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ORIGINAL PAPER

Haematological changes and metabolic alterations in SARS-CoV-2 infected patients hospitalised at an Infectious Diseases Center, Ibadan, Nigeria

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ABSTRACT

Introduction and aim. Following the use of repurposing drugs to successfully manage coronavirus disease 2019 (COVID-19) patients in an Infectious Diseases Center (IDC) in Nigeria, it was imperative to assess haematological changes and metabolic alterations in these patients which may inform recommendations for future use.

Material and methods. Blood samples of admitted COVID-19 Nigerian patients during therapeutic management were analysed for haematological- (total white blood cells (WBC), lymphocyte, monocyte, neutrophil, eosinophil, basophil and neutrophil:lymphocyte ratio) and blood chemistry- parameters [total protein, total and conjugated bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), albumin, urea, creatinine, total cholesterol, triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), PO_4^{3-}, Ca^{2+}, uric acid, Na^+, K^+, Cl^- and HCO_3^-] using autoanalysers. The percentages of patients having values below, within and above reference ranges were compared using Chi-square test while the mean values at admission were compared with mean values at discharge using Student *t*-test.

Results. The mean values of total protein, albumin, Na^+, HCO_3^-, uric acid, Ca^{2+}, WBC, platelets, lymphocytes, eosinophils and basophils were significantly increased in COVID-19 patients at discharge compared with COVID-19 patients at admission. Also, more percentages of COVID-19 patients at discharge compared with COVID-19 patients at admission had albumin, ALP, total bilirubin, HDL, Na^+, K^+, Cl^-, HCO_3^-, urea, creatinine, WBC, lymphocytes, neutrophils, monocytes, eosinophils and basophils within normal reference intervals.

Conclusion. This study showed that most metabolic and haematological derangements were normalised by repurposing drugs in most of the COVID-19 patients at this IDC, thus supporting the continuous use of this therapeutic option.

Keywords. COVID-19, laboratory tests, reference interval, repurposed drugs, therapeutic action

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Introduction

Studies have reported abnormal systemic or cellular metabolisms in several disorders, but such report is not available in COVID-19 Nigerian patients before and after therapeutic intervention.[1,2] Metabolic functions which are integrated, balanced and homeostatic pathways have been demonstrated to be altered by several virus infections, leading to viral escape from immune attack, causing tissue inflammation and multiple organ damage in COVID-19 patients.[2,3] The entry of SARS-CoV-2 depends on the interaction between virus Spike protein and angiotensin-converting enzyme 2 (ACE2) receptors on epithelial cells in the lungs and other organs with ACE2 receptors to become a multi-organ disease.[4] The exact mechanisms involved in multi-organ disease during COVID-19 have not been clear, but probably multi-factorial.

It was reported that viral infection affects lipid signalling and synthesis which adversely distorts protective host immune response and other metabolisms.[5] Another study demonstrated that interruption in lipid synthesis impairs virus replication, suggesting that lipid pathways can represent a relevant target in the investigation of viral disorders.[6] Higher levels of diglycerides, free fatty acids, and triglycerides were identified in higher amounts in the severe COVID-19 patients.[4] Furthermore, studies reported increased viral replication in cells with excessive intracellular lipid accumulation.[7] These findings suggested altered systemic and lipid metabolisms in COVID-19 patients.[5,6]

The index case of the COVID-19 in Nigeria was recorded on the 27 February 2020 and within 5 months (by the 7 July 2020), all 36 states and the Federal Capital Territory (FCT) had reported at least a case of the disease, with a total number of 29,789 cases and 669 deaths. As at 10 August, 2022, the total cases of COVID-19 in Nigeria was 262,000 with 3,157 deaths while there were 586,000,000 cases and 6,400,000 deaths worldwide.[7]

A recent study showed that between 3.4% to 51.7% newly admitted COVID-19 Nigerian patients had one or more abnormal values of renal function parameters.[8] Most COVID-19 patients (51.7%) had abnormal levels of chloride and 37.8% of the patients had abnormal levels of creatinine.[8] Another study recorded that COVID-19 patients with elevated creatinine had a significantly higher rate of AKI and associated mortality.[9] These observations suggested an association between COVID-19 severity and renal injury.[8,9]

Abnormal liver function tests (LFTs) are frequently observed in patients with COVID-19, of which the underlying pathogenesis is incompletely understood. Though LFTs are not necessarily liver specific but it has been suggested that elevated aminotransferases in COVID-19 could originate from myositis rather than liver injury.[10] Hypoalbuminemia reported in 55% of hospitalised COVID-19 patients was associated with disease severity to predict mortality.[3] Plasma ALT and AST were elevated more frequently and to a greater extent in patients with severe COVID-19 compared to those with mild disease and are associated with increased disease severity and mortality, whereas other studies did not find an association with mortality, disease progression, ICU admission, or length of hospital stay.[3,11] Several case reports have described severe LFT abnormalities or acute or chronic liver failure in patients with COVID-19. Elevated ALP was reported in 2%-5% of patients, and elevated GGT was reported in 13%-54% of patients (weighted average: 23%).[12] The prevalence of total bilirubin elevations ranged between 1% and 18% of patients with COVID-19 on admission.[3] The prevalence of total bilirubin elevations ranged between 1% and 18% of patients with COVID-19 on admission.[3] Despite extensive studies of LFTs in COVID-19 patients, the prognostic value of abnormal LFTs in COVID-19 is unclear.

COVID-19 is a respiratory disease which also affects multiple systems including the cardiovascular, neurological, haematopoietic and immune system among others.[3,10] Leukopenia, leukocytosis, neutropenia and lymphopenia are among the most common laboratory abnormalities in COVID-19.[13,14] Tan et al. showed that lymphocyte percentage was inversely related to the severity and prognosis of COVID-19 patients.[13] Although, reports on haemocytometric changes in COVID-19 infection at different stages of the disease are many, the roles of these changes in indicating disease prognostication is still poorly understood especially, in Nigerian COVID-19 patients.[15]

Demographic- and immune- parameters in COVID-19 Nigerian patients were previously reported. Moreso, repurposed medication used for COVID-19 patients at IDC, Ibadan, Nigeria are combinations of vitamin D, vitamin C, Zn, azithromycin, hydroxychloroquine and chloroquine (as an alternative to hydroxychloroquine).[14,16-20] The treatment options for COVID-19 at this center are largely supportive as there is no universally agreed protocol of care. In addition, these medications were not used in the context of a trial but the positive outcomes encouraged their continued use. However, it was reported that despite patient's toleration of these medications without a risk of bacterial and malarial super-infection, there is need for further evaluation.[20] However, Arinola and Edem emphasised the need for public enlightenment on the dangers inherent in micronutrient supplement abuse.[21]

Aim

The present study therefore assessed the haematologic- and metabolic- alterations in admitted COVID-19 patients on repurposing drugs in an IDC in Ibadan, Nigeria.

Material and methods

The study was conducted after obtaining institutional ethical approval (UI/EC/20/0233) and informed consent from each study participant. Confirmed cases of COVID-19 (n=195) were recruited from an Infectious Diseases Centre, Olodo, Ibadan, Nigeria between May 2020 and August 2020. They were confirmed to be infected with SARS-CoV-2 using nucleic acid Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) on nasal and pharyngeal swab specimens.[8] On the day of admission, the patients were commenced on the following medication: vitamin D, vitamin C, Zn^{2+}, azithromycin, hydroxychloroquine and chloroquine (as an alternative to hydroxychloroquine). Ten ml of blood sample was collected from each patient and analysed for haematological parameters (total white blood cell (WBC), lymphocyte, monocyte, neutrophil, eosinophil, basophil, red blood cell and platelets counts as well as haematocrit and haemoglobin levels) using haematology autoanalyser (Sysmex XN-450, Norderstedt, Germany). Blood chemistry parameters such total-bilirubin (Bil-T) and conjugated- bilirubin (Bil-D), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), albumin, urea, uric acid creatinine (Cr), total cholesterol (TC), sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO_3^-) using an autoanalyser (Erba XL-200, Mannheim, Germany). The values of biochemical- and haematological- parameters obtained were compared with normal reference ranges to determine the proportion of patients having parameters below, within and above reference ranges. Data were represented as frequencies and percentages. Chi-square test was used to determine the differences between the frequencies while the differences in the mean of variables of COVID-19 patients at admission and discharge were compared using Student *t*-test on the SPSS statistical software (IBM, Chicago, IL, USA), version 21 for windows. *p*-value less than 0.05 was considered as statistically significant.

Results

As shown in Table 1, most COVID-19 patients (69%) in IDC, Olodo, Ibadan were males, 63% of the patients were less than 40 years of age, 47% were privately employed, 70% of them spent less than 10 days on admission, none had severe COVID-19 and 68% of them were hypertensive.

As shown in Figure 1, the mean values of total protein and albumin were significantly increased while ALT, AST, ALP, GGT, total bilirubin and direct bilirubin were not significantly different in COVID-19 patients at discharge compared with COVID-19 patients at admission.

As shown in Table 2, the TC, triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were significantly increased in COVID-19 patients at discharge compared with COVID-19 patients at admission. The mean levels of Na^+, HCO_3^-, uric acid and Ca^{2+} were significantly increased in COVID-19 patients at discharge compared with COVID-19 patients at admission (Figure 2).

Table 1. Demography and length of hospital admission of COVID-19 patients

Variables	Frequency	Percentage (%)
Gender		
Male	134	69
Female	61	31
Age (years)		
<40 years	122	63
≥ 40 years	73	37
Occupation		
Self employed	53	27
Private	91	47
Civil servant	31	16
Unemployed	29	10
Days on admission		
≤10 days	137	70
>10 days	58	30
Severity		
Mild	117	60
Moderate	78	40
Severe	0	0
Co-morbidity types		
Hypertension	134	68
Peptic Ulcer Disease	39	20
Diabetes mellitus	19	10
Sickle Cell Disease (HbSS)	3	2

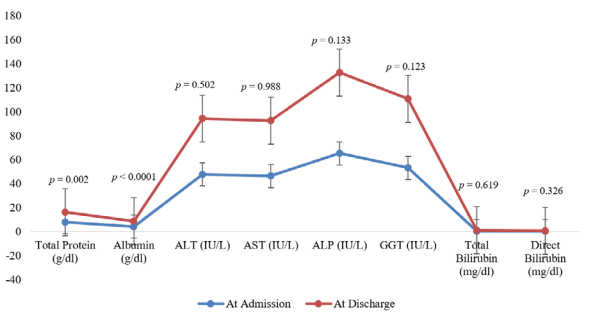

Fig. 1. Liver function parameters in COVID-19 patients at admission and at discharge, AST – aspartate transferase; ALT – alanine transferase; ALP – alkaline phosphatase; GTT – gamma-glutamyl transferase

Table 2. Mean values of lipid profile in COVID-19 patients at admission compared with values at discharge*

Parameters	At Admission	At Discharge	t	p
TC (mg/dl)	176.49±49.50	196.98±49.18	7.835	<0.0001
TG (mg/dl)	103.06±49.21	126.04±64.03	4.366	<0.0001
HDL (mg/dl)	49.72±16.54	56.64±15.06	5.973	<0.0001
LDL (mg/dl)	104.08±41.33	111.46±43.13	2.991	0.003

*TC– total cholesterol; TG– triglycerides; HDL– high density lipoprotein; LDL– low density lipoprotein

As shown in Figure 3, the mean values of WBC, platelets, lymphocytes, eosinophils and basophils

were significantly increased while HGB, HCT, neutrophils, monocytes and NLR were significantly reduced in COVID-19 patients at discharge compared with COVID-19 patients at admission.

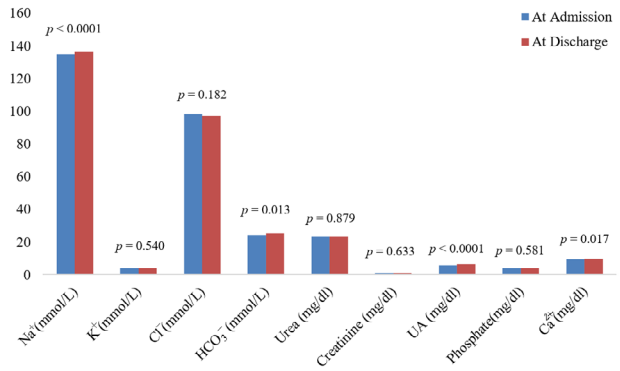

Fig. 2. Renal function parameters in COVID-19 patients at admission and at discharge

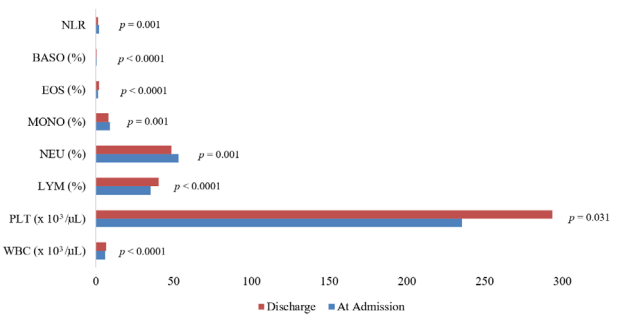

Fig. 3. Haematological parameters in COVID-19 patients at admission and at discharge, WBC – white blood cells; PLT – platelets; LYM – lymphocytes; NEU – neutrophils; EOS– eosinophils; BASO – basophils; NLR –netrophil:lymphocyte ratio

More percentages of COVID-19 patients at discharge compare with COVID-19 patients at admission had albumin, ALP, total bilirubin, HDL, Na^+, K^+, Cl^-, HCO_3^-, urea, creatinine, WBC, lymphocytes, neutrophils, monocytes, eosinophils and basophils (88%vs. 86%, 88% vs. 85%, 94% vs. 93%, 91% vs. 80% , 93% vs. 88%, 89% vs. 72%, 79% vs. 77%, 82% vs. 81%, 87% vs. 76%, 96% vs. 92%, 89% vs. 65%, 61% vs. 43%, 80% vs. 57%, 73% vs. 55%, 42% vs. 27% and 100% vs. 99% respectively) within normal reference intervals (RI). However, more percentages of COVID-19 patients at admission compare with COVID-19 patients at discharge had total protein, ALT, AST, GGT, direct bilirubin, total cholesterol, triglycerides, LDL, uric acid, PO_4^{3-}, calcium and platelets (58% vs. 46%, 45 vs. 41%, 39% vs. 28%, 52% vs. 46%, 96% vs. 95%, 72% vs. 51%, 86% vs. 83%, 40% vs. 37%, 77% vs. 68%, 84% vs. 83%, 75% vs. 68%and 78% vs. 76% respectively) within normal reference intervals (Tables 3-6).

Table 3. The frequencies (percentages) of COVID-19 patients at admission compared with frequencies (percentages) of COVID-19 patients at discharge having liver function parameters within and outside normal reference intervals*

Parameters	Category	At Admission n (%)	At Discharge n (%)	Chi-square	p
Total protein	Below RI	2 (1.1)	0 (0)	4.127	0.127
	Within RI	109 (57.7)	87 (46)		
	Above RI	78 (41.3)	102 (54)		
Albumin	Below RI	21 (11.1)	8 (4.2)	42.471	<0.0001
	Within RI	163 (86.2)	166 (87.8)		
	Above RI	5 (2.6)	15 (7.9)		
ALT	Below RI	0 (0)	0 (0)	13.187	<0.0001
	Within RI	85 (45.2)	77 (41)		
	Above RI	103 (54.8)	111 (59)		
AST	Below RI	0 (0)	0 (0)	0.192	0.662
	Within RI	57 (30.3)	52 (27.7)		
	Above RI	131 (69.7)	136 (72.3)		
ALP	Below RI	3 (1.8)	2 (1.2)	42.843	<0.0001
	Within RI	138 (84.7)	144 (88.3)		
	Above RI	22 (13.5)	17 (10.4)		
GGT	Below RI	1 (0.8)	0 (0)	32.665	<0.0001
	Within RI	63 (51.6)	56 (45.9)		
	Above RI	58 (47.5)	66 (54.1)		
Total Bil	Below RI	0 (0)	0 (0)	13.899	<0.0001
	Within RI	175 (93.1)	176 (93.6)		
	Above RI	13 (6.9)	12 (6.4)		
Direct Bil	Below RI	0 (0)	0 (0)	37.696	<0.0001
	Within RI	181 (95.8)	180 (95.2)		
	Above RI	8 (4.2)	9 (4.8)		

* ALT – alanine transferase; AST – aspartate transferase; ALP – alkaline phosphatase; GTT – gamma-glutamyl transferase; Bil - bilirubin

Table 4. The frequencies (percentages) of COVID-19 patients at admission compared with frequencies (percentages) of COVID-19 patients at discharge having lipid profile within and outside normal reference intervals*

Parameters	Category	At Admission n (%)	At Discharge n (%)	Chi-square	p
TC	Below RI	0 (0)	0 (0)	42.831	<0.0001
	Within RI	136 (72)	97 (51.3)		
	Above RI	53 (28)	92 (48.7)		
TG	Below RI	0 (0)	0 (0)	11.846	0.001
	Within RI	162 (85.7)	156 (82.5)		
	Above RI	27 (14.3)	33 (17.5)		
HDL	Below RI	33 (17.5)	13 (6.9)	19.318	0.001
	Within RI	151 (79.9)	171 (90.5)		
	Above RI	5 (2.6)	5 (2.6)		
LDL	Below RI	54 (29)	46 (24.7)	77.808	<0.0001
	Within RI	75 (40.3)	69 (37.1)		
	Above RI	57 (30.6)	71 (38.2)		

* TC – total cholesterol; TG – triglycerides; HDL – high density lipoprotein; LDL – low density lipoprotein

Table 5. The frequencies (percentages) of COVID-19 patients at admission compared with frequencies (percentages) of COVID-19 patients at discharge having renal function parameters within and outside normal reference intervals

Parameters	Category	At Admission n (%)	At Discharge n (%)	Chi-square	p
Na^+	Below RI	19 (10.9)	10 (5.7)	9.486	0.050
	Within RI	153 (87.9)	162 (93.1)		
	Above RI	2 (1.1)	2 (1.1)		
K^+	Below RI	32 (18.4)	16 (9.2)	6.124	0.190
	Within RI	126 (72.4)	155 (89.1)		
	Above RI	16 (9.2)	3 (1.7)		
Cl^-	Below RI	35 (20.1)	35 (20.1)	43.795	<0.0001
	Within RI	134 (77)	138 (79.3)		
	Above RI	5 (2.9)	1 (0.6)		
HCO_3^-	Below RI	22 (12.6)	13 (7.5)	2.353	0.671
	Within RI	141 (81)	143 (82.2)		
	Above RI	11 (6.3)	18 (10.3)		
Urea	Below RI	26 (13.8)	15 (7.9)	48.896	<0.0001
	Within RI	144 (76.2)	165 (87.3)		
	Above RI	19 (10.1)	9 (4.8)		
Creatinine	Below RI	1 (0.5)	1 (0.5)	16.459	0.002
	Within RI	174 (92.1)	182 (96.3)		
	Above RI	14 (7.4)	6 (3.2)		
Uric acid	Below RI	1 (0.5)	0 (0)	16.470	<0.0001
	Within RI	145 (77.5)	127 (67.9)		
	Above RI	41 (21.9)	60 (32.1)		
Phosphate	Below RI	3 (3.4)	1 (1.1)	12.280	0.015
	Within RI	73 (83.9)	72 (82.8)		
	Above RI	11 (12.6)	14 (16.1)		
Ca^{2+}	Below RI	0 (0)	0 (0)	2.674	0.102
	Within RI	21 (75)	13 (67.9)		
	Above RI	7 (25)	9 (32.1)		

Table 6. The frequencies (percentages) of COVID-19 patients at admission compared with frequencies (percentages) of COVID-19 patients at discharge having haematological parameters within and outside normal reference intervals*

Parameters	Category	At Admission n (%)	At Discharge n (%)	Chi-square	p
WBC	Below RI	41 (21.7)	7 (3.7)	60.024	<0.0001
	Within RI	123 (65.1)	164 (86.8)		
	Above RI	25 (13.2)	18 (9.5)		
PLT	Below RI	26 (13.8)	13 (6.9)	20.201	<0.0001
	Within RI	148 (78.3)	144 (76.2)		
	Above RI	15 (7.9)	32 (16.9)		
LYM	Below RI	45 (23.8)	11 (5.8)	22.185	<0.0001
	Within RI	81 (42.9)	115 (60.8)		
	Above RI	63 (33.3)	63 (33.3)		
NEU	Below RI	45 (23.8)	31 (16.4)	24.577	<0.0001
	Within RI	107 (56.6)	152 (80.4)		
	Above RI	37 (19.6)	6 (3.2)		
MONO	Below RI	15 (7.9)	14 (7.4)	10.380	0.034
	Within RI	104 (55)	137 (72.5)		
	Above RI	70 (37)	38 (20.1)		
EOS	Below RI	130 (70.3)	98 (53)	39.523	<0.0001
	Within RI	50 (27)	79 (42.7)		
	Above RI	5 (2.7)	8 (4.3)		
BASO	Below RI	0 (0)	0 (0)	0.011	0.917
	Within RI	183 (98.9)	184 (99.5)		
	Above RI	2 (1.1)	1 (0.5)		

* WBC – white blood cells; PLT – platelets; LYM – lymphocytes; NEU – neutrophils; MONO – monocytes; EOS – eosinophils; BASO – basophils

Discussion

Our study is the first and most comprehensive report on the haematologic- and biochemical-alterations in Nigerian patients with COVID-19managed at an Infectious Disease Center (IDC) in Oyo State. Previous reports demonstrated the importance of metabolism on the outcomes of viral infections.[1,2,11] The severity of COVID-19 has been reported to be high in patients with underlining metabolic conditions and this demonstrated that metabolic changes could be related to the prognosis of COVID-19.[1,16] It is well established that one of the critical phases of COVID-19 is the cytokine storm generated by the host response, causing extreme inflammatoryprocess.[14,19,22] Patients with existing chronic inflammation often present with accentuated cytokine storm, causing physiological imbalance and aggravated health problems in different organs.[22] An *in silico* study demonstrated the interaction of the spike protein (S protein) from SARS-CoV-2 with human innate immune receptor (Toll-like receptors, TLRs).[23] TLR4 activation has been associated with inflammatory conditions and alteration of lipid and glycolytic homeostasis.[23] The molecular mechanisms involved in metabolic dysfunction in COVID-19 are still sparsely described and not completely understood.[11] Since S protein of SARS-CoV-2 interacts with host TLR to induce inflammation, therefore raised NLR, monocyte and neutrophil counts in our COVID-19 patients at admission may be indicators of the existence of inflammatory phenomenon, and thus alterations in metabolic parameters are expected. This present study revealed one form of abnormal metabolic parameters in 11–72% of COVID-19 patients and haematologic derangements in 1–73% of COVID-19 patients.

Besides altered glycolytic and gluconeogenic pathways in COVID-19, lipid and mitochondrial metabolisms is also affected, demonstrating that altered energy metabolism in renal cell may lead to development of kidney injury affecting systemic metabolism during SARS-CoV-2 infection.[14,24,25] Fatty acids act as mitochondrial substrates for oxidative metabolism and lipid excess induces reactive oxygen species production, apoptosis, inflammation, profibrotic factors release, and organelle damage.[26] Excess LDL levels in 31% of COVID-19 patients at admission and in 32% COVID-19 of patients at discharge may account for inflammation and production of reactive oxygen species in COVID-19 patients as previously pointed out.[11,14,18,19]

Patients infected with SARS-CoV had dysregulated levels of serum free fatty acids and altered lip-

id profile was observed in SARS-CoV infection, even 12 years after recovery from the disease.[3] However, cellular and molecular mechanisms that orchestrate lipid metabolism during SARS-CoV-2 infection are poorly described as recently pointed out.[28] It has also been observed that lipid bodies are localised in monocytes from SARS-CoV-2 infected patients and these lipid bodies are sources of energy and inflammatory mediators for virus replication and virus escape from immune system elimination.[30,31] Thus, normal lipid profile is found in 14–60% of COVID-19 patients at admission compared with 11–72% COVID-19 patients at discharge.

Liver function tests include measures of hepatocyte injury (AST and ALT), bile duct injury or cholestasis (ALP and GGT), markers of hepatic clearance/biliary secretion capacity (bilirubin), as well as measures of synthetic capacity (prothrombin time and albumin). Lower levels of pre-albumin in patients with severe COVID-19 were reported, suggesting decreased hepatic synthesis[3], thus supporting higher level of albumin in COVID-19 patients at discharge compared with COVID-19 patients at admission. In the context of inflammation, hypoalbuminemia in COVID-19 at admission may also reflect albumin extravasation as a consequence of increased capillary permeability. Additional factors that could explain the observed hypoalbuminemia in newly admitted COVID-19 patients are increased catabolism and malnutrition. Plasma ALT and AST were elevated more frequently and to a greater extent in patients with severe COVID-19 compared to those with mild disease.[11] This was also the case for the present study where ALT and AST above normal reference limits were found in 54.8% vs. 59% and 69.7% vs. 72.3% of COVID-19 patients at admission and discharge respectively.

SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) as docking and entry receptor on host cells while Transmembrane serine protease 2 (TMPRSS2) is also involved in its cellular entry.[4] ACE2 is highly expressed on the brush border of small intestinal enterocytes and SARS-CoV-2 infection was observed in human small intestine organoids.[32] SARS-CoV-2 nucleocapsid was detected in the cytoplasm of intestinal biopsies of a patient with COVID-19.[32] Theoretically, direct virus-induced cytopathic effects could play a role in LFT abnormalities in COVID-19. Assuming brisk viral replication in the intestine, it appears plausible that viruses could enter the portal circulation to reach the liver. Hepatic Kupffer cells would attempt to clear the virus and initiate an inflammatory response. It is also possible that inflammatory mediators from the intestine could enter the portal system and sinusoids.

Previous reports suggested that some changes in the peripheral blood in COVID-19 patients provide clue or guidance for diagnosis, treatment, and prognosis of the disease.[11,14,27] A study indicated that the total number of peripheral white blood cells was normal or the lymphocyte count was reduced in patients at the early stage of COVID-19 and that lymphocyte percentage was inversely related to the severity and prognosis of patients with COVID-19.[13] Other studies reported that WBC, neutrophil count, NLR and PLR in the severe group were significantly higher than those in the moderate group; meanwhile, lymphocyte number and eosinophil count in the severe group were significantly lower than those in the moderate group.[11,14,29] The present finding of lymphopenia in our COVID-19 patients at admission may be related to persistent infection and prolonged hypoxia leading to compensatory hyperplasia of the bone marrow to release more granulocytes or continuous lymphocytes destruction through invasion by SARS-CoV-2 and constant removal of these destroyed lymphocytes by the spleen and other immune organs. However, lymphopenia was conjectured to because by depletion of T lymphocyte subpopulation.[33]

Both NLR and platelet lymphocyte ratio in patients with severe COVID-19 disease were significantly higher and showed that this is probably the best single parameter for differential diagnostic efficacy.[34] Liu et al. also suggested that NLR was helpful for early detection of severe COVID-19 patients with high prediction accuracy, which is consistent with the conclusion of Wang et al.[11,27,34] Our present study showed significantly increased NLR in newly admitted COVID-19 patients compared with discharged patients, thus supporting the usefulness of NLR in COVID-19 prognostication.

The findings of this study has implication on the potential mechanisms of hepatic-kidney injury in patients with SARS-CoV-2, which is capable of binding specifically to ACE2 on hepatocytes, bile duct cells, and liver endothelial cells to cause viral injuries. Besides apoptotic liver cells, fatty change is more frequent in COVID-19 patients while immune-mediated inflammation and drug toxicity may also lead to hepatic injuries. Thus, the risk of severe COVID-19 could be higher for liver transplant recipients using immunosuppressive drugs and especially for those with metabolic complications.[35] In addition, SARS-CoV-2 could infect kidney tissues by ACE2, CD147, and GRP78 with the assistance of TMPRSS2 and furin-like cleavage on spike protein directly to induce kidney injury. Acute kidney disease during COVID-19 can also be caused by activation of Complement system, cytokine storm, abnormal coagulation, rhabdomyolysis or possible direct injury to renal vascular caused by virus.[35] Dysregulation of intestinal microbiota, increase risk of cytokine storms and damage to mucosal immune system were proposed possible mechanisms of injury caused by SARS-CoV-2 on gastrointestinal tract causing abdominal pain and vomiting.[36]

Conclusion

This study found that the repurposed drugs used for the management of COVID-19 patients at the IDC, Olodo, Ibadan, Nigeria normalised certain haematological- and metabolic-parameters which were abnormal at the point of admission. Thus, the use of these repurposed drugs in the management of COVID-19 patients show promising results and should be considered for use in poor resource settings.

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Declarations

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Author contributions

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Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL PAPER

Clinico-epidemiological and vaccination profile of patients attending flu clinic of a tertiary health care institution in Eastern India during the third wave of COVID-19 pandemic

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ABSTRACT

Introduction and aim. With the third wave of COVID-19 hitting the country, there is an urgent need to systematically document the clinical-epidemiological and vaccination details of the patients to formulate evidence-based decisions. So, this study was planned to describe the profile of patients attending the flu clinic of a tertiary care hospital in eastern India.

Material and methods. This hospital-based cross-sectional study was done for 6 weeks (Jan-Feb 2022) among 623 patients using a pre-tested, structured questionnaire related to COVID-19. An unadjusted odds ratio was calculated and statistical significance was attributed to a p-value <0.05.

Results. Out of 623 patients, almost 90% of the patients were vaccinated against COVID-19 with at least one dose of any vaccine. Cough (57.8%) was the most common complaint. Patients aged > 60 years and those having one or more than one comorbidity suffered from moderate-severe COVID-19 infection when compared to their counterparts (p<0.001). Also, 2.1% of fully vaccinated, 3.8% of one dose vaccinated and 10.9% of unvaccinated patients suffered from moderate-severe COVID-19.

Conclusion. During the third wave of the COVID-19 pandemic, a smaller number of elderlies compared to the previous two waves were affected indicating age shifting. The severity of COVID-19 was less among vaccinated individuals compared to unvaccinated highlighting the importance of COVID-19 vaccination.

Keywords. COVID-19, epidemiology, mutation, pandemic, SARS-CoV-2, vaccination

Introduction

Coronavirus disease 2019 (COVID-19), originated in Wuhan, China has been there for more than 2 years with multiple waves.[1] COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a single-stranded RNA virus of the family coronaviridae with a varying incubation period of 2-14 days.[2,3] To date world has seen 50,75,01,771 confirmed cases and 62,20,390 deaths due to COVID-19.[4] India is the second most affected country in the pandemic and has reported almost 4,30,60,086 confirmed cases and 5,22,223 deaths as of April 2022.[5] Since the beginning of the COVID-19 pandemic, the coronavirus has mutated, resulting in variants of the virus such as delta variant and most recently the newer identified "omicron". Each new variant brings a new wave of COVID-19 cases.[6] Besides the evolution of SARS-CoV-2 into new strains, several other factors such as the effectiveness of vaccines, COVID-19 appropriate behaviour, and herd immunity have had an impact on whether new COVID-19 cases are increasing or declining in particular locations.[7] Moreover, India also recognised a surge in the COVID-19 cases in January 2022

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hinting toward the third wave of the COVID-19 pandemic. The health and wealth of the population of the world including India have drained as a result of multiple waves of COVID-19.[7]

Many pieces of literature have shown several obvious differences between the first and the second wave epidemiology, and the clinical profile of COVID-19 patients presenting to the designated health centre.[8–10] Aggarwal et al., New Delhi, India in 2020 showed that the median age of those who were hospitalized due to COVID-19 was 54.5 years with 68.8% having one or more comorbidities and 90.6% having dyspnoea as presenting complaint.[11] Guan et al. in Wuhan, China 2020 did a cross-sectional study among 1099 lab-confirmed COVID-19 positive patients and showed that the most common symptom was fever, followed by cough and around 56% had ground-glass opacity on CT chest.[12] Another study from New Delhi, India in 2020 showed that a significant proportion of patients were asymptomatic (44.4%) and among the symptomatic, cough (34.7%) was the most common symptom followed by fever (17.4%) and 3.5% patients required oxygen supplementation, 2.8% patients had severe disease requiring intensive care.[13] Thus, with the plausibility of third-wave hitting the country, there is an urgent need to systematically document the clinical-epidemiological and vaccination profile of the COVID-19 patients to formulate evidence-based decisions.[10]

Aim

With this background, this current study was planned to assess the clinico-epidemiological characteristics and its association with vaccination profile among patients attending the flu clinic of a tertiary care hospital in eastern India during the third wave of the COVID-19 pandemic.

Material and methods

Ethical approval

This study was approved by Institute Ethics Committee, AIIMS Patna (Ref: AIIMS/Pat/IEC/2022/859). We adhered to the principles of ethics thereafter throughout the study.

Study design and participants

This was a hospital-based observational study done for the duration of 6 weeks (the first week of January 2022 to the third week of February 2022) at the flu clinic of All India Institute of Medical Sciences, Patna. AIIMS Patna is a 950 bedded tertiary health care institution under the Ministry of Health and Family Welfare, Government of India in the eastern state of Bihar catering for the population of Bihar and neighbouring states. AIIMS Patna was declared a dedicated COVID-19 hospital during all waves of the COVID-19 pandemic. Flu Clinic, established by the Department of Community and Family medicine was the first point of contact for all influenza-like illness (ILI) patients, COVID-19 suspects and COVID-19 confirmed patients for further management in the hospital. The study participants included all the documented ILI patients, suspects and confirmed COVID-19 cases coming to the flu clinic during the above-mentioned period.

Inclusion criteria

The study included all the documented patients (COVID-19 suspects and laboratory-confirmed COVID-19 cases) who attended the flu clinic during the study period and gave consent to participate in the study. A well-informed written consent was taken from the eligible patients. If the patient was unable to give consent, the attendant of such patients were asked for written consent. The consent was taken by the co-investigators who aided in data collection process. The details like name and other confidentiality details were not revealed during the time of analysis.

Exclusion criteria

Patients less than 18 years of age were excluded as COVID-19 vaccination of <18 years in India started in January 2022 only. Also, the patients refusing to participate were not considered in this study

Sample size calculation

The representative target sample size needed, to achieve the study objectives and sufficient statistical power, was calculated with a sample size calculator.[14] The sample size was calculated to be 377, using a margin of error of 5%, a confidence level of 95%, and a 50% response distribution. However, all the patients who attended the flu clinic during the study period were included in the study. A consecutive sampling method was used to arrive at the sample size.

Data collection method

A pretested, structured questionnaire/study tool was designed to collect the relevant information from the patients attending the flu clinic through a face-to-face interview. The questionnaire was incorporated into "google forms" and was administered by the residents and interns posted at the flu clinic.[15] The residents and interns were trained on the study tool and data collection process by the investigators of the study.

Questionnaire design and validation

The questionnaire was divided into three sections. Section A captured the basic details of the patients including sociodemographic details like age, gender, occupation, education, and possession of ration cards. Section B included details regarding comorbidity(s), details regarding COVID-19 vaccine status, history of COVID-19 and

any history of coming in contact with COVID-19 confirmed case without protection and any history of travel to high risk/containment areas designated by the Government of India in last two weeks. Section C contained details regarding present COVID-19 status whether the patient is a suspect or laboratory-confirmed case, presenting symptoms, the status of oxygen saturation and whether the patient was ambulatory or non-ambulatory at the time of flu clinic visit and advice given at the flu clinic whether to home isolate or admit in the hospital. The questionnaire developed in English was translated to Hindi (the local language) and pretested in a sample of patients just before the data collection and necessary changes were made and back-translated to English. The final English version of the questionnaire was incorporated into the "Google forms". Few copies of the Hindi version were kept at the flu clinic for uniformity in administration. The questionnaire had a good internal consistency (Cronbach's alpha- 0.7).

Outcome variable

The main outcome of the study was to assess the clinical severity (asymptomatic/mild/moderate/severe) of the COVID-19 suspects/confirmed cases during the third wave of COVID-19.

Explanatory variables

Variables like age, gender, education, occupation, socioeconomic status, contact with COVID-19 confirmed cases without protection and COVID-19 vaccination status were used to explain the outcome of COVID-19 patients.

Data management and statistical analysis

The information collected was entered in MS Excel and analysis was done using IBM SPSS version 22. (SPSS Inc., Chicago, IL, USA) Results were either tabulated or represented graphically wherever necessary. The quantitative variables like the age of the patient were expressed as Mean (SD) after checking the normality. The categorical variables like gender, education, occupation, history of travel to high risk/crowded place, COVID-19 vaccination status, history of COVID-19, co-morbidities, the status of the patient at flu clinic, and present COVID-19 status were expressed as proportions and percentages. For this study, education status was categorised into illiterate and literate, occupation was classified into health care workers (HCW) and non-HCW/general population and the interval between 2nd dose of COVID-19 vaccination and flu clinic visit was < 6 months and >6 months, comorbidities as present or absent and presenting complaints as asymptomatic, non-ILI symptoms, ILI symptoms. The severity of COVID-19 was classified based on SPO2 status as jointly given by the Indian council of medical research, AIIMS New Delhi and Ministry of Health and Family Welfare, Government of India guidelines updated on January 2022.[16] Patients were classified as mild (SPO2 >93%), moderate (SPO2 ≤93 to >90%) and severe COVID-19 (SPO2 ≤90%). For ease of analysis, asymptomatic-mild cases and moderate-severe cases were clubbed. A simple binary logistic regression analysis was done to find out whether the unadjusted odds and values of $P < 0.05$ were considered statistically significant.

Results

Weekly trend

A total of 623 patients were enrolled in the study over six weeks (8 January to 19 February 2022). The week-wise trend of patient attendance at the flu clinic for screening and admission has been depicted in Figure 1. The line diagram reflects a declining trend for suspected as well as confirmed COVID 19 cases, with a peak number of cases during the first week (Suspected- 210, confirmed-120) which gradually touched the lowest numbers during the 6th week (Suspected- 07, confirmed-03) (Figure 1).

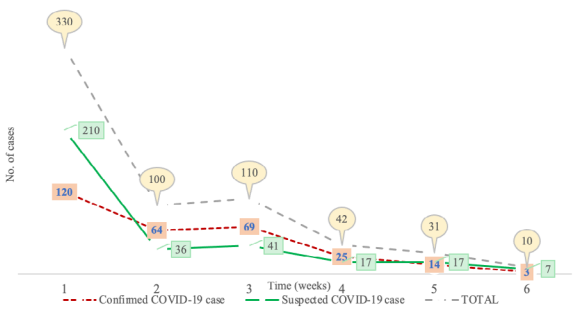

Fig. 1. Week-wise trend of patient attendance at Flu Clinic (n=623)

Socio-demographic details of the patients

The age of the patients ranged from 19 to 90 years with the mean age being 35.4 ± 14.7 years. The majority (54.6%) were male with a male to female ratio of 1.2:1. Of all the cases, 372 (59.7 %) were healthcare workers. Majority patients [570, (91.5%)] were literate.

A total of 239 (38.4%) cases had a history of travel to high-risk/ crowded places and 399 (64%)

were exposed to laboratory-confirmed COVID-19 cases. Nearly, a quarter [158 (25.4%)] of patients reported suffering from COVID-19 in the past (Table 1).

Vaccination details

Almost 90% of the patients were vaccinated against COVID-19 with at least one dose and almost 69% had taken both the doses of COVID-19 vaccine. Two third (66.8%) were vaccinated with Covaxin, 19.9% by Covishield. Almost 10% were not vaccinated with any of the COVID-19 vaccines (Figure 2).

Table 1. General characteristics of patients attending flu clinic (n=623)

Variables	Category	n	%
Age (in years)	<60	559	89.7
	≥60	64	10.3
Gender	Female	283	45.4
	Male	340	54.6
Education	Illiterate	53	8.5
	Literate	570	91.5
Occupation	HCW	372	59.7
	Non-HCW (General population)	251	40.3
History of travel to high-risk/ crowded places	Yes	239	38.4
	No	384	61.6
COVID-19 vaccination status	Not vaccinated	64	10.3
	Vaccinated with one dose	131	21
	Vaccinated with Two doses	428	68.7
History of any contact with confirmed COVID-19 case	Don't Know	79	12.7
	No	145	23.3
	Yes	399	64
History of suffering from COVID-19 in the past (confirmed by lab test)	No	465	74.6
	yes	158	25.4
The interval between flu clinic visit and 2nd covid vaccine dose*(in months)	0- 6	136	31.8
	>6	292	68.2
Comorbidity	Absent	483	77.5
	Present	140	22.5

*n= 428 as 132 who did not remember the vaccination month or are not eligible for 2nd dose were excluded from the analysis; HCW – health care worker

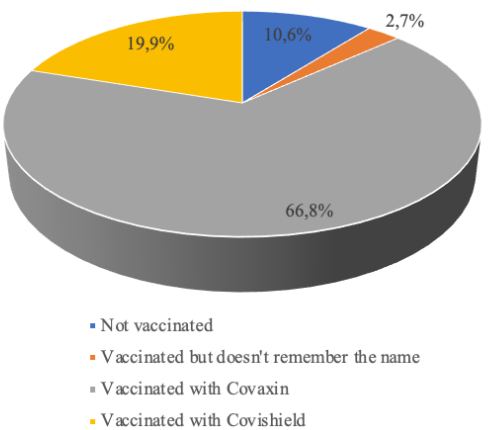

Fig. 2. Vaccination status of patients as per the type of vaccine (n=623)

Clinical details

Comorbidities were present in 140 (22.5%) patients. Out of those 140 cases, hypertension and cancer were the most common comorbidity i.e., 42 (30%) each; followed by diabetes mellitus (38, 27.1%), heart disease (19, 13.5%), kidney diseases (10, 7.1%) and other comorbidities (22, 15.7%).

Almost half of the total patients (47.4%) were laboratory-confirmed COVID-19 cases and the rest (52.6%) were suspected cases of COVID-19. The mean (SD) CT value of the lab-confirmed COVID-19 cases was 22.9 (5.3).

Majority (84.4%) had ILI symptoms such as cough (360, 57.7%), fever (359, 57.3%), sore-throat (202, 32.4%) etc. Whereas, 2.9% had non- ILI symptoms such as loss of smell and taste (11, 1.7%), vomiting (9, 1.4%) (Figure 3).

The majority (81.3%) were ambulatory and the admission rate was only 14.1%. Only 3.4% (21) were suffering from moderate-severe COVID-19 infection. The mean(SD) oxygen saturation of mild and moderate-severe COVID-19 infection was 98.2 (0.9) % and 88.8 (7.6) % respectively (Table 2).

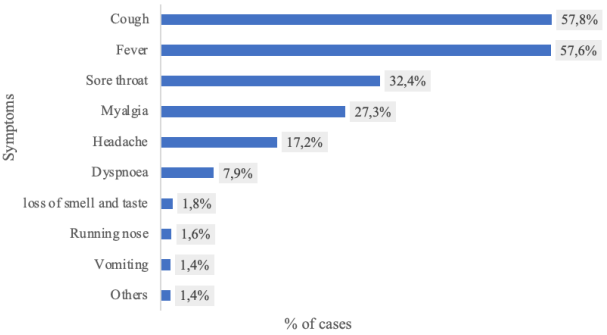

Fig. 3. Distribution of patients as per the presenting symptoms (n=623)*

*NB: ILI symptoms – fever, cough, sore throat, running nose, headache, myalgia, dyspnoea; Non-ILI symptoms – loss of smell and taste, vomiting; Others – abdominal pain, loose stools, chest pain, loss of consciousness

Table 2. Distribution of patients as per status assessed at flu clinic (n=623)*

Characteristics	Frequency	%
Presenting symptoms		
Asymptomatic	79	12.7
Non- ILI symptoms	18	2.9
ILI symptoms	526	84.4
Status of Patient		
Ambulatory	506	81.3
Non- ambulatory	117	18.7
Present COVID-19 status		
Confirmed COVID-19 case	295	47.4
Suspected COVID-19 case	328	52.6
Advice given at the flu clinic		
Admission to the COVID-19 ward	88	14.1
Home isolation	535	85.9

*NB: ILI-Influenza-like illness, ILI symptoms – fever, cough, sore throat, running nose, headache, myalgia, dyspnoea; Non-ILI symptoms – loss of smell and taste, vomiting; Others – abdominal pain, loose stools, chest pain, loss of consciousness

Patients aged > 60 years ($p<0.001$) and those having one or more than one comorbidity ($p<0.001$) suffered from moderate-severe COVID-19 infection more than their counterparts. Also, 2.1% of patients who were fully vaccinated, 3.8% of those with one dose vaccinated and 10.9% of unvaccinated patients suffered from mod-

erate-severe COVID-19. This difference in the severity of COVID-19 among vaccinated and unvaccinated patients was statistically significant (p<0.003). Moderate-severe COVID-19 infection was reported only among the general population whereas all the HCW reporting to the flu clinic were either asymptomatic or had a mild infection (Table 3).

Table 3. Association of general characteristics of patients with severity of COVID-19 (n=295)*

Characteristics	COVID 19 severity		Unadjusted Odds ratio (95 % CI)	Test statistics (p-value)
	Asymptomatic/ mild (n=278)	Moderate-severe (n=17)		
Mean age (± SD)	40.4 (17)	61.9 (18.9)	–	5.01 (<0.001)
Age category				
< 60 years	229 (97.5)	6 (2.5)	1	19.1 (<0.001)
≥ 60 years	49 (81.7)	11 (18.3)	8.4 (3–25.8)	
Gender				
Female	132 (94.9)	7 (5.1)	1	0.065 (0.8)
Male	146 (93.6)	10 (6.4)	1.3 (0.5–3.6)	
Education				
Illiterate	46 (92)	4 (8)	1	0.169 (0.5)
literate	232 (94.7)	13 (5.3)	0.64 (0.2–2.38)	
Occupation				
Health care worker	108 (100)	0 (0)	–	–
Non- Health care worker	170 (90.9)	17 (9.1)		
Comorbidity				
Absent	174 (99.4)	1 (0.6)	1	19.06 (<0.001)
Present	104 (86.7)	16 (13.3)	26.7 (3.4–204.8)	
Have you taken any COVID-19 vaccine?				
Not vaccinated	57 (89.1)	7 (10.9)	–	13.44 (0.003)
Vaccinated with one dose	126 (96.2)	5 (3.8)		
Vaccinated with two doses	419 (97.9)	9 (2.1)		
The interval between flu clinic visit and 2nd covid vaccine dose*				
0-6 months	69 (93.2)	5 (6.8)	0.2 (0.03–1.5)	1.27 (0.241)
> 6 months	94 (97.9)	2 (2.1)	1	
History of any contact with confirmed COVID-19 case?				
No	90 (90.9)	9 (9.1)	0.38 (0.11–1.1)	2.1 (0.09)
Yes	131 (96.3)	5 (3.7)	1	
Past H/O suffering from COVID-19 (confirmed by lab test)				
No	228 (93.8)	15 (6.2)	0.6 (0.09–2.4)	0.106 (0.745)
Yes	50 (96.2)	2 (3.8)	1	
History of travel to high risk/containment areas/to a crowded place				
Yes	91 (96.8)	3 (3.2)	2.2 (0.68–10.1)	1.05 (0.284)
No	187 (93.1)	14 (6.9)	1	

*n= 428, Values are expressed as n (%) unless specified.
SD – standard deviation; IQR – interquartile range

Discussion

Considerable disparities in demographic and clinical patterns have been observed across three consecutive COVID-19 pandemic waves. This study demonstrated the clinical and vaccination profile of COVID-19 patients from eastern India during the third wave pandemic. Understanding the profile of patients and the nature of severity of the disease will provide direction for the policymakers and better preparedness against subsequent waves of COVID-19.

Socio-demography

In this study, there was a male predominance which is analogous to other national and international studies by Aggarwal et al. (New Delhi), Guan et al. (China), Hasan et al. (Bangladesh).[11,12,17] Few factors which could be speculated to account for this gender gap are the outdoor engagement of males, lesser propensity to seek health care among females in the society and differences in biology.[10,18]

Clinical details

In this study, there were more symptomatic patients than asymptomatic patients (87.3% vs. 12.7 %). Since the study setting is a tertiary health care centre, more symptomatic patients seek medical assistance than high-risk asymptomatic contacts.

Consistent with our study findings, Mohammad Jahid Hasan found that 13.09% of COVID-19 patients were asymptomatic.[17] While Mizumoto et al. found a relatively higher prevalence (34.6%) of asymptomatic on board the Diamond Princess cruise ship, Japan.[19]

The most common symptoms reported during this third wave were fever, cough and sore throat. The Gastrointestinal symptoms (vomiting, loose stools) which were added in the second wave when the delta variant of COVID was dominant were minimal.[20,21] Furthermore, the more frequently reported symptoms of loss of smell and taste during the first covid wave were almost negligible during this omicron dominant third wave.[22]

In our study, we found that the third wave was less serious in terms of oxygen requirement and severity of COVID-19 when compared to the first and second waves of the COVID-19 pandemic. Baseline oxygen saturation [mean (SD)] was higher for the third wave [97.7 %] when compared with the second wave [84 (13.4) %] and first wave [91.9 (7.4) %] [2122]. Furthermore, we found that only 5.7% of cases were severe COVID-19 in the third wave compared to 70.2 % in the second wave and 37.5% in the first wave.[23]

Vaccination details

The hybrid immunity acquired through vaccination and high prior exposure to the Delta variant might be accountable for keeping the severe illness proportion low during the third wave.[24]

This fact is also evident from our study findings as vaccinated patients were less like to suffer severe covid

infection as compared with the unvaccinated ones. However, Rahman et al. investigated the clinical features of COVID-19 infection among infection-naïve, vaccinated, and post-infection-vaccinated individuals. They concluded that the naturally infected individuals were less likely to be reinfected by COVID-19 infection than the infection-naïve and vaccinated individuals. The low number of vaccinated individuals in their study might be a limitation to correlate the vaccination status with hospitalisation or severity.[25] Furthermore, a study by Moghadas et al. established the notable impact of vaccination as well.[26] They found that vaccination considerably reduced adverse outcomes with non-ICU hospitalizations, ICU hospitalizations, and deaths by 63.5%, 65.6%, and 69.3% respectively.

Comparison between the first, second and third waves of the COVID-19 pandemic

The elderly population was found to be less compared to others in this study as compared with the first wave (32.5%) and second wave (27.8%).[26,27] This indicates age shifting phenomenon. One possible reason could be higher vaccination coverage among the elderly. This also implies we should be more vigilant about infection among young individuals and others. Also, our study highlighted that geriatric age had a significant association with moderate/severe infection. Extreme ages are at augmented risk of developing any severe infection which possibly explains this finding. This observation is parallel to other studies.[10,28–30]

Furthermore, an undersized proportion (13.3%) of patients presented with any comorbidity in our study, compared with 21.7 % and 21.1% of patients with the coexisting condition reported during the first and second wave respectively.[27] Although Moderate/Severe Covid infections were higher in patients with one or more comorbidities. Backing our study findings, Vardhan H mentioned in their study paper that the SARS-CoV-2 infection causes more severe illness in patients with comorbid diseases such as diabetes and hypertension.[30] Sharing similar findings, Zhang et al. studied 633 COVID-19 patients in China, of whom 247 patients had at least one comorbidity and were more likely to exhibit a more severe form of COVID-19 illness.[31] Similarly, another study conducted in Spain had more severe illnesses among patients with comorbidities.[32]

Limitation and strength of the study

Because this is a hospital-based study, the actual proportion of COVID-19 patients based on various severity categories could not be evaluated in a way that could be contemplated to the general population. Only status on arrival was assessed and the final severity outcome could not be assessed due to lack of follow-up. There is a possibility of a change in severity status. Despite these limitations, our study's merit lies in establishing the association of age, comorbidity and vaccination with the severity of COVID-19.

Conclusion

During the third wave of the COVID-19 pandemic, a smaller number of elderlies compared to the previous two waves were affected indicating age shifting. The severity of COVID-19 was less among COVID-19 vaccinated individuals compared to unvaccinated highlighting the importance of COVID-19 vaccination. People with comorbid conditions were found to be suffering from severe COVID-19 more compared to people without any comorbidities. Necessary vigilance about the people with comorbid conditions and strict adherence to COVID-19 appropriate behaviour among them is needed. This observation from our study might be useful in case a plausible fourth COVID-19 pandemic wave emerges in India in the coming future due to the mutant nature of the SARS-CoV-2 virus.

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Declarations

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Author contributions

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

Conflicts of interest

The authors declare no competing interests and no conflict of interests.

Data availability

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

Ethics approval

This study was approved by Institute Ethics Committee, AIIMS Patna (Ref: AIIMS/Pat/IEC/2022/859). We adhered to the principles of ethics thereafter throughout the study.

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ORIGINAL PAPER

Effect of the prognostic nutritional index and systemic immune-inflammatory index in predicting short-term mortality in geriatric patients with SARS-CoV-2 infection

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ABSTRACT

Introduction and aim. We aimed to investigate whether systemic immune inflammatory index (SII) and prognostic nutritional index (PNI) were associated with short-term mortality in geriatric patients with SARS-CoV-2.

Material and methods. Our study was designed retrospectively. The data of patients that presented to a single center. The primary outcome of the study was the diagnostic value of SII and PNI in predicting 28-day mortality in geriatric patients with SARS-CoV-2 pneumonia.

Results. 272 geriatric patients with SARS-CoV-2 included. The median PNI was 42.5, and the median SII was 687.6 (430–1404.2). In univariant analysis, PNI and SII has a significant relationship with mortality ($p<0.001$ and $p=0.008$, Mann-Whitney U test). PNI had an area under the curve (AUC) value of 0.680, which was significantly higher than that of SII (AUC: 0.6). The odds ratio of PNI (>40.1) and SII (<1.267) for 30-day mortality were determined as 1.12 , and 1.

Conclusion. In conclusion, the blood tests used to calculate PNI and SII are routinely included in complete blood count and biochemistry tests that can be performed in every hospital. According to the results of the current study, the mortality group had significantly higher SII values and significantly lower.

Keywords. mortality, SARS-Cov-2 infection, PNI

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) started in 2019 in Wuhan, China. It soon became a pandemic, resulting in the death of more than five million people. SARS-CoV-2 causes an inflammatory disease transmitted by droplets and progresses from clinically asymptomatic to acute respiratory syndrome in the lungs. It has a more mortal course in geriatric patients, associated with weakened immunity, poor nutrition, and comorbidities in this patient population.[1,2] The prognostic nutritional index (PNI), calculated using the lymphocyte count and serum albumin levels, is an indicator of nutrition and immunity.[3] Some studies have shown PNI to be a useful marker for predicting long-term outcomes in patients receiving chemotherapy.[4] The systemic immune inflammatory index (SII) is a new leukocyte-based inflammatory index developed in the last decade. It has been suggested that SII can be a prognos-

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tic determinant in patients.[5] SII has been reported to be associated with mortality in various cancers, including pancreatic, hepatocellular, lung, stomach, and esophageal cancers. SII was also found as mortality predictor in coronary artery disease.[6] Studies have been conducted in which SII was able to predict poor outcomes in patients with venous sinus thromboses.[3]

Patients infected with SARS-CoV-2 have increased inflammation, which affects blood parameters, such as lymphocyte, neutrophil, thrombocyte, and albumin values.

Aim

In this study, we aimed to investigate SII and PNI in terms of their possible association with short-term mortality in geriatric patients with SARS-CoV-2.

Material and methods

Study design and ethical approval

This study was designed retrospectively and conducted in a single center; at the emergency department of the University of Health Sciences Umraniye Training and Research Hospital. The data of patients that presented to our hospital between November 01, 2021, and March 01, 2022, and were confirmed to have SARS-CoV-2 based on a positive reverse transcription-polymerase chain reaction (RT-PCR) test were screened. Ethical approval was obtained from the local ethics committee on March 31, 2022 and number 119. All the stages of the study followed the tenets of the Declaration of Helsinki.

Study population

The patients included in our study were all aged over 65 years and presented to the emergency department with symptoms of SARS-CoV-2 (weakness, myalgia, cough, fever above 37.5 °C, shortness of breath, loss of taste and smell, diarrhea, sore throat, and nausea) and were admitted to the inpatient ward or intensive care unit. The RT-PCR test results of all the patients were positive. Outpatients and patients with missing data were excluded from the study.

Data collection

All the patients were brought to the emergency department via outpatient or emergency ambulance services. Patient data were obtained from the hospital computer-based data system. The patients' demographic data and blood parameters, including hemoglobin, whole blood cell count, neutrophil count, thrombocyte cunt, lymphocyte count, hematocrit, albumin, lactate, C-reactive protein, blood urea nitrogen (BUN), platelet, mean platelet volume, and plateletcrit values were recorded. In addition, 28-day all-cause mortality data were obtained from the hospital computer-based data system and the national mortality reporting system. SII was calculated using the following formula: platelets × neutrophils/lymphocytes, and PNI was calculated as follows: 10 × serum albumin (g/dL) + 0.005 × total lymphocyte count. Lastly, the C-reactive protein/albumin ratio (CAR) and the BUN/albumin ratio (BAR) were determined.

Outcomes

The primary outcome of the study was the diagnostic value of SII and PNI in predicting 28-day mortality in geriatric patients with SARS-CoV-2 pneumonia.

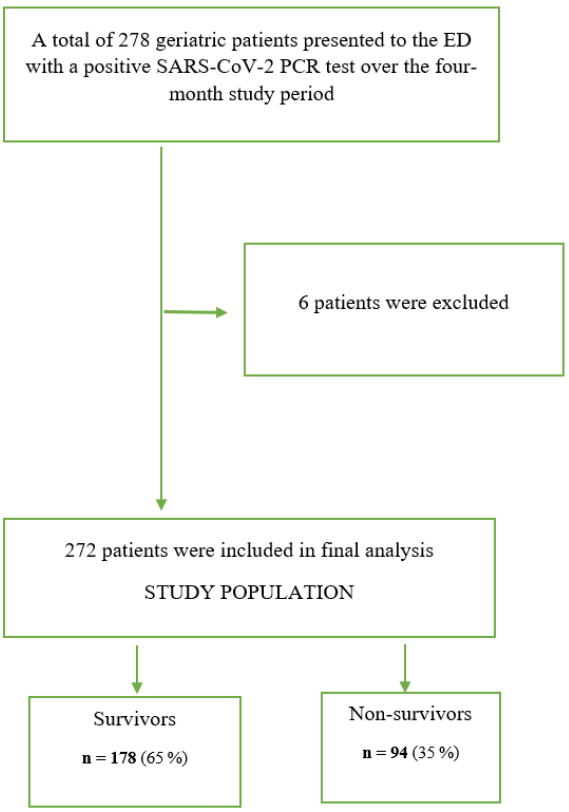

Fig. 1. A flow diagram of the study

Statistical analysis

Data were analyzed using Jamovi (Version 2.3.12.0; The Jamovi Project, 31 May 2022; R Core Team, 2022). The conformity of continuous variables to the normal distribution was examined using the Shapiro-Wilk test. Categorical variables were expressed as percentages, and continuous variables as median (interquartile range) values. The differences between the survivor and non-survivor groups were compared using the Mann–Whitney U test for non-normally distributed quantitative variables. A p value of <0.05 was considered statistically significant. The receiver operating characteristic (ROC) analysis was performed to determine the power of statistical mortality-related parameters in predicting mortality, and the results of this analysis were shown with area under the curve (AUC), cut-off, sensitivity, and specificity values, and 95% confidence intervals (CIs). Odds ratios (OR) were calculated by using two-by-two frequency tables of ratios. ORs were used to compare the ability of the pa-

rameters to predict mortality. A p value of <0.05 was considered statistically significant.

Results

A total of 278 geriatric patients presented to the emergency department of our hospital due to SARS-CoV-2. Of these patients, six were excluded using the study criteria (Figure 1).

As a result, 272 patients were included in the sample. The median (25^{th}–75^{th} percentile) age value was 77 (71–84) years, and 146 (53.7%) of the patients were female. Baseline characteristics of the enrolled patients and their comparison between the survivor and non-survivor groups are shown in Table 1, and the laboratory parameters of the enrolled patients and their comparison between the survivor and non-survivor groups are given in Table 2.

Table 1. Baseline characteristics of the enrolled patients and their comparison between the survivor and non-survivor groups

	Total (n=272)	Survivor (n=176)	Non–survivor (n=96)	p value
Age (years)	77 (71–84)	75 (70–82)	81.5 (74–87)	<0.001
Female	146 (53.7%)	100 (56.2%)	46 (48.9%)	0.312
Male	126 (46.3%)	78 (43.8%)	48 (51.1%)	
Comorbidities				
Hypertension	206 (75.7%)	135.0 (76.7%)	71 (74%)	0.614
Diabetes mellitus	109 (40.1%)	68.0 (38.6%)	41.0 (42.7%)	0.513
Malignancy	26 (9.6%)	16.0 (9.1%)	10.0 (10.4%)	0.722
Hypothyroidism	11 (4.0%)	7 (4%)	4.0 (4.2%)	0.940
Hyperthyroidism	3 (1.1%)	0 (0%)	3.0 (3.1%)	0.018
Hyperlipidemia	124 (45.6%)	75 (42.6%)	49 (51%)	0.182
Obesity	2 (0.7%)	0 (0%)	2.0 (2.1%)	0.055
Alzheimer's disease	23 (8.5%)	13 (7.4%)	10.0 (10.4%)	0.391
Epilepsy	17 (6.2%)	11 (6.2%)	6.0 (6.2%)	0.999
Chronic obstructive pulmonary disease	49 (18.0%)	31 (17.6%)	18.0 (18.8%)	0.816
Coronary heart disease	101 (37.1%)	63 (35.8%)	38.0 (39.6%)	0.537
Asthma	51 (18.8%)	33 (18.8%)	18.0 (18.8%)	0.999
Congestive heart failure	31 (11.4%)	17 (9.7%)	14.0 (14.6%)	0.222
Chronic kidney disease	23 (8.5%)	14 (8%)	9.0 (9.4%)	0.687

For the whole sample, the median neutrophil–to–lymphocyte ratio (NLR) was 3.8 (2.5–7.5), the median PNI was 42.5 (39.2–45.8), and the median SII was 687.6 (430.0–1404.2). Mortality occurred in 94 (35%) patients within 28 days. PNI, SII, NLR, CAR, and BAR has a significant relationship with mortality (p<0.001, p=0.008, p<0.001, p<0.001, and p<0.001, respectively). The ROC curve analysis revealed that a low PNI had potential predictive value in the prognosis of geriatric patients (Figure 2).

Figure 2 present the AUC values of PNI for short–term mortality according to the ROC analysis. PNI had an AUC value of 0.680 (95% CI: 1.07–1.18), which was significantly higher than that of SII (AUC: 0.600). The OR of PNI (>40.1) and SII (<1.267) for 30-day mortality were determined as 1.12 (95% CI: 1.07–1.18), and 1.0 (95% CI: 1–1), respectively.

Table 2. Laboratory parameters of the enrolled patients and their comparison between the survivor and non–survivor groups

Variables	Total n=272 (%, 25^{th}–75^{th} percentile)	Non-survivors n=94 (35%) (%, 25^{th}–75^{th} percentile)	Survivors n=178 (65%) (%, 25^{th}–75^{th} percentile)	p values
Lactate (mmol/L)	1.7 (1.3–2.1)	1.6 (1.2–2)	1.8 (1.3–2.2)	0.027
Albumin (g/dl)	36.7 (34.1–39.1)	37.5 (34.8–39.7)	35.2 (32.8–37.5)	<0.001
C–reactive protein (mg/dl)	5.1 (1.8–10)	4.0 (1.1–7.4)	8.5 (4.0–15.9)	<0.001
Blood urea nitrogen (mg/dl)	44.9 (34.2–71.2)	44.9 (34.2–63.7)	55.6 (38.5–88.8)	0.004
White blood cell count (10^3/µl)	6.5 (4.9–8.5)	6.2 (4.9–8.0)	7.4 (4.8–9.8)	0.029
Neutrophil count (10^3/µl)	4.6 (3.4–6.5)	4.3 (3.3–6.0)	5.4 (3.6–7.6)	0.004
Lymphocyte count (10^3/µl)	1.1 (0.8–1.6)	1.2 (0.8–1.7)	1.0 (0.7–1.3)	0.013
Hemoglobin (g/dl)	12.8 (11.4–13.8)	12.8 (11.6–13.8)	12.9 (11.1–13.7)	0.77
Hematocrit (%)	38.5 (34.9–41.5)	38.1 (35.5–41.5)	38.8 (34.2–42)	0.984
Platelet count (10^3/µl)	185 (149–245)	189.0 (152–247.8)	177.5 (148.2–211)	0.157
Mean platelet volume (fl)	9.8 (9–10.8)	9.8 (9–10.8)	10 (9.1–10.7)	0.591
Plateletcrit (%)	0.2 (0.1–0.2)	0.2 (0.2–0.2)	0.2 (0.1–0.2)	0.236
Platelet distribution width (%)	16.3 (16–16.6)	16.3 (16.0–16.5)	16.3 (16.1–16.6)	0.561
Neutrophil/lymphocyte ratio	3.8 (2.5–7.5)	3.3 (2.3–5.9)	5.5 (2.7–9.4)	<0.001
Platelet/lymphocyte ratio	164.9 (120.9–257.4)	157.3 (118.7–243.8)	189.0 (122.2–278.5)	0.092
C-reactive protein/albumin ratio	0.1 (0–0.3)	0.1 (0–0.2)	0.2 (0.1–0.4)	<0.001
Blood urea nitrogen/albumin ratio	1.3 (0.9–2.1)	1.1 (0.9–1.8)	1.6 (1.1–2.6)	<0.001
Prognostic nutritional index	42.5 (39.2–45.8)	40.3 (36.8–43.9)	43.6 (40.8–47.1)	<0.001
Systemic immune–inflammation index	687.6 (430–1404.2)	861.6 (492.8–1785.7)	667.0 (383.1–1200.7)	0.008

Table 3 present the accuracy of PNI and SII in predicting short term mortality in geriatric patients with SARS-CoV-2 infection.

Discussion

In this study, we evaluated the laboratory parameters of geriatric patients with SARS-CoV-2 at the time of their presentation to the emergency department. We investigated the predictive power of PNI and SII in short–term mortality. We determined that the PNI and SII values were associated with mortality (p<0.001 and p=0.008, respectively). An increase in the SII value and a decrease

in the PNI value were affected the results. The cut-off value was calculated as 40.1 for PNI and 1,267 for SII.

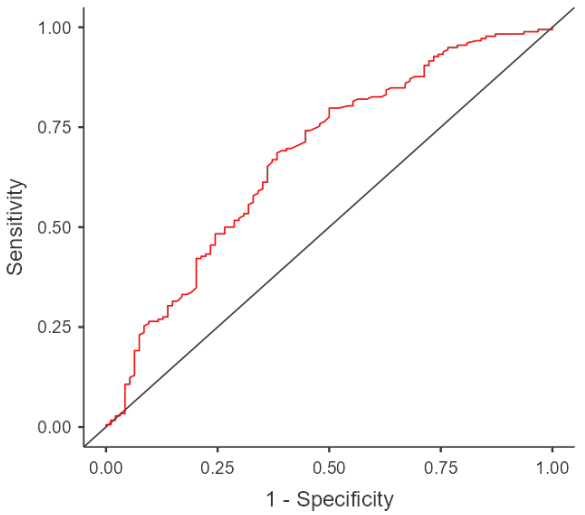

Fig. 2. ROC curve of the PNI in predicting in short term mortality of geriatric patients

Table 3. Accuracy of prognostic nutritional index and systemic immune-inflammation index in predicting short term mortality in geriatric patients with SARS-CoV-2 infection

	Prognostic nutritional index	Systemic immune-inflammation index
Sensitivity	50 %	37 %
Specificity	80 %	79 %
Accuracy	69 %	64 %
Prevalence	35 %	35 %
Positive Predictive Value	57 %	48 %
Negative Predictive Value	75 %	70 %
Positive Likelihood Ratio	2.47	1.74
Negative Likelihood Ratio	0.63	0.8
Area under the curve	0.68	0.6
95% confidence interval values	0.538–0.678	0.618–0.752
Cut-off	40.1	1,267

Due to the high levels of inflammatory markers in the elderly, their frailty increases, and defense against external stimuli and diseases decreases.[7] SARS-CoV-2 creates an inflammatory disease and affects older individuals to a greater extent, and as a result mortality is higher in the geriatric population.[8]

It has been shown that PNI can be used to predict the poor outcomes of various diseases.[9] Proinflammatory cytokines and albumin level affected by nutrition are used in the calculation of PNI. Studies have found that PNI predicts prognosis in patients with malignancies.[10] Matsuda et al. showed that PNI was significant in predicting the risk of postoperative complications (AUC=0.609, cut-off=50, p=0.008).[11] In another study aiming to predict the prognosis with PNI in patients diagnosed with COVID-19, Hu et al. evaluated 122 patients and associated a low PNI score with a poor prognosis (odd ratio: 0.797; p=0.03).[12] Ikeya et al. showed that PNI was a useful marker in predicting long–term outcomes in patients receiving chemotherapy (p=0.001).[3] Soeters et al. concluded that albumin was associated with inflammation, reporting a decrease in albumin mass and half–life in diseases causing inflammation, which was also associated with mortality.[13] Baldemir and Alagöz recommended starting nutritional support early in geriatric patients with chronic obstructive pulmonary disease considering that the albumin level was affected by nutritional status and reduced albumin was associated with mortality.[14] Since SARS-CoV-2 is an inflammatory disease, a decrease in the albumin level is expected. In our study, there was a positive correlation between low albumin and mortality (p<0.001). Similar to previous studies, there was a significant correlation between mortality and PNI in geriatric patients in our study (AUC=0.68, p<0.001, odds ratio: 1.12, 95% CI: 1.07–1.18). Inflammation that develops during the SARS-CoV-2 disease process coupled with inflammation observed in the elderly affect the parameters that constitute PNI. This can explain the significant relationship between PNI and mortality due to SARS-CoV-2 in geriatric patients.

Lymphopenia predicts clinical worsening in SARS-CoV-2 disease.[15, 16, 17, 18] NLR is calculated using lymphocyte values. Özdemir et al. showed that NLR could predict short–term mortality in their study with more than 2000 SARS–CoV–2 infected patients.[2] Yang et al. showed that NLR was associated with a poor prognosis in patients with SARS-CoV-2.[17] In our study, there was a significant relationship between NLR and mortality (p<0.001).

Platelet, neutrophil, and lymphocyte values change in SARS-CoV-2 disease according to the severity of inflammation.[18] SII is calculated using these values. Fois et al. showed a significant correlation between SII and mortality in patients with SARS-CoV-2 (p=0.039).[19] In our study, a high SII level was also associated with mortality (p<0.001), which is consistent with the literature.

Limitations of the study

There were some important limitations to our study. First, our number of patients was limited and the sample represented patients that presented to the hospital over a certain period. Second, during the COVID–19 pandemic, the virus underwent several mutations, and patient mortality and adverse outcomes varied according to the nature of the virus. Third, our study was single–centered, and therefore the data obtained from our study may differ from domestic and international data. Multicenter controlled randomized studies with a larger number of patients should be conducted.

Conclusion

In conclusion, the blood tests used to calculate PNI and SII are routinely included in complete blood count and biochemistry tests that can be performed in every hos-

pital. As a result, PNI and SII are easily accessible and easy to calculate. According to the results of the current study, the mortality group of geriatric SARS-CoV-2 infected patients had significantly higher SII values and significantly lower PNI values. Multicenter controlled randomized studies with a larger number of patients should be conducted to verify our results.

Declarations

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Author contributions

Conceptualization, A.Ö; Methodology, A.Ö., M.İ.; Software, S.E.; Validation, G.A.; Formal Analysis, A.Ö; Investigation, A.Ö.; Resources, A.Ö.; Data Curation, S.E.; Writing – Original Draft Preparation, H.A.; Writing – Review & Editing, A.Ö.; Visualization, S.E.; Supervision, S.E.

Conflicts of interest

The authors declare no competing interests.

Data availability

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

Ethics approval

Study was approved by the institutional review board, and a waiver of authorization was given (Ethics Committee decision no. 119, date: 31/03/2022).

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ORIGINAL PAPER

Determination of post-traumatic growth status of frontline infection control nurses in the COVID-19 pandemic – a cross-sectional study

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ABSTRACT

Introduction and aim. It is very important for nurses to experience post-traumatic growth in order to protect their mental health after traumatic events such as a pandemic**.** The aim of this study is to determine the post traumatic growth status of infection control nurses, who play an important role in health services in the COVID-19 pandemic.

Material and methods. This study is a cross-sectional, descriptive study. The study was conducted with 170 infection control nurses working in infection control committees of hospitals in Turkey. "Nurse Descriptive Information Form" and "Post Traumatic Growth Inventory (PTGI)" were used as data collection tools in this study.

Results. As a result of this research, the mean PTGI total score of the infection control nurses was 70.73±23.03, and it was determined that they experienced moderate growth from the sub-dimensions of the scale. Also it was determined that there was a statistically significant difference between the changes in philosophy of life sub-dimension scores of PTGI according to the age and marital status of the nurses. In addition, it was determined that there was a significant difference between the total PTGI scores according to the year of working as an infection control nurse and the loss of a relative of the healthcare worker due to the COVID-19 disease.

Conclusion. In this study, it can be said that infection control nurses experienced a moderate post-traumatic growth after the COVID-19 pandemic. Age, marital status, working year and loss of a healthcare worker friend during the pandemic period seem to affect nurses' post-traumatic growth. It is very important to determine the mental health of infection control nurses working on the front lines in the pandemic.

Keywords. COVID-19 pandemic, mental health, nurses, posttraumatic growth

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Introduction

The Coronavirus 2019 (COVID-19), which emerged in Wuhan, China in December 2019 and spread rapidly in many countries and continents, has come to the fore as a world epidemic that has shaken the world deeply in many ways.[1,2] As the geographical area of the rapidly spreading virus expanded, there has been a rapid increase in the number of virus-related deaths.[3] It is seen that the rapid spread of the COVID-19, resulting in death, affects the mental and physical health of people and the economy on a global scale significantly.[4] Especially globally, the health systems of many countries have come to the point of collapse under the sudden and rapid effect of COVID-19, and health workers are among the groups most affected by this pandemic.[4] The World Health Organization reported that more than 35,000 healthcare workers worldwide have been infected with the COVID-19 virus, and some have died while caring for COVID-19 patients.[2]

When COVID-19, which is a contagious disease, first emerged, there were uncertainties about the way of transmission, treatment and prevention of the disease. Due to the rapid increase in COVID-19 cases in hospitals, an important burden and responsibilities have emerged, especially for healthcare professionals working in infection control units, in order to protect patients, patient relatives and healthcare workers from disease and to prevent the spread of the disease.[4,5] In the pandemic process, diagnosis and treatment processes of patients diagnosed with COVID-19 are carried out in a multidisciplinary manner, following the hospital isolation processes, protecting the health of hospital staff, planning the distribution of personnel to be assigned to the hospital units during the pandemic period, taking the infection control measures to be followed in the hospital for the pandemic, and intermittent monitoring of the personnel in this regard. Infection control units took an active role in the planning of inspection and training, and they were under an intense overtime including long working hours.[5] In this process, especially nurses among the health workers working on the front lines, besides their intense working hours, were more exposed to the risk of virus transmission, faced with more risks, being away from their families for a long time, experiencing stigma, and dying more among their colleagues and in the patients they care for. had to testify.[4] All these situations have greatly affected the mental health of the nurses and caused them to experience stress.[6]

Coping responses to stress are a continuum of adaptive and maladaptive responses to stress. It is seen that maladaptive reactions arise from working during the pandemic in nurses providing healthcare services. These reactions include exposure to trauma that can lead to post-traumatic stress disorder, eating and socializing problems, sleep problems (such as nightmares involving negative experiences in the hospital during the COVID-19 period that is flashbacks), exhaustion, frustration, anger and depression.[6–8] However, not everyone who experiences the pandemic elicits maladaptive responses.[4,6,7] In addition to these negative consequences, traumatic events can positively change individuals in a process known as post-traumatic growth.[9] Nurses can also improve post-traumatic growth by saving lives and improving patient outcomes.[7]

The concept of "post-traumatic growth" was first introduced by Tedeschi and Calhoun in 1996.[10] Post-traumatic growth refers to the emergence of a significant positive mental change in the life of a person who survived a forcing or traumatic event such as an earthquake or tsunami.[9,10] Previous studies have found that nurses can experience psychological stress responses when working on the front lines of an infectious disease epidemic.[8,11] The COVID-19 pandemic has caused a traumatic experience especially for nurses due to its devastating effect.[11,12] It is stated that individuals can grow as a result of trauma or stressful events, which means that individuals can realize post-traumatic growth.[12] It is stated that the realization of post-traumatic growth in post-pandemic nurses can protect nurses from the psychological effects of the pandemic or improve the psychological effects that may occur after trauma.[4,7,13]

When the studies conducted after the COVID-19 pandemic are examined, it is seen that there are several studies examining the post-traumatic growth status of healthcare workers.[4,7,13,14] Studies with nurses, on the other hand, seem to have been conducted with nurses in China, although there is limited evidence.[7,12,14,15]

Aim

No study has been found to determine the post-traumatic growth status of infection control nurses working on the front lines, especially during the pandemic period. Therefore, the aim of this study is to examine the post-traumatic growth status of infection control nurses, who play an important role in health services in infectious diseases such as pandemics. It is thought that the data obtained from this study will shed light on the interventions to be made to ensure the psychological empowerment of infection control nurses.

Material and methods

Ethical approval

Ethics committee approval dated 29.08.2022 and numbered 205981 was obtained from the Human Research Ethics Committee of Zonguldak Bulent Ecevit University to conduct the research. Informed consent was obtained from the infection control nurses participating in the study online.

Study design

This study is a cross-sectional, descriptive study. The research was carried out with infection control nurses

working in infection control committees of hospitals in Turkey in September 2022.

Participants

The population of the study consists of all infection control nurses in Turkey. There is no number of infection control nurses in official sources and records in Turkey. For this reason, snowball sampling method, which is one of the non-probability sampling methods, was used as a sampling method. In this method, after the reference people related to the subject of the study were selected, other people with similar characteristics were reached through these people. The reason for preferring this method is that the number of nurses working actively as infection control nurses in Turkey has not been reached and it is thought that there will be less difficulty in reaching nurses with this method. In addition, this method was used in order to provide the expected benefit in the results to be obtained from this research as soon as possible and to obtain the results quickly. In this direction, infection control nurses who took an active role in the field during the pandemic period were taken as reference through online platforms, and other infection control nurses were tried to be reached through social media, whatsapp and telegram. In this direction, 170 infection control nurses who agreed to participate in the study at the time the research data were collected were reached.

Inclusion criteria: Being between the ages of 18-65, working as an infection control nurse during the pandemic period. Exclusion criteria; refusing to participate in the research.

Instruments

"Nurse Descriptive Information Form" and "Post Traumatic Growth Inventory" were used as data collection tools in the research.

Nurse Descriptive Information Form: This form, which was prepared by the researchers by scanning the literature, includes questions such as age, gender, education level, marital status, having a child, working year as a nurse, working year as an infection control nurse, working time in the pandemic.[12–14]

Post Traumatic Growth Inventory (PTGI): The scale is one of the most well-known psychometric tools that measure positive changes after trauma. It was first developed by Tedeschi and Calhoun in 1996.[10] The original form of the scale consists of 21 items, 6-point Likert type and 5 sub-dimensions (new possibilities, relating to others, personal strength, spiritual change, and appreciation of life).[10] The Turkish validity and reliability study of the scale was performed by Kağan et al. (2012). The Turkish version is a total of 21 items and a 6-point Likert-type (0,1,2,3,4,5) scale. In this scale, change in self-perception (lowest 0-highest 50 points; items 5, 10, 11, 12, 13, 15, 16, 17, 18, 19), change in philosophy of life (lowest 0-highest 35 points; items 1, 2, 3, 4, 7, 14) and change in relationships (lowest 0-highest 25 points; items 6, 8, 9, 20, 21).[16] The lowest and highest score that can be obtained from the scale total score is between 0 and 105. High scores obtained from the total score of the scale and in the sub-dimensions indicate that the person has experienced a high level of growth after the traumatic experience. The cronbach's alpha coefficient is 0.92, and the internal consistency of the subscales varies between 0.77 and 0.88.[16] The Posttraumatic Growth Inventory (PTGI The cronbach alpha value in this study is 0.94.

Data collection

This study data were collected by contacting infection control nurses on online platforms such as social media, whatsapp groups, and telegram, after obtaining the permission of the ethics committee. Nurses were invited to participate in the research by sharing study information, study aim and survey links on online platforms. Each infection control nurse reached was asked to reach another infection control nurse.

Statistical analysis

The data obtained in this study were evaluated in SPSS 22.0 statistical program (IBM, Armonk, NY, USA). Frequency and percentage analyzes were used to determine the descriptive characteristics of the nurses participating in the study, and mean and standard deviation statistics were used in the analysis of the scale. Kurtosis and Skewness values were examined to determine whether the research variables showed a normal distribution. It was determined that the study variables did not show a normal distribution. Non-parametric methods were used in the analysis of the data. Man Whitney U test and Kruskal Wallis analyzes were used to examine the differences in scale levels according to the sociodemographic characteristics of the nurses. The results were evaluated at the 95% confidence interval, at the $p<0.05$ level of significance.

Results

Of the infection control nurses participating in the study, 97.1% were over 30 years old, 98.2% were female, 80.0% were married, 80.6% had children and 65.5% had a bachelor's degree. While 91.2% of the nurses have been working as nurses for more than 10 years, 56.5% have been working as infection control nurses for less than 10 years. Considering the weekly working hours during the pandemic period; there was an increase in the working hours of 75.3% of the nurses. 52.4% of the infection control nurses intervened in the patient diagnosed with Covid-19, and 63.5% were diagnosed with Covid-19 disease. Of the infection control nurses participating in the study, 17.1% lost a family member due to illness during

Table 1. Personal characteristics of infection control nurses (n=170)

Characteristics of Infection Control Nurses	n	%
Age		
≤ 30	5	2.9
>30	165	97.1
Gender		
Female	167	98.2
Male	3	1.8
Education status		
Associate degree	8	4.7
Bachelor's degree	113	65.5
Master degree	46	27.1
PhD degree	3	1.8
Marital status		
Married	136	80
Single	34	20
Status of having children		
Yes	137	80.6
No	33	19.4
Years of work as a nurse		
≤ 10	15	8.8
>10	155	91.2
Years of work as an infection control nurse		
≤ 10	96	56.5
>10	74	43.5
Has there been an increase in weekly working hours during the pandemic period?		
Yes	128	75.3
No	42	24.7
Intervention of a patient diagnosed with COVID-19 during the pandemic period		
Yes	89	52.4
No	81	47.6
Getting diagnosed with COVID-19 during the pandemic	108	63.5
Yes	62	36.5
No		
Losing a family member due to COVID-19 during the pandemic period		
Yes	29	17.1
No	141	82.9
Losing a healthcare worker relative due to COVID-19 during the pandemic period		
Yes	55	32.4
No	115	67.6

Table 2. PTGI scores and PTGI sub-dimension score of infection control nurses (n=170)

Post Traumatic Growth Inventory	$\bar{X} \pm SD$	min–max
PTGI total score	70.73±23.03	21–115
PTGI sub-dimension scores		
Change in self-perception	35.83±12.66	10–58
Change in philosophy of life	20.69±6.14	6–34
Change in relationships	14.20±6.27	5–28

the pandemic period, and 32.4% lost a healthcare worker relative during the pandemic period (Table 1).

The mean PTGI score of the infection control nurses was 70.73±23.03. Nurses' PTGI scale sub-dimension scores are given in Table 2.

The comparison of nurses' personal characteristics with PTGI total score and sub-dimension score is given in Table 3.

Nurses over the age of 30 had statistically significantly higher scores on the PTGI change in philosophy of life sub-dimension scores than those of nurses under the age of 30 (U=156.500, p=0.018); it was determined that the change in philosophy of life sub-dimension score of married nurses were statistically significantly higher than the scores of single nurses (U=1723,000, p=0.022) (Table 3).

It was determined that there was a statistically significant difference between the PTGI total score and sub-dimension scores according to the year of work as an infection control nurse ($p<0.05$). It has been determined that my nurses who have worked for less than 10 years, PTGI total score and all sub-dimension scores are statistically significantly higher than the scores of those who have worked for more than 10 years ($p<0.05$) (Table 3).

It was determined that the PTGI total score and the change in self-perception sub-dimension scores of the nurses who lost their relatives due to the COVID-19 disease during the pandemic period were statistically significantly higher than the scores of the nurses who did not lose their relatives due to the COVID-19 disease during the pandemic period ($p<0.05$) (Table 3).

Discussion

The sudden and emergency occurrence of the COVID-19 pandemic can be considered as a traumatic event that may trigger mental problems such as post-traumatic stress disorder for nurses.[9,17] Post-traumatic growth is a positive psychological indicator for nurses after the COVID-19 pandemic, which is a traumatic situation. Post-traumatic growth is critical for frontline nurses during the COVID-19 pandemic.[12] In this study, it was aimed to determine the post-traumatic growth status of infection control nurses, who played an important role in health services in the COVID-19 pandemic. Since there is no study in the literature with infection control nurses working on the front lines after the covid 19 pandemic, it is thought that the results of this study are very valuable as the first results obtained in this field. When the results obtained from the study were examined, it was seen that the infection control nurses experienced moderate post-traumatic growth, and the post-traumatic growth score was affected by age, marital status, working year as an infection control nurse, and losing a healthcare worker friend due to COVID-19.

Table 3. Comparison of personal characteristics of infection control nurses with PTGI total scores and sub-dimension scores

Personal characteristics		PTGI total score $\bar{X} \pm SD$	PTGI sub-dimension scores		
			Change in self-perception $\bar{X} \pm SD$	Change in philosophy of life $\bar{X} \pm SD$	Change in relationships $\bar{X} \pm SD$
Age					
≤ 30		54.8±14.72	30±10.95	14.8±2.48	10±3.8
>30		71.21±23.09	36.01±12.69	20.87±6.13	14.33±6.29
	U	230	293.5	156.5	246.5
	p	0.092	0.272	0.018	0.125
Gender					
Female		54.80±14.72	30±10.95	14.8±2.48	10±3.8
Male		71.21±23.09	36.01±12.69	20.87±6.13	14.33±6.29
	U	209	203.5	245.5	234
	p	0.623	0.578	0.953	0.845
Education status					
Associate degree		65±25.57	32.25±14.21	19.00±6.21	13.75±6.73
Bachelor's degree		69.99±23.34	35.51±12.75	20.47±6.3	14.00±6.32
Master degree		72±21.61	36.43±12.22	21.26±5.7	14.30±5.96
PhD degree		94.66±21.03	48.33±8.32	24.66±7.02	21.66±6.02
	X^2	3.609	3.992	2.657	3.85
	p	0.307	0.262	0.448	0.278
Marital status					
Married		72.14±23.34	36.53±12.76	21.16±6.23	14.44±6.48
Single		65.08±21.13	33.02±12.01	18.82±5.43	13.23±5.33
	U	1874.5	1865	1723	2072
	p	0.088	0.081	0.022	0.349
Status of having children		71.62±22.85	36.36±12.45	20.94±6.08	14.31±6.36
Yes		67.06±23.73	33.63±13.46	19.66±6.36	13.75±5.95
No					
	U	2041.5	2006	1917.5	2157.5
	p	0.388	0.316	0.176	0.684
Years of work as a nurse		70.06±22.10	36.53±12.32	20.26±6.26	13.26±5.49
≤ 10		70.80±23.18	35.76±12.73	20.73±6.14	14.29±6.35
>10					
	U	1116.5	1137.5	1070	1053
	p	0.8	0.891	0.611	0.547
Years of work as an infection control nurse		74.59±22.42	38±11.89	21.50±6.28	15.09±6.38
≤ 10		65.72±22.99	33.02±13.14	19.64±5.82	13.05±5.98
>10					
	U	2757.5	2758.5	2884	2877
	p	0.013	0.013	0.036	0.034
Has there been an increase in weekly working hours during the pandemic period?					
Yes		70.89±23.02	36.05±12.60	20.78±6.29	14.06±6.29
No		70.23±23.31	35.16±12.96	20.42±5.69	14.64±6.27
	U	2592	257	2540.5	2552.5
	p	0.729	0.672	0.594	0.624
Intervention of a patient diagnosed with COVID-19 during the pandemic period					
Yes		72.77±20.95	37.04±11.41	21.30±5.93	14.42±5.95
No		68.44±25.06	34.50±13.85	20.02±6.33	13.96±6.63
	U	3309.5	3305.5	3195.5	3436.5
	p	0.357	0.351	0.201	0.599
Getting diagnosed with COVID-19 during the pandemic					
Yes		70.88±22.94	36.04±12.77	20.44±6.12	14.39±6.15
No		70.46±23.36	35.46±12.55	21.12±6.18	13.87±6.51
	U	3339	3272	3137	3147.5
	p	0.977	0.807	0.494	0.515
Losing a family member due to COVID-19 during the pandemic period					
Yes		69.24±22.22	36.13±12.26	19.79±5.99	13.31±5.93
No		71.04±23.25	35.77±12.78	20.87±6.17	14.39±6.34
	U	1909.5	2042	1782.5	1841.5
	p	0.576	0.992	0.277	0.399
Losing a healthcare worker relative due to COVID-19 during the pandemic period					
Yes		76.56±20.73	38.98±11.72	22.05±5.33	15.52±5.94
No		67.94±23.63	34.33±12.86	20.04±6.40	13.57±6.35
	U	2524	2484.5	2609.5	2591
	p	0.033	0.024	0.065	0.056

As a result of the study, it was determined that the after pandemic post-traumatic growth status of the infection control nurses was moderate (70.73±23.03). This finding suggests that infection control nurses working on the front lines of the pandemic experienced possible post-traumatic growth. Although a similar study with infection control nurses has not been found in the literature, it is seen that there are similar results in a small number of studies conducted in China with nurses from other fields.[12,14,15,17] The after pandemic post-traumatic growth status of the infection control was moderate above in the study of Cui et al. (70.53±17.26), in the study of Peng et al. (65.65±11.5), and in the study of Li et al. (63.28±23.41). In another study conducted with nurses in China, it was determined that nurses showed a high level of growth (96.26±21.57).[14] It is very important for nurses to experience posttraumatic growth as a result of traumatic situations such as pandemics, even if at a moderate level, in terms of protecting mental health. In order to increase this post-traumatic growth, nurse managers' awareness of the posttraumatic growth status of nurses and what factors affect this will shed a great light on the interventions to be planned.

As a result of this study, when the results of the PTGI sub-dimension of the nurses were examined, the change in self-perception sub-dimension was above the medium level (35.83±12.66), from the change in philosophy of life sub-dimension above the medium level (20.69±6.14), and from the change in relationships sub-dimension, a moderate growth (14.2±6.27). have been detected. Studies with nurses after the pandemic are limited in the literature. Since different versions of the posttraumatic growth inventory are used in these studies, the sub-dimensions of the version used in this study differ. For this reason, it is not sufficient to compare the sub-dimension scores obtained from this study with the results in the literature. However, as a result of this study, it is seen that nurses experienced moderate posttraumatic growth in terms of sub-dimensions. In future research, nurses should be evaluated comprehensively in this dimension.

As a result of this study, nurses over the age of 30 compared to the nurses under the age of 30; It was determined that married nurses had significantly higher changes in philosophy of life sub-dimension scores than single nurses. It is seen that PTGI scores according to gender and age are compared in very few of the limited studies conducted during the pandemic period in the literature.[15,17] As a result of the study of Li et al. (2022), it was determined that being married is an important factor that positively affects posttraumatic growth. The reason why married nurses experience more positive posttraumatic growth may be related to the fact that they have more social and moral support from the family, because social support has been found to play a vital role in balancing and protecting.[15,17] In another study conducted in China, it was determined that there was no significant relationship between age and marital status and posttraumatic growth scores.[17] In this sense, it is very important to increase social support resources and strengthen family dynamics in order to strengthen the psychological empowerment of nurses.

As a result of this study, it was determined that the PTGI total score and all sub-dimension scores of the nurses who worked as an infection control nurse for less than 10 years were higher than the scores of those who worked for more than 10 years. In a similar study conducted with nurses in China, the opposite result was determined, and it was determined that nurses with more than 10 years of work had higher PTGI scores.[12] In another study, it was determined that the working year did not affect the PTGI score.[17] The reason why the result of this study is different from the studies conducted in China can be explained as the fact that nurses with high working years may have experienced burnout and fatigue due to working for many years, and that nurses with more working years have other burdens and responsibilities (home, children, etc.) in their lives that may affect their post-traumatic growth. It is a fact that the working year affects post-traumatic growth. However, considering these different results, the reasons why the working year affects the growth of nurses should be determined, and interventions that will strengthen nurses psychologically should be planned for these reasons.

As a result of this study, it was determined that the scale total score and the Change in Self-Perception sub-dimension scores of "nurses who lost their relatives due to COVID-19 disease during the pandemic period" were higher than the nurses who did not lose their relatives. This finding suggests that the loss of a healthcare worker's relative affects posttraumatic growth. When the limited studies conducted with nurses during the pandemic period were examined, it was seen that the post-traumatic growth status of the nurses who lost their relatives was not examined. Losses experienced during the pandemic process are a traumatic experience for individuals. The loss of colleagues by nurses during this period may further increase the trauma caused by the pandemic. Face-to-face with death in the immediate environment may have created a trauma for nurses, and this trauma may have contributed to them becoming stronger than before the trauma period. In addition, it provided an opportunity for nurses who lost a loved one to question the meaning of life and develop their coping skills based on experiencing the stressful event after trauma. may have spurred growth.

Limitations

This study provides new evidence about the posttraumatic growth status of nurses for interventions to strengthen the mental health of infection control nurses. However, there are several limitations in the research. First, the collection of research data in online environments is a limitation of the research. There is no institution in Turkey where infection control nurses can be reached. For this reason, although the online data collection method was preferred, this data collection method may have caused response bias. Respondents may have more positive views of posttraumatic growth than nonresponders. The lack of information about non-responders is a limitation of this study. Secondly, the number of male nurses working as infection control nurses is quite low in the country where this study was conducted. The low number of male nurses may have affected the findings. The third limitation is that this study was a cross-sectional type and evaluated posttraumatic growth status only at a certain time, without longitudinal observation of nurses.

Conclusion

As a result of this study, it can be said that infection control nurses experienced a moderate growth after the trauma of the COVID-19 pandemic. It is seen that

age, marital status, working year and loss of a healthcare worker friend during the pandemic period affect the post-traumatic growth of nurses. Since there is no study in the literature with infection control nurses working on the front lines after the COVID-19 pandemic, it is thought that the results of this study are very valuable as the first results obtained in this field. It is very important to determine the mental health of nurses working on the front lines during the pandemic. In this direction, it is necessary to plan interventions that will improve the mental health of infection control nurses. Nursing leaders should pay attention to posttraumatic growth and its influencing factors and offer solutions to protect the mental health of nurses. Nursing administrators should be aware of the mental state of nurses, strengthen psychological interventions such as psychological guidance and mental health protective training, especially for nurses with more working years, increase social support resources, take into account the psychological experiences of nurses comprehensively to support psychological growth and reduce post-traumatic psychological burden. should be maintained regularly. In addition, multicenter longitudinal studies with larger sample sizes are needed to evaluate posttraumatic growth and the factors affecting it, including infection control nurses, in future studies.

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Author contributions

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

Conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability

Data available on request from the authors.

Ethics approval

Ethical approval to conduct the study was obtained from the Human Research Ethics Committee of Zonguldak Bulent Ecevit University (decision number: 205981, Decision date: 29.08.2022). Informed consent was obtained from the infection control nurses participating in the study online.

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ORIGINAL PAPER

Comparison of triglyceride-glucose index and HOMA-IR as indicators of insulin resistance in obese women with subclinical hypothyroidism

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ABSTRACT

Introduction and aim. Thyroid hormones play an important role in glucose metabolism as in many metabolic events. The aim of our study is to evaluate the relationship between subclinical hypothyroidism (SCH) and insulin resistance, especially in obese women.

Material and methods. Newly diagnosed SCH patients with body mass index (BMI) ≥30 who applied to our outpatient clinic between March 2021 and October 2021, and euthyroid obese women who applied for routine control were included in the study. In this study, we used homeostasis model assessment of insulin resistance (HOMA-IR) and triglyceride glucose (TyG) indexes, which are noninvasive, simple and useful methods for evaluating insulin sensitivity.

Results. The study included 78 female patients between the ages of 19 and 64. A correlational analysis was performed between thyroid stimulating hormone (TSH) and HOMA-IR, TyG, and BMI. The results showed that TSH levels were positively correlated with HOMA-IR (R=0.297, p=0.008), TyG (R=0.316, p=0.005) and BMI (R=0.307, p=0.006). This relationship was stronger for TyG compared to the other variables. As another finding, BMI was positively correlated with HOMA-IR (R=0.359, p=0.001) and TyG (R=0.404, p<0.001). This relationship was stronger for TyG than HOMA-IR.

Conclusion. These results show that patients with SCH are at risk of developing diseases that accompany insulin resistance, such as metabolic syndrome and cardiovascular disorders. The most important finding of our study is that the TyG index gives more significant results than HOMA-IR, especially in obese women.

Keywords. HOMA-IR, insulin resistance, subclinical hypothyroidism, triglyceride-glucose index

Introduction

Subclinical hypothyroidism (SCH) is defined as a serum thyroid-stimulating hormone (TSH) level above the upper limit of normal despite normal levels of serum free thyroxine.[1] Its incidence in general population surveys varies between 3% and 8%.[2] It is likely to go undiagnosed for a long time, as it usually does not cause significant symptoms in patients. Even if SCH does not cause significant discomfort in patients, it is metabolically associated with many conditions. It has been shown that it may increase the risk of obesity and SCH can cause cardiovascular diseases due to hyperuricemia, and heart failure may develop due to decreased myocardial contractility.[3,4] In addition, previous studies suggest that SCH raises blood pressure and cholesterol levels, impairs insulin secretion, and increases the risk of peripheral neuropathy, peripheral arterial disease, and diabetic

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nephropathy by damaging both micro and macrovascular function.[5]

Thyroid hormones play an important role in glucose metabolism as in many metabolic events. In animal studies performed during the fetal period, it has been shown that free T3 levels have a significant effect on hepatic and cardiac glycogen stores.[6] Increased T3 concentrations are associated with an increase in glucose turnover and an increase in insulin-mediated glucose flux into skeletal muscle and adipocytes, through the insulin-sensitive glucose transporter-4.[7]

Many studies have shown that there is a strong relationship between overt hypothyroidism and metabolic syndrome, type 2 diabetes mellitus and obesity.[8,9] Although most of the studies show that SCH also increases the risk of these disorders, there are also publications suggesting that there is less relationship between SCH and these diseases.[10] Insulin resistance is defined as a decreased response to insulin at normal concentrations in the circulation. Insulin resistance can be seen in non-diabetic obese individuals and in type 2 diabetic patients.[11] The pathophysiological reasons underlying insulin resistance have not been adequately clarified yet. Generally, insulin resistance is seen as a result of insulin activity defect.

Aim

The aim of our study is to evaluate the relationship between SCH and insulin resistance, especially in obese women who are in the risk group for metabolic diseases. In this study, we used Homeostasis model assessment of insulin resistance (HOMA-IR) and triglyceride glucose (TyG) indexes, which are noninvasive, simple and useful methods for evaluating insulin sensitivity. This is the first study showing the relationship between newly diagnosed SCH obese women and insulin resistance evaluated with TyG in our knowledge.

Material and methods

Study was approved by the institutional review board, and a waiver of authorization was given (Ethics Committee decision no: 02, date: 26.08.2022).

This retrospective study included 78 subjects as a patient group of 48 females and control group of 30 females. Newly diagnosed SCH patients with body mass index (BMI) ≥30 who applied to our outpatient clinic between March 2021 and October 2021, and euthyroid obese women who applied for routine control were included in the study. Demographic data and assay results of the patients were evaluated retrospectively. Patients with diabetes mellitus, dyslipidemia, hypertension, rheumatological disease, malignancy, pregnancy and known thyroid disease were excluded from the study. Patients with infection, high acute phase markers, and patients using drugs such as corticosteroids with known effects on insulin resistance were not included in the study. The TyG index was calculated as ln[fasting triglycerides (mg/dL) × fasting glucose (mg/dL)/2]. The HOMA-IR index was calculated using the following formula: HOMA-IR = fasting insulin (μU/mL) × fasting glucose (mg/dL)/405.

Statistical analyses of the data obtained in the study were made using SPSS version 26.0 software (IBM, Armonk, NY, USA). Conformity of the data to normal distribution was examined visually (histogram and probability graphs) and with the analytical method of the Kolmogorov-Smirnov test. Results for quantitative variables are expressed as mean ± SD for normally distributed data; and mean (interquartile range) for non-parametric data. Comparisons of quantitative variables between the groups were made using the Student's t-test or the Mann Whitney U-test according to the conformity of the data to normal distribution. The Spearman's correlation coefficient was applied to measure the correlation between various parameters. A two-sided value < 0.05 was considered statistically significant.

Results

The study included 78 female patients between the ages of 19 and 64. The mean age of the patients was 40.2±10.7. Both groups were similar in terms of age (p=0.087). Patients compared to HOMA-IR and TyG index scores, the median HOMA-IR (p=0.044) and TyG index scores (p=0.016) were higher in the SCH group. When the groups were compared in terms of BMI levels, SCHs had higher BMI levels than controls (p=0.037). According to the laboratory parameters, the mean values of Triglyceride (Tg) were higher in the SCH group (p=0.029), and no difference was determined in respect of fasting glucose (p=0.078), insulin (p=0.062), and LDL (p=0.223) levels. The demographic and clinical characteristics of all study patients are shown in Table 1.

Table 1. Characteristics of the study sample: study group vs. control group*

	Total, n=78	Control group, n=30	Study group, n=48	p
Age (Years)	40.2±10.7	37.6±10.0	41.8±10.9	0.087
BMI (kg/m^2)	33 (32–35)	32 (31–34)	34 (32–36)	0.037
Fasting glucose	96 (89–104)	93 (87–100)	98 (91–105)	0.078
Insulin (uIU/mL)	9.3 (6.4–15)	8.4 (5.2–11.6)	10.9 (7.1–15.6)	0.062
HOMA-IR	2.3 (1.5–3.4)	1.8 (1.1–2.7)	2.6 (1.7–3.9)	0.044
TyG index	4.7 (4.5–4.9)	4.6 (4.4–4.7)	4.8 (4.5–5)	0.016
LDL (mg/dL)	93 (68–113)	88 (66–109)	100 (69–116)	0.223
Tg (mg/dL)	122 (92–164)	109 (81–140)	140 (96–197)	0.029
TSH (mIU/L)	6.3 (2.3–7.8)	2 (1.6–4)	7.5 (6.5–8)	<0.001

*LDL – low-density lipoprotein; BMI – body mass index, TyG – triglyceride-glucose; Tg – triglyceride; TSH – thyroid stimulating hormone

A correlational analysis was performed between TSH and HOMA-IR, TyG, and BMI. The results showed

that TSH levels were positively correlated with HOMA-IR (R=0.297, p=0.008), TyG (R=0.316, p=0.005) and BMI (R=0.307, p=0.006). This relationship was stronger for TyG compared to the other variables. As another finding, BMI was positively correlated with HOMA-IR (R=0.359, p=0.001) and TyG (R=0.404, p<0.001). This relationship was stronger for TyG than HOMA-IR. Correlations for study variables are shown in Table 2.

Table 2. Correlation matrix of study variables[a]

		HOMA-IR		TyG index		TSH		BMI
HOMA-IR	R	—						
	p	—						
TyG index	R	0.274	*	—				
	p	0.015		—				
TSH	R	0.297	**	0.316	**	—		
	p	0.008		0.005		—		
BMI	R	0.359	**	0.404	***	0.307	**	—
	p	0.001		<0.001		0.006		—

* p<0.05, ** p<0.01, *** p<0.001; TyG – triglyceride-glucose; BMI – body mass index; TSH – thyroid stimulating hormone

Discussion

Hyperinsulinemic euglycemic clamp technique is the gold standard method for determining insulin sensitivity, but this method is an expensive and invasive procedure and can only be used for research purposes.[11] Therefore, non-invasive methods have been developed to measure insulin resistance. One of the most commonly used methods for this purpose is HOMA-IR. Recently, TyG index, which is calculated by Tg level and fasting glucose level, has been proposed as a reliable and simple indicator of insulin resistance in many studies. It has been shown in some studies that the TyG index is more significant than HOMA-IR and even correlates with the hyperinsulinemic euglycemic clamp method.[12,13] In this study, we evaluated the relationship between SCH and insulin resistance using both these methods.

Pergola et al., in their study on euthyroid obese women, progressive central fat accumulation is associated with a parallel increase in FT3 levels, and it was thought that this might be due to a thermogenic phenomenon.[14] In the same study, it is stated that as the BMI level increases, the feedback effect of free thyroid hormones on TSH may be impaired.[14] In the study by Singh et al., the BMI values of patients with both SCH and overt hypothyroidism were found to be higher than the control group.[15] In our study, a significant positive correlation was found between TSH level and BMI.

Overt hypothyroidism has been shown to be associated with glucose intolerance and insulin resistance in many studies, and it has been found that these disorders ameliorate as a result of conversion to euthyroid state with levothyroxine treatment.[16,17] There are studies showing that the mechanism of development of insulin resistance in hypothyroidism occurs as a result of decreased sensitivity to insulin in skeletal muscle and adipose tissue.[18] However, this relationship could not be revealed so clearly in SCH. Conflicting results have been observed in previous studies. While Shanta et al. found a significant relationship between TSH and insulin resistance in female patients with SCH, another study by Owecki et al. found no significant relationship between TSH level and HOMA-IR.[19,20]

Choi et al. study on 5727 patients showed that the association between SCH and TyG index was only statistically significant in females with hypothyroidism. In female patients, association is evident with subclinical thyroid dysfunction.[21] In the same study, the authors also evaluated the relationship between SCH and the HOMA-IR and they found that TSH was not significantly correlated with HOMA-IR.[21] In our study, we found that both HOMA-IR and TyG indices were statistically correlated with TSH values. However, this relationship was more significant for TyG. We think that the difference between the HOMA-IR values in these two studies is due to the inclusion of only obese patients in our study. Both Choi et al.'s study and our study suggest that the TyG index is more significant than HOMA-IR in demonstrating insulin resistance in SCH patients. The TyG index is a better indicator of peripheral IR as it mainly reflects the IR in the muscle. In contrast, HOMA-IR reflects IR mainly in the liver and is a better indicator for hepatic IR. Therefore, these differences can be observed.[22,23]

The relationship between SCH and lipid parameters has been evaluated in many studies before. Although there are conflicting results in studies between SCH and LDL, it is seen that SCH increases the Tg level in many studies. While no significant relationship was found between TSH level and LDL in the study by Pergola et al., a statistically significant relationship was found between SCH and LDL in the study conducted by Ebrahimpour et al.[9,14] In the review published by Fatourechi, it was stated that levothyroxine replacement in SCH patients did not have a significant effect on LDL.[2] Since the Tg value, which is also used in the calculation of the TyG index, shows a positive correlation with TSH, it is statistically significant as a result of our study. This correlation seems to be compatible with previous studies.

This work had some limitations. First, this study was a retrospective and single-centre analysis with a limited sample size. In addition, due to the retrospective nature of the study, free T3 levels of many patients could not be reached.

Conclusion

As a result, it was observed that patients with SCH had increased insulin resistance and Tg levels. BMI levels of SCH patients were also found to be higher than the con-

trol group. These results show that patients with SCH are at risk of developing diseases that accompany insulin resistance, such as metabolic syndrome and cardiovascular disorders. In our opinion, the most important finding of our study is that the TyG index gives more significant results than HOMA-IR, especially in obese women. Initiation of levothyroxine replacement therapy may be considered in patients with SCH in order to reduce the risk of metabolic diseases mentioned above. More comprehensive prospective studies are needed in this regard.

Declarations

Funding

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Author contributions

Conceptualization, C.O.K. and H.A.F.; Methodology, C.O.K.; Software, H.A.F.; Validation, V.S. and C.O.K.; Formal Analysis, H.A.F.; Investigation, C.O.K. and V.S.; Resources, C.O.K.; Data Curation, H.A.F.; Writing – Original Draft Preparation, C.O.K. and V.S.; Writing – Review & Editing, C.O.K. and H.A.F.; Visualization, V.S.; Supervision, H.A.F.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

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

Ethics approval

Study was approved by the institutional review board, and a waiver of authorization was given (Ethics Committee decision no: 02, date: 26.08.2022).

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ORIGINAL PAPER

The role of erythrocyte distribution width in predicting poor outcomes in geriatric patients with acute pancreatitis

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ABSTRACT

Introduction and aim. In our study, our aim was to evaluate the relationship between red cell distribution width (RDW) values and prognosis in geriatric patients with acute pancreatitis.

Material and methods. Patients over the age of 65 and diagnosed with acute pancreatitis who applied to the Emergency Department of Ümraniye Training and Research Hospital between 16.07.2021 and 15.05.2022 were included in our retrospective study. RDW levels were recorded using the hospital data system.

Results. Our study included 184 patients, 19 (10.3%) of which died. Sixty-five percent of our patients were women. The mean hospital stay was 5 days (from 3 to 9). A statistically significant relationship was also observed between high RDW and mortality (p=0.006). The diagnostic test performance analyses of CRP, and RDW in predicting mortality revealed that they were statistically significant in predicting mortality, with the AUC value being calculated as 0.66 (0.6061–0.7368) for CRP, with a cut-off value of 22; and 0.69 (0.6909–0.7368) for RDW, with a cut-off value of 14.5 (p=0.019, p=0.006, respectively).

Conclusion. Hematological parameters can help predict a prognosis in patients with acute pancreatitis. Although RDW is not statistically more significant than CRP, it can be used as a prognostic marker in patients with acute pancreatitis.

Keywords. acute pancreatitis, CRP, RDW

Introduction

Acute pancreatitis is a sudden onset inflammatory disease of the pancreas that can develop due to many illnesses, especially gallstones and alcohol use.[1,2] In the inflammatory process, an increase in the number of neutrophils may be expected, as well as a decrease in lymphocytes in conjunction with physiological disorders.[2-4] Acute pancreatitis can occur locally or cause widespread organ dysfunction. Mortality can reach up to 50% in patients with severe acute pancreatitis. The reason for this is multiple organ dysfunction syndrome (MODS).[5,6] It is possible, however, to prevent organ failure and mortality with early and effective treatment.[6]

In order to predict the prognosis, studies on albumin, white blood cell (WBC), and C-reactive protein (CRP) were performed in patients with pancreatitis.[7-9] Hematological parameters have been the subject of studies on acute pancreatitis and many other inflammatory diseases.[2,9] Red cell distribution width (RDW), which may increase due to inflammation, ischemia, and hypoxia, is a measure of the mean corpuscular/erythrocyte volume calculated using the standard deviation of erythrocyte volume heterogeneity.[10,11] Some studies have

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previously been conducted to evaluate the association of RDW with a poor prognosis in patients with pancreatitis, mostly comparing patients with pancreatitis with patients without pancreatitis.[2,6,9,12-14] The relationship of RDW with mortality was also investigated in patients with acute coronary syndrome, acute appendicitis with inflammation similar to acute pancreatitis, infective diseases such as COVID-19, and malignancies.[11,15-17]

It can be quite difficult to predict the prognosis of acute pancreatitis, which has many causes, including idiopathic, especially in geriatric patients. Geriatrics may not be able to apply to the hospital with common symptoms, and this may prolong the clinician's time to make the diagnosis. In studies investigating the relationship of RDW with prognosis in patients with acute pancreatitis, the geriatrics group was not evaluated separately.[15] In addition, we found that CRP, an inflammatory parameter, was investigated in patients with acute pancreatitis, and again, it was not evaluated separately in the geriatric patient group.[9]

Although it has been predicted that RDW may be effective in ischemic diseases and inflammatory processes, we think that evaluating the relationship of RDW with a prognosis in geriatrics with acute pancreatitis and its comparison with CRP in the same study will contribute to the literature.

Aim

In our study, our primary aim was to evaluate the relationship between RDW and prognosis in geriatric patients diagnosed with acute pancreatitis, and our secondary aim was to compare the relationship between RDW and CRP and prognosis in geriatric patients diagnosed with acute pancreatitis.

Material and methods

Ethical approval

The instant study was carried out with the permission of the University of Health Sciences, Ümraniye Education and Research Hospital Ethics Committee (Date: 23/06/2022, Decision No: B.10.1.TKH.4.34.H.GP.0.01/215).

Study design

Patients who applied to the Emergency Department of Ümraniye Training and Research Hospital between 16.07.2021 and 15.05.2022 were included in our retrospective study.

Study population

The study included patients over the age of 65 who were admitted to the emergency department and were considered to have acute pancreatitis, who were confirmed clinically and via laboratory and radiologically, whose hemogram parameters were measured and registered in the emergency department. Those with acute pancreatitis findings on tomography were included in the study, while those with only clinical findings and only elevated amylase and/or lipase parameters but no findings on tomography were excluded from the study. Patients with incomplete data on mortality, with chronic pancreatitis, under 65 years of age, and who refused to participate in the study were excluded.

Data collection

Age (year), comorbid diseases, WBC count, neutrophil count, monocyte count, lymphocyte count, platelet count, hemoglobin, hematocrit count, RDW, mean platelet volume (MPV), platelet distribution width (PDW), and sodium, potassium, glucose, blood urea nitrogen (BUN), creatinine, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), CRP, amylase, lipase levels were recorded. Length of hospital stay, ward, intensive care stays, and 30-day mortality rates were also recorded. Length of hospital stay, and intensive care unit admission rates were recorded using the hospital data system. According to their survival status, the patients were divided into two groups – survivors and those who died – according to the National death notification system in Turkey. The examinations and data of patients who attended the emergency department were used.

Statistical analysis

The categorical data was done using the fisher exact test and chi-square test. Quantitative variables were presented as median and interquartile range (IQR, 25th-75th percentile) values, and the Mann-Whitney test was used in analyzing the paired groups. During this analysis, the area under the curve (AUC) values were calculated, and the sensitivity, specificity, accuracy, and 95% confidence interval (CI) data were analyzed. The AUC values of the parameters were calculated and tested mutually for significance with the DeLong quality test. Statistical analysis was performed using SPSS v. 26.0 (IBM, Chicago, IL, USA). Statistical significance was accepted as $p<0.05$.

Results

Our study included 184 patients, 19 (10.3%) of which died. Sixty-five percent of our patients were women. The mean hospital stay was 5 days (between 3 and 9). There was a statistically significant correlation between hospitalized patients and mortality (p=0.001). Comorbid disease was present in 84% of the patients. While age values were found to be higher in the non survivor group (p=0.023), there was no statistically significant relationship between comorbidity and mortality (p=0.19). Amylase and lipase values were higher in living patients (p=0.039, p=0.01, respectively). A statistically significant correlation was observed between high BUN and

creatinine and mortality (p=0.004, p=0.001, respectively) (Table 1).

Table 1. The relationship of demographic characteristics of geriatric patients with acute pancreatitis with mortality*

Characteristic	n=184	Survivor n=165 (89.7%)	Non-Survivor n=19 (10.3%)	p-value
Age	76 (70, 83)	75 (70, 82)	83 (78, 86)	0.023
Gender, n (%)				0.026
Female	120 (65%)	112 (68%)	8 (42%)	
Male	64 (35%)	53 (32%)	11 (58%)	
LOHS	107 (54, 206)	112 (54, 204)	91 (60, 218)	0.78
Length of Stay Days	5 (3, 9)	5 (3, 9)	4 (3, 10)	0.72
LOHS_Dicotom, n (%)				0.6
<= 7 Days	126 (68%)	114 (69%)	12 (63%)	
> 7 Days	58 (32%)	51 (31%)	7 (37%)	
ED Outcome, n (%)				0.001
Disposition	25 (14%)	25 (15%)	0 (0%)	
Admission to Services	146 (79%)	134 (81%)	12 (63%)	
Admission to ICU	13 (7.1%)	6 (3.6%)	7 (37%)	
Comorbidities, n (%)	155 (84%)	141 (85%)	14 (74%)	0.19
Hypertension	132 (72%)	120 (73%)	12 (63%)	0.38
Diabetes mellitus	65 (35%)	62 (38%)	3 (16%)	0.06
Malignancy	25 (14%)	22 (13%)	3 (16%)	0.73
Hyperlipidemia	88 (48%)	81 (49%)	7 (37%)	0.31
Alzheimer	13 (7.1%)	11 (6.7%)	2 (11%)	0.63
COPD	27 (15%)	23 (14%)	4 (21%)	0.49
Ischemic heart disease	66 (36%)	59 (36%)	7 (37%)	0.93
Asthma	28 (15%)	27 (16%)	1 (5.3%)	0.32
Heart failure	24 (13%)	22 (13%)	2 (11%)	>0.99
Chronic renal failure	22 (12%)	19 (12%)	3 (16%)	0.71
Cerebrovascular disease	19 (10%)	18 (11%)	1 (5.3%)	0.7

* LOHS – length of hospital stay; ED – emergency department; ICU – intensive care unit; COPD – chronic obstructive pulmonary disease

There was a statistically significant relationship between low hemoglobin and hematocrit and mortality (p=0.012, p=0.005, respectively). A statistically significant relationship was also observed between high RDW and mortality (p=0.006). No statistically significant correlation was observed between hematological parameters other than hemoglobin, hematocrit and RDW, and mortality. A statistically significant correlation was found between low albumin and high CRP and mortality (p=0.001, p=0.019, respectively) (Table 2).

The diagnostic test performance analyses of albumin, CRP, and RDW in predicting mortality revealed that they were statistically significant in predicting mortality, with the AUC value being calculated as 0.78 (0.5789–0.8606) for albumin, with a cut-off value of 35.8; 0.66 (0.6061–0.7368) for CRP, with a cut-off value of 22; and 0.69 (0.6909–0.7368) for RDW, with a cut-off value of 14.5 (p=0.001, p=0.019, p=0.006 respectively) (Table 3, Fig. 1, Fig. 2).

Table 2. Relationship between laboratory parameters and mortality in geriatric patients with acute pancreatitis*

Characteristic Median (IQR); n (%)	n=184	Survivor n=165 (89.7%)	Non–Survivor n=19 (10.3%)	p–value
ALT (IU/L)	80 (26, 202)	82 (27, 201)	62 (22, 308)	0.94
Albumine (g/dL)	40.1 (36.9–42.3)	40.7 (37.3–42.7)	35.2 (33.1–38.5)	0.001
Amylase (U/L)	712 (299–1.502)	741 (324–1.545)	348 (209–878)	0.039
AST (IU/L)	130 (40–277)	133 (42–253)	125 (28–511)	0.87
CRP (mg/L)	13 (4–47)	11 (3–44)	37 (16–58)	0.019
Glucose (mmol/L)	137 (110–181)	137 (111–181)	133 (108–174)	0.55
BUN (mg/dL)	41 (33–62)	40 (32–60)	62 (51–126)	0.004
Creatinine (mg/dL)	0.96 (0.77–1.42)	0.93 (0.76–1.27)	1.80 (1.02–2.57)	0.001
Lipase (U/L)	1.437 (606–3.566)	1.698 (641–4.123)	696 (409–1.364)	0.01
Potasium (mEq/L)	4.4 (4.1–4.72)	4.4 (4.1–4.7)	4.5 (4.2–5.1)	0.22
Sodium (mEq/L)	138 (136–140)	138.9 (136–140)	137 (133.8–138)	0.063
Total bilirubin (mg/dL)	1.29 (0.63–2.78)	1.27 (0.62–2.59)	1.58 (1.00–3.66)	0.38
Direct bilirubin (mg/dL)	0.63 (0.22–1.44)	0.62 (0.24–1.42)	0.71 (0.13–2.2)	>0.99
Indirect bilirubin (mg/dL)	0.56 (0.28–1.01)	0.56 (0.28–1)	0.81 (0.28–1.46)	0.32
WBC (10^3µ/L)	10.7 (8–13.9)	10.5 (8.0–13.3)	12.4 (8.5–16)	0.3
Neutrophil (10^3µ/L)	8.6 (6.1–12.1)	8.5 (6.1–11.5)	10.7 (6.1–15.3)	0.31
Monocyte (10^3µ/L)	0.48 (0.33–0.65)	0.48 (0.32–0.65)	0.47 (0.36–0.69)	0.66
Lymphocyte (10^3µ/L)	1.11 (0.66–1.64)	1.15 (0.68–1.64)	0.87 (0.5–1.5)	0.17
Hemoglobin (g/dl)	12.60 (11.3–13.60)	12.70 (11.5–13.70)	11.20 (9.55–12.9)	0.012
Hematocrit (%)	38.5 (34.8–41.6)	38.9 (35.5–41.6)	33.5 (29.6–39)	0.005
RDW (fl)	14.05 (13.50–14.93)	14.00 (13.50–14.60)	15.30 (14.25–15.95)	0.006
Platelet (10^3µ/L)	251 (194–308)	251 (194–304)	253 (214–317)	0.29
MPV (fl)	9.5 (8.8–10.6)	9.5 (8.8–10.6)	9.10 (8.55–10.9)	0.61
Pct (µg/L)	0.24 (0.19–0.29)	0.24 (0.19–0.29)	0.23 (0.21–0.31)	0.31
PDW (fl)	16.1 (15.9–16.5)	16.1 (15.9–16.5)	16.2 (15.95–16.45)	0.87

* ALT – alanine aminotransferase; AST – aspartate aminotransferase; CRP – C-reactive protein; BUN – blood urea nitrogen; WBC – white blood cell; RDW – red cell distribution width; MPV – mean platelet volume; PDW – platelet distribution width

Table 3. ROC analysis for labaratuary parameters for 30-day mortality

	Cut-off point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	p-value
Albumin (g/dL)	35.8	86.06%	57.89%	94.67%	32.35%	0.78	0.001
Amylase (U/L)	482	63.64%	68.42%	94.59%	17.81%	0.64	0.039
Lipase (U/L)	871	69.7%	63.16%	94.26%	19.35%	0.68	0.01
Hemoglobin (g/dl)	10.5	91.52%	42.11%	93.21%	36.36%	0.68	0.012
Hematocrit (%)	34.9	78.79%	63.16%	94.89%	25.53%	0.7	0.005
RDW (fl)	14.5	73.68%	69.09%	21.54%	95.8%	0.69	0.006
Creatinine (mg/dL)	1.78	52.63%	86.06%	30.30%	94.04%	0.74	0.001
BUN (mg/dL)	49.22	78.95%	63.03%	19.74%	96.30%	0.7	0.004
CRP (mg/L)	22	73.68%	60.61%	17.72%	95.24%	0.66	0.019

* RDW – red cell distribution width; BUN – blood urea nitrogen; CRP – C-reactive protein

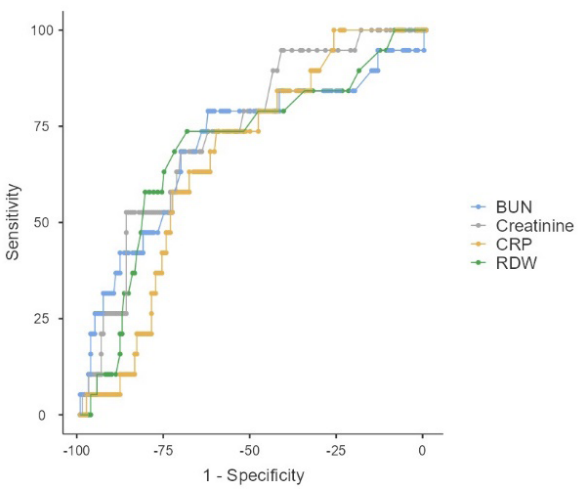

Fig. 1. Parameters associated with mortality as their values increase

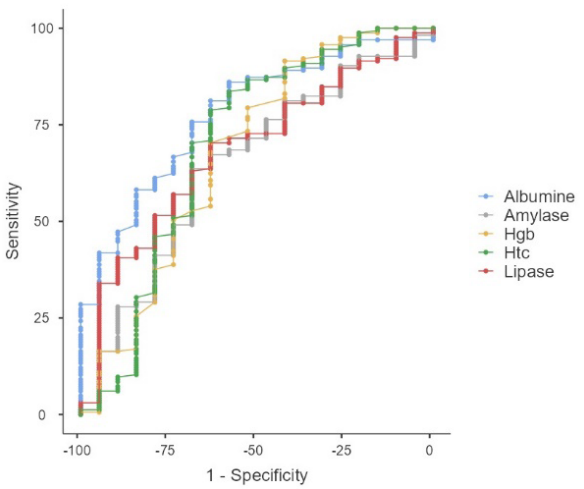

Fig. 2. Parameters associated with mortality as their values decrease

The effect of laboratory parameters on mortality according to cut-off values was also evaluated separately and is shown in Table 4.

In the cox regression analysis performed with age and laboratory parameters, there was a statistically significant relationship between albumin, hemoglobin, hematocrit, RDW and BUN and mortality in the univariant analysis (p=0.002, p=0.026, p=0.036, p=0.028, p=0.004 respectively) (Table 5).

Table 4. Comparison of the effects of laboratory values on cut-off values and mortality*

	n=184	Survivor n=165 (89.7%)	Non-Survivor n=19 (10.3%)	Odds Ratio	95% CI Lower	95% CI Upper	p-value
Albumine < cut-off point (g/dL)	45 (24,5%)	33 (20%)	12 (63.15%)	6.86	2.5	18.77	<0.001
Amylase < cut-off point(U/L)	74 (40.2%)	61 (37%)	13 (68.42%)	3.69	1.34	10.22	0.008
Lipase < cut-off point (U/L)	63 (34.23%)	51 (31%)	12 (63.15%)	3.83	1.43	10.30	0.005
Hemoglobin < cut-off point (g/dl)	48 (26.08%)	38 (23%)	10 (52.63%)	3.71	1.41	9.80	0.005
Hematocrit < cut-off point (%)	39 (21.2%)	28 (16,9%)	11 (57.9%)	6.73	2.48	18.24	<0.001
RDW > cut-off point (fl)	58 (31.5%)	45 (27.27%)	13 (68.42%)	5.78	2.07	16.12	<0.001
Creatinine > cut-off point (mg/dL)	61 (33.15%)	48 (29.09%)	13 (68.42%)	5.28	1.90	14.70	<0.001
BUN > cut-off point (mg/dL)	76 (41.3%)	61 (37%)	15 (78.94%)	6.39	2.03	20.14	<0.001
CRP > cut-off point (mg/L)	64 (34.78%)	52 (31.51%)	12 (63.15%)	3.73	1.39	10.01	0.006

* RDW – red cell distribution width; BUN – blood urea nitrogen; CRP – C-reactive protein

Table 5. Cox regression analysis of age and laboratory parameters*

	Univariate				Multivariate			
	Hazard Ratio	95% CI Lower	95% CI Upper	p value	Hazard Ratio	95% CI Lower	95% CI Upper	p value
Age	1.04	0.985	1.099	0.16	1.008	0.948	1.073	0.798
Albumin (g/dL)	0.863	0.785	0.948	0.002	0.899	0.792	1.02	0.098
Amylase (U/L)	0.999	0.999	1	0.125	1	0.999	1.001	0.727
Lipase (U/L)	1	0.999	1	0.078	1	0.999	1	0.399
Hemoglobin (g/dl)	0.748	0.579	0.966	0.026	0.639	0.215	1.905	0.422
Hematocrit (%)	0.912	0.838	0.994	0.036	1.089	0.755	1.572	0.648
RDW (fl)	1.202	1.02	1.415	0.028	1.142	0.917	1.423	0.236
Creatinine (mg/dL)	1.137	0.958	1.35	0.142	0.74	0.471	1.162	0.191
BUN (mg/dL)	1.005	1.002	1.009	0.004	1.008	1	1.017	0.061
CRP (mg/L)	1.004	0.997	1.012	0.259	1	0.989	1.011	0.968

* RDW – red cell distribution width; BUN – blood urea nitrogen; CRP – C-reactive protein

Discussion

In our study, we found that RDW with CRP can predict a prognosis in patients with acute pancreatitis. When we looked at the AUC values, we found that RDW was superior to CRP in predicting mortality. There was no statistically significant relationship between neutrophil and lymphocyte mortality, which are inflammatory markers. In the Cox regression analysis, which included age and laboratory parameters, RDW and CRP were not statistically significant in the multivariate analysis; We observed that RDW can be used as a mortality marker in univariant analysis. CRP was not statistically significant in the univariant analysis. As far as we could detect, there was no study comparing RDW and CRP in predicting mortality. Since acute pancreatitis is an inflammatory disease, CRP; albumin and kidney function tests because it can cause fluid and electrolyte disorders, and AST, ALT, bilirubin and amylase, lipase values due to biliary tract diseases were examined in our study. While hemoglobin and albumin were significantly lower in non-survivors; BUN and creatinine were significantly higher as we expected. Many studies have been conducted to investigate the effect of RDW on prognosis in acute pancreatitis.[2,6,8] In a study examining patients with acute pancreatitis due to gallstones, it was found that the RDW level was statistically significantly higher in patients with severe acute pancreatitis.[2] A study in which Pian et al. examined 169 acute pancreatitis patients showed that, in addition to high neutrophils and low lymphocyte levels in patients with severe acute pancreatitis, high RDW values also creates a statistically significant difference in mild acute pancreatitis. In the same study, low albumin was also found to be associated with a poor prognosis.[6] In another retrospectively planned study, low albumin, high WBC, and high RDW were associated with a poor prognosis.[8] Hao et al., in an acute pancreatitis study of 210 patients, showed that high RDW and low albumin may be indicators of a poor prognosis.[12] Another meta-analysis stated that RDW could be used as an easily measurable parameter in predicting the prognosis of acute pancreatitis.[13] In another study examining hematological parameters and inflammatory markers, neutrophil RDW and CRP were statistically significantly higher in patients with severe acute pancreatitis with a poor prognosis.[9] This is different than our study as it found that CRP was more determinative than RDW in severe acute pancreatitis. Lymphocytes and albumin were statistically significantly lower.[9] In another study investigating the effect of CRP on prognosis in acute pancreatitis, it was found that CRP was higher in patients with local complications present on computed tomography, but it was not statistically significant.[14] In our study, there was a statistically significant relationship between high RDW and CRP and mortality, but although high neutrophil and low lymphocyte levels were evident in non-surviving patients, there was no statistically significant relationship between high neutrophil levels and low lymphocytes and mortality. There was a statistically significant correlation between low albumin and mortality.

We thought that the rate of comorbid disease had an important place in geriatric patients, but our study found no statistically significant relationship between comorbidity and mortality.

In the literature, there were studies investigating the relationship of RDW with prognosis in different diseases.[11,15-17] In a study conducted in patients with acute coronary syndrome, there was no statistically significant difference between RDW and mortality.[11] A meta-analysis found that there was no statistically significant difference in RDW level in patients with acute appendicitis compared to patients without a diagnosis of acute appendicitis.[15] In a study conducted in patients with a diagnosis of COVID-19, there was a statistically significant relationship between high RDW and mortality.[16] In a study conducted in patients with colorectal cancer, the rate of development of infectious complications was statistically significantly higher in patients with high RDW.[17] In a meta-analysis conducted in psoriasis patients, it was observed that the RDW level was significantly higher in patients with psoriasis, but no statistically significant correlation was found between disease severity and RDW.[18] Considering the results obtained in previous studies in inflammatory diseases and acute pancreatitis, we think that studies should be conducted in which RDW is compared with different parameters.

Limitations of the study

In our study, application data of acute pancreatitis patients were obtained, laboratory data were not obtained during follow-up, and re-admission evaluations could not be made. Since chronic pancreatitis patients who have not been diagnosed yet are not know were evaluated as having acute pancreatitis.

Conclusion

Hematological parameters can help predict a prognosis in patients with acute pancreatitis. High CRP together with low hemoglobin and hematocrit can predict the prognosis of patients with acute pancreatitis. Although RDW is not statistically more significant than CRP, it can be used as a prognostic marker in patients with acute pancreatitis.

Declarations

Funding

Authors have no commercial interest and financial interest. The costs of the research were covered by the researchers.

Author contributions

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

Conflicts of interest

All authors declare that there are 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

The instant study was carried out with the permission of the University of Health Sciences, Ümraniye Education and Research Hospital Ethics Committee (Date: 23/06/2022, Decision No: B.10.1.TKH.4.34.H.GP.0.01/215).

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ORIGINAL PAPER

Zinc in fibromyalgia patients: relationship with body mass index and sleep quality

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ABSTRACT

Introduction and aim. Given the potential relationship between oxidative stress and fibromyalgia and well-documented antioxidant efficacy of zinc, the present study aimed to determine serum zinc concentration in FM patients as compared to healthy controls, as well as to identify the correlation of serum zinc concentration with the body mass index (BMI) and sleep quality,

Material and methods. In this case-control study, 54 fibromyalgia patients were consecutively recruited between October 01, 2021 and December 01, 2021. The control group consisted of 54 age- and sex-matched healthy controls.

Results. Fibromyalgia group had significantly lower zinc concentration, higher body mass index, and lower sleep quality scores as compared to the healthy control group. The correlation analysis revealed a significantly negative correlation between serum zinc concentration and body mass index and a significantly positive correlation between serum zinc concentration and sleep quality both in fibromyalgia and healthy control groups.

Conclusion. Our results both support the hypothesis that low serum zinc concentration plays a role in the pathophysiology of fibromyalgia and indicate that fibromyalgia may lead to weight gain and poor sleep quality, which needs to be confirmed in large-cohort studies.

Keywords. body mass index, fibromyalgia, sleep quality, zinc

Introduction

Fibromyalgia (FM) is a disease characterized by widespread musculoskeletal pain of unknown etiology.[1,2] Its prevalence is reported between 2% and 5% depending on age, sex and specific populations, with women being 2-3 times more likely to have this disease as compared with men.[2,3] In our country, the prevalence of FM ranges from 3.6% in adult females to 31% in geriatric population.[4-6]

The diagnosis of FM is usually established using the American College of Rheumatology (ACR) criteria, which requires presence of pain in at least 11 of the 18 tender points throughout the body on palpation for at least 3 months, with the combination of patient history, physical examination and laboratory analysis contributing to the diagnosis.[7,8]

In addition to widespread pain, patients with FM also present with accompanying conditions such as sleep disorder, mood disorder, cognitive disorder, headache, restless leg and fatigue. Pain, fatigue, and sleep disorder are the most common conditions that every patient with FM presents with.[1,7]

Although the etiology and pathogenesis of FM is yet to be clarified, interaction between genetic, immunologic, neuroendocrine, environmental, psychological and nutritional factors is thought to be involved in its pathogenesis.[1,8] The role of oxidative stress in the pathogenesis of FM is well documented. Low serum levels of antioxidant elements, particularly those involved in the redox process such as magnesium, zinc, and selenium, have been demonstrated in some FM

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patients. Moreover, serum magnesium and zinc levels are also associated with clinical parameters in FM, indicating their potential role in the pathogenesis of the disease.[9-12]

Zinc is an essential element required for various systems to function properly. It naturally exists in some foods and is also available as a dietary supplement. Recommended daily amount of zinc is 11 mg for adult males and 8 mg for adult females.[13] It is involved in the catalytic activity of nearly 100 enzymes as a cofactor and plays a role in numerous cellular metabolisms with its well-documented antioxidant role.[11,14]

Aim

Given the potential relationship between oxidative stress and FM and well-documented antioxidant efficacy of zinc, the present study aimed to determine serum zinc concentration in FM patients as compared to healthy controls, as well as to identify the correlation of serum zinc concentration with the body mass index (BMI) and sleep quality, both of which are frequently encountered in patients with FM.

Material and methods

This single-center case-control prospective study was carried out between October 01, 2021 and December 01, 2021 in the Istanbul Medipol University School of Medicine, Department of Internal Medicine, Istanbul Turkey. The study was approved by the Non-Interventional Clinical Research Ethics Committee of Istanbul Medipol University (Date 14/10/2021, Decision No:1009).

The study population consisted of 108 subjects aged ≥18 years [consecutively recruited 54 FM patients who met the ACR 2016 criteria and were otherwise healthy, and 54 age- and sex-matched healthy controls (HCs)].[15]

All subjects were informed about the purpose and design of the study in detail, and those who agreed to participate in the study and signed the informed voluntary consent form were enrolled.

A detailed medical history was obtained from all participants, and their physical examination including measurement of height and body weight was performed. The height was measured using a stadiometer with accuracy of 0.1 cm, and the body height was measured using a Tanita scale with accuracy of 0.1kg (Tanita Body Composition Analyzer, MC-780MA-N, Japan). The BMI was calculated as the body weight in kilograms divided by the square of the height in meters (kg/m^2). The patients in each group were then classified according to their BMI defined by the World Health Organization as normal weight (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2) and obese (≥30 kg/m^2).[16] Study exclusion criteria for all subjects were the history of chronic diseases, drug use, alcohol consumption, smoking and drug abuse, pathological findings on physical examination, and laboratory analyses indicating presence of a comorbid condition.

Blood samples for complete blood count, routine biochemical analyses [alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, thyroid-stimulating hormone (TSH), fasting blood glucose, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, triglyceride], and measurement of serum zinc concentration were collected from all study participants in the morning after 12-hour fasting period.

Serum zinc concentration was measured using the Randox colorimetric assay for zinc (United Kingdom) and then compared between the FM and HC groups. For the measurement of serum zinc concentration, 6 mL blood sample was taken into the heavy metal-free trace element tubes containing heparin. The blood was centrifuged at 2500 rpm for 10 minutes. Zinc concentrations were expressed in μg/dL.

Sleep quality in both groups was assessed using the Richards Campbell Sleep Questionnaire (RCSQ), which showed excellent internal consistency and moderate correlation with polysomnographic recording -the gold standard in measuring sleep quality. The RCSQ is a five-item self-administered questionnaire used to assess perceived sleep depth, sleep latency, efficiency of sleep, sleep quality and the number of awakenings during sleep. Subsequently, a 6th item -night-time noise- was included in the RCSQ. Each of the sleep parameters is rated from 0 (the worst possible sleep) to 100 (the best sleep) on a visual analogue scale. The total score ranges between 0 and 600, which is then divided by six to obtain a mean total score for each patient (0-25: very poor sleep quality, 75-100 good sleep quality).[17,18] The validity and reliability study of the Turkish version of RCSQ was performed in 2015 by Ozlu and Ozer.[19]

Statistical analysis

Data was analyzed using the MedCalc® Statistical Software Program version 19.7.2 (MedCalc Software Ltd, Ostend, Belgium). Descriptive statistics (mean, standard deviation, median, minimum and maximum) were presented for continuous variables. The normality of continuous variables was analyzed by Shapiro-Wilks test. Two independent normally distributed variables were compared using the Student t-test, whereas Mann-Whitney U test was used for the comparison of two independent non-normally distributed variables. The relationship between categorical variables was analyzed using the Chi-square test, in combination with Yates Continuity Correction Chi-Square, where available. The relationship between two variables was determined using the Pearson's correlation coefficient for normally distributed variables and using the Spearman's rho correlation for non-normally distributed variables.

The level of statistical significance was set at p <0.05. Univariate and multivariate logistic regression analyses including serum zinc, RCSQ sleep score and BMI were performed.

Results

A total of 108 subjects (54 in each of the FM and HC groups) were included in the study. Overall, 91 (84.3%) of the study population were female and 17 (15.7%) were male. There was no difference between the FM and HC groups in terms of gender distribution (FM group: 46 [85.2%] females and 8 [14.8%] males; HC group: 45 [83.3%] females and 9 [16.7%] males; p=1.00). The mean age of the study population was 42±10.3 years (range, 24-63 years). The FM and HC groups were comparable in terms of the mean age of the participants (42.6±9.8 years [range, 25-61 years] and 41.4±10.9 years [range, 24-63 years], respectively; p=0.559). The mean serum zinc concentration of the study population was 79.6±17.1 ug/dL (range, 42-128 μg/dL), with significantly lower concentrations detected in the FM group vs. the HC group (73.6±15.1 μg/dL [range, 42-102 μg/dL] vs. 85.7±17 ug/dL [range, 49-128 μg/dL]; p≤0.001). The mean BMI of the whole study group was 26.1±4.5 kg/m^2 (range, 18.2-37.5 kg/m^2). The FM group had significantly higher BMI compared to the HC group (FM group: 27.3±4.6 kg/m^2 [18.2-37.5 kg/m^2] and HC group: 24.8±4.2 kg/m^2 [18.2-32.8 kg/m^2]; p=0.003). With regard to the mean sleep quality score, it was 63.0±12.7 in the whole study population, and it was significantly lower in the FM group than the HC group [58.1±11.3 vs. 67.8±12.3; p≤0.001], indicating poorer sleep quality in the FM group. The general characteristics of the whole study population and according to FM and healthy control groups are demonstrated in Table 1.

Table 1. General characteristics of the whole study population and according to study groups[a]

Characteristics	Study population	FM Group	HC Group	p
	n=108	n=54	n=54	
Sex				
Female, n (%)	91	46 (85.2)	45 (83.3)	1.00*
Male, n (%)	17	8 (14.8)	9 (16.7)	
Age, years, mean±SD	42±10.3	42.6±9.8	41.4±10.9	0.559**
Zinc, ug/dL, mean±SD	79.6±17.1	73.6±15.1	85.7±17	<0.001**
RCSQ Score, mean±SD	63.0±12.7	58.1±11.3	67.8±12.3	<0.001***
BMI, kg/m^2, mean±SD	26.1±4.5	27.3±4.6	24.8±4.2	0.003***

[a] *Yates continuity correction, **Student's t-test, ***Mann-Whitney U test; FM – fibromyalgia; HC – healthy control; SD – standard deviation; BMI – body mass index; RCSQ – Richards Campbell Sleep Questionnaire

The correlation analysis between serum zinc concentration and BMI revealed a significantly negative correlation in the whole study population (r=-0.719, p<0.001) and in the FM and HC groups (r=-0.726, p<0.001 and r=-0.648, p<0.001, respectively).

The correlation of serum zinc concentration with sleep quality and BMI is demonstrated in Table 2.

Table 2. Correlation of serum zinc concentration with sleep scores and body mass index in the whole study population and according to study groups[a]

	Sleep score		BMI	
Serum Zinc	r	p	r	p
Study population	0.824**	<0.001	-0.719*	<0.001
FM Group	0.751*	<0.001	-0.726*	<0.001
HC Group	0.861**	<0.001	-0.648**	<0.001

[a] *Pearson's correlation coefficient, **Spearman's correlation coefficient; FM – fibromyalgia; HC – healthy control; BMI – body mass index

Regarding the correlation between serum zinc concentration and sleep quality, a significantly strong correlation was detected both in the overall study population and in the HC group (r=0.824, p<0.001 and r=0.861, p<0.001, respectively), and a good correlation was detected in the FM group (r=0.751, p<0.001). Univariate and multivariate regression analyzes are shown in Table 3.

Table 3. Univariate and multivariate regression analyzes

	Univariate Logistic Regression			Multivariate Logistic Regression		
	Sig	OR	95 % CI	Sig	OR	95 % CI
Zinc	<0.001	0.954	0.93–0.98	0.438		
Sleep Score	<0.001	0.989	0.98–0.99	<0.001	0.989	0.98–0.99
BMI	0.005	1.141	0.04–1.25	0.944		

Univariate regression analysis revealed that fibromyalgia was associated with both zinc and RCSQ sleep score and BMI. However, when the multivariate logistic regression analysis was examined, it was seen that this relationship was only between fibromyalgia and RSCQ sleep score.

Discussion

The present study, which investigated serum zinc concentrations in FM patients compared to the controls and its relationship with sleep quality and BMI, found significantly lower zinc concentration in the FM group and showed significant correlation with sleep quality and BMI.

Fibromyalgia is a painful condition affecting every population across the world. FM presents itself not only with pain, but also with various clinical conditions including mood disorders (such as anxiety, depression),

sleep disorders, restless leg syndrome, and chronic fatigue syndrome (CFS), each of which impairs the patient quality of life.[1] Thereby, prevention and treatment of this disorder is of great importance for patient quality of life. However, being aware of the pathogenesis of the disease is essential to establish accurate preventive measures.

Although the pathophysiology of FM is yet unclear, several mechanisms have been proposed, including the oxidative stress. Altindag and Celik reported significantly lower total plasma antioxidant capacity in FM patients than in healthy controls, supporting the role of antioxidants in the pathogenesis of FM.[20] Given that some trace elements, such as magnesium, zinc, and selenium are essential for some antioxidant enzymes and that oxidative stress is one of the mechanisms in the pathophysiology of FM, it has been suggested that deficiency of these elements might be involved in the pathophysiology of FM and related clinical conditions.[2,9-11,21-24]

Zinc is the second most important element for life following iron, and it is found in all tissues, fluids and secretions with total amount to be approximately 2–4 g.[13] Along with well-documented antioxidant efficacy of zinc, the role of serum zinc concentration in the etiology of FM and its relationship with clinical conditions has recently become the subject of interest.[11]

In a manner supporting the hypothesis that zinc plays a role in the pathophysiology of FM due to its antioxidant effect, a study reported lower zinc concentrations and higher levels of oxidative stress markers in the FM patients compared to the healthy controls. In that study, zinc showed strong negative correlation with the number of tender points, where magnesium showed moderate negative correlation. Moreover, oxidative stress markers also showed negative correlation with zinc and magnesium.[25]

In our country, significantly lower serum zinc and magnesium concentrations were determined in FM patients compared to the healthy controls, whereas no significant difference was found for serum selenium concentration. A significant correlation was demonstrated between zinc concentration and the number of tender points, suggesting that these elements might play a role in the pathogenesis of FM.[9,12,26] However, in the present study, we did not investigate the oxidative status of the FM patients or the association between zinc concentration and tender points, which may be considered a limitation for the present study.

Serum zinc concentration is low also in the patients with CFS and myofascial pain syndrome.[27,28] In addition, the erythrocyte concentration of zinc was found to be associated with pain in the patients with myofascial pain syndrome.[12] All of these findings support the impact of low zinc concentration on pain in FM patients.

A prospective study conducted in 60 Iraqi FM patients diagnosed based on the ACR criteria found significantly lower serum magnesium, calcium, and zinc concentrations in the patients with FM than healthy controls. They concluded that levels of these elements may be a good indicator to evaluate this disease.[14]

Contrary to the studies demonstrating low serum zinc concentrations in FM patients than in the controls,[9,12,14,24] Rosborg et al., who compared the concentrations of 30 elements in the whole blood, urine, and drinking water in female patients with FM and matching controls, found higher serum zinc concentrations in FM patients.[21] The results of this study did not support the hypothesis that trace elements including zinc play a role in the development of FM.[23] Likewise, a systematic review and meta-analysis of 5 randomized controlled trials and 40 observational studies investigating mineral and vitamin deficiencies in the patients with FM and with CFS failed to find sufficient evidence to support the hypothesis that vitamin and mineral deficiencies play a role in the pathophysiology of CFS or FM syndrome.[29]

These contradictory results reported in the studies investigating the role of trace elements in the pathogenesis of FM might be due to the differences between the studies in terms of sample size, study methods, specimens (such as whole blood, serum, and erythrocyte) used for the measurement of zinc concentration or the criteria used for the diagnosis of FM. In addition, time of blood sample collection for zinc measurement is also important since zinc concentration is affected by many factors.

In the present study, we demonstrated significantly lower serum zinc concentration in the FM group and supported that zinc was involved in the pathogenesis of FM, as was confirmed in many studies.

Obesity is also one of the clinical conditions that are frequently encountered in FM patients. Although the relationship between obesity and chronic musculoskeletal pain remains unclear, the prevalence of obesity in people with chronic musculoskeletal pain is high as 37-65%.[30] Such a high prevalence of obesity in the patients with chronic musculoskeletal pain including FM may result from the oxidative stress playing a role in the pathophysiology of obesity and FM or may be due to the pain itself, which restricts the activities of daily living and results in weight gain.[31] This was confirmed also in the present study, where the mean BMI value of the FM group was significantly higher than that of the HC group, even though BMI values in the FM group indicated rather overweight than obesity.

Zinc not only plays an important role in many biochemical and metabolic processes as a cofactor of some enzymes including those involving in the pathophysiology of obesity, but also participates in carbohydrate,

protein, and lipid metabolisms indicating its potential role in the pathophysiology of obesity.[32] The relationship between zinc and obesity, particularly in the presence of inflammation and oxidative stress, has been demonstrated in earlier studies.[32,33] Serum zinc concentration was found low in obese people, showing a negative correlation with BMI, particularly when compared with those with normal weight.[34] Moreover, a negative correlation was demonstrated also between low serum zinc concentration and insulin resistance in perimenopausal obese women.[35] In a study, no relationship was demonstrated between serum zinc concentration and BMI; however, an inverse relationship was determined between erythrocyte zinc concentration and BMI, suggesting that erythrocyte concentration of zinc might be a more reliable parameter in assessing zinc status.[36] Despite normal serum zinc concentrations both in the obese group and in the control group, a study reported lower serum zinc concentration in the obese group, which did not reach the level of statistical significance. However, a significantly negative correlation was demonstrated between serum zinc concentration and BMI.[37] In the present study, similarly, serum zinc concentration was within the normal range in both groups regardless of the BMI value. The correlation analysis revealed significantly negative correlation between zinc concentration and BMI in the whole study population and in both groups regardless of the pain status. The results indicating a negative correlation between zinc concentrations and BMI values suggest that FM patients have higher BMI values because of, at least in part, low serum zinc concentrations as well as limited activity due to pain.

The interaction between obesity, pain, and poor sleep quality is well-documented.[38,39] Sleep is essential for our body and mind to rest at night and to wake up in the morning as refreshed; thus, poor sleep or sleep quality has a considerable impact on daily life as well as well-being. FM is usually accompanied by sleep disorders due not only to obesity but also to the widespread pain, causing the people to hardly perform and maintain the activities of daily living. Considering that zinc plays a role in the pathophysiology of both obesity and FM, we hypothesized that it might be playing a role also in sleep quality in FM patients.

Although a Chinese cohort study failed to demonstrate any effect of blood zinc concentration on sleep quality at preschool age, lower blood zinc concentration in preschool age was predictive of poor sleep quality at adolescence and higher blood zinc concentration was associated with better sleep quality, underlining potential importance of zinc concentrations in early childhood.[40] In contrast, a study found no significant difference between the hemodialysis patients with and without sleep disturbances in terms of blood zinc, manganese, copper, and lead levels, but low blood selenium levels were associated with severe sleep disturbance.[41]

In addition to the limited number of studies investigating the effects of zinc concentrations in various body fluids on sleep quality, several studies also investigated the effect of zinc consumption or zinc supplements on sleep quality. A study investigating sleep quality in female students based on zinc consumption found no difference in sleep quality of those consuming adequate vs. inadequate amount of zinc. However, zinc intake showed a significant association with sleep delay and mental quality of sleep.[42] Effect of melatonin plus zinc supplement (M+Z) on sleep parameters in the patients with CFS was investigated in a randomized, double-blind, placebo-controlled study. In that study, sleep quality and sleep latency improved with treatment in both M+Z and placebo groups; however, sleep latency worsened after treatment discontinuation in the M+Z group. No difference was determined between the groups in any parameters of Pittsburgh Sleep Quality Index.[43] Conflicting results reported in the studies might have arisen from different scales used to assess sleep parameters.

The results of our study showed that there is a direct relationship between fibromyalgia and sleep quality. This is understandable given that fibromyalgia patients have lower zinc levels and a higher BMI and both of these factors affect sleep quality. Detection of lower zinc levels, worse sleep quality and higher BMI in fibromyalgia patients compared to the control group is due to the close relationship between these 3 conditions.

Our study has some limitations. First, low sample size and cross-sectional design of the study make it difficult to draw a definite conclusion. Besides, the major limitation was the fact that zinc concentration was studied only in the serum. Erythrocyte concentration of zinc may give more accurate results, because the majority of zinc in the body is found intracellularly.

Conclusion

Our results do not only support the hypothesis that low serum zinc concentration plays a role in the pathophysiology of FM but also indicate that FM may lead to weight gain and poor sleep quality, which needs to be confirmed in large-cohort studies. Studies investigating zinc concentrations in other tissue samples including erythrocytes (intracellular concentration) and showing an improvement in pain and related clinical conditions after treatment with zinc supplements are required to draw further and more precise conclusion.

Declarations

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Author contributions

Conceptualization, E.Y.; Methodology, E.Y.; Formal Analysis, E.Y.; Investigation, E.Y.; Resources, E.Y.; Writing – Original Draft Preparation, E.Y.; Writing – Review & Editing, E.Y.; Visualization, E.Y.; Supervision, E.Y.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

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

Ethics approval

The study was approved by the Non-Interventional Clinical Research Ethics Committee of Istanbul Medipol University (Date 14/10/2021, Decision No:1009).

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ORIGINAL PAPER

The role of hematological parameters in differentiating *Plasmodium falciparum* and others – a study from Somalia

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ABSTRACT

Introduction and aim. Accurate identification of Plasmodium species is important because of the differences in their treatment. We aimed to investigate the role of hematological and biochemical parameters in the differentiation of *Plasmodium falciparum* and other plasmodium species.

Material and methods. This is a retrospective study. Patients admitted to the emergency department with signs and symptoms of malaria were included into the study. Patients with malaria were grouped as *P. falciparum* and others. Hematological parameters of two groups were compared by univariate and multivariate analysis. Statistical analysis was performed using the Jamovi.

Results. A total of 107 patients were included in the study. According to univariant and multivariant analysis there was no difference in between two groups in the terms of blood urea nitrogen, aspartate aminotransferase, total bilirubin, hemoglobin, hematocrit, white blood cell count, platelet count, and mean platelet volume (in univariate analysis p values were 0.029, 0.011, 0.019, 0.171, 0.870, 0.307, 0.042, and 0.276, respectively and in multivariate analysis p values for blood urea nitrogen, aspartate aminotransferase, total bilirubin, hemoglobin, and platelet count were 0.100, 0.535, 0.328, and 0.213, respectively).

Conclusion. The investigated hematological and biochemical parameters were found to be not valuable in predicting type of malaria. On the other hand, we recommend confirming the results of our study with larger samples and multicenter studies.

Keywords. malaria, Plasmodium falciparum, Plasmodium vivax, platelets

Introduction

Malaria is a disease that has been known since ancient times and is seen in 300-500 million people per year in developing countries in tropical regions. Among the infectious diseases, death due to malaria ranks 6th to 8th.[1] It is estimated that in Africa, there are more than 12,000,000 malaria cases, and 155,000 –310,000 malaria-related deaths per year attributable to epidemics if control options are not implemented or well-timed, that is equal to about 4% of estimated annual malaria cases worldwide and 12–25% of estimated annual worldwide malaria-related deaths, including up to 50% of the estimated malaria mortality (annual worldwide) in adults.[2]

The traditional diagnosis of malaria is made by examining thin smear and thick drop preparations and this is accepted as the gold standard. However, several disadvantages of this method are known. Peripheral smear examination fails during periods of low parasitemia. In order to diagnose with the traditional method, it is necessary to examine a large number of microscopic fields, which is very laborious, causes loss of time and requires experienced personnel.[3] The type of parasite, the negligence

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of the microscopy, the mistakes made during the preparation and staining of blood smears sometimes cause the inadequacy of traditional diagnostic methods. For this reason, many research laboratories are trying to develop alternative methods for the diagnosis of malaria.[4]

Accurate identification of Plasmodium species is important because of the differences in their treatment. *Plasmodium falciparum* infections can be fatal in a very short time, *Plasmodium vivax* and *Plasmodium ovale* hypnozoites can remain latent in liver cells and cause relapses. The appearance of gametocytes in untreated individuals indicates active infection, and in treated individuals' persistent infection. It should be noted that persistent infection can also be seen after successful treatment.[5] Hemogram analysis is an easily accessible and inexpensive examination. We speculated that the hematological parameters may contribute to the differentiation of plasmodium species or to support the diagnosis.[6]

Aim

In this study, our aim is to investigate the role of hematological and biochemical parameters in the differentiation of *P. falciparum* and other plasmodium species.

Material and methods

Ethical approval

Approval for the study was obtained from the ethics committee of Mogadishu Somalia-Turkish Training and Research Hospital with 11 November 2021 date and 450 number. Informed consent was not obtained because the study did not include the personal information of the patients, within the knowledge of the ethics committee.

Study design

This analytical study was conducted retrospectively in a tertiary hospital emergency room with a total of 300 beds, 50 of which were intensive care units, in a sub-Saharan city Mogadishu located in the coastal Banaadir region on the Indian Ocean. During the study period, there were 350 emergency applications per day from the center where the study was conducted.

Study population

Patients who applied to the emergency department with malaria symptoms and signs between January 1, 2021, and January 1, 2022, were included in the study. Patients with haemoglobinopathy or hematological malignancy and patient with missing data were excluded from study. Patients' data were obtained from written documents and hospital computer-based laboratory system. Rapid Diagnostic Kit-Rapid Diagnostic Test (RDT) (SD Bioline Malaria Ag Pf/Pan™ RDT) (Batch No. 60952) for malaria was used for the diagnostic test of the patients. RDT is using to diagnose malaria by detecting evidence of malaria parasites (antigens) in human blood. These tests require a drop of peripheral blood. Visual readings are classically available in 20 minutes. Test results are reported as *P. falciparum* and other. The patients were divided into two groups according to these results.

Data collection

Demographics, signs, laboratory parameters and clinics of the patients were documented. Signs were recorded as splenomegaly and hepatomegaly, icterus, vomiting, diarrhea, cough, headache, and pallor. The laboratory parameters were recorded as total bilirubin, indirect bilirubin, direct bilirubin, creatinine, hemoglobin, platelet count, mean platelet volume, blood urea nitrogen, aspartate transaminase, alanine transaminase, hematocrit, and white blood cell count. The clinics of the patients were recorded as spontaneous bleeding, pulmonary edema, cerebral malaria, shock, severe acidosis, severe malarial anemia, clinical evidence of jaundice renal failure, vital organ dysfunction, and minor symptoms.

Statistical analysis

Jamovi (Version 1.6.21.0; The Jamovi Project, 2020; R Core Team, 2019) was used for statistical analysis. The similarity of the data to the normal distribution was evaluated with the Shapiro Wilk test. Categorical data were presented by number and percentage, and continuous data with median and 25th and 75th percentiles. For comparison between *P. falciparum* and other groups, chi-square test was used in categorical data and Mann Whitney U test was used in continuous data. Likelihood ratios (LRs) were calculated using sensitivity and specificity values in the evaluation of relationship between plasmodium species and laboratory parameters. Values below 0.05 were used for the significant p value.

Results

A total of 107 patients were included in the study. Seventy-five (70.1%) of the patients were male. The median age was 31 (25th and 75th percentiles: 25–50). The three most common symptoms were fever (42.1%), headache (30.8%), and vomiting (20.6%). Baseline characteristics of the enrolled patients and their distribution according to the type of malaria are shown in Table 1.

The comparisons of hematological parameters of the *P. falciparum* and other plasmodium type groups are shown in table1. In univariant analysis significant differences were identified between hematological parameters of the *P. falciparum* and other plasmodium type groups as; headache (8 (18.6%) versus 25 (39.1%), p=0.025), blood urea nitrogen (15.5 (range: 9–35) versus 10 (range: 7–22) mg/dL, p=0.029), aspartate aminotransferase (43.5 (range: 30.8–65.27) versus 28 (range: 21–48) U/L, p=0.011), total bilirubin (1.43 (range: 0.61–3.84) versus 0.56 (range: 0.33–1.28) mg/L,

Table 1. Baseline characteristics of the enrolled patients and their distribution according to the type of malaria

	Total	*Plasmodium falciparum*	*Other Plasmodium*
	n=107	n=43 (40.1%)	n=64 (59.9%)
Age, years	31 (25–50)	31.0 (25.5–51.5)	31.5 (24–48)
Gender			
Male	75 (70.1%)	30 (69.8%)	45 (70.3%)
Female	32 (29.9%)	13 (30.2%)	19 (29.7%)
Signs and symptoms			
Fever	45 (42.1%)	15 (34.9%)	30 (46.9%)
Headache	33 (30.8%)	8 (18.6%)	25 (39.1%)
Vomiting	22 (20.6%)	9 (20.9%)	13 (20.3%)
Cough	15 (14.2%)	7 (16.7%)	8 (12.5%)
Diarrhea	36 (34.0%)	15 (35.7%)	21 (32.8%)
Pallor	13 (12.1%)	6 (14.0%)	7 (10.9%)
Icterus	19 (17.8%)	7 (16.3%)	12 (18.8%)
Hepatomegaly	15 (14.0%)	9 (20.9%)	6 (9.4%)
Splenomegaly	6 (5.6%)	3 (7.0%)	3 (4.7%)
Laboratory findings			
Aspartate aminotransferase (U/L)	32 (22–57)	43.5 (30.8–65.27)	28 (21–48)
Alanine aminotransferase (U/L)	23 (15–46.5)	23.68 (17.62–45)	22 (11–52)
Total bilirubin (mg/dL)	0.66 (0.43–2.45)	1.43 (0.61–3.84)	0.56 (0.33–1.28)
Direct bilirubin (mg/dL)	0.35 (0.14–1.91)	0.73 (0.32–2.47)	0.29 (0.12–0.58)
Creatinine (mg/dL)	0.80 (0.50–1.12)	0.90 (0.43–1.39)	0.77 (0.55–1.01)
Blood urea nitrogen (mg/dL)	13 (8–29)	15.5 (9–35)	10.0 (7–22)
Hemoglobin (g/dL)	12.0 (10.1–13.2)	11.7 (9.6–13.2)	12.1 (10.2–13.9)
Hematocrit (%)	35.0 (28.3–40.4)	35.5 (25.1–40.7)	34.6 (31.2–40)
White blood cell count (10^3/μL)	6.18 (4.80–8.00)	6.04 (5.32–8.53)	6.20 (4.6–8)
Platelet count (10^3/μL)	212 (102–294)	166 (102–218)	118 (116–334)
Mean platelet volume (fL)	8.0 (7.2–9)	8.2 (7.3–10.2)	8.0 (7–9)
Platelet mass index	1760.0 (1037.9–2513.5)	1552.5 (1029.2–2196.0)	1920.0 (1242–2679.1)

p=0.019), and platelet count (166 (range: 102-218) versus 118 (range: 116-334) 10^3/μL, p=0.042). On the other hand, according to multivariant analysis there was no statistically significant difference in the terms of headache, blood urea nitrogen, aspartate aminotransferase, total bilirubin, and platelet count. Univariate and multivariate logistic regression analyses of patients with *P. falciparum* and others are presented in Table 2.

Table 2. Univariate and multivariate logistic regression analyses of patients with *P. falciparum* and others*

	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	p	OR (95% CI)	p
Age, years		0.136		0.546
Age, ≥50 vs. <50	1.123 (0.458–2.752)	0.801		
Gender		0.952		
Headache	0.357 (0.142–0.893)	0.025	0.406 (0.079–2.09)	0.406
Blood urea nitrogen (mg/dL)		0.029	1.029 (0.995–1.06)	0.1
Aspartate aminotransferase (U/L)		0.011	0.995 (0.981–1.01)	0.535
Total bilirubin (mg/dL)		0.019	1.079 (0.926–1.26)	0.328
Hemoglobin (g/dL)		0.171		
Hematocrit (%)		0.870		
White blood cell count (10^3/μL)		0.307		
Platelet count (10^3/μL)		0.042	0.996 (0.989–1)	0.213
Mean platelet volume (fL)		0.276		

*OR – odds ratio; CI – confidence interval

The ROC curve analysis was performed to determine the predictive ability of the blood urea nitrogen, total bilirubin, aspartate aminotransferase, and platelet count for predicting type of malaria. The cut-off values of these parameters according to the best Youden's index, as well as their sensitivity, specificity, AUC, and 95% confidence interval values are presented in Table 3.

Discussion

In current study, we evaluated role of hematological parameters in the differentiation of *P. falciparum* and other plasmodium species. According to the results of this study, there was a statistically significant difference between the *P. falciparum* and other plasmodium species groups for all mentioned parameters.

In our analysis, first, nonparametric comparison tests were used to determine the relationship between scoring systems and mortality. There was significantly difference between groups in the term of headache, blood urea nitrogen, aspartate aminotransferase, total bilirubin, and platelet count. A second analysis was

Table 3. Accuracy of the blood urea nitrogen, total bilirubin, aspartate aminotransferase, and platelet count in predicting type of malaria*

	AUC	Cut–off	Sensitivity	Specificity	+LR	–LR	+PV	–PV	Accuracy	95% CI	p
Blood urea nitrogen (mg/dL)	0.626	≤12	64.3%	59.0%	1.57	0.61	51.9	70.6	61.2	51.1–70.7	0.025
Total bilirubin (mg/dL)	0.684	≤0.61	76.0%	58.1%	1.68	0.44	57.6	73.9	64.3	50.3–76.7	0.009
Aspartate aminotransferase (U/L)	0.656	≤30.8	81.6%	55.6%	1.84	0.33	56.4	81.1	66.3	55.7–89.7	0.007
Platelet count (10^3/μL)	0.619	≤220	53.2%	75.6%	1.62	0.346	76.7	51.7	62.1	52.0–71.5	0.033

*AUC – area under the curve; PV – predictive value; CI – confidence interval; LR – likelihood ratio

performed based on the ROC curve to determine the laboratory parameters' ability to distinguish whether a patient infected with *P. falciparum* or other plasmodium species. AUC values less than 0.5 were evaluated as indistinguishable from random, while those close to 1 were considered close to the perfect model.[7,8] It has been reported that the AUC value should be greater than 0.8 for a model to predict mortality well.[7,8] In the discriminatory power analysis, we determined the AUC value of laboratory parameters lower than 0.7, which was considered to be unacceptable. Thus, our retrospective, comparative study, was demonstrated that laboratory parameters that we investigated were not a predictor of plasmodium species, according to ROC analysis. On the other hand, LRs supply the clearest data on the way in which laboratory parameter can be used reliably. Ratios >5 or < 0.2 provide of strongest evidence.[9,10] In our study group, LR values of laboratory parameters were not in this range. A further analysis was performed based on multivariant analysis. Confirming the ROC analysis results, the multivariate analysis showed that the parameters with a significant difference compared to the univariant analysis result were not sufficient to say that they were statistically independent predictors of the difference between the groups.

Malaria is a disease that is transmitted to humans by the bite of a parasitic mosquito, which can be fatal if not treated in time, and causes fever and chills in seizures. Rare ways of transmission are congenital, blood transfusion, shared injector use, organ transplantation, nosocomial transmission.[11] The simple, effective, and short-term method used in the diagnosis of malaria is the Giemsa stain. In this method, thin smears and thick drops are made from the blood sample taken from the fingertip and after staining with Giemsa, the evolutionary periods of the parasite are searched. If the diagnosis is not made at the first examination, 3 consecutive days of examination are recommended.[12]

Changes in hemoglobin, platelet and leukocyte counts are used to determine the severity of the disease.[13-15] Among the hematological parameters, hemoglobin is the most frequently affected in severe disease. Severe anemia has been reported in 5.5 to 15% of cases. It has been reported that this change may be a change related to geography.[16-18] Platelet count is affected in severe malaria infections, as in sepsis. Disseminated intravascular coagulation, immune mechanisms, and hypersplenism are some examples of mechanisms that could be mechanism of platelet reduction in malaria patients as sepsis and septic shock.[19,20] The transient increase in band cells observed in infection indicates a stronger stimulus for neutrophil production during the acute phase of infection. In the acute phase, premature release of neutrophils from the bone marrow occurs, resulting in an increased proportion of younger, less well-differentiated neutrophils into the circulation. Although this alteration is widely recognized in other acute infectious diseases, few studies have investigated these disorders for neutrophil in malaria infection.[21] Rodrigues-da-Silva et al. compared erythrogram and leucogram of *P. falciparum* and *P. vivax* infections. They evaluated white blood cell counts and red blood cell counts, hemoglobin, hematocrit, reticulocyte, lymphocyte, eosinophil, platelet, segmented neutrophil, band cell, monocyte, and basophil counts. They reported that there was no difference between the *P. falciparum* and *plasmodium vivax* groups in terms of all hematological parameters, except for platelet count.[22] Arévalo-Herrera et al. compared hemoglobin and platelet count of *P. falciparum* and *plasmodium vivax* groups with severe malaria. They found that there was no difference between the two groups in terms of hemoglobin and platelet count. In the current study, it was seen that there was no difference between *P. falciparum* and other *Plasmodium* groups in terms of all hematological parameters.[23] The results of both mentioned studies were compatible with current study. The main difference between the current study and mentioned literature was difference in the methodology of the studies. While thick smear was used as a diagnostic test in both studies, RDT was used in our study. The rapid diagnosis kit could not distinguish between plasmodium types other than *P. falciparum*.

The most important limitation of the current study was its retrospective design. The second limitation is that microscopy was not used to distinguish between *P. falciparum* and other plasmodium species. A third limitation is inability to distinguish other plasmodium species is the inability to perform subgroup analysis. A fourth limitation is that we could not evaluated possible confounders such as bacterial infections. A last limitation is the single-center study and the relatively limited sample size. We suggest that our study results be validated with multicenter studies with larger samples.

Conclusion

The present study was done to test the ability of the hematological parameters to differentiating *P. falciparum* and others. Based on all the observations from the present study, it was concluded that the there was no statistically significant difference between the *P. falciparum* and other *Plasmodium* species groups for all mentioned parameters, total bilirubin, indirect bilirubin, direct bilirubin, creatinine, hemoglobin, platelet count, mean platelet volume, blood urea nitrogen, aspartate transaminase, alanine transaminase, hematocrit, and white blood cell count. These mentioned parameters were found to be not valuable in predicting type of malaria. On the other hand, we recommend confirming the results of our study with larger samples and multicenter studies.

Declarations

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Author contributions

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

Conflicts of interest

We declare no conflict of interest.

Data availability

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

Ethics approval

Study was approved by the institutional review board, and a waiver of authorization was given (Ethics Committee decision no. 450, date: 11.22.2021).

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ORIGINAL PAPER

The relationship between women's childbirth experiences and their maternal attachment and the risk of postpartum depression

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ABSTRACT

Introduction and aim. This descriptive study was conducted to examine the effects of the childbirth experiences of mothers on their maternal attachment and postnatal depression.

Material and methods. The study was conducted with 315 mothers who agreed to participate in the study in Obstetrics and Gynecology Hospital between 2 September 2019 and 25 February 2020. A "Personal Information Form", "Childbirth Expectation and Experience Questionnaire (CEEQ)", "Maternal Attachment Inventory (MAI)", and "Edinburgh Postnatal Depression Scale (EPDS)" were used to collect data. The data were analyzed with the IBM SPSS v23 program. Independent-samples t test, one-way analysis of variance (ANOVA), and Pearson's correlation test were used in the analyses.

Results. The mean postpartum depression mean score of the participants was 4.3±5.9, and 23% of them were at risk of depression. The mean maternal attachment score of participants was determined as 85.2±4.6, and their mean birth experience score was 3.0±3.1. A positive and significant relationship was found between more than half of the items of Childbirth Expectation and Experience Questionnaire and general satisfaction ($p \leq 0.05$). The childbirth experience scores of the participants were not significantly related to their maternal attachment and postpartum depression scores ($p > 0.05$).

Conclusion. In this study, no significant relationship was found between the childbirth experiences of women and their maternal attachment or postpartum depression levels.

Keywords. childbirth experience, childbirth satisfaction, maternal attachment, postpartum depression

Introduction

Childbirth is defined as a multifaceted experience. Feelings of safety and control, pain experienced at labor, personal support systems, nursing/midwifery care that is received, preterm delivery, mode of delivery, use of analgesia, and women's participation in the decision-making process affect their childbirth experience.[1,2] Women's childbirth experiences have positive or negative outcomes in terms of quality of life, well-being, and health, both in the short term and in the long term.[3]

The potential effect of childbirth on the mother is related to the mother's delivery experience.[4] While a positive childbirth experience is remembered as an empowering life event affecting the transition to motherhood, a negative experience can lead to postpartum depression, fear of future childbirth, and demand for cesarean section.[3,5-

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[10] Positive childbirth experiences increase the self-confidence of women, promote their relationships with their children, and contribute to their future childbirth planning processes.[11] Women with negative childbirth experiences may have posttraumatic stress disorder, sexual dysfunction, inability to adapt to their parental role, insufficiency in maternal attachment, postpartum depression, increased breastfeeding problems, inability to make a decision to have another baby, and fear of future childbirth.[11-12] Postpartum depression is a mental health issue that has a prevalence of 5-60.8% worldwide.[13] During this period, the mother-baby interaction may be affected by lack of joy, low energy and self-esteem, sleep disorders, ambivalence, changes in appetite, fear of injury, anxiety about the baby, sadness, crying, doubt, difficulty in concentration, apathy, and thoughts of death and suicide.[14] It has been stated that the stress experienced by mothers may also have a negative effect on the mother-infant attachment process.[15-17] Additionally, some studies have stated that postpartum depression leads to inadequate maternal attachment.[14,18] Nevertheless, some studies have reported contradictory results about the effects of childbirth experiences on mother-infant attachment.[4,19]

The postpartum period is the period when the mother is the most likely to establish a close relationship with her baby, and the most attachment is experienced.[20] The mother's attachment to her baby in the first few months of the postpartum period can positively affect her attachment attitudes in her next pregnancy and attachment patterns.[21-24] The first attachment experience that the baby will have forms the basis of the attachment they will experience in the following years.[25]

Aim

Childbirth is a significant life experience, and it can affect the perception of future childbirth processes. For this reason, determining the positive and negative experiences of mothers and intervening with these experiences will prevent problems that may occur in later life periods. Mothers with negative childbirth experiences or early depression due to these experiences should be noticed by nurses during follow-up and be provided with necessary consultations. This study was conducted to determine the relationship between women's childbirth experiences and their maternal attachment and postpartum depression levels.

Material and methods

Ethical approval

Permissions were obtained from the researchers who performed the validity and reliability studies of the scales. Additionally, written permission was obtained from Ege University Ethics Committee (Decision number: 19-6.1T/25) and the Buca Obstetrics and Gynecology Hospital in İzmir. The mothers who participated in the study provided verbal consent.

Study design and participants

This is a descriptive study. The population of the study consisted of women who gave birth in the Buca Obstetrics and Gynecology Hospital. The total number of women who gave birth (via vaginal delivery or cesarean section) in the hospital in 2017 was 6667. The G*Power 3.1.9.2 software was used to conduct a power analysis to determine the required sample size. With 1–β error probability, 80% power was considered sufficient.[26] Post hoc power was determined as 100% for a sample size of 315 participants. The women to be included in the sample were determined using the random sampling method. Women who were able to read, understand, and speak Turkish, did not have a mental illness, had not received any psychiatric diagnosis before, and agreed to participate in the study were included. Women who were or whose babies were taken to the intensive care unit during delivery owing to a complication and those whose babies had to be transferred to another hospital were excluded.

Collection of data

The data were collected between 2 September 2019 and 25 February 2020 by the researchers using a "Personal Information Form", the "Childbirth Expectation and Experience Questionnaire", the "Maternal Attachment Inventory", and the "Edinburgh Postnatal Depression Scale".

Data collection tools

Personal Information Form

This form (22 questions) was prepared by the researchers in line with the literature.[27,28] The form included questions on the sociodemographic (17 questions) and obstetric (10 questions) characteristics of the participants including age, educational status, employment status, marital status, number of pregnancies and births, age of the husband, occupation, income level, and social security status.

Childbirth Expectation and Experience Questionnaire (CEEQ)

This scale was developed in 2008 by Tanglakmankhong and consists of two parts CEEQ-1 and CEEQ-2. The first part of the scale, which evaluates the expectations of pregnant women from delivery in the intrapartum period, consists of 36 questions. The expressions of these questions were formed by using the future tense (CEEQ-1). There are 37 questions in the second part of the scale to evaluate the satisfaction of postpartum women's expectations (CEEQ-2). The Childbirth expectations are questioned in the first part, and childbirth experiences are questioned in the second part. CEEQ-1 is a binary likert type scale designed as "Yes" and "No" for each question item. For example, in questions about the woman's thoughts on the events that will occur during

childbirth, the woman who will give birth is asked to evaluate the statement "Item 1: I got medication to reduce pain". CEEQ-2was administered after women gave birth and this scale consists of past tense expressions of the first 36 items in CEEQ-1. In this study, the second part (CEEQ-2), including only birth experiences, was used. The response options for the first 36 items evaluating childbirth experiences are "Yes" and "No". Item 37 of the scale has a 4-point Likert-type scoring system with the response options of "not satisfied", "satisfied", "moderately satisfied", and "very satisfied". The total score of the scale is calculated by adding the scores of the first 36 items, and the score of the 37th item is not included. Cronbach's alpha coefficient of the scale was reported as .94.[29] Its validity and reliability analyses in Turkish were performed by Muslu and Yanıkkerem (2020), and its Cronbach's alpha value was found to be .89. In this study, the Cronbach's alpha value was calculated as .90.[28]

Maternal Attachment Inventory (MAI)

This scale was developed by Müller (1994), and its Cronbach's alpha coefficient was found to be .85 among women who had just become mothers.[30] It was adapted to Turkish by Kavlak and Şirin (2009) who reported the Cronbach's alpha values of the scale as .77 and .82 among mothers with one-month-old babies and those with four-month-old babies, respectively. In this study, the Cronbach's alpha value of MAI was determined as .71. MAI is a four-point Likert-type scale consisting of 26 items. The items consist of direct statements scored between 1 and 4 (never, sometimes, often, and always). The minimum and maximum scores to be obtained from the scale are 26 and 104. Higher the scores indicate higher maternal attachment levels.[22]

Edinburgh Postnatal Depression Scale (EPDS)

This scale, which was developed by Cox et al., was prepared to determine the risk of postpartum depression in women.[31] Its adaptation to Turkish was performed by Engindeniz et al.[32] This 4-point Likert-type scale consists of 10 items. The response options are scored between 0 and 3. The minimum and maximum score that can be obtained from the scale are 0 and 30. Items 1, 2, and 4 are directly scored, while items 3, 5, 6, 7, 8, 9, and 10 are inversely scores. The internal consistency coefficient of the scale was reported to be .79, its split-half reliability coefficient was .80, its sensitivity was .84 when the cut-off point was taken as 12/13, its specificity was .88, its positive predictive value was .69, and its negative predictive value was .94. In this study, the Cronbach alpha value of the scale was determined to be .73. The cut-off point of the scale was calculated as 12, and the participants with a scale score of 12 or higher were considered to be at risk of postnatal depression.

Data collection process

Data collection was carried out in two stages. In the first stage, the women who agreed to participate in the study were interviewed in their rooms 12 hours after they gave birth, and the "Personal Information Form" and the "Childbirth Expectation and Experience Questionnaire" were applied to them. Their phone numbers were saved, and the second interview was performed by phone with the same women 4-6 weeks after their delivery. The "Maternal Attachment Inventory" and the "Edinburgh Postnatal Depression Scale" were applied to the women on the phone.

Data analysis

The data were analyzed using the SPSS software (IBM, Chicago, IL, USA). Descriptive statistics such as frequencies, percentages, arithmetic means, and standard deviations were used in the analyses. As the data were normally distributed, parametric tests were used in the analyses. Independent-samples t-test was used in the comparison of the mean results of two independent groups, one-way analysis of variance (one-way ANOVA) was used in the comparison of more than 2 independent groups, and Pearson's correlation test was used in the analyses of the relationships between the scale scores of the participants. All test results were evaluated at the significance level of ≤0.05.

Results

The mean age of the women who participated in the study was 23.4±3.8, and approximately two-thirds (70.5%) were 25 years old or younger. Based on their self-reports, 97.5% of the participants had social security, the income of 53.7% was less than their expenses, and 90.2% lived in the city. Also all participants were married.

Table 1. Scale scores of the participants (n=315) *

	n		SD	Min.	Max.
MAI	315	85.2	4.6	78	101
EPDS	315	4.3	5.9	0	23
CEEQ-2	315	3	3.1	2.1	3.6

*MAI – Maternal Attachment Inventory; EPDS – Edinburgh Postnatal Depression Scale; CEEQ-2 – Childbirth Expectation and Experience Questionnaire 2

The mean gestational week of the participants at childbirth was 39.1±1.1 (min.: 37, max.: 42). While 89.9% (n=315) stated that it was their first pregnancy, 8.6% (n=315) had experienced a miscarriage, and 2.5% (n=315) had had an abortion. It was found that 83.5% (n=315) of the participants had a wanted pregnancy, 69.5% (n=315) had received support from their husbands during pregnancy, 11.4% had support from their husband's sister, 10.8% had support from their mothers-in-law, 3.5% (n=315) had support from their mothers, and 4.8% (n=315) had support from their sisters-in-

law. The mean MAI, EPDS, and CEEQ-2 scores of the participants were determined as 85.2±4.6, 4.3±5.9, and 3±3.1, respectively (Table 1).

Table 2. Relationships between birth experience scale item scores and childbirth satisfaction scores (n=315)

	General Childbirth Experience	
	R	P
1. I got medication to reduce pain	0.111*	0.049
2. I got medication to induce labor	0.213**	<0.0001
3. I had special instruments for checking my baby's health	0.186**	0.001
4. I had a vaginal examination for checking cervix dilatation	0.154**	0.006
5. I had intravenous fluids	0.237**	<0.0001
6. I had food and fluids withheld during labor and birth	0.137*	0.015
7. I had other laboring women stay in the same room during labor	-0.028	0.617
8. I had a relative by my side during labor	-0.024	0.677
9. I had my husband by my side during labor	-0.016	0.781
10. I was able to contact my family during labor	0.157**	0.005
11. I got supportive care from nurses during labor	0.528**	<0.0001
12. I received information from nurses about methods of pain relief	0.532**	<0.0001
13. I received information from nurses about my progress of labor	0.489**	<0.0001
14. I had my legs strapped on metal stirrups during delivery	0.200**	<0.0001
15. I was in a private delivery room during delivery	0.005	0.935
16. I had a nurse coaching during delivery	0.730**	<0.0001
17. I was delivered by a nurse	0.696**	<0.0001
18. I was delivered by a doctor	0.581**	<0.0001
19. I was informed immediately when something is wrong with me or my baby	0.631**	<0.0001
20. I was involved in decision making about my care and treatments during the delivery process	0.562**	<0.0001
[a]21. I was assisted with forceps or vacuum instruments when I could no longer push	–	–
[a]22. I had an operation to deliver my baby if I had any complications	–	–
23. I had an episiotomy	0.128*	0.024
24. I had anesthetic medication before the episiotomy	0.312**	<0.0001
25. Doctor was ready to help at any time when something was wrong with me during delivery	0.596**	<0.0001
26. Student nurses took care of me during my labor and birth	0.593**	<0.0001
27. Nurses spoke to me politely	0.664**	<0.0001
28. Nurses treated my family politely	0.660**	<0.0001
29. Nurses helped me talk with doctor	0.743**	<0.0001
30. Nurses contacted the doctors for me when I wanted to consult the doctors	0.719**	<0.0001
31. Nurses were happy to help me	0.626**	<0.0001
32. Nurses were busy and may not have time to take care me	0.643**	<0.0001
33. Nurses brought my baby to me immediately after birth	-0.010	0.856
34 Nurses took a very good care of my baby after birth	-0.022	0.693
35. My baby and I were safe during labor and birth	0.193**	0.001
[a]36. My husband and my family had a chance to hold my baby after birth	–	–

[a] 21, 22, 36: The correlation coefficient and p-value could not be measured since the responses given to items 21, 22, and 36 were fixed; * There is a weak positive correlation between CEEQ-2 and the item of birth satisfaction; ** There is a strong positive relationship between CEEQ-2 and the item of birth satisfaction.

There was no significant relationship between the scores of the participants obtained from the three scales, indicating no significant relationship between the childbirth experiences of the participants and their maternal attachment and postpartum depression levels (p>0.05) (Table 3).

Table 3. Relationship between the scale scores of the participants*

		CEEQ-2	MAI	EPDS
CEEQ-2	R	1		
	P			
MAS	R	-0.057	1	
	P	0.315		
EPDS	R	-0.064	-0.038	1
	P	0.255	0.499	

*CEEQ-2 – Childbirth Expectation and Experience Questionnaire-2; MAI – Maternal Attachment Inventory; EPDS – Edinburgh Postnatal Depression Scale

Discussion

The act of delivery is affected by individuals' previous life negative birth experiences can have everlasting effects on the physical and mental health of the woman throughout her life. The negative birth experience may affect in the near future or in the following years, women should be examined at regular intervals after birth, and their satisfaction and birth experiences should be questioned. There is still no standard assessment in the evaluation of the relationship between the birth experience of mothers and postpartum depression and maternal attachment.

In this study, the mean birth experience scale score of the participants was determined as 3±3.1. Among their participants Tanglakmankhong found the mean CEEQ-2 score to be 3.5, which was higher than that in this study.[29] Muslu and Yanıkkerem, on the other hand, reported a lower mean score of CEEQ-2. In the study conducted by Tanglakmankhong and this study included both primiparous and multiparous women and both women who had cesarean section deliveries and those who had vaginal deliveries.[29] The lower mean score of CEEQ-2 in the study by Muslu and Yanıkkerem was thought to be due to the fact that the study was conducted only with primiparous women who gave birth through vaginal delivery.[28] A positive and significant relationship was found between more than half of the birth experience scale items (27 items) and general satisfaction. The items that were not found significantly related to general satisfaction were: "Item 7: I had other laboring women stay in the same room during labor," "Item 8: I had a relative by my side during labor," "Item 9: I had my husband by my side during labor," "Item 33: Nurses brought my baby to me immediately after birth," and "Item 34: Nurses took a very good care of my baby

after birth." The deficiency in mother- or baby-friendly practices for intrapartum care services may be the reason for the lack of significance in the relationships of these items to general satisfaction with childbirth. In this context, although the hospital where the study was conducted is a mother-baby-friendly hospital, it may be stated that there are problems in the implementation of practices such as accompanying the woman during the childbirth process, bringing the mother and baby together immediately, and ensuring the participation of the husband in the delivery process. Similarly, in the study conducted by Muslu and Yanıkkerem, no statistically significant relationship was determined between childbirth satisfaction and the statements of the same scale in items 7, 8, 9, 34, or 35. In the same study, in addition to these items, no statistically significant relationship was found between general satisfaction with childbirth and the following: "Item 15: I was in a private delivery room during delivery" and "Item 21: I was assisted with forceps or vacuum instruments when I could no longer push."[28] In contrast, Tanglakmankhong found significant positive relationships between general satisfaction and all items of CEEQ-2. These differences may be explained by different modes of delivery of the women included in different studies. While women who had either vaginal delivery or cesarean section delivery were included in the study conducted by Tanglakmankhong and in our study, the sample of Muslu and Yanıkkerem included only women who had vaginal delivery. In another study, it was found that pregnant women from Germany had a more negative childbirth experience compared to Belgian pregnant women, and women giving birth at home had more positive birth experiences than those giving birth in hospital.[33] Additionally, the individual childbirth experiences of women and differences in the implementations practiced in the intrapartum period may have caused this variation in results, as these factors vary from country to country.

In this study, the mean EPDS score of the participants was 4.3±5.9, and 23% of them were at risk in terms of depression. The mean EPDS scores of women in other studies conducted in Turkey were higher than that in our study.[34-36] In studies conducted in other countries, the rates of antenatal depression have varied between 7% and 30.9%.[5-39] The rate of postpartum depression may vary from culture to culture and region to region. The differences in different studies may have been affected by multifactorial variables such as the individual childbirth experiences of women, their mode of delivery, the socio-cultural structure of the society they live in, the planning status of their pregnancies, their perceptions of motherhood, their history of depression, partner support, physical changes, and interruption of work life. Parents contented with the introduction of a new baby in the family may not notice the symptoms of depression at an early stage. Therefore, postpartum depression assessment measures should be evaluated in a longer term, not shortly after birth.

The MAI scores of the participants of our study were lower than those reported in the literature.[22,40-42] Maternal attachment that begins between the mother and the baby during pregnancy and continues after delivery is influenced by many factors such as the personal and obstetric characteristics of mothers, the perception of motherhood and social support, childbirth experiences, mode of delivery, prenatal education, culture, the involvement of the partner, and postpartum depression.

In this study, no significant relationship was identified between the participants' childbirth experiences and their maternal attachment and postpartum depression levels. In Turkey, there have been no studies conducted on this subject. While it has been reported in eleven studies conducted in other countries that there is a statistically significant relationship between childbirth experiences and postpartum depression, no such relationship was stated in four studies.[43-46] Trauma and stress experienced during and after childbirth are known to cause psychological effects in postpartum women.[48] In the study conducted by Ünsal Atan et al., nearly half women reported their birth experiences as poor, bad, and very bad.[36] In the same study, the risk of postpartum depression was found to be higher in mothers who had vaginal deliveries, those who had not received training on modes of delivery during pregnancy, and those who underwent interventions during delivery (enema, oxytocin induction, and amniotomy). It was also stated that unfavorable childbirth experiences and postpartum stress can negatively affect mother-infant attachment.[48] Practices in Turkey such as electronic fetal monitoring, enema, oxytocin induction, amniotomy, episiotomy, intravenous hydration, and restrictions on food and beverage intake are routinely used without medical necessity cause postpartum depression and the interruption of mother-infant attachment.

Limitations of the study

It was a limitation that this study was conducted in a single hospital in one city, and the results of the study cannot be generalized to the entire society.

Conclusion

The process of childbirth is affected by individuals' previous experiences and negative experiences can have everlasting effects on the physical and mental health of the woman throughout her life. In our study, more than half of the participants had positive childbirth experiences. There was no significant relationship between the childbirth experiences of the participants and their maternal attachment and postpartum depression levels. However, it is known that a stressful life increases the risk of postpartum depression. Therefore, it is important that

nurses/midwives provide mothers with a positive birth experience, which can reduce the risk of postpartum depression and strengthen the bond between the mother and her baby.

In this context, nurses should provide supportive care that ensures women's confidence, privacy, collective decision-making, and safety. There is still no standard assessment in the evaluation of the relationship between the childbirth experiences of mothers and their postpartum depression and maternal attachment statuses. Since a negative childbirth experience may show its effect in the near future or in the following years, women should be examined at regular intervals after childbirth, and their satisfaction and birth experiences should be considered. The timing of the measurements of these factors is also important in postpartum evaluations that are recommended to be carried out. In evaluations made immediately after childbirth, the relaxation of women and their joy of having a healthy baby may replace negative emotions and lead the actual experience to be overlooked. Therefore, it is recommended that the childbirth experiences of women be evaluated in both short and long terms, negative birth experiences be revealed, and changes be made in health services for the desirable outcomes.

Declarations

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Author contributions

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

Conflicts of interest

The authors have no conflict of interest. Additionally, there is no relationship of interest with any company in the study we are responsible for. No support was received from any project or company for the research.

Data availability

The data have not been made public, but are kept with the authors, if necessary.

Ethics approval

Study was approved by Ege University Ethics Committee (Decision number: 19-6.1T/25) and the Buca Obstetrics and Gynecology Hospital in İzmir.

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ORIGINAL PAPER

Risk and associates of tobacco, alcohol and cannabis use among undergraduate university students – a Pan-India cross-sectional study

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ABSTRACT

Introduction and aim. Substance abuse and its associated problems are a global concern. Young adults, particularly college-going students, remain among the highest at-risk groups for various substance use disorders. So, this study was conducted to find out the prevalence of substance use and its correlates among undergraduate (UG) university students.

Material and methods. We did an online cross-sectional survey among 1003 undergraduate university students across India using a pre-structured, self-reported questionnaire consisting of basic demographic details, standard tool (WHO-ASSIST), and the results were tabulated. A multivariable binary logistic regression analysis was performed to find out the correlates of substance use and Pearson correlation to find a correlation between ASSIST scores. Significance was attributed to a p-value <0.05.

Results. A total of 320 (31.9%), 167 (16.7%), and 125 (12.5%) among 1003 students used alcohol, tobacco, and cannabis respectively. 70 (21.9%), 116 (69.5%), and 62 (49.6%) were at moderate-high risk of abuse for alcohol, tobacco, and cannabis respectively. There was a strong positive statistically significant (p<0.001) correlation between all three substance-specific scores (Pearson's Coefficients r = 0.643, 0.763, and 0.725 respectively).

Conclusion. One, two, and three out of every ten students used cannabis, tobacco, and alcohol respectively. Many of them fall into the moderate-high risk category. The data suggest that a student at high risk for any one substance is also at a higher risk of using another substance as well. This calls for an integrated 'bundle' approach to focus on all substances together as one unit.

Keywords. alcohol, cannabis, India, substance use, tobacco, WHO-ASSIST

Introduction

Substance abuse and its associated problems are a global concern. WHO estimates published in 2022, report that around 3 million deaths every year result from harmful use of alcohol, tobacco use kills more than 8 million people each year and about 0.5 million deaths annually are attributable to drug use.[1–3] These are important contributors to the global disease burden with studies showing that around 4.2% of all DALYs (disability-adjusted life-years) are attributable to alcohol use, and 1.3% of all DALYs are attributable to drug use as a risk factor.[4] According to the Global Adult Tobacco Survey (GATS-2), India report, 28.6 percent of all Indian adults currently either smoke tobacco and/or use smokeless tobacco; khaini and bidi being the most commonly used tobacco products.[5,6] The report on 'Magnitude of Sub-

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stance Use in India 2019' estimated that 14.6% of the population of India (between the ages of 10 and 75) uses alcohol and 2.8% of the population uses cannabis products either in the legal form (bhang) or in illegal forms (ganja and charas).[7]

Substance use disorder (SUD) is the persistent use of drugs despite substantial harm and adverse consequences. They are characterized by an array of mental/emotional, physical, and behavioral problems such as chronic guilt; an inability to reduce or stop consuming the substance(s) despite repeated attempts; driving while intoxicated; physiological withdrawal symptoms.[8,9]

Adolescence is the period when the initiation of these habits usually takes place. During adolescence, students are more vulnerable due to increased academic pressure, peer group influence, and increased popularity and availability of substances. Understanding the pattern and circumstances leading to substance abuse will help guide appropriate interventions to protect young adults from substance abuse and its consequences like dependence and injuries.[10–12]

Educational institutes around the globe have long tried various ways to restrict or decrease the prevalence of substance use on their campuses but their success is highly questionable. This may be attributed to the lack of proper data that makes the formulation of appropriate interventions a shot in the dark for the authorities. Moreover, most strategies have a telescopic narrow approach to tobacco/alcohol/cannabis control, by focusing only on one substance, but maybe it will be more effective if all the substances were targeted together as one unit to bring down the risk of abuse for each substance.

Systematic reviews and meta-analysis studies have tried to gauge the effectiveness of various types of interventions for reducing substance use, and while some strategies seem to show minor improvements, the need for more research that identifies the most effective combinations of intensive behavioral, pharmacologic and newer interventions for these substance use disorders, has been reported,[13,14] which bears the weight for the collection of data on the determinants influencing the use of such substances. Studies conducted worldwide have estimated a prevalence rate of substance abuse to be around 20-40 percent among university students; however, these restrict themselves to tobacco or alcohol use or are stream specific and many of these are gender-biased. Additionally, not many multi-center studies have been conducted in developing countries.[15] So, unlike previous studies, we tried to focus on all undergraduate students from various educational streams across India and living in different places.

There are only limited studies in India (8-10) that have estimated the prevalence of substance abuse among university students.[16,17]

Aim

Hence, the present study was designed to estimate the prevalence of substance abuse (mainly alcohol, tobacco, and cannabis), determine the prevalence of risk associated with these substance use, and identify factors that may have an association with the different substances used among university students from various streams across India.

Material and methods

Study design and duration

This was an online cross-sectional study that was carried out for a duration of 3 months (November 2021 to January 2022). All participants gave their informed consent for inclusion before they participated in the study. The approval from the Institute Ethics Committee (IEC) was taken (AIIMS/Pat/IEC/2022/930).

Study setting

This study was primarily conducted at All India Institute of Medical Sciences (AIIMS) Patna, a 960 bedded tertiary care hospital & medical college and an institute of national importance under the Ministry of Health and Family Welfare, Government of India.

The study was conducted via online mode with undergraduate university students from different streams participating from all over India.

Study population

The study population included all undergraduate university students from various streams across India. The study tool was shared with students of AIIMS Patna and undergraduate university students from all over India. All the students above 17 years (age for admission into undergraduate courses) were included and students who did not consent to participate in the study were excluded. (Figure 1)

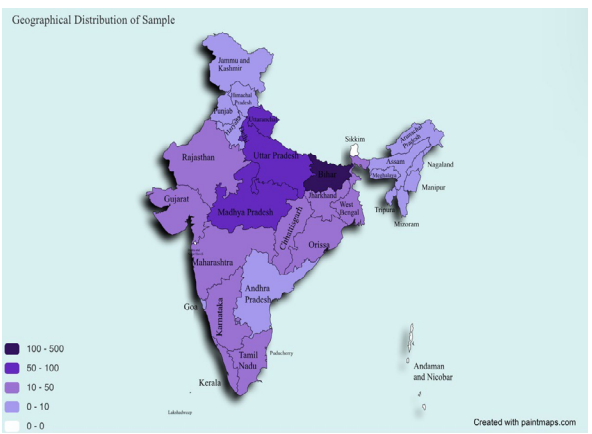

Fig. 1. Distribution of study participants across India (n=1003)[18]

Sample size and sampling technique

Considering the prevalence of tobacco use to be around 28%, with absolute precision of 5%, the mini-

mum sample size was calculated to be 310 at 95% confidence intervals (CI).[5] Nonetheless, we intended to include all eligible students in the study.

The list of all students of AIIMS Patna and their contact numbers were obtained from the AIIMS Patna administration section and the study tool was shared with them over WhatsApp/email and they were asked to participate voluntarily. They were also asked to share the study tool among their contacts from various streams and also the study tool was sent to universities all over India via emails of student unions/official WhatsApp groups/Instagram handles and all undergraduate students were urged to participate if they were willing. A total of 1003 students participated across India.

Study tool and technique

Information was collected using a predesigned, structured, standard questionnaire on "Google forms" that was sent to all students via WhatsApp and email. Digital consent was encrypted into the Google form link, and participants could proceed only after giving consent. 'ASSIST version 3.0' given by WHO was used.[19]

The questionnaire was divided into two sections and all questions were in the English language. The first section included socio-demographic details of the students like the basic details (age, gender and place of stay), educational stream, substance use habits (alcohol, tobacco, and cannabis), and associated drives behind them. The second section included questions related to the prevalence of substance use mainly alcohol, tobacco, and cannabis, and the risk associated with them using the WHO-ASSIST (version 3.0) questionnaire.[19]

In the ASSIST questionnaire, specific substance involvement scores were given.

A 6-item tool for tobacco products with 4 items on a 5-point Likert scale ranging from 'never' to 'daily/almost daily' and 2 items on a 3-point Likert scale ranging from 'no-never' to 'yes, in the last three months', with the scores ranging from 0–31 with 0–3, 4–26 & 27+ scores signifying low, moderate & high-risk levels respectively. For alcoholic beverages, a 7-item tool was used with 5 items on a 5-point Likert scale ranging from 'never' to 'daily/almost daily' and 2 items on a 3-point Likert scale ranging from 'no-never' to 'yes, in the last three months' with the scores range from 0–39 with 0–10, 11–26 & 27+ scores signifying low, moderate & high-risk levels respectively. For cannabis, a 7-item tool was used with 5 items on a 5-point Likert scale ranging from 'never' to 'daily/almost daily' and 2 items on a 3-point Likert scale ranging from 'no-never' to 'yes, in the last three months' scores range from 0–39 with 0–3, 4–26 & 27+ scores signifying low, moderate & high-risk levels respectively.

The tool had good concurrent, construct, predictive and discriminant validity, including the development of cut-off scores for 'lower', 'moderate', and 'high' risk.[19] According to WHO-ASSIST, the moderate-high risk category signifies hazardous use/at risk of dependency for a particular substance and warrants brief interventions and/or treatment to prevent progression to substance abuse.[20]

The questionnaire has been validated in an Indian setting and was found to have a good internal consistency (Cronbach's alpha: 0.8–0.86 for alcohol, tobacco, and cannabis).[20,21]

Statistical analysis

The collected information was entered in MS Excel and statistical analysis was done by Jamovi software[22] and IBM SPSS ver. 22.0 (IBM Corp., Armonk, NY, USA). The results were tabulated or represented as figures wherever necessary. A descriptive analysis was done to describe the socio-demographic details of the students. The continuous variables like age, age of initiation of substance use, and ASSIST scores were expressed as mean (SD) after checking the normality of the data.

The categorical variables like different streams, place of stay, and risk quantification of ASSIST scores were shown as frequencies and proportions. A univariate analysis was performed and the variables with p value less than 0.2 were considered for multivariate analysis model. The crude odds ratio (OR) with 95% CI was reported. A multivariable binary logistic regression analysis was done to find out the associates of substance use among students. We used 'Enter Method' in SPSS to build the models and the model fit was assessed by Hosmer-Lemeshow goodness of fit test and the model variability was given by Nagelkerke's R^2 (pseudo R square). An adjusted odds ratio was calculated with a 95% CI. Statistical significance was attributed to $p<0.05$.

Results

Out of 1003 participants, it was found that the maximum number, i.e. 806 (80.3%) belonged to the 20-25 year age category, with the mean (SD) age of 21 (1.65) years. Almost two-thirds, 632 (63%) were males. We found that 719 (71.7%) participants resided in hostels. It was seen that a maximum of 668 (66.6%) belonged to the medical and allied fields (Table 1).

The mean (SD) age of initiation for alcohol, tobacco, and cannabis was found to be 19.6 (1.94), 19.6 (2.25), and 20.6 (2.21) years respectively.

Among the sample of 1003 participants, 320 (31.9%, 95% CI: 29.07–34.85%) self – reported that they consumed alcohol, 167 (16.7%, 95% CI: 14.47–19.08%) used tobacco products, and 125 (12.5%, 95% CI: 10.56–14.65%) engaged in the use of cannabis products (Figure 2).

Table 1. Socio-demographic details of students (n=1003)

Variable	Category	n (%)
Age (years)	<20	184 (18.3)
	20-25	806 (80.4)
	>25	13 (1.3)
Gender	Male	632 (63)
	Female	371 (37)
Place of Stay	Hostel	719 (71.7)
	own residence	211 (21)
	Rental Apartment	73 (7.3)
Stream/Course*	Medical field & allied	668 (66.6)
	Sciences & allied	63 (6.3)
	Engineering	209 (20.8)
	Commerce & allied	18 (1.8)
	Arts	27 (2.7)
	Law	18 (1.8)
Academic year of college	First year	260 (25.9)
	Second year	206 (20.5)
	Third Year	334 (33.3)
	Fourth year	138 (13.8)
	Fifth year	13 (1.3)
	Internship	52 (5.2)

*Medical & allied – MBBS, nursing, and dental students; Sciences & allied – basic science stream, home science, and life science students; Commerce & allied – commerce, economics, management, and business administration students

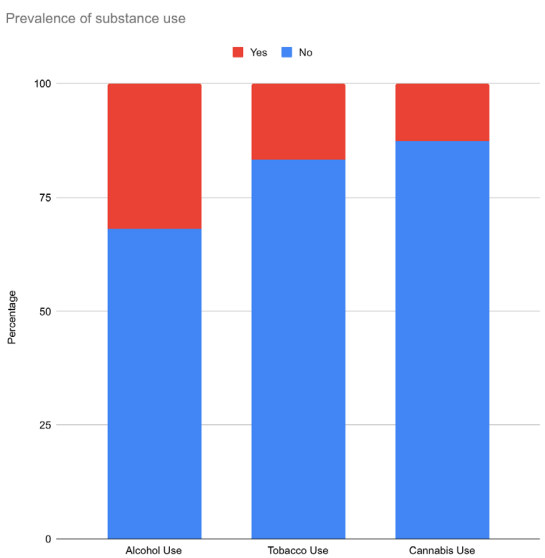

Fig. 2. Prevalence of substance use among students (n=1003)

Upon calculation of substance – specific risk for abuse using ASSIST, for alcohol, out of 320 students, 70 (21.9%, 95% CI: 17.69–26.72%) fell into the category of moderate – high risk; for tobacco, out of 167 students, 116 (69.5%, 95% CI: 62.1–75.94%) fell into the category of moderate – high risk; and for cannabis, out of 125 students, 62 (49.6%, 95% CI: 40.98–58.24%) fell into the category of moderate – high risk (Table 2).

Table 2. Risk stratification of various substance use (WHO-ASSIST)

Variable	Category	n (%)
Alcohol risk (n=320)	Lower Risk	250 (78.1)
	Moderate Risk	54 (16.9)
	Higher Risk	16 (5)
Tobacco risk (n=167)	Lower Risk	51 (30.5)
	Moderate Risk	105 (62.9)
	Higher Risk	11 (6.6)
Cannabis risk (n=125)	Lower Risk	63 (50.4)
	Moderate Risk	48 (38.4)
	Higher Risk	14 (11.2)

In the univariate analysis, for alcohol, age (crude OR, 1.390; 95% CI, 1.274–1.515), male gender (crude OR, 1.540; 95% CI, 1.160–2.044), living in a hostel (crude OR, 1.630; 95% CI, 1.195–2.223), and later academic year (second year: crude OR, 2.783; 95% CI, 1.803–4.295, third – year: crude OR, 2.535; 95% CI, 1.702–3.776, fourth – year: crude OR, 3.259; 95% CI, 2.031–5.231, and fifth year/internship: crude OR, 7.605; 95% CI, 4.197–13.778) were found to be significant predictors of substance use.

For tobacco, age (crude OR, 1.454; 95% CI, 1.314–1.608), male gender (crude OR, 2.338; 95% CI, 1.582–3.455), living in a hostel (crude OR, 1.669; 95% CI, 1.113–2.501), and later academic year (second year: crude OR, 2.618; 95% CI, 1.5–4.567, third – year: crude OR, 1.874; 95% CI, 1.101–3.19, fourth – year: crude OR, 3.018; 95% CI, 1.664–5.472, and fifth year/internship: crude OR, 7.242; 95% CI, 3.739–14.027) were found to be significant predictors of substance use.

For cannabis, age (crude OR, 1.417; 95% CI, 1.271–1.579), male gender (crude OR, 3.497; 95% CI, 2.127–5.747), living in a hostel (crude OR, 2.118; 95% CI, 1.297–3.459), studying in the medical field (crude OR, 0.623; 95% CI, (0.405 – 0.956) and later academic year (second year: crude OR, 3.724; 95% CI, 1.812 – 7.652, third – year: crude OR, 3.735; 95% CI, 1.896 – 7.358, fourth – year: crude OR, 2.981; 95% CI, 1.342 – 6.618, and fifth year/internship: crude OR, 11.628; 95% CI, 5.262 – 25.693) were found to be significant predictors of substance use.

These variables were considered in the multivariable logistic regression.

Age (for alcohol: AOR, 1.215; 95% CI, 1.089–1.356, for tobacco: AOR, 1.328; 95% CI, 1.173–1.503, for cannabis: AOR, 1.179; 95% CI 1.02–1.362), male gender (for alcohol: AOR, 1.415; 95% CI, 1.050– 1.907, for tobacco: AOR, 2.184; 95% CI, 1.456–3.275, for cannabis: AOR, 3.323; 95% CI, 1.990–5.546), living in hostels (for alcohol: AOR, 1.554; 95% CI, 1.079–2.239, for tobacco: AOR, 1.634; 95% CI 1.068–2.501, for cannabis: AOR, 1.923; 95% CI, 1.098–3.368) and later academ-

Table 3. Associates of various substance use among students (n=1003)*

Parameter	Categories	Alcohol use (n=320)		Tobacco use (n=167)		Cannabis use (n=125)	
		Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
Age (years)		1.390 (1.274–1.515)	1.215 (1.089–1.356)	1.454 (1.314–1.608)	1.328 (1.173–1.503)	1.417 (1.271–1.579)	1.179 (1.02–1.362)
Gender	Male	1.54 (1.16–2.044)	1.415 (1.05–1.907)	2.338 (1.582–3.455)	2.184 (1.456–3.275)	3.497 (2.127–5.747)	3.323 (1.99–5.546)
	Female	1	1	1	1	1	1
Place of stay[a]	Hostel	1.63 (1.195–2.223)	1.554 (1.079–2.239)	1.669 (1.113–2.501)	1.634 (1.068–2.501)	2.118 (1.297–3.459)	1.923 (1.098–3.368)
	Others	1	1	1	1	1	1
Stream[b]	Medical field & allied	1.256 (0.943–1.672)	0.825 (0.565–1.203)	0.826 (0.576–1.185)	–	0.623 (0.405–0.956)	0.737 (0.427–1.273)
	Non-medical fields	1	1	1	–	1	1
Academic year	First Year	1	1	1	1	1	1
	Second Year	2.783 (1.803–4.295)	2.455 (1.559–3.868)	2.618 (1.5–4.567)	2.000 (1.127–3.55)	3.724 (1.812–7.652)	3.109 (1.474–6.558)
	Third year	2.535 (1.702–3.776)	2.09 (1.325–3.297)	1.874 (1.101–3.19)	1.207 (0.686–2.126)	3.735 (1.896–7.358)	3.062 (1.453–6.454)
	Fourth year	3.259 (2.031–5.231)	2.740 (1.549–4.845)	3.018 (1.664–5.472)	1.895 (0.998–3.597)	2.981 (1.342–6.618)	2.611 (1.047–6.515)
	Fifth year & internship*	7.605 (4.197–13.778)	4.023 (1.943–8.329)	7.242 (3.739–14.027)	2.611 (1.159–5.883)	11.628 (5.262–25.693)	6.633 (2.466–17.84)
	Nagelkerke R Square	0.128		0.14		0.164	

* OR – odds ratio; AOR – adjusted OR; CI – confidence interval; [a] Place of stay was clubbed into hostel and others (which included home and rental apartment); [b] The stream was clubbed into medical fields and non-medical fields (which included sciences and allied, engineering, arts, commerce, and allied and law)

ic year (for alcohol: from second year – AOR, 2.455; 95% CI, 1.559–3.868 to fifth year/internship – AOR, 4.023; 95% CI, 1.943–8.329, for cannabis: from second year – AOR, 3.109; 95% CI, 1.474–6.558 to fifth year/internship – AOR, 6.633; 95% CI, 2.466–17.840, and for tobacco: only for second year – AOR, 2.000; 95% CI, 1.127– 3.550 and for fifth year/internship – AOR, 2.611; 95% CI, 1.159–5.883) were found to be independent predictors for substance use (Table 3).

There was a strong positive correlation between all three substance-specific scores, i.e., for alcohol, tobacco, and cannabis (as suggested by the Pearson's Coefficients of 0.643, 0.763, and 0.725) and all correlations were statistically significant (p<0.001). (Table 4)

Table 4. Correlation matrix for various substances used

Variables	ASSIST Alcohol Score Correlation coefficient (p-value)	ASSIST Tobacco Score Correlation coefficient (p-value)	ASSIST Cannabis Score Correlation coefficient (p-value)
ASSIST Alcohol Score	1		
ASSIST Tobacco Score	0.643 (<0.001)	1	
ASSIST Cannabis Score	0.763 (<0.001)	0.725 (<0.001)	1

For alcohol, social events were the leading reason (reported by 50.3% of alcohol users), followed by personal desire and interest (45.3%) and inexperience and curiosity (33.8%).

For tobacco products, personal desire and interest were the leading drivers (reported by 44.3% of tobacco users), followed by social events (39.5%) and stress (34.7%).

For cannabis products, social events and personal desire and interest were both the leading causes (both reported by 50.4% of cannabis users), followed by inexperience and curiosity (37.6%) and stress (23.2%). (Figure 3)

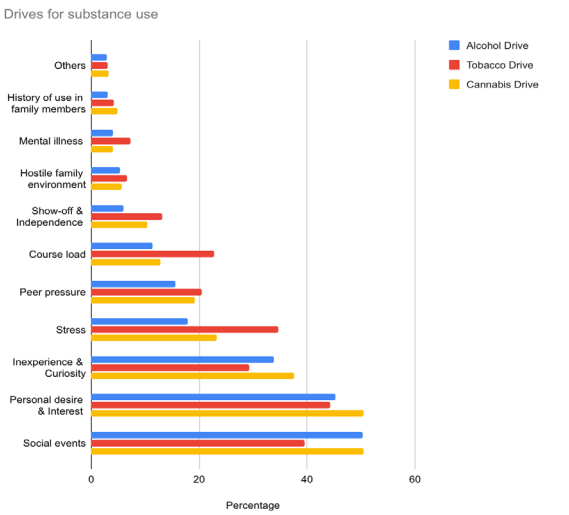

Fig. 3. Drives for alcohol, tobacco, and cannabis use (self-reported)

Discussion

The prevalence of alcohol, tobacco and cannabis use among undergraduate university students was found to

be 31.9%, 16.7% and 12.5% respectively. Out of those who use these substances, 21.9%, 69.5% and 49.6% users were at moderate-high risk of abuse for alcohol, tobacco and cannabis respectively. Age, male gender, living in hostels and later academic years were found to be significant predictors of substance use. A strong positive correlation between all three ASSIST substance specific scores was observed. Social events, personal desire & interest, inexperience & curiosity and stress were reported as the leading drives which lead to substance use.

In the National Drug Dependence Treatment Centre (NDDTC), All India Institute of Medical Sciences (AIIMS), New Delhi's 2019 report on 'Magnitude of Substance Use in India', the prevalence of alcohol use among males was 27.3%,[7] which is close to the prevalence of 31.9% that we found in our study. In the study conducted by Baba et al.[23] the prevalence of substance use they found i.e. 31.3% was remarkably close to our finding of alcohol use being prevalent in around 31.9% of students. Although contrary to their findings, we found that alcohol and not tobacco was the most commonly used substance among undergraduate students.

Compared to the GATS-2 India report,[5] the prevalence of tobacco use we found, i.e. 16.7% was much lower than the 28.6% prevalence of tobacco use in the Indian population aged 15 and above. Again, the prevalence was far less than the 55.6% found in adolescent males by Saddichha et al.[24] This can maybe be explained by observational bias, or inadequacy of samples equally from all parts of India, or maybe the prevalence among this age group and demographic is less, but this could not be verified due to the non-availability of specific data.

Upon risk stratification using ASSIST, we observed that 21.9% of alcohol users, 69.5% of tobacco users, and 49.6% of cannabis users fell into the moderate-high risk category, which is close to findings by McNeely et al.[25] for alcohol (24.8% users) but significantly higher compared to 34.6% tobacco users and 23.8% cannabis users falling into the moderate-high risk category as reported by them. Similar comparisons can be drawn with other previous studies as well which report a lower proportion of users falling into the moderate-high risk category.[26] This may be explained by the difference in calculation of the proportion (i.e. inclusion of participants who reported no lifetime use of any substance for calculating the proportion), the difference arising due to selection of participants, reporting bias or it is also possible that the risk is higher in this population i.e. the undergraduate university students.

In our study, it was observed that age and male gender were predictors of substance use, consistent with the conclusions of many studies.[27–30] We found that males were 2-3 times more likely to use substances compared to females, same as reported by Obadeji et al.[31]

Our finding that location/place of stay (at home or hostel) is a predictor of substance use, was similar to that of Jinyoung et al. and Muskoya et al.[11,32] It was observed that students living in hostels were 1.5 times more likely to indulge in alcohol consumption, 1.6 times more likely to use tobacco products, and almost twice as likely to use cannabis. Thus, regular raids and checks, more stringent vigilance by campus security, installation of smoke detectors, prohibiting the sale of such substances inside campus premises, etc. are all steps that can be taken to reduce the prevalence of these substances in hostels.

A Arora et al.[15] and their finding that the prevalence was higher among medical students in later academic years was also corroborated in our study, and we found that it held true for other educational streams as well. In our study, it was observed that compared to the first year, students in later academic years were 2-6 times more likely to engage in substance use. This may be due to the exposure they get with age and peer pressure as they move to higher classes. This points to the need to strengthen the efforts to control substance use in later academic years.

The most significant finding was the strong positive correlation observed between all three ASSIST substance-specific scores. This kind of association between the use of all three substances was also reported by Simon et al.[27] This points toward the need for an '*integrated combined approach*' for controlling substance use. A student who is at high risk for tobacco abuse is more likely to show high-risk behavior for alcohol and cannabis use as well. Therefore, strategies must be formulated to include not just tobacco but other substances as well. Thus, it can be reasoned that if the use of all the substances is considered and targeted as one unit, the prevalence of each substance might be brought down. Conversely, it also points to the possibility that maybe the current control strategies for decreasing an individual substance's use, like tobacco or alcohol, have been less successful, due to a 'narrow' approach and a lack of a broader, more well-rounded approach that focuses on many risk factors simultaneously.

Social events, inexperience & curiosity, and personal desire were the three leading drives behind substance use. This finding that occasional celebrations (social events) were among the leading reasons for substance use, was also similar to observations by Baba et al.[23] Peer pressure and academic load/performance were also identified as causes leading to substance use, similar to findings by Tomczyk et al.[33] Stress was also reported as a major drive behind substance use, especially for tobacco products.

This gives us a potential way to decrease the use of substances by targeting these drives; like chaperones at school sanctioned social events might discourage their use, early education about the risks associated with using them may help curb the curiosity & desire to try them and providing university students with effective

tools/ strategies to deal with stress like ensuring easy access to a therapist, yoga and meditation sessions, might stop students from turning to substances as a coping mechanism.

The study has potential limitations which may help explain some of the discrepancies in our findings compared to previously established reports and future studies should address them. In order to get a pan-India sample, the data was collected through online mode for a wider reach, but the selection of participants depended on our ability to gain access to the geographical scope of the participants, which was limited. Although we did get participants from every state of India, the contribution from each state is not equal or in proportion to its population size. The sampling technique in this study was not random but a mixture of snowball sampling (as students of AIIMS Patna were asked to forward the Google form to their friends in other colleges and other streams) and convenient sampling (as the authors sent the link to participate in the study to other universities via emails, official WhatsApp groups and Instagram accounts of college administrators/student unions), which is a potential selection bias. Despite assuring the participants that no personal identifiers will be collected and that all data will be kept confidential, there exists a possible reporting bias due to the inherent taboo associated with substance use, especially with cannabis use, since most forms of it are illegal in India, which could have compelled some subjects to withhold information. Also, we couldn't eliminate the bias administered due to self-administered nature of the study tool.

Conclusion

One, two, and three out of every ten undergraduate students were found to use cannabis, tobacco, and alcohol, respectively. Two out of ten alcohol users, seven out of ten tobacco users, and five out of ten cannabis users were identified to fall into the category of hazardous use/at risk of dependent use.

It was seen that age, male sex, living in hostels (compared to homes or rental apartments), and later/ higher academic years (second, third, fourth, and fifth compared to the first year) were significant predictors of substance use. These findings substantiate the need for creating control strategies for college-going students focusing on these factors. A strong positive correlation was seen between the usages of all three substances, which calls for a 'bundle' approach to control them, instead of focusing on only one substance.

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Declarations

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Author contributions

Conceptualization, R.B. and R.Rao; Methodology, R.B. and R.Rao; Software, R.Rao and R.J.; Validation, R.Rao and R.; Formal Analysis, R.B., R.J. and R.Rao; Investigation, R.B. and R.J.; Resources, R.Rao and R.; Data Curation, R.B., R.Rajan, R. and S.S.; Writing – Original Draft Preparation, R.B.; Writing – Review & Editing, R.J., R.Rao, R.Rajan, R.G., R. and S.S.; Visualization, R.J., R.G. and S.S.; Supervision, R.Rao; Project Administration, R.Rajan and R.G.; Funding Acquisition, Not applicable.

Conflicts of interest

The authors do not have any conflicts of interest to declare.

Data availability

The datasets are not publicly available but are available from the corresponding author on reasonable request.

Ethics approval

All participants gave their informed consent for inclusion before they participated in the study. The approval from the Institute Ethics Committee (IEC) was taken (AIIMS/Pat/IEC/2022/930).

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REVIEW PAPER

Correlation between rheumatoid arthritis and periodontitis

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ABSTRACT

Introduction and aim. The association between periodontitis (PD) and rheumatoid arthritis (RA) has been analyzed and described in literature. Periodontal pathogens, such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* are pointed as the common factors for both diseases. In this work we demonstrate that treatment of dental and oral diseases is an unconditional requirement for patients with RA.

Material and methods. PubMed was searched with the keyword "rheumatoid arthritis" and "periodontitis" from May 1999 to January 2022, showed 181 articles. Ultimately 72 articles were included in the review.

Analysis of the literature. The above mentioned pathogens exhibit multiple mechanisms that disturb immune and inflammatory responses of the human organism. Those mechanisms lead to the periodontal disease (PD) that may activate the systematic reactions which in turn lead to intensification of systematic diseases such as rheumatoid arthritis (RA). *P. gingivalis* has the ability to expresses PAD enzyme (peptidylarginine deiminase) and activates the citrullization process. Moreover, the bacterium produces gingipain cysteine proteinases, which degrade the mechanisms of immunological system. The latter pathogen, *A. actinomycetemcomitans,* expresses hypercitrullization in neutrophils.

Conclusion. Both pathogens influence inflammatory response of the organism, through the common pro-inflammatory mediators for periodontitis and rheumatoid arthritis, intensify the clinical manifestations of both diseases.

Keywords. *Aggregatibacter actinomycetemcomitans*, periodontitis, *Porphyromonas gingivalis,* rheumatoid arthritis, risk factors

Introduction

The association between periodontitis (PD) and rheumatoid arthritis (RA) has been analyzed and described in literature.[1-3] Periodontal pathogens, such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* are pointed as the common factors for both diseases. The above mentioned pathogens exhibit multiple mechanisms that disturb immune and inflammatory responses of the human organism. Those mechanisms lead to the periodontal disease (PD) that may activate the systematic reactions which in turn lead to intensification of systematic diseases such as rheumatoid arthritis (RA).[4,5] *P. gingivalis* has the ability to expresses PAD enzyme (peptidylarginine deiminase) and activates the citrullization process. Moreover, the bacterium produces gingipain cysteine proteinases, which degrade the mechanisms of immunological system.[6] The latter pathogen, *A. actinomycetemcomitans,* expresses hypercitrullization in neutrophils.[7] Both pathogens influence inflammatory response of the organism, through the common pro-inflammatory mediators for periodontitis and rheumatoid arthritis, intensify the clinical manifes-

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tations of both diseases.[5] In this work we demonstrate that treatment of dental and oral diseases is an unconditional requirement for patients with RA.[8]

Rheumatoid arthritis (RA) is a chronic, systematic disease with an inflammatory and autoimmunological background of unclear etiology. The pathogenesis of RA consists of not only genetic, but also environmental factors such as smoking. The disease can cause symmetrical cartilage and bone damage mainly in hands and feet. The clinical manifestations include pain, swollen and inflammatory exudate in joints and periarticular tissues. Additionally, patients complain about morning stiffness and limited range of motion. The course of illness is variable with remission and exacerbation periods. RA appears more frequently in females than males, especially in the elderly. It can lead to systematic extra-articular changes, disability, premature death and socioeconomic burdens.[9,10]

Periodontitis (PD) is a chronic, inflammatory disease which is associated with dysbiosis of the oral microbiota (dental plaque). It relates to the supporting structures of the teeth – the gingiva, bone and periodontal ligament. The potential results include tooth loss and systemic inflammation. To the risk factors, which promote the development of the disease are: inappropriate oral hygiene, interaction with the immune defense of the host, dysbiosis of oral microbiota, environmental risk factors (smoking) and genetic susceptibility.[11-14]

Many authors who in their studies indicate a possibility of simultaneous occurrence of RA and PD emphasize the existence of the association between these diseases.[1-3] They indicate common features such as: pathogenesis, inflammatory mediators profile, environmental factors (smoking, low socioeconomic status), genetic factors (*HLA-DRB1* allele of the MHC class II molecules), clinical manifestations (Figure 1).

We could say, that PD is a risk factor for RA.[15] The risk is especially pronounced within patients with a severe and seropositive RA where a stronger clinical course of PD has been observed. Coexistence of RA and PD can lead to intensification of either or both of the diseases. Active periodontal disease is associated with higher RA disease symptoms. Consequently, the treatment of one disease may have influence on the other.[16]

Aim

The aim of the study was to analysis of the currently available literature related to periodontitis and rheumatoid arthritis. In this work, we focus on the periodontal pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actimomycetemcomitans* which are pointed out as the comman factors for both diseases.

Material and methods

In this work we analyzed literature, related to the connection between periodontitis and rheumatoid arthritis. We focused on the latest available literature by searching electronic database – PubMed (NCBI). 181 articles were found from the period of May 1999 until January 2022. Finally, 72 titles were considered for analysis. We selected the articles on periodontitis and rheumatoid arthritis in which two periodontal pathogens (*P. gingivalis, A. actinomycetemcomitans)* were described together with their influence on rheumatoid arthritis.

Analysis of the literature

Porphyromonas gingivalis

As described in the literature, *P. gingivalis* – a gram-negative, anaerobic bacterium involved in periodontal disease, could be also predisposing factor for RA development or exacerbation. The bacterium disturbs the immune and inflammatory responses of the human organism and causes inflammation in the dental pockets. This inflammation in turn could affect systematic reactions which intensify the systematic diseases such as RA.[4,14,17-19]

Citrullination is a physiological process in healthy tissues that regulates apoptosis and inflammatory processes through the human enzyme PAD (peptidylarginine deiminase (PAD).[17] *P. gingivalis* is a pathogen, which has the ability to express its own PAD enzyme and causes citrullization process.[6,20] Liao et al. formulate a hypothesis that *P. gingivalis*, the major pathogen in PD and only pathogen which expresses PAD enzyme, could be involved in the pathogenesis of RA through citrullination of RA autoantigen (i.e. fibrin in synovium).[21]Additionally, it has the ability to change free arginine in the way independent on calcium. Citrullination, that is stimulated by *P. gingivalis* PAD enzyme, increas-

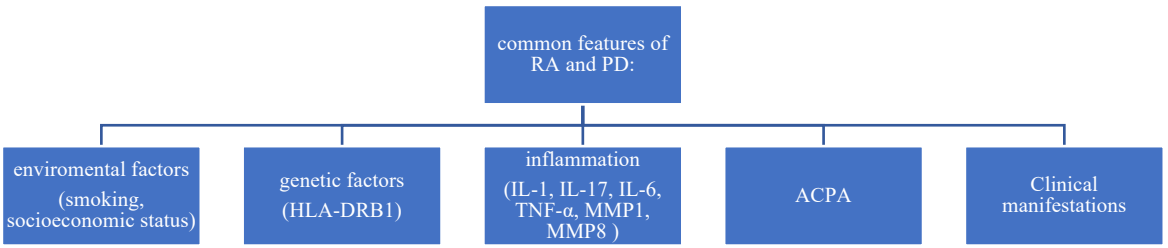

Fig. 1. Common features of rheumatoid arthritis and periodontitis

es the adaptation and ability for survival of the pathogen in humans.[22] The immune system recognizes the generated citrullinated peptides as foreign antigens. As a result, it stimulates the production of anti-citrullinated protein antibodies which could initiate inflammatory process in RA patients.[23-25] Citrullinated proteins generate the epitopes which break immunological tolerance in genetically predisposed patients. Several authors, including Makrygiannakis et al., have demonstrated the presence of some anti-citrullinated protein antibodies in synovial fluid in RA patients as compared to the healthy ones.[26] The antibodies are a highly specific diagnostic tool towards RA. Many studies show that a higher level of positive anti-cyclic citrullinated peptide antibodies (anti-CCP) is associated with a more aggressive course of RA and the bone destruction. The increasing level of anti-CCP appears also in an aggressive PD. Wagner et al. have observed similar relations. They suggest a "two-hit" model of RA. Citrullinated peptides provided by *P. gingivalis in inflamed gingival tissues spread epitopes* to the citrullinated proteins in the inflamed synovial joint. This could lead to an aggressive and chronic reaction, characteristic for RA.[27-30] Smit et al. showed an increased activity of RA within the patients with severe periodontitis which corresponded to a higher level of antibodies against *P. gingivalis* as compared to severe periodontitis patients without RA.[31]

P. gingivalis produces virulent factors, i.e. gingipain cysteine proteinases, specific for arginine or lysine. Some factors, such as cytokines, chemokines, immunoglobulins, complement proteins and host cell receptors, involve the destruction of host proteins. They also degrade periodontal tissues (collagen and proteins of basement membrane). Stathopoulou et al. demonstrated that the lack of secondary cytokine response is caused by *P. gingivalis* protease degradation process. The authors conclude that *P. gingivalis* could change protective immune response to pathogenic one, because of changing cytokine profile to unfavorable by lysine gingipains associated degradation of cytokines.[32]

Recent researches showed that *P. gingivalis* could change the adaptive immune response through the interaction with dendritic cells. This response in turn promotes a cytokine release, that stimulates development of T helper 17 cells (Th17) with simultaneous downregulation of Th1 cells. Moreover, *P. gingivalis* stops gingival epithelial cells production of Th1 stimulating chemokines. As a result the pathogen influences balance between Th1 and Th17 lymphocytes, supporting the line Th17 responsible for inflammation.[33-37]

Many authors emphasize the effect of *P. gingivalis* on the complement. The bacterium provides both activating as well as inhibiting influence. This pathogen generates gingipains, which degrade complement components such as the C3, C4 and C5, what leads to the inhibition of the complement activation (regardless of the pathway of its activation) and compromises the immune response of the organism. It is suggested that *P. gingivalis* has the ability to inactivate the complement in order to protect the other periodontal bacteria. On the other hand, the capacity of activation of the complement, provided by *P. gingivalis,* cause the local inflammatory response ensuring necessary nutrients for the whole microbiome. Consequently, periodontal bacteria create own mechanism managing the inflammation to reach crucial benefits. Periodontitis is the disorder between homeostasis of the organism and microbiome which strongly affects etiology and modulation of other systematic diseases, such as RA.[38-41]

According to many studies, complement and toll-like receptors (TLR) form an important link between the infection and the local or systematic inflammatory/autoimmunological reactions such as RA or PD.[42] Literature has described, that *P. gingivalis* is able to avoid recognition by the TLRs particularly TRL2.[43,46] *P. gingivalis* with complement C5 convertase-like activity, increases cyclic adenosine monophosphate (cAMP) concentrations, resulting in suppression of macrophage function and enhanced pathogen survival. This synergy is orchestrated by TLR2 signaling, a pertussis toxin – and thapsigargin-sensitive C5a receptor pathway, with protein kinase A and glycogen synthase kinase-3b as downstream effectors. The blockade of the C5a receptor could have therapeutic implications for periodontitis and atherosclerosis.[47]

Abe et al. in their original studies performed with the use of mouse model demonstrated that *P. gingivalis* abolishes C5a receptor inactivating the immune system. In the process it releases an inflammatory response which depends on the immune complement system. The response leads to the destruction of the alveolar bone.[48] Curtis et al. presented the results of investigation showing that increased temperature at the site of inflammation in the periodontium may alter the modification of *P. gingivalis* lipid A and its interaction with TLR4, which influences the interaction of this pathogen with the innate host defense (Figure 2).[49]

Studies indicate that RA and PD express a similar profile of inflammatory mediators. RA is represents the inflammation of the synovial membrane and the destruction of bones and cartilage. In PD we can observe the destruction of the periodontal ligament and the alveolar bone. *P. gingivalis* deregulate the inflammation reactions leading to the above-mentioned processes.

Notably, *P. gingivalis* induces increased level of IL-17 in serum of periodontitis patients. A chronic activation of the IL-17R could potentially switch the acute inflammatory process into a chronic one, associated with RA.[34,50-52] In the same studies, IL-17 is demonstrated to be a significant mediator regulating immune response, produced by subset of T cells and has a strong impli-

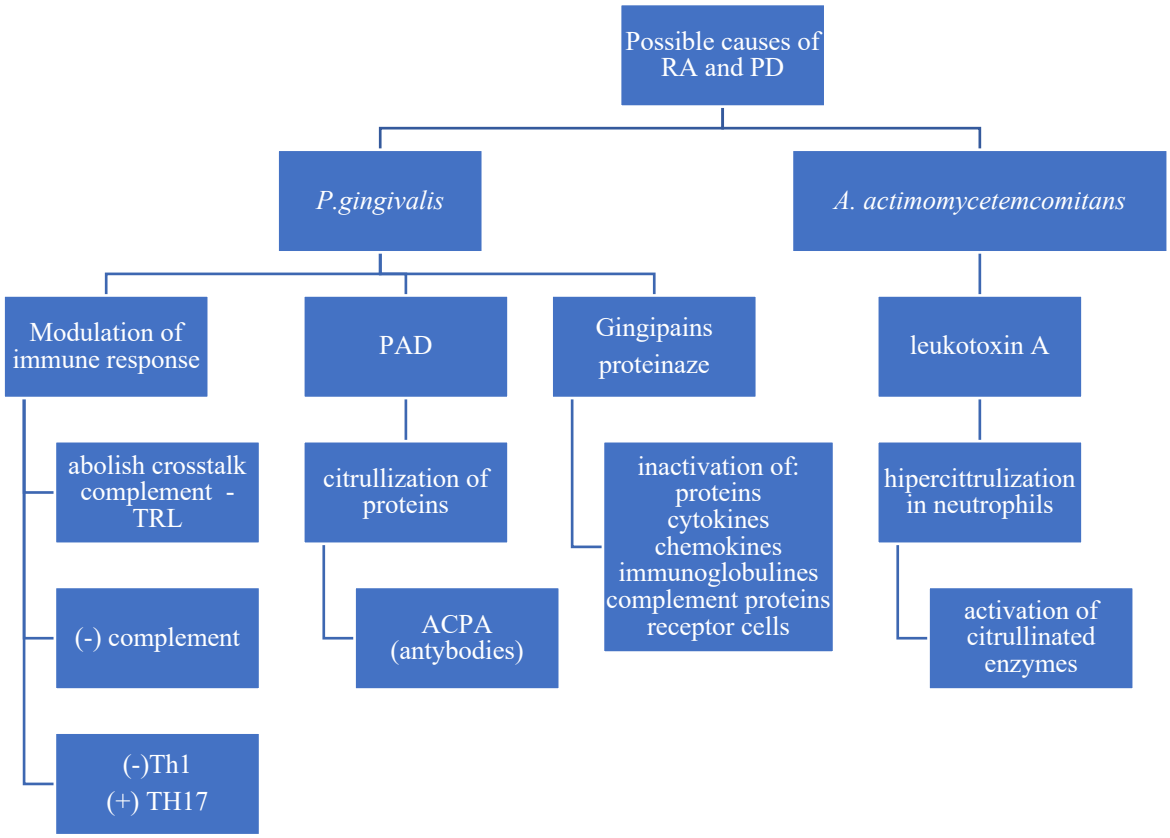

Fig. 2. Possible causes of rheumatoid arthritis and periodontitis

cation in autoimmunity and inflammation.[53] Increasing expression of the IL-17 is observed in autoimmune diseases, such as RA. Moreover, in case of periodontitis, its growth induces inflammation of gingival tissue and alveolar bone loss.[53-55]

TNF-α plays a key role in inflammatory reactions in RA and PD. This cytokine is locally produced by neutrophils as a result of innate immune response.[56,57] Kato et al. presented that, T*NF-α* increases the activity of *P. gingivalis* in human gingival epithelial cells. This situation causes a persistent infection of *P. ginigvalis* and a continuation of immune responses in periodontal tissues.[58] In other studies Palm et al. showed that *P. gingivalis* induces apoptosis of fibroblasts, that is a characteristic feature of periodontitis and periodontitis is associated with a decreased number of fibroblasts. A variety of fibroblast-derived inflammatory mediators including TNF- α are inactivated by *P. gingivalis* due to proteolytic activities of gingipains. Because of this, bacteria can create a more favorable microenvironment where it can evade the host immune response and promote its own growth and establishment.[59]

While analyzing inflammatory mediators the IL-6 should be mentioned. It is produced mainly by macrophages. However, fibroblasts, monocytes, T lymphocytes and endothelial cells also contribute to the production of IL-6. The main functions of this mediator include: removing of infection factors and regeneration of damaged tissues. What is important, IL-1 and T*NF-α stimulate the production of this interleukin.*[60] *An increased level of IL-6 is found in synovial fluid and serum of RA patients. It has been proven, that IL-6 is associated with a risk and pathogenesis of PD. It plays a significant role in the initiation of the acute phase of PD.*[61,62] According to some authors, significant increase the IL-6 production in human gingival fibroblasts is caused by lipopolysaccharide, produced by *P. gingivalis*.[63-65.]

Recent studies suggest that neutrophils play an increasingly significant role in chronic inflammatory diseases, such as rheumatoid arthritis or periodontitis. Accumulation of neutrophils can be found in inflamed and osteolytic lesions in PD. LPS generated by *P. gingivalis* could have the ability to activate osteoclastogenesis through the interaction between neutrophils and osteoclasts.[33,72]

Aggregatibacter actinomycetemcomitans

In addition to *P. gingivalis, A. actinomycetemcomitans* is mentioned as a common risk factor for RA and PD. It is a gram-negative, anaerobic bacterium, involved in the chronic and aggressive periodontitis.[66,67] This pathogen induces hypercitrullization in neutrophils through toxin leukotoxin A (LtxA) which could change the morphology of neutrophils, mimicking extracellular trap forma-

tion and this process results in the hypercitrullinated autoantigen release, triggering autoimmune response in rheumatoid arthritis patients.[5] Leukotoxin A disturbs citrullination by human PAD enzymes in host neutrophils. Hipercitrullinated proteins resemble citrullinated proteins in joints. They are also observed in gingival crevicular fluid (GCF) of patients with periodontal disease.[68] Konig et al. demonstrated that *A. actinomycetemcomitans* has the ability to deregulate protein citrullization in host's immune cells and acts as a potential inducer of cellular hypercitrullination as it expresses a potent inducer of cellular hypercitrullination as well as citrullinated RA autoantigens. In comparison to another periodontal pathogens *A. actinomycetemcomitans* might stimulate the release of citrullinated autoantigens without the participation of citrullinated enzymes.[37,67] New insights into this matter were provided by a recent study showing that *A. actinomycetemcomitans* could express a production of ACPA in individuals genetically predisposed to RA. The presence of anti-LtxA antibodies was significantly correlated with ACPA and RF positivity (Figure 2).[7,68]

Leukotoxin A produced by *A. actinomycetemcomitans*, activates the secretion of IL-1β from human macrophages, which stimulate the bone loss process in the periodontal disease and RA.[69,70] IL-1 plays an important role in the inflammation processes and stimulates the autoimmune reactions which lead to the destruction of tissues in RA and PD. Higher level of this cytokine is observed in synovial and gingival fluid in RA patients.[71]

Conclusion

Because of many clinical and experimental studies, which have suggested a connection between periodontal disease (PD) and rheumatoid arthritis (RA), there is a significant need of cooperation between physicians, dentists and dental hygienists. Local control of periodontal diseases and inflammation processes, followed by non-surgical periodontal treatment, decrease the systematic inflammation and may prove beneficial in reducing the severity of RA .The non-surgical treatment is the essential therapy in the case of periodontal disease, which may improve the oral condition in patients with RA. It consists of dental plaque control, supragingival scaling and root planning. As a result, the systematic level of inflammatory mediators and periodontal pathogens is decreased. This decrease leads to the reduction of RA activity. A routine oral examination should be conducted in patients diagnosed with RA. Within patients with active PD the basic periodontal treatment should be applied. Many studies highlight the need for complex and adequate dental care for the RA patients aiming to improve the oral health. The necessity of an interdisciplinary collaboration between doctors and dentists is undeniable, so as to the RA patients have the possibility of an interdisciplinary treatment.

Declarations

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Author contributions

Conceptualization, A.Z; Methodology, A.Z.; Investigation, A.Z.; Writing – Original Draft Preparation, A.Z.; Writing – Review & Editing, J.T.

Conflicts of interest

The authors declare no competing interests.

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REVIEW PAPER

Obesity-diabetes-endocrinopathy – the metabolic connection

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ABSTRACT

Introduction and aim. The article outlines the mechanisms of interrelationships between obesity, type 2 diabetes, and certain disorders of the endocrine system. The paper explains how insulin resistance develops, which is a key link between obesity and several related disorders, how hypercortisolemia leads to the development of obesity and glucose intolerance, why thyroid dysfunctions are bidirectionally associated with metabolic disturbances, in what way excessive body weight leads to the hypogonadism in men, or how menopause promotes the development of abdominal obesity, carbohydrate intolerance and, in some cases type 2 diabetes.

Material and methods. Scientific articles were reviewed by searching for information using the online database with scientific articles, including PubMed, Google Scholar and other available scientific databases.

Analysis of the literature. The huge prevalence of obesity, diabetes, and hormonal disorders (e.g., autoimmune thyroid disease, female and male hypogonadism) over the contemporary world together with the serious health consequences of these conditions makes up a specific triangle of metabolic connections, increasingly absorbing the human, organizational and financial resources of health systems.

Conclusion. Recognizing the relationship between the components of this triangle and understanding the risks arising from this phenomenon may allow to effectively reduce its impact on our health.

Keywords. diabetes, endocrinopathy, hypogonadism, insulin resistance, obesity

Introduction

Obesity, often referred to as the tsunami of the 21st century is such closely associated with another pandemic of our time - type 2 diabetes, that many use the term "diabesity" to describe both diseases together. On the other hand, many disorders of the endocrine system, e.g., hypothyroidism that occurs with a frequency of about 5% in women and less often in men, menopause that affects all women after 50 years, or looking wider – all kinds of male and female hypogonadism leading to an increase in body weight and glucose metabolism disturbances.1-5

The etiopathogenetic interconnectedness, the very high prevalence and the serious health and socioeconomic consequences resulting from all these diseases make it possible to connect them in a kind of specific metabolic triangle with an increasing impact on health systems in today's world.

Aim

The paper explains how insulin resistance develops, which is a key link between obesity and several related disorders, how hypercortisolemia leads to the development of obesity and glucose intolerance, why thyroid dysfunctions are bidirectionally associated with metabolic disturbances, in what way excessive body weight leads to the hypogonadism in men, or how menopause promotes the development of abdominal obesity, carbohydrate intolerance and, in some cases type 2 diabetes.

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Material and methods

Scientific articles were reviewed by searching for information using the online database with scientific articles, including PubMed, Google Scholar and other available scientific databases.

Analysis of the literature

Obesity

Obesity is a chronic disease characterized by excessive accumulation of fat mass, increasing the risk of many other diseases, including the cardiovascular, metabolic and cancers. Adipose tissue that accumulates fat based on its morphology can be categorized as white (WAT), brown (BAT), or beige. Depending on the location WAT can be classified as visceral (central, abdominal) and subcutaneous. Moreover, in obesity fat mass can increase ectopically as intrahepatic, epicardial, perivascular, mesenteric, omental, and retroperitoneal. Brown adipose tissue is characterized by its morphology and function, with concentrated mitochondria giving it a characteristic brown appearance. Beige fat represents a class of adipose tissue, in which brown adipocytes appear within classical WAT depots.[1]

Excessive WAT leads to the development or to the progression of many closely related metabolic disorders and diseases such as dyslipidemia, diabetes, asthma, chronic obstructive pulmonary disease, obstructive sleep apnea, hypertension, cardiovascular disease, esophageal reflux disease, non-alcoholic fatty liver disease, polycystic ovary syndrome, hypogonadism, infertility, osteoarthritis, cancers (esophagus, small intestine, colon, liver, gallbladder, pancreas, kidneys, breasts, uterine, prostate), depression, stress incontinence.[2,3]

According to the World Health Organization (WHO) obesity is now reaching epidemic proportions, being mainly a result of rapidly changing demographic and socioeconomic conditions. In 2016 1.9 billion adults over the world were overweight (body mass index (BMI) >25 kg/m^2) and 650 million (13% of the total population) were obese (BMI ≥30 kg/m^2). Unfortunately, the problem no longer concerns only adults, but also increasingly touches children and young people. In 2018 year 40 million children before the age of 5 were overweight or obese. Only in Africa the number of overweight children increased by 50% over the last two decades. In Asia in 2018 year every second child before 5 years was overweight or obese. This must result in a further increase in the number of obese adults by 5–15% over the next 10-15 years, which in turn will result in a 100,000 additional events of coronary artery disease.[1,4]

Obesity is most often of primary nature. It means, that it develops under the influence of environmental factors that overlap the underlying genetic background. More than 400 genes that may be involved in excessive accumulation of fat tissue have been described. A genetic predisposition may be result of:

- a single mutation, e.g., of a leptin molecule or its receptor (very rare, leads to the development of extreme obesity),
- chromosomal aberration, e.g., Prader-Willi syndrome (much more common, among the other symptoms unstoppable hunger and huge obesity are observed),
- polymorphisms of many different genes (polygenic obesity, the most common).

An increasing role in the pathophysiology is currently also attributed to epigenetic changes.

Among the environmental factors, the most important are the reduction of physical activity and poor nutrition: hypercaloric meals rich in saturated fats, cholesterol, and simple carbohydrates, but poor in polyunsaturated fatty acids and fiber. Normal composition of the intestinal microflora, which significantly affects the energy balance is also important, and in the case of adverse changes inadequate microbiome may promote the accumulation of body fat.[5] A great role is attributed to proper nutrition during fetal life. Among newborns with low-birth-weight childhood obesity and the cardiovascular diseases later in adulthood are significantly more common.[6]

Secondary obesity may be the result of hormonal disorders, of the effects of certain drugs (phenothiazine derivatives, H1-receptor antagonists, oral contraceptives, antidepressants, antiepileptic drugs, antidiabetic drugs, glucocorticoids, β-blockers) and less often of the other causes.

Obesity – insulin resistance – diabetes mellitus

It has been known for a long time that there is a strong relationship between weight gain and the risk of prediabetes and type 2 diabetes. It has been even estimated that every kilogram more in body weight means increase in a chance of diabetes of 4.5% over 10 years. A one-unit higher BMI (approx. 2.7–3.6 kg) equals the 12.1% higher risk of diabetes.[7] In effect, obesity accounts for about half of the new cases of type 2 diabetes in today's world.[8]

Insulin resistance is the key factor of the pathway from obesity to diabetes. With a high BMI, large, fat-filled adipocytes react less well to insulin and are no longer able to accumulate energy in the form of lipids. Fats therefore begin to accumulate in other organs, where they bring on lipotoxicity, which also contributes a local decrease in insulin sensitivity. In adipose tissue itself, altered adipocytes and accumulating mononuclear cells become a source of many hormones, large amounts of free fatty acids (FFA) and many cytokines and adipokines. Biologically active lipid particles – long-chain acylo-CoA esters (LCACoA) formed from the connection of fatty acid and coenzyme A, diacylglycerols and

ceramides generate insulin resistance in peripheral tissues.[9] Released in excess FFA inhibit glucose uptake, hindering the translocation of glucose transporter 4 (GLUT 4) in skeletal muscles and other organs.[10] In addition, free fatty acids inhibit the secretion of insulin by β-cells worsening glucose metabolism also in that way.[11]

Adipokines and cytokines that increase insulin resistance include leptin, resistin, lipocalin 2, interleukin 6 (IL-6), tumor necrosis factor α (TNFα) and others.

Leptin is a hormone that inhibits appetite and reduces the amount of food absorbed in the digestive tract. Generally, this hormone increases the sensitivity of cells to insulin, however it was proved that in patients with prediabetes there is resistance to leptin action leading to increased insulin levels.[12] In consequence insulin and leptin levels are high in obese patients, and it was demonstrated experimentally that insulin stimulates the production of leptin by the adipose cells.[13] On the contrary, high insulin levels impair the physiological hypothalamic response to leptin for reducing appetite; weight loss improves this response.[14] Thus, overweight and obesity themselves generate weight accumulation leading to a vicious cycle. It was shown that high levels of leptin were associated with decreased insulin sensitivity in prediabetes patients.[15] Under certain physiological conditions, leptin increases insulin sensitivity, thereby confirming the existence of an altered mechanism of action of this protein in patients with prediabetes, obesity or overweight. Such associations have been reported in earlier studies, leading to high level of leptin to be considered a predictor of type 2 diabetes developtment, contrary to all the physiological roles that leptin fulfils in normal weight normoglycemic patients.[12]

Resistin is produced in adipose tissue and by immune cells. It seems that its main physiological role is to maintain glucose levels during starvation. While an increase in insulin resistance (as a result of the increase in gluconeogenesis and glycogenolysis with consequential hyperglicemia) under the influence of this factor was clearly observed in animal studies, this effect in humans has not been unambiguously confirmed.[16]

Lipocalin 2 is a protein derived from adipose tissue and liver involved in the immune response to infection. Many studies point to a relationship between its levels and the intensity of the inflammation that accompanies obesity and insulin resistance. A relationship between the amount of lipocalcin 2 in visceral adipose tissue and the severity of the inflammatory process, serum insulin levels, and Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) has been established in humans.[17]

Interleukin 6 (IL-6) produced by epithelial cells, macrophages, and fibroblasts in adipose tissue and in the immune system reduces the expression of insulin receptors in peripheral tissues and inhibits signal transmission from these receptors. IL-6 restrains adipogenesis and causes a decrease in levels of adiponectin – metabolically beneficial adipokine.[18]

Tumor necrosis factor α (TNFα) is a pro-inflammatory cytokine produced by mononuclear cells in adipose tissue and skeletal muscles. Acting in auto- and paracrine manner it reduces insulin sensitivity mainly by inactivation of insulin receptor-associated insulin receptor substrate 1 (IRS1), inhibition of tyrosine kinase activity and, as a result, by stop the translocation of the GLUT 4 in the cell. The production of TNFα in humans is positively correlated with obesity, insulin levels and insulin resistance.[19]

Reduced insulin sensitivity usually precedes the onset of type 2 diabetes for many years. This is because decrease in insulin sensitivity is initially compensated by a higher secretion of this hormone by pancreatic β-cells. However, it should be kept in mind, that despite apparent normoglycemia, the risk of atherosclerosis during this period is comparable to that of overt diabetes.[20] Later, with the progressive dysfunction of β-cells prediabetes and subsequently diabetes appears. Given the prevalence of obesity all over the world, the number of cases of diabetes, which is currently estimated on 463 million worldwide, with prognosis to almost double by 2030 should be not surprising.[21] Diabetes is the main cause of blindness, chronic renal disease, myocardial infarctions, strokes, and lower limb amputations nowadays.[22] Of course, the number of people with prediabetes is much greater, although more difficult to estimate precisely.

Diabetes treatment requires, first of all, implementation of lifestyle modification – reducing of body weight and increasing physical activity. Loss weight by 5-10% leads to a disproportionately reduction in visceral fat mass, and thereby improve insulin sensitivity with subsequent further metabolic and clinical benefits.[23] Among antidiabetic drugs the first-line medicine - metformin reduces insulin resistance and promotes, albeit to a small extent, weight reduction. Also, the so-called incretin drugs and sodium-glucose co-transporter 2 (SGLT2) inhibitors either do not affect body weight or lead to its decrease, which is sometimes even used for the treatment of obesity (liraglutide, semaglutide). However, many traditional drugs, with insulin among them, cause further weight gain.

Bariatric operations, originally designed to promote weight loss powerfully treat type 2 diabetes, causing remission in most cases, through diverse mechanisms additional to the secondary consequences of weight loss. Large observational studies demonstrated that bariatric (now called "metabolic") surgery is associated with reductions in cardiovascular risk factors, macro- and microvascular diabetes complications, cancer and death. Clinical trials, directly comparing various surgical vs non-surgical interventions for type 2 diabetes, clearly demonstrate the for-

mer to be superior for improvements in glucose control, as well as other metabolic endpoints. The safety profiles of modern laparoscopic bariatric/metabolic operations are similar to those of elective laparoscopic hysterectomy or knee arthroplasty.[24]

Obesity and endocrine disorders (endocrinopathies)

Among the endocrine system disorders that lead to weight gain and predispose to the development of obesity can be mentioned:

- Cushing syndrome
- growth hormone deficiency
- hyperprolactinemia
- thyroid diseases
- alleged hypothyroidism
- insulinoma
- hypogonadism
- polycystic ovary syndrome (PCOS)

Adrenocorticotropic hormone (ACTH)-dependent and ACTH-independent Cushing syndrome are rare diseases. The pituitary tumor, which is responsible for 70% of cases of endogenous hypercortisolemia occurs at a frequency of 30/million. The annual incidence of the adrenal origin disease is 2-5/million. Incomparably more frequently hypercortisolemia is a result of the treatment with glucocorticoids (GCS), which have been broadly used in the therapy of many autoimmune, respiratory, gastrointestinal, and other diseases. In Cushing syndrome, a frequency of hyperglycemia is estimated at 53%, and diabetes in 36% of patients.[25] Excess cortisol and its derivatives affect glucose metabolism in many ways. Activation of GCS receptors present on β-pancreatic cells switch on the genomic mechanisms responsible for inhibition of glucose uptake and a decrease in insulin secretion. Impact of incretin hormones (glucagon-like peptide 1, GLP-1) on β-cells is weakened.[26] The effect of glucocorticoids on insulin-producing cells is important as it enables to increase the production of this hormone in conditions of insulin resistance resulting from hypercortisolemia. However, the most important effect of GCS is the strong anti-insulin activity in the liver, skeletal muscles, and adipose tissue.[27] In muscles and adipose tissue insulin is responsible for glucose uptake and its storage as a glycogen. Insulin also inhibits lipolysis and reduces the release of free fatty acids into the blood. In the liver it inhibits gluconeogenesis and glycogenolysis. These processes are significantly disrupted under conditions of hypercortisolemia. This is a result of the restrain of the insulin receptor signal due to effects on IRS-1, phosphatidylinositol 3 kinase (PI3K) and protein kinase B (PKB).[27] Glucocorticoids also inhibit the activity of the enzyme responsible for glycogen synthesis in the muscles and stimulate proteolysis increasing the amino acid pool, which further interferes with the transmission of the signal from the insulin receptor. Naturally, GCS-induced visceral obesity through the mechanisms already described contributes to insulin resistance with a subsequent hyperglycemia.

European Society of Endocrinology (ESE) recommends that testing for hypercortisolism should not be routinely applied in obesity, but only in patients with clinical suspicion of hypercortisolism and in candidates for bariatric surgery. Also, patients using GCS no need such tests. If hypercortisolism testing is considered a 1 mg overnight dexamethasone suppression test as first screening tool is recommended. In case of positive result as a second line test either 24-h urine cortisol or late-night salivary cortisol should be performed. It is worth remembering that treatment of proven endogenous hypercortisolism is not normalizing BMI in most cases.[28]

Growth hormone deficiency syndrome (GHD) is a set of signs and symptoms resulting from impaired growth hormone secretion by pituitary somatotropic cells. Its incidence among adults is estimated at 37.5-42.5/100,000. The most common causes are pituitary tumors (44%) and craniopharyngiomas (11%), less often GHD occurs after radiotherapy of brain tumors (7%), brain injuries, Sheehan syndrome and is caused by lymphocytic pituitaritis. Growth hormone significantly affects metabolism. It has anabolic and lipolytic properties. Its impact on carbohydrate metabolism is more complex. On the one hand, acting directly, antagonistically to insulin, GH inhibits the transport of glucose to tissues and its oxidation, and intensifies gluconeogenesis in the liver. On the other hand, GH indirectly, through its mediator – insulin-like growth factor-1 (IGF-I) exhibits insulin-like activity.[29]

One of the apparent symptoms of GHD is a change in body composition: central obesity and a decrease in lean body mass, in this in skeletal muscle mass. BMI is usually not apparently altered. The consequences of this body composition modification are insulin resistance, an increase in fasting insulin levels, a higher incidence of type 2 diabetes. Substitution therapy with recombinant human growth hormone (rhGH) initially further reduces insulin sensitivity, but during longer treatment beneficial metabolic effects secondary to reduction in visceral fat begin dominating.[30] In clinical practice testing for IGF1/GH is not routinely applied in obesity and should be performed only in patients with suspected hypopituitarism. If tested a GH-stimulation test should be performed. Growth hormone should not be used to treat obesity in patients with normal GH levels.[28]

Hyperprolactinemia can be caused by a pituitary tumor (prolactinoma), but much more often is of physiological origin and occurs in pregnancy, during breastfeeding, in stressful situations, after exercise or during irritation of the mammary glands. Many medications can also cause elevated prolactin levels. Apart

from the effects of on lactation and gonadal function prolactin have significant clinical implications on metabolism. Association between hyperprolactinemia and insulin resistance as well as with metabolic syndrome has been proved. The likely pathogenesis of weight gain in hyperprolactinemia includes decreased dopaminergic tone, leptin resistance, reduction in adiponectin levels, high hypothalamic pressure, and hypogonadism. It can also lead to increased low-density lipoproteins and triglycerides and reduced high-density lipoproteins levels, which is likely the result of reduced lipoprotein lipase activity. This can lead to further weight gain and increased risk of cardiovascular diseases. Testing for hyperprolactinemia should not be routinely performed in obesity. The finding of high prolactin levels first of all requires the exclusion of pregnancy and other physiological causes of this condition. Only when accompanied by clinical features use of small doses of dopamine receptor agonists (DA) to decrease prolactin secretion can be considered. In case of prolactinoma DA should be used for treatment. Therapy can cause weight loss by improving insulin and leptin sensitivity and the lipid profile.[31]

Thyroid hormones – triiodothyronine (T3) and thyroxine (T4) have great impact on energy balance, as they increase the basal metabolic rate (BMR) by stimulating thermogenesis, affect the food ingestion, as well as influence glucose and lipid metabolism. Hypothyroidism leads to the decrease in BMR and reduces thermogenesis, contributing to increase in body weight. The inverse relationship between free thyroxine (fT4) levels and BMI has been demonstrated, so weight reduction usually is accompanied by normalization of hormonal changes.[32] Free triiodothyronine (fT3) levels in persons with a high BMI are normal or elevated.[33] Heightened T4 to T3 conversion observed in such cases may be considered as a compensatory mechanism to prevent further energy gain. Leptin seems to be the mediator in this process. This hormone is produced in larger amounts in overdeveloped adipose tissue and stimulates deiodination of T4 to T3. Leptin also acts centrally, increasing the secretion of thyrotropin-releasing hormone (TRH) and, consequently, TSH and T3 levels. This mechanism also can be thought of as compensatory, preventing further accumulation of energy in a form of a fat storage. The results of the studies on TSH levels in obese subjects are not entirely conclusive. The increase in TSH, observed in majority of the trials is, inter alia, the result of chronic inflammation associated with the obesity. The cytokines produced in such cases (TNFα, interleukins) inhibit the sodium iodine symporter mRNA expression and consequently the uptake of iodide by thyrocytes, which may trigger a compensatory increase in TSH levels.[34] Inversely, the thyrotropic hormone has been shown to directly stimulate adipocytes to produce leptin through receptors present in adipose tissue.

The relationship between thyroid function and the risk and the course of diabetes has been established for a long time. Epidemiological studies confirm a higher incidence of thyroid dysfunction in patients with diabetes, especially type 1 (up to 31.4% of women) compared to persons without this disease.[35] This relationship is explained by the existence of common genes: *HLA, CTLA-4, PTPN22, FOXP3*, responsible for both the development of type 1 diabetes and autoimmune thyroid disease.[36]

Thyroid dysfunction alters glucose metabolism through several mechanisms. Overproduction of thyroid hormones leads to an increase in the degradation rate and to shortening of the half-life of insulin, as well as inhibits the transition of proinsulin into the active hormone.[37] Intestinal absorption of glucose and its production in the liver (gluconeogenesis) are increased. This is partly due to a greater influx of FFA in conditions of augmented lipolysis caused by catecholamines under the influence of overproduction of thyroid hormones. Growth hormone and glucagon secretion increases.[38,39] All these phenomena lead to the hyperglycemia. In turn, in hypothyroidism hepatic glucose production decreases and insulin requirements go down.[40] On the other hand, resistance to this hormone occurs and glucose utility in peripheral organs is impaired.[41]

The effects of T3 and T4 on glucose metabolism are also the results of their interactions with hormones involved in energy balance regulation. Hyperthyroidism leads to a decrease in leptin levels, while in hypothyroidism usually increase in the production of leptin is seen, although the results of the studies on this subject are not entirely conclusive. Reciprocally, as it was already mentioned, leptin is a factor that increases triiodothyronine levels by the impact on type 1 deiodinase. A similar, but inverse relationship occurs between thyroid function and the ghrelin levels. Reduced levels of this peptide are observed in obesity, type 2 diabetes, as well as in hyperthyroidism, which can be consider as a state of negative energy balance. The return of normal thyroid function usually normalizes ghrelin levels.[35] T3 and T4 also affect glucose metabolism by thermogenesis regulation, acting both at the central level in the hypothalamus and locally stimulating the activity of an uncoupling proteins in the brown adipose tissue.[42,43] In diabetes, especially poorly controlled, a fall in the production of thyroid hormones is observed. This is the result of a reduced TSH response to TRH, as well as suppressed conversion of thyroxine into triiodothyronine.[44] The increased insulin levels resulting from resistance to this hormone lead to an enlarging of the thyroid gland, with a tendency to form nodules.[45]

ESE recommends that all patients with obesity should be tested for thyroid function. Testing should be based on TSH and fT4 measurements. Overt hypothyroidism (elevated TSH and decreased FT4) should be

treated in obesity, but hyperthyrotropinemia (elevated TSH and normal FT4), should not be treated with the aim at reducing body weight. ESE recommends against the use of thyroid hormones to treat obesity in case of normal thyroid function.[28]

Insulinoma although very rare is the most common functioning neuroendocrine tumor of the pancreas and is the main cause of endogenous hyperinsulinemic hypoglycemia. The most common clinical manifestations are neurovegetative and neuroglycopenic symptoms secondary to hypoglycemia. Progressive weight gain is also an important clinical feature, due to the anabolic action of insulin and the need to feed periodically to reduce hypoglycemia. Testing for insulinoma should not be routinely performed in obesity. In patients with high BMI and hypoglycemic symptoms blood glucose, insulin, C-peptide 72-h supervised fast may be useful as a first diagnostic procedure.[28]

Male hypogonadism may be defined as a set of signs and symptoms resulting from abnormal gonadal function including impaired gametogenesis and/or the secretion of gonadal hormones.[46] In men, primary (hypergonadotropic) and secondary (hypogonadotropic) hypogonadism are usually distinguished. The most common cause of the former is Klinefelter syndrome, and a latter are pituitary tumors. Rarely is observed so-called "peripheral" hypogonadism, which is a consequence of gene polymorphism for the androgen receptor.

The relationship between fall in the testosterone levels and the development of obesity is bidirectional, however it seems that the impact of body weight on testosterone is stronger than the reverse relationship. Obesity caused by testosterone deficiency should be understood rather as an excess in visceral fat mass (to a lesser extent subcutaneous fat mass) than increased BMI, because hypogonadism, like GH deficiency, alters body composition. Fat mass grows up and a decrease in lean body mass (including muscle mass) is observed, without a marked change in BMI.

Obesity disturbs pulsating gonadotropin-releasing hormone (GnRH) secretion, and consequently decreases luteinizing hormone (LH) and finally testosterone production. Levels of main binding testosterone protein in circulation – sex hormone-binging globulin (SHBG) are also diminished. The effect of excess body weight on testosterone has for a long time been linked mainly to increased aromatization, but currently this mechanism as a crucial process is questioned. At present a significant role is attributed to leptin, which under the obesity conditions stops stimulating production of the kisspeptin – a protein in the hypothalamus necessary for the GnRH secretion.[47] Furthermore, leptin inhibits stimulating effect of LH on Leydig cells.[48] Testosterone treatment reduces leptin production. This effect is indirect, being a consequence of a fat mass decline, although testosterone also acts directly, what indicates a strong relationship between these hormones in terms of testosterone secretion and fat mass regulation. In addition to leptin, also hyperinsulinemia and increased cytokine production play an important role in the genesis of obesity-related hypogonadism. The negative relationship between pro-inflammatory cytokines and testosterone secretion has been demonstrated.[49] E. g. in older men, even a slight increase in interleukin 2 levels results in a marked inhibition of GnRH secretion and a decrease in LH production.[50] In animal experiments, hypogonadism caused by a high-fat diet was associated with the state of inflammation in the hypothalamus, an increase in the expression of pro-inflammatory cytokines and a decrease in kisspeptin receptor expression.[51] The lack of receptors for insulin, leptin, and androgens on neurons responsible for GnRH production indicates that the regulation of the activity of these neurons is indirect.

Low testosterone levels are associated with a higher risk of type 2 diabetes. Inversely, men suffer from this disease have been shown to have lower testosterone than their healthy counterparts.[52] Both insulin resistance and diabetes are more common in men who are hormonally treated for prostate cancer, as well as after testosterone substitution therapy withdrawn.[53,54] The mechanisms responsible for these associations are of central and peripheral origin. In mice with a knockout of the insulin receptor in the central nervous system decrease in GnRH secretion with the subsequent hypogonadotropic hypogonadism occurs.[55] On the other hand, testosterone deficiency leads to the development of insulin resistance, which is at least in part the result of abnormal androgen receptor function in peripheral organs. It has been found, that one of the causes of this phenomenon is a decreased activity of the α-coactivator of the peroxisome proliferator-activator receptor gamma (PPARγ).[56] Testosterone treatment improves insulin sensitivity, which is associated with an increase in the expression of genes responsible for transmitting the signal from insulin receptor in adipose tissue.[57,58]

According to ESE guidelines in males with clinical features of hypogonadism but not routinely in all obese patients measuring total and free testosterone (or calculated), SHBG, FSH and LH is suggested. Weight loss is considered of most importance to restore normal testosterone secretion in obese patients with hypogonadism. If weight loss cannot be achieved and if clinical and biochemical hypogonadism persists, treatment with testosterone can be considered in individual cases; contra-indications should be considered, and other causes of hypogonadism should have been ruled out. Testosterone should not be used to treat obesity in patients with normal testosterone levels.[28]

Hypogonadism in women can be caused by the functional or organic disorders of the hypothalamus

or pituitary gland, as well as of diseases of the ovaries themselves, such as gonadal agenesis, chromosomal defects, or steroidogenesis disturbances. Irradiation, chemotherapy, autoimmune diseases leading to premature ovarian failure, nutritional deficiencies, injuries, certain medications and, finally, menopause can also cause a deficiency or complete absence of female sex hormones.[59,60]

Estrogens are responsible for the characteristic distribution of adipose tissue in women, that is, in the gluteal and femoral regions. They operate through estrogen receptor 1 and 2 (ER1 and ER2), which are in fact transcription factors regulating the expression of genes that affect metabolic processes. The non-genomic mechanisms involve the activation of receptors present on the surfaces of target cells. Androgens also affect distribution of body fat, but they are responsible for the abdominal type of obesity. Estrogen deficiency creates a state of relative hyperandrogenism, what explains the redistribution of body fat observed in such conditions in women. Obesity due to lack of estrogen, similarly as it is in the case of hypogonadism in men, rather alters body composition (increases and redistributes fat mass, decreases lean body mass) than changes BMI. Reduced SHBG production, related to lack of estrogen, further increases exposure on androgens. On the other hand, intensified aromatization in obese premenopausal women increases estrogen levels, what results in a characteristic female-type distribution of excess body fat. However, after menopause, when overweight is accompanied by a lack of estrogen, abdominal obesity usually dominates, along with its metabolic consequences – insulin resistance, chronic inflammation, lipid disorders and an increased risk of diabetes and cardiovascular diseases.[61]

Estrogens play an important role in regulating of the energy balance. They significantly affect metabolism of the fat tissue. Under conditions of estrogens deficiency mononuclear cells infiltrate fat tissue, the production of pro-inflammatory cytokines increases, and insulin resistance appears, potentiating the cardiometabolic risk. Another important feature of female sex hormones is the "browning" of white body fat. This process, which makes possible to remove excess energy in the form of heat emission, without putting it in high-energy compounds ceases under conditions of scarcity of these hormones.[62] Estrogens inhibit appetite, what can be observed for example during the menstrual cycle, when the amount of food consumed decreases gradually from the follicular phase to the time before ovulation. The mechanisms of action of estrogens in this regard are very complex and involve several processes in the central nervous system and in peripheral organs.

Estrogens easily cross the blood-brain barrier and operate in many regions responsible for appetite control. Their activity within the hypothalamus is particularly important, as they inhibit the expression of neuropeptide Y through ER1 receptors and Gq-coupled membrane-estrogen-receptors.[63] An additional mechanism is the inhibition of production of ghrelin – the strongest orexigenic peptide mainly derived from the stomach.[64] Hence, estrogens deficiency increases appetite and contributes to the accumulation of energy. A relationship between estrogens levels and leptin production and the participation of this hormone in the mechanisms of estrogen-related regulation of energy balance are not clearly established.[65] Estrogens also affect resting energy expenditure (REE). In the absence of these hormones a decrease in REE occurs, and the substitution therapy in women before menopause restores the correct energy balance.[66] In animal studies estrogens also restored energy expenditure associated with physical activity, but this feature has not been confirmed in humans.[67]

Female sex hormones are involved in carbohydrate metabolism. Although skeