.¹ This type of degeneration is characterized by trans-synaptic

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degeneration resulting from the blocking of afferent impulses in the ION, which is distinct from HOD.¹

The hypertrophic appearance of HOD is caused by enlargement of neuronal bodies and vacuolization in the ION.¹ Three-dimensional T₂-weighted and fluid-attenuated inversion recovery imaging clearly demonstrate these changes, including enlarged ION and augmented signals on the affected side.^{2,3}

HOD is frequently caused by a lesion in the dentato-rubro-olivaris pathway, also known as the Guillain-Mollaret triangle (GMT). This pathway involves afferent fibers from the dentate nucleus, which travel through the dentarubral tract to the upper part of the cerebellum, cross the midline, and reach the red nucleus on the contralateral side of the brain.⁴ Any lesion involving any segment of this pathway from the dentate nucleus to the ION can lead to HOD. The olivo-dentatus tractus fibers enter the lower peduncle of the cerebellum and do not cause HOD because they are non-afferent fibers that originate from the ION to the contralateral dentate nucleus.⁴

HOD can be bilateral or unilateral. Bilateral HOD can result from various genetic and anatomical abnormalities.⁵ A Chinese study found that out of 151 patients, nine had HOD related to genetically determined nervous system lesions, one patient had unilateral HOD, and the other eight had mitochondrial diseases caused by POLG and SURF1 gene mutations.²

The underlying cause of HOD remains unknown in many patients.^{2,6,7} In most cases, patients exhibit soft palatal tremors and progressive ataxia.⁸ Similar findings were observed in patients with bilateral HOD without any new structural damage to the GMT. The majority of these patients did not undergo genetic testing for SURF1 and POLG mutations, and further studies are needed to evaluate genetic neurological diseases. All the patients in these studies had bilateral idiopathic HOD due to undiagnosed hereditary disorders. However, POLG and SURF1, which are associated with Alexander's disease,⁹ and autosomal dominant spinocerebellar ataxia type 20 (SCA20),¹⁰ can cause bilateral HOD.

Aim

Further research is needed to understand the epidemiology and etiology of HOD, including its unilateral and bilateral variations. Although the mechanism and treatment efficacy are unclear, continued research on this condition is essential. In this report, we present a clinical case of bilateral HOD that was identified and confirmed by neuroimaging two months after pontine hemorrhage.

Description of the case

A male patient, aged 52, was admitted to the stroke department of the university clinic on April 29, 2023, com-

plaining of sudden onset of nausea, dizziness, tinnitus, and noticeable gait imbalance, as well as difficulty swallowing and speech. Upon neurological evaluation, the patient exhibited cognitive impairment, with psychomotor excitation, disorientation, visual hallucinations, full vertical and horizontal gaze paresis, mild left nasolabial asymmetry, decreased pharyngeal reflexes, dysarthria, dysphonia, dysphagia, and moderate neck stiffness.

Computed tomography and magnetic resonance imaging (MRI) without contrast revealed a hematoma measuring 18×15×24 mm in size in a pontine projection. MR angiography revealed normal magistral blood vessels within the brain (Fig. 1). Laboratory tests, including complete blood count, urinalysis, and blood chemistry panel, revealed nonspecific inflammatory changes, hyperlipidemia, and impaired kidney function (GFR 40 mL/min/1.73 m²). Carotid Doppler ultrasonography showed atherosclerotic plaques in the internal carotid artery with 68% right-sided stenosis and 30% left-sided stenosis. Transthoracic echocardiography and chest radiography revealed atherosclerotic degeneration of the ascending aorta and aortic valve, and dilatation and hypertrophy of the left ventricle.



Fig. 1. MR angiography showed normal magistral blood vessels within the brain

After a week, the patient exhibited partial recovery, enabling him to stand and walk with assistance. However, walking remains challenging owing to diplopia, oscillations, and trunk imbalance. A repeat neurological examination revealed partial recovery of symmetric eye movements, with sequelae of right-sided convergent strabismus, vertical and horizontal divergence of the eyeballs, and limited rightward abduction of gaze. A neurosurgeon, vascular surgeon, and rehabilitation specialist advised the patient not to perform surgical interventions for long-term indications. The in-hospital management involved treatment with dehydration, antipsychotics, and hypotensive medications. The patient was discharged with recommendations for hypotensive, hypolipidemic, antiplatelet, and sedative medication.

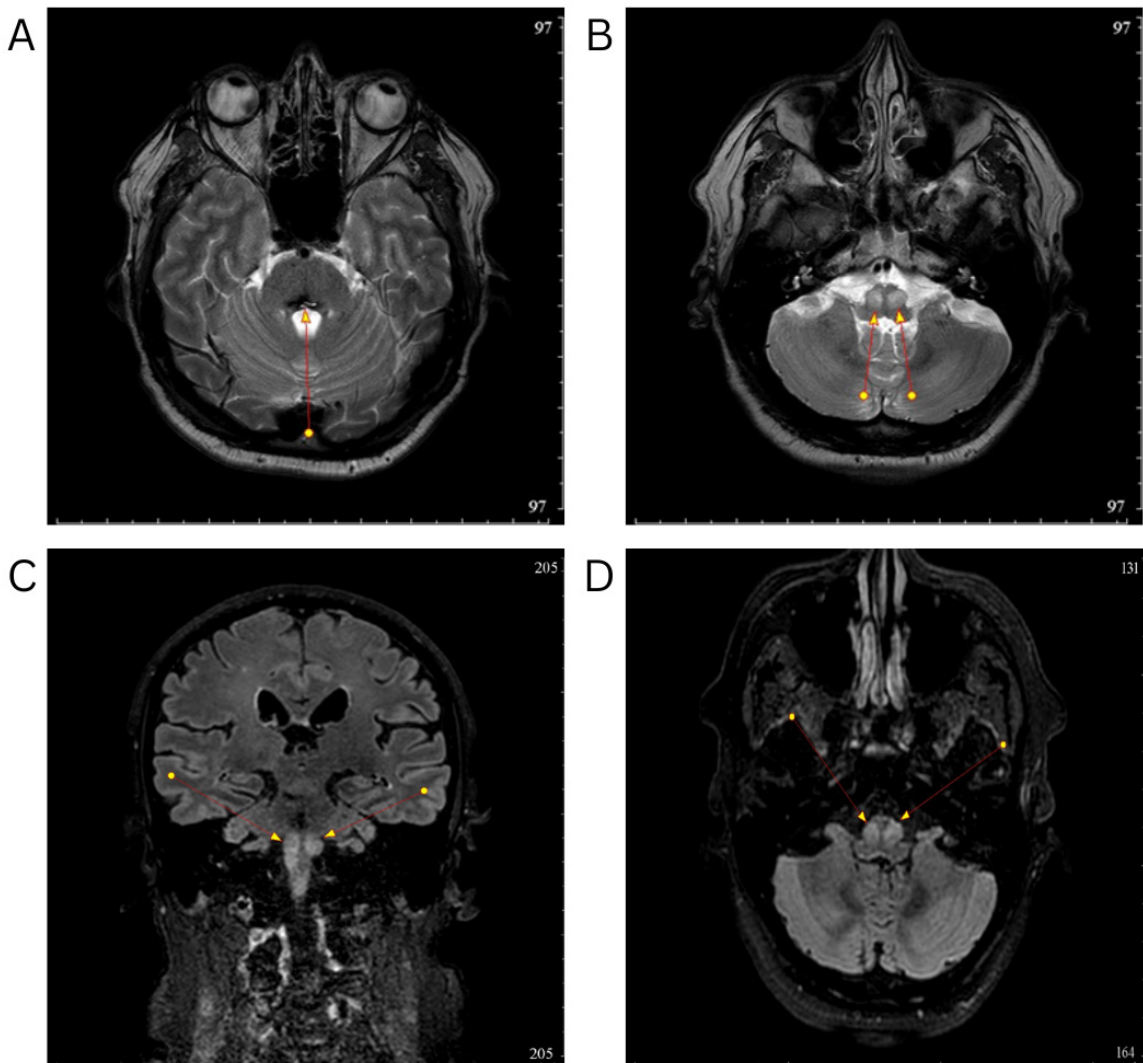


Fig. 2. A: MRI showed images in coronal T1w, B: frontal T1w, C: coronal T2w, and D: frontal T2w, pons is decreased in size and post-hemorrhagic cystic mass (9×3×10 mm) with hemosiderin deposits in the tegmentum and bilateral area of high signal intensity observed in the T2w MR image (15×6 mm)

At the 10-week follow-up, the patient still experienced diplopia, right convergent strabismus, limited rightward abduction of gaze, and a positive Romberg sign without severe dysmetria. Vertical pendular nystagmus was observed in all directions of ocular motion, and was not suppressed by fixation. Visual acuity remained unchanged from right to left, and the pupils were uniform and responsive to light. Fundoscopy revealed hypertensive microangiopathy. The saccades, convergence, and smooth horizontal and vertical tracking were normal. The ocular-cephalic reflex and head jerk test results were within normal ranges. The patient could not walk independently because of oscillopsia and diplopia. Dysarthria, decreased throat reflexes, and lingual and pharyngeal paresthesia were observed with no palatal myoclonia. No abnormalities in somatosensory sensitivity or reflexes were detected.

Permanent pendular vertical nystagmus was also present with no suppression of gaze fixation during ocu-

lar motion in any direction. The saccades, convergence, and horizontal and vertical tracking were unaffected. The patient continued to experience severe oscillopsia and diplopia, which prevented independent walking.

Repeated MRI with contrast revealed the presence of a post-hemorrhagic cystic mass with hemosiderin deposits in the pons and bilateral hyperintense foci of size 15×6 mm in the medulla oblongata, as detected by T₂-weighted and fluid-attenuated inversion recovery imaging. These findings were indicative of Wallerian degeneration (Fig. 2).

Discussion

This particular case involved clinical and neuroimaging examinations of a post-stroke patient with HOD. Bilateral post-pons hemorrhage HOD is uncommon and exhibits diverse clinical symptoms and MRI results, necessitating individualized patient monitoring and MRI assessment changes. In a retrospective study, 76% of patients exhibited bilateral HOD.¹¹

Despite an unknown cause, bilateral HOD is disproportionately more prevalent in males. In some cases, both prominent and unexpectedly identified asymptomatic forms of HOD were detected by MRI.¹² Prominent forms of HOD were identified in 23% of cases with unilateral lesions and 39% with bilateral lesions.¹³

The precise causes of the clinical signs associated with HOD remain unknown.¹⁴ The number of functional neurons in the ION and their afferent fibers, as well as the severity of the underlying illness, can influence the onset, severity, and progression of these symptoms.

Patients with HOD degeneration often experience palatal tremor, cerebellar ataxia (dysmetria, indistinct speech, and walking ataxia), nystagmus, ophthalmoplegia, ocular myoclonia, and other involuntary eye movements.¹⁵ In our clinical case, HOD presented with ophthalmoplegia, nystagmus, dysarthria, and ataxia.

MRI is the most effective diagnostic tool for validating and confirming the diagnosis of HOD, as it provides a thorough delineation of the morphopathologic structure of the affected areas.¹⁶

To date, patients with bilateral HOD have been found to harbor various gene mutations. POLG mutations have been linked to a range of clinical signs, including bilateral HOD.⁵ Leigh syndrome, a severe neurological disorder characterized by cytochrome C oxidase deficiency that typically affects neonates, is caused by SURF1 mutations. Bilateral HOD is often observed on MRI in patients with mutations in SURF1, TTC19, and AIFM1.^{11,17} In the absence of other clear causes, mitochondrial dysfunction may be the reason for bilateral HOD, particularly in cases of hearing loss.¹⁸ This is particularly true when both conditions occur simultaneously. Patients with SCA20 also exhibited signs of bilateral HOD.¹⁹ Genetic testing can aid in the precise diagnosis of patients with bilateral HOD who have a family history of the disease or present with neurological and physiological signs indicative of SCA or mitochondrial disease.

As previously mentioned, HOD is characterized by the microscopic features of neuronal body enlargement and vacuolization, astrocytic hyperplasia, and proliferation with gliosis and demyelination.¹ These characteristics are indicative of trans-synaptic degeneration resulting from the loss of afferent fibers in the hilum of the ION.¹

The literature on HOD consists mostly of case studies.^{16,20} Previous studies have reported that HOD can occur unilaterally or bilaterally.^{6,7,21,22} However, recent research has challenged the idea that HOD is consistently associated with GMT lesions. In fact, many patients with HOD do not have lesions in the GMT, and a small number of patients may have non-lesional HOD.^{6,21} Some studies suggest that non-lesional HOD is mostly bilateral.^{6,21,22} However, there is limited documentation of these cases. Therefore, we present a patient with bilat-

eral HOD who exhibited a clinical picture that, although not entirely unique, had sparse documentation in larger investigations and not in individual case reports.

The standard therapy for HOD comprises gabapentin, memantine, botulinum toxin injections, and deep brain stimulation, with psychotherapy being beneficial for patients with chronic illnesses. However, some patients may show self-limited recovery, necessitating avoidance of overtreatment. Oculomotor or nonsensory tremors often persist throughout life, but some may experience improvement after several years.²³ The efficacy of HOD treatment is unclear because of its unclear pathological mechanisms. Our patient underwent rehabilitation for pontoon hemorrhage and later received pregabalin and clonazepam without symptom improvement. Further research is needed to clarify the pathological mechanism of HOD, particularly in cases of recurrent hemorrhage and bilateral HOD.

The results of this study did not include diffusion tensor imaging and MRI data, which are necessary to verify changes in brain structures within the GMT when MRI findings are inconclusive. Additional prospective monitoring of the patient, in conjunction with biopsy analysis, is needed to confirm the observation and uncover the pathological basis of the neuroimaging nature.

Conclusion

In this report, we describe an unusual case of pontine hemorrhage with early onset HOD in the brainstem. Contrast MRI is required to confirm HOD, whereas non-contrast MRI is the only method used for imaging and is not always reliable because it can cause neoplasia, infarction, demyelinating disorders, infections, and other similar problems. Remarkably, this type of degeneration results in hypertrophy, rather than atrophy. A case of HOD with radiological diagnosis may have many pathological phenotypes.

The literature on HOD reveals inconsistencies and gaps, particularly in the areas of epidemiology, illness comprehension, and therapy. Despite the patient's intricate medical history, this case showed a broad spectrum of symptoms experienced by a patient with HOD. Here, we discussed possible alternative diagnosis and uncertainties surrounding this condition. Further studies are required to address this issue.

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Author contributions

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

Conflicts of interest

The authors declare no competing interests.

Data availability

Not applicable.

Ethics approval

The authors reported that they acquired the necessary informed consent form from the patient, who consented to the publication of their photo, video materials, and other clinical information. The patient was informed that confidentiality would be ensured.

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CASUISTIC PAPER

Cryptogenic stroke in a young patient after COVID-19 infection – a case report

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ABSTRACT

Introduction and aim. Cryptogenic stroke (CS) is a type of cerebral ischemia in which the cause is unknown or unclear. It can be difficult to determine the cause of CS because of various factors, such as incomplete investigations or the transient or reversible nature of the event. Infections can increase the risk of stroke by causing localized inflammation of the meninges and cerebral parenchyma, systemic inflammation, coagulation, and endothelial dysfunction. COVID-19-related cerebrovascular events can happen due to a hypercoagulable state from systemic inflammation and cytokine storm, post-infectious immune-mediated responses, and direct viral-induced endothelitis, which can lead to angiopathic thrombosis.

Description of the case. A 25-year-old male was diagnosed with mild semantic aphasia. The patient had a history of moderate COVID-19 infection. An angiography revealed that the M2 segment of the middle cerebral artery in the left hemisphere was not contrasted, indicating an occlusion. MRI and CT scans showed evidence of ischemic changes in the left hemisphere of the brain. The patient was treated with several drugs, including antiplatelet and neuroprotective drugs.

Conclusion. Our case demonstrates that autoimmune antibody formation, specifically antinuclear antibodies, can cause vasculopathy, leading to thrombus formation and stroke. It suggests a potential link between autoimmune antibody formation and stroke in COVID-19 patients.

Keywords. antinuclear antibody, case report, COVID-19, cryptogenic stroke, etiology

Introduction

Since December 2019, COVID-19, caused by SARS-CoV-2, has spread across the globe and has become a pandemic, infecting over 81 million individuals and resulting in over 1.7 million deaths.¹ While respiratory symptoms are the most commonly reported, there is an increasing understanding of neurological symptoms, with a range of 36% to 56%, based on a comprehensive series of hospitalized patients, with a small yet clinically important risk of acute ischemic stroke.^{2–6}

Cryptogenic ischemic strokes are cerebral infarcts that manifest with symptoms, for which no probable cause can be identified after conducting an adequate

diagnostic evaluation.⁷ The determination of a stroke as cryptogenic is contingent on the extent and quality of the etiological investigation and current knowledge regarding stroke mechanisms. A significant proportion of ischemic strokes are currently classified as cryptogenic or with an undetermined etiology.⁸ The incidence of CS varies depending on several factors, including the age of the patients, the definition of CS used, and the range of the etiological investigation. Among young adults, the percentage of strokes caused by undetermined causes decreases from more than 60% in the 15–19 age group to 25% in the 45–49 age interval.⁹

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In populations and hospital records of elderly patients, the prevalence of ischemic stroke ranges from 20% to 40% when using the TOAST criteria (Trial of Org in Acute Stroke Treatment). This percentage has remained consistent over time. The Rochester Epidemiology Project conducted between 1985 and 1989 revealed an incidence rate of 36.1% (164/454). The male sex is associated with an undetermined stroke etiology (odds ratio 1.50; 95% confidence interval 1.08–2.08; $p=0.015$).^{10,11} There are few studies that compare the incidence of cryptogenic stroke between different races. The Greater Cincinnati/Northern Kentucky Stroke Study, which is a biracial population study, found that black patients had a greater prevalence of cryptogenic stroke than white patients (54% to 49%).¹²

In comparison to strokes that have a determined cause, CS usually lead to less severe initial neurological deficits, lower mortality rates, and less final disability.¹³ While not all long-term follow-up studies show this, in most cases, patients who have experienced cryptogenic ischemic stroke have a lesser risk of recurrence compared to those who previously had a stroke with an identified cause.^{14–16}

The interpretation of CS is conventionally relies on the elimination of other firmly settled causes of stroke.¹⁷ Three classification systems, namely, the Trial of Org 10172 in Acute Stroke treatment system (TOAST), the Atherosclerosis, Small vessel disease, Cardiac causes, other, and Dissection scheme (ASCOD), and the Causative Classification of Stroke System (CCS), have frequently been used to enumerate mechanisms or subtypes of ischemic stroke. However, none of these systems formally details CS.

A comprehensive assessment of CS requires brain imaging, such as magnetic resonance imaging (MRI)/computed tomography (CT) or, MR angiography (MRA), transcranial Doppler, neurovascular imaging with CT angiography (CTA), and cardiac evaluation using echocardiography, and in some patients hypercoagulable testing, rapid plasmin reagin (RPR), genetic analysis or other tests for atypical causes. Nonetheless, a broader consensus is required to define the features and criteria for diagnosing cryptogenic stroke.¹⁸ In this context, CS are symptomatic cerebral infarcts for which no specific cause is identified after adequate diagnostic evaluation. An accurate and consistent classification of CS is essential for appropriate clinical management and improving patient outcomes.⁷

Managing cryptogenic stroke poses a significant challenge in secondary stroke prevention, particularly in the choosing of suitable antithrombotic therapy. While the administration of oral anticoagulation in the prophylaxis of cardioembolic strokes is a well-established in practice, CS are increasingly being acknowledged as sharing many attributes with cardioembolic

strokes. However, there is no well-defined protocol for ideal long-term treatment currently. The American Stroke Association and the American College of Chest Physicians suggest that antiplatelet agents are the preferred option for non-cardioembolic ischemic strokes. According to a recent global survey conducted across 48 countries, it has been observed that the overwhelming majority (94%) of hospitals frequently prescribed antiplatelet therapy for secondary prophylaxis of CS.¹⁹ However, there are mounting evidence that cryptogenic stroke patients may aid from anticoagulation.

Aim

In this article, we present a case of an individual with CS who had a history of COVID-19 infection that was presented to neurology department with several cerebrovascular events. This report is to offer a comprehensive view of the diagnostic process and detailing the advancing from the onset of signs and symptoms to the recognition of the disease. By presenting this case report, we hope it will serve as a valuable resource for medical practitioners.

Description of the case

A 25-year-old East Slavic male, was admitted to the emergency department of the Neurology unit at the Regional Hospital of Grodno, Belarus. The patient presented with speech difficulties, which had developed two weeks after a moderate COVID-19 infection which has been showed positive test result for RT-PCR test.

Clinical findings: At admission, the patient underwent a neurological examination, revealing semantic aphasia. Rest examination was normal, alerted and oriented (Person, place, and time). The Glasgow Coma Scale (GSC) was 15b, while the National Institute of Stroke Scale (NIHSS) was 2b. The pupils were equal, round, and reactive to light, while the visual fields were intact to confrontation. Ocular movements were also intact. The face was symmetric at rest and with activation, with intact sensation throughout. The muscles of the tongue and palate activated symmetrically, while muscle bulk and tone were average. Strength was 5/5 in all extremities (both hands & legs), and both proximally and distally. Fine motor movements were intact bilaterally, and sensation was normal to light touch, vibration, pinprick, and proprioception. The Romberg test was negative, and there was no dysmetria on finger-nose-finger or heel-knee-shin. The patient displayed a narrow-based gait with a good arm swing bilaterally and normal stride length.

Diagnostic assessment: According to the results of angiography, it has been observed that the M2 segment of the middle cerebral artery (MCA) in the left hemisphere is not contrasted, which indicates that there is a blockage or occlusion in that segment.

The results of the MRI and CT scans conducted on the brain indicate evidence of ischemic changes that

have occurred within the left hemisphere of the brain, as illustrated in Figures 1 and 2.

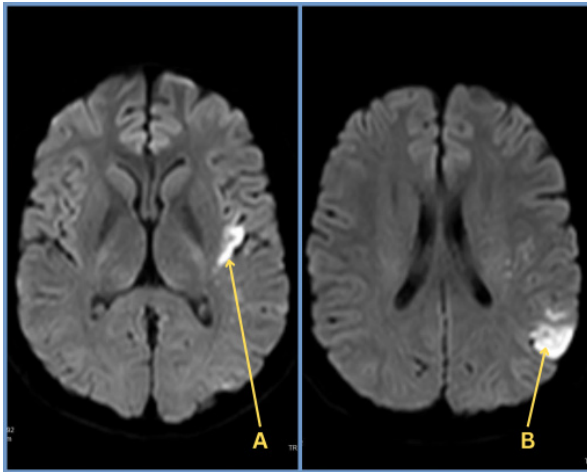


Fig. 1. MRI of the brain showing: A: diffusion-weighted imaging (DWI), a hyperintense area corresponding to acute ischemic changes in insula, B: diffusion-weighted MRI (DWI-MRI) showed a hyperintense area in the left parietal lobe (cortical infarction) and insula

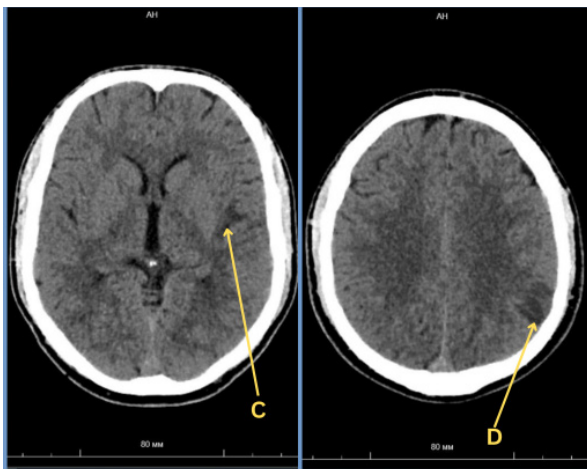


Fig. 2. CT scan of the brain showing: C: lesion in insula, D: lesion in parietal lobe

In the CT scan of the brain on the left in the parietal lobe, subcortically, a hypodense area with unclear contours, a cross-section of about 19×28 mm was identified and a similar area was visualized in the subcortical nuclei about 8×25 mm. CT scan shows a significant signs of ischemic changes in the left hemisphere of the brain.

The electrocardiogram revealed sinus bradycardia, while the complete blood count showed an increase in segmented neutrophils which was 81% (norm 45-70)% (neutrophilia) and a decrease in lymphocytes which was 10% (norm 18-40)% (lymphopenia). Biochemical blood testing results were unremarkable, and the blood lipid profile showed normal glucose and complement lev-

els. Hemostasiogram results indicated elevated levels of D-dimer. ELISA analysis showed normal levels of beta 2 glycoprotein, while the antinuclear antibody (ANA) screen Ig (A, G, M) returned a positive result showing 88.4 U/ml (norm 0-40)U/ml. ANA-2 and ANA-3 tests were not performed. The SS-B (La) (Sjögren syndrome type B antigen) and SS-A (Ro) (Sjögren's-syndrome-related antigen A) were both normal, while the DsDNA (double-stranded DNA) Screen yielded normal results. Immunoglobulin titer showed a result of, IgA 2.2 g/L (norm 0.7-7) g/L; IgG 11 g/L (norm 7-16 g/L), and IgM 0.6 g/L (norm 0.2-5 g/L) which were normal.

Therapeutic intervention: a conservative approach was taken in regards to treatment, with a focus on neuroprotection. A solution of magnesium sulfate (20) and potassium chloride (10) in saline solution (250) was administered through IV drips, along with glycine (0.1) in tablet form three times a day, and alpha choline (1000 mg) with Ringer's solution (500) through IV drops once a day. For antiplatelet purposes, Aspicaard (75 mg) was prescribed to be taken daily with lunch. Sol. Emoxypini (0.5%-100 mL) was administered as an antihypoxant, with IV drops once a day. For lipid-lowering purposes, atorvastatin (20 mg) was prescribed. To ensure gastroprotection, omeprazole (20 mg) was prescribed to be taken on an empty stomach in the morning. Additional recommendations were provided to the patient, such as monitoring salt and animal fat intake, avoiding easily digestible carbohydrates, and regularly checking blood pressure and lipid profile. An annual ultrasound of the brachiocephalic artery was also advised.

Discussion

CS are cerebral infarcts that have symptomatic manifestations and lack a discernible cause after thorough diagnostic evaluation.⁷ Cryptogenic ischemic strokes are typically associated with less severe neurological deficits, lower mortality, and less severe final disability when compared to strokes with identified origins.¹³ Our patient presented mild neurological deficits, including semantic aphasia, and made a complete recovery without any disability at the time of discharge from the hospital. The possible sources of cerebral embolism in patients with CIS include the heart, veins of the lower extremities and pelvis, non-stenotic atherosclerosis of the brachiocephalic arteries (BA), atheromas of the aortic arch, paradoxical embolism, non-atherosclerotic vasculopathy, monogenic diseases, and hypercoagulable conditions, etc.²⁰ The etiology of the patient's condition may have been autoimmune antibody formation (ANA), which could lead to vasculopathy and the formation of thrombi, ultimately resulting in stroke. ANAs, which can be track down in many autoimmune diseases and viral infections, might be to blame for the vasculopathy. Infections might cause autoimmune reactions through

mechanisms such as epitope spreading, molecular mimicry, cryptic antigens, and bystander activation. ANAs, which are typically not associated with autoimmune diseases, have been documented to be caused by transient auto-reactive B and plasma cell reactivation due to infection.^{21,22}

Autoantibodies, such as ANAs, lupus anticoagulant, anti- β 2 glycoprotein 1, anti-Ro/SSA, and anti-cardiolipin antibodies, have been discovered in individuals infected with SARS-CoV-2.^{23,24}

Various studies conducted on patients with SARS-CoV-2 reveal the prevalence of ANAs between 18% and 57.5%.²⁵ Our patient also tested positive in the ANA screening test.

During the acute stage of the illness, the emergence of autoantibodies is linked to SARS-CoV-2 infections, which supports COVID-19's pathophysiology. Autoantibodies increase in the weeks following recovery but resolve, with a notable reduction in average autoreactivities at 12 months, with some ANAs still detectable.²⁶⁻²⁹

Cryptogenic stroke is a diagnosis that is reached by excluding known causes. The patient's history and physical examination are particularly informative in this regard, and a carefully planned strategy for laboratory and imaging workup is crucial.⁷ In our case, the diagnosis was made in accordance with standard guidelines. The physical examination done upon admission revealed only speech impairment. Laboratory diagnostics showed an increase in segmented neutrophils (neutrophilia), a decrease in lymphocytes (lymphopenia), elevated levels of D-dimer, and positive test results for ANA screen Ig (A, G, M).

In modern medical practice, diagnosing and evaluating ischemic stroke patients involves multiple factors. Medical professionals use MRI and CT scans of the brain to determine the location, volume, and number of infarcts. Diffusion sequences in MRI scans are particularly useful for detecting small lesions and those in the brain stem and cerebellum.⁷ In our patient's case, the MRI revealed lesions in both the insula and parietal lobe, while the CT scan showed a subcortical hypodense area with unclear brain contours in the left parietal lobe.

Regarding the treatment of stroke, the administration of oral anticoagulant (OAC) is recommended for secondary prophylaxis of stroke. This is indicated in cases of paroxysmal atrial fibrillation, as well as when the morphological pattern of stroke suggests an embolic cause or when there is intracardiac thrombus formation. As per the recent guidelines, OAC is considered reasonable for high-risk patients only if they have other indications for OAC. In most cases, antiplatelet treatment is suggested to prevent a recurrent event.³⁰ In the case of our patient, antiplatelets have been prescribed for the purpose of treatment, along with neuroprotective drugs.

Conclusion

This report outlines the case of a young male patient who presented with cryptogenic stroke. The report demonstrates a correlation between an increased titer of Ig of ANA test and a history of COVID-19 infection, suggesting evidence of autoimmune antibody production in the patient. Based on this finding, it may be hypothesized that ANAs can cause vasculopathy, leading to thrombus formation and ultimately, stroke. Given the potential link between autoimmune antibody formation and stroke in COVID-19 patients, we recommend that autoimmune antibody tests be conducted in patients with stroke who have a history of COVID-19 infection.

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Declarations

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Author contributions

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

Conflicts of interest

None declared.

Data availability

Not applicable.

Ethical approval

Patient signed informed consent was taken regarding publishing their data. And the study was approved by the Institutional Ethics Committee.

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PROTOCOL PAPER

Doctoral dissertations in nursing – research protocols in a multicenter comparative study

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ABSTRACT

Introduction and aim. Nursing doctoral research is vital for advancing the field, improving patient care, and addressing complex healthcare challenges. It contributes to evidence-based practices, innovative interventions, and the development of skilled nurse researchers and leaders, shaping the future of healthcare through enhanced knowledge and informed policy decisions. This scoping review aims to map the landscape of PhD dissertations in the nursing domain.

Inclusion criteria: This scoping review will consider doctoral studies in nursing domain published in Dutch, English, Finnish, Hebrew, Portuguese, Polish, Slovak, Swedish, published between 2020–2023.

Material and methods. The search strategy was to find published literature in the following electronic databases: CINAHL, PubMed, SCOPUS, PsycINFO, Web of Science, Psychology & Behavioral Sciences Collection, and ERIC. Additionally, national repositories of several countries was explored, and contact established with universities represented by the members of this research team. The screening process entailed two steps carried out by two independent reviewers: firstly, screening by title and abstract and secondarily by full text. Data describes the literature according to the review research questions that were defined following the PPC mnemonic. Data will be presented graphically when possible and accompanied by a narrative that describes the Ph.D. dissertation characteristics.

Conclusion. Identifying areas of research in nursing contributes to the development of nursing as a science.

Keywords. doctoral dissertations, education, nursing, nursing science, nursing study

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Introduction

Nurses play a pivotal role in addressing the World Health Organization's (WHO) assertion that research is indispensable for resolving public health challenges. Their contribution involves addressing diseases of poverty, responding to the rise in chronic diseases, and ensuring access to safe delivery practices.^{1,2} This is achieved through substantial investment in their educational development, including pursuit of the doctorate degree.³ The importance of research conducted by nurses with doctoral preparation is indisputable, underscoring the necessity for a robust scientific education.^{4,5}

The global landscape of nurses' doctoral education reflects diverse traditions and varies based on the region where they develop professionally. For example, in the United States of America (USA), nurses began their doctoral path at the beginning of the 20th century, initially as Doctorates of Education (DEd) and later as Doctorates of Nursing Science (DNSc), followed by the Doctorates of Philosophy (PhD). Several decades later, European nurses started pursuing doctoral studies through the PhD degree. Due to the historical unavailability of a doctorate in nursing in Europe, nurses ended up seeking doctoral education in other disciplines, such as sociology, psychology, and education.^{6,7} Nursing-oriented doctoral programs began emerging in the 1970s^{8,9} and in some European countries the impulse was made by the redesign of the Bologna Process's educational cycle.¹⁰

In recent years, the professional doctorates as the Doctorates of Nursing Practice (DNP) has emerged in nursing as alternatives to the traditional PhD. The DNPs are often tailored to nurses in clinical or managerial roles, but is not too common in Europe.⁷ Although the DNPs increased significantly in the USA, compared to PhDs, and is established in USA and the United Kingdom (UK). The WHO (WHO, 2020) reports that the shortage of PhD prepared faculty internationally is an impediment to establishing higher degree nursing education programs.¹ Around the world, the research PhD is seen as the gold standard of doctoral education.^{8,9}

In Europe, earlier studies have scrutinized doctoral dissertations in nursing science, spanning the years 1990 to 2019. These reviews investigated dissertations published between 1982 and 2018, with the majority focusing on timelines ending in 2014 and 2015. Explored countries included Finland, Denmark, Norway, Portugal, Sweden, Turkey, and the United Kingdom.¹⁰⁻¹⁹ These studies predominantly delved into specific methodologies or themes, such as nursing education research, palliative care, geriatrics, treatment of postoperative pain and non-pharmacologic practices in nursing, research ethics, theoretical approaches, and phenomenology in nursing research.¹²⁻²⁰ Some studies aimed to identify general doctoral dissertation topics but in specific locations such as in Nordic countries, or in Finland, but lim-

ited to 1984–2015 or across countries until 2019, with a search conducted only in three databases and with data analyzed through a specific framework.^{11,14,21}

Especially since 2020, after the most destructive and life-altering global pandemic in modern history and its far-reaching consequences on individuals, groups, and societies, there may have been changes in the guidelines shaping health research within nursing doctoral dissertations. Mainly because nurses were called upon to highlight their importance in the fight for equality in the health of populations that takes place in practice, in academia, in science and in politics.^{22,23}

In our initial review, no studies were found specifically focusing on nurses' PhD dissertations in Europe since 2020. Consequently, we deem it essential to summarize and analyze PhD dissertations in nursing between 2020–2023 in Europe. Through this systematic examination of the doctoral landscape in nursing, we aim to pinpoint the primary areas of research undertaken by nurses in their doctoral journey, identify their targeted populations and settings where they occur, discern prevalent scientific methodologies, and, if feasible, comprehend the principal outcomes of each dissertation. Thus, this scoping literature review aims to describe an overview of the nursing research in PhD dissertations in nursing domain.

Aim

The aim was established by following the Participants Concept Context (PCC) mnemonic as recommended by the Joanna Briggs Institute for scoping reviews.²⁴ In December 2023, a preliminary search of the Cochrane Database of Systematic Reviews, the JBI Evidence Synthesis, OFS, PubMed and CINAHL, was conducted and no current or in progress reviews on the topic were identified in the contexts in which the authors propose.

Review questions

What are the current research interests in PhD dissertations within nursing science?

This review will seek to describe PhD by answering the following sub-questions:

- What are the most prolific research themes in PhD nursing dissertations?
- What is the main target population in the PhD nursing dissertations?
- What methodologies have been used in the PhD nursing dissertations?
- What settings are the dissertations focused on?
- What are the main conclusions of the PhD nursing dissertations?

Eligibility criteria

Population

This review encompasses literature on PhD dissertations conducted by nurses throughout their doctoral journey,

encompassing diverse populations without limitations. For the purpose of this study, dissertations authored by nurses or midwives will be included, but the term ‘nurses’ will be consistently used throughout this document to maintain terminological uniformity.

We will include studies involving all type of target populations, including but not limited to patients, clients, healthcare professionals or systems, nursing students, etc. Only in countries where there is no PhD in Nursing and nurses must obtain their PhD degree in other scientific areas, their dissertations will be included in this study. The PhD dissertations without an explicit positive opinion from an accredited ethics committee will be excluded.

Concept

This review focuses on PhD dissertations that seek to enhance nursing sciences. As explained in the previous point, in the countries where nurses cannot pursue the PhD in Nursing, we will consider PhD dissertations made by nurses in other scientific areas if they seek to enhance nursing sciences.

For the purposes of this review, PhD dissertations constitute the literature reporting on doctoral theses within the realm of intervention or research, regardless of the methodologies employed which contribute to the development or improvement of the nursing sciences domains. Mapping and analyzing the identified PhD dissertations will serve as the foundation comprehending their characteristics, including domains, research themes, target populations, settings, methodologies employed, and outcomes, thereby informing the nature of doctoral dissertations.

Context

This review considered PhD dissertations in nursing or made by nurses that enhance nursing sciences irrespective of the settings, including but not limited to academia, clinical practice, etc. This study will be focused but not limited to in the following countries: England, Finland, Israel, The Netherlands, Poland, Portugal, Slovakia and Sweden. In this group, Poland is the only country where there is no PhD in nursing, Polish nurses obtain a PhD in health sciences or other disciplines. The selection of countries is based on the nationalities of the authors involved in this study, who are members of the Research Committee of Sigma Theta Tau – European Region, an internationally recognized top honor society for nursing dedicated to advancing global health. Although Israel is not geographically located in Europe, it is a member in many European transnational federations, frameworks and takes part in many European events and associations such as Sigma. Literature will be included irrespective of geographic location without a specific racial- or gender-based criteria.

Types of sources

This scoping review will encompass primary studies employing all kind of designs, including, for instance, the publication of project protocols, a practice that is increasingly common among PhD students. These studies will be considered for inclusion across the databases outlined in the search strategy section. Additionally, national repositories will be explored, and data will be gathered or verified directly through email with universities of the selected countries, when it is not possible to obtain the information through previous means.

Material and methods

The proposed scoping review will be conducted in accordance with the JBI methodology, chapter 11, for scoping reviews, which encompasses the work of Arksey and O’Malley with refinements by Levac, Colquhoun and O’Brien.²⁴ The development of the protocol for the current review complies with the Extension for Scoping Reviews of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-ScR).²⁵ A detailed timeline of the research steps and planned work is provided in Table 1.

Table 1. Timeline and planned work

Phase	Date	Activities
Preliminary search and protocol development	December 2023 – February 2024	– Conduct preliminary searches in databases. – Develop and refine the search strategy. – Finalize the review protocol in accordance with the PRISMA-ScR guidelines.
Comprehensive literature search	March 2024 – April 2024	– Execute the search strategy across selected databases. – Identify and compile PhD dissertations from national repositories in the selected countries. – Contact universities directly if necessary to obtain missing data.
Study selection	May 2024 – July 2024	– Screen titles and abstracts by two independent reviewers. – Conduct full-text reviews to assess eligibility against inclusion criteria – Resolve disagreements through discussion or with a third reviewer.
Data extraction and analysis	July 2024 – September 2024	
Reporting and dissemination	October 2024 – March 2025	

Search strategy

The search strategy will aim to locate PhD dissertations in the open-access national repositories and databases, as well, if necessary, with the universities in the eight selected countries.

To identify the focus, timelines, and the principal articles about PhD dissertations in the earlier reviews concerning nursing doctoral dissertations, a preliminary search was conducted in December of 2023 to the databases PubMed, CINAHL, Google Scholar and Science Direct. The text keywords contained in the titles and ab-

stracts of relevant articles, and the index terms used to describe the articles, were used to develop a full search strategy CINHALL, PubMed, SCOPUS, PsycINFO, Web of Science, Psychology & Behavioral Sciences Collection, and ERIC (Table 2). Articles published in Dutch, English, Finnish, Hebrew, Polish, Portuguese, Slovak, Swedish, with a time range 2020–2023, will be included.

Table 2. Search strategy

Search	Query	Records retrieved
#1	"doctor* dissertation*" or "PhD dissertation*"	
#2	"nurs*"	
#3	#1 AND #2	
Limited to 2020–2023: Dutch, English, Finnish, Hebrew, Polish, Portuguese, Slovak, Swedish		

The terms will also be used in the national repositories search provided by each author of this protocol. For example, in Portugal theses are available in the open-access digital repositories of the University of Lisbon (<https://repositorio.ul.pt>), the Catholic University of Portugal (<https://repositorio.ucp.pt>), the University of Porto (<https://repositorio-aberto.up.pt>) and University of Coimbra (<https://estudogeral.uc.pt>). In Finland, all the dissertations can be found in repository Tiedejatutkimus.fi.²⁶

Despite searching through databases and national repositories in the eight countries previously proposed within this study, if obtaining the required information proves challenging, universities offering doctoral programs in nursing or related scientific fields where nurses pursue their doctorates will be contacted via email to acquire the necessary data. In instances where information is unclear or inconsistent, direct outreach to universities will also be considered to confirm and share the PhD dissertations completed between 2020–2023.

Study/Source of evidence selection

All the identified records will be collated and uploaded into EndNote™ X8 (Clarivate Analytics, PA, USA) and duplicates removed. From the national repositories and/or information from direct contacts with universities, a list will be generated for each country, organizing PhD dissertations alphabetically by title. All information will be analyzed to check if there are duplicates between databases, national repositories or information from universities, and these will be removed.

Following a pilot test, titles and abstracts will then be screened by two independent reviewers for assessment against the inclusion criteria for the review to redefined, if necessary, any criteria. The full text will be assessed in detail against the inclusion criteria by two independent reviewers. Reasons for exclusion of full-text documents that do not meet the inclusion criteria will be recorded and reported in the scoping review. Any disagreements that arise

between the reviewers at each stage of the selection process will be resolved through discussion or with a third reviewer. The results of the search will be reported in full in the final scoping review and presented in a Preferred Reporting Items for Systematic Reviews and Meta-analyses for Scoping Reviews (PRISMA-ScR) flow diagram.²⁵

Table 3. Data extraction instrument

Study ID	1. Study Number	
	2. Author	
	3. Year	
	4. Title	
	5. Country	
	6. Source	
Reason for inclusion or exclusion	7. PhD dissertation in nursing	1: Yes (proceed to question 9) 2: No – (proceed to question 8)
	8. PhD dissertation made by a nurse or a midwife.	1: Yes 2: No – exclude
	9. Are there other reasons for exclusion?	1: Yes – exclude (proceed to question 9.1) 2: No (proceed to question 10)
	9.1. Specify other reason for exclusion	Specify in own words reason for exclusion
Characteristics of the PhD dissertation abs	10. Research Themes/Topics	Specify
	11. Target populations	Specify
	12. Methodologies used (use the information on the abstract)	Specify
	13. Settings	
	13.1. Academia	
	13.2. Clinical practice	
	13.3. Other	Specify the setting
	14. Main Conclusions	Specify briefly
	14.1. Results	
	14.2. Future Studies	
	Comments to review from reviewers	Describe them.

Data extraction

Data will be extracted by one or two reviewers and accordingly the language limitations were overcome with a data extraction tool developed by the reviewers. The data extracted will include specific details about PhD dissertations including all kind of populations, regarding all the research in nursing domain, irrespectively of settings, where dissertations focus their development and other relevant findings to the review question. The data will be exported to Microsoft Excel® (Redmond, Washington, USA). A draft extraction tool is provided (Table 3). The draft data extraction tool will be modified and revised as necessary during the process of extracting data from each included dissertation. Modifications will be detailed in the full scoping review. Any disagreements that arise between the reviewers or doubts that emerge in the process will be resolved through discussion or with a third reviewer. Authors of papers will be contacted to request missing or additional data, where required.

Data analysis and presentation

Results will be reported graphically with tables when possible. Tables will be developed and refined throughout the data extraction to reflect the purpose and objective of the review. The results will be classified under the following categories: study identification (ID); reasons for inclusion or exclusion; characteristics of study population/paper, regarding the research themes, target populations, methodologies used, settings, and main outcomes of the PhD dissertations made by nurses in nursing or other scientific areas in countries where it is not possible to pursue PhD in nursing.

A narrative will accompany the result tables and will describe the characteristics of the body of literature and data collected related to PhD dissertations in any context.

Conclusion

Identifying the topics in nursing doctoral dissertations will be helpful for identifying areas of research in nursing science in the future. It will also allow future research to be directed towards strictly nursing topics.

Declarations

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Author contributions

Conceptualization, L.T-S.; M.C.; D.C.; M.N.; H.P.; S.S.; S.W.; and M-L.L.; Methodology, L.T-S.; M.C.; D.C.; M.N.; H.P.; S.S.; S.W.; and M-L.L.; Software, L.T-S.; M.C.; D.C.; M.N.; H.P.; S.S.; S.W.; and M-L.L.; Validation, L.T-S.; M.C.; D.C.; M.N.; H.P.; S.S.; S.W.; and M-L.L.; Formal Analysis, L.T-S.; M.C.; D.C.; M.N.; H.P.; S.S.; S.W.; and M-L.L.; Investigation, L.T-S.; M.C.; D.C.; M.N.; H.P.; S.S.; S.W.; and M-L.L.; Resources, L.T-S.; M.C.; D.C.; M.N.; H.P.; S.S.; S.W.; and M-L.L.; Data Curation, L.T-S.; M.C.; D.C.; M.N.; H.P.; S.S.; S.W.; and M-L.L.; Writing – Original Draft Preparation, L.T-S.; M.C.; D.C.; M.N.; H.P.; S.S.; S.W.; and M-L.L.; Writing – Review & Editing, L.T-S.; M.C.; M.N.; H.P.; S.S.; S.W.; J.S. and M-L.L.; Visualization, L.T-S.; M.C.; M.N. and M-L.L.; Supervision, L.T-S.; M.C.; D.C.; M.N.; H.P.; S.S.; S.W.; and M-L.L.; Project Administration, L.T-S.; M.C.; D.C.; M.N.; H.P.; S.S.; S.W.; J.S. and M-L.L.; Funding Acquisition, L.T-S.; M.C.; D.C.; M.N.; H.P.; S.S.; S.W.; J.S. and M-L.L.;

Conflicts of interest

The author asserts that they have no conflicts of interest.

Data availability

Not applicable.

Ethics approval

Not applicable.

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CORRIGENDUM

Corrigendum: Morbidity profile and outcome of new-born admitted in sick newborn care units of Uttar Pradesh, India

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A Corrigendum on

Morbidity profile and outcome of new-born admitted in sick newborn care units of Uttar Pradesh, India by Singh SP, Paliwal HC, Shekhar S, Kushwaha P. *Eur J Clin Exp Med.* 2023;21(3):590–594. doi: 10.15584/ejcem.2023.3.27.

In the published article, an error occurred in Table 2. The fifth line should read “≥2.6” instead of “≥2.5.” This was an unintentional editing mistake. The corrected Table 2 appears below.

The authors apologize for this error and confirm that it does not affect the scientific conclusions of the article in any way. The original article has been updated.

Table 2. Outcome of neonates admitted in SNCU among different birth weight groups (n=22904)

Weight (kg)	Outcome				Total
	Discharged	Expired	LAMA	Referred	
0–1.5	1246 (50%)	654 (26.3%)	244 (9.8%)	340 (13.7%)	2484 (100%)
1.6–2	2696 (72.3%)	359 (9.6%)	243 (6.5%)	426 (11.4%)	3724 (100%)
2.1–2.5	4332 (76.4%)	389 (6.9%)	315 (5.6%)	631 (11.1%)	5667 (100%)
≥2.6	8354 (75.6%)	581 (5.3%)	701 (6.3%)	1393 (12.6%)	11029 (100%)
Total	16628 (72.5%)	1983 (8.6%)	1503 (6.6%)	2790 (12.2%)	22904 (100%)

$\chi^2 = 1334.2$, $df = 15$, $p < 0.001$





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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. The abstract should not contain any undefined abbreviations or unspecified references. **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. *Comput 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.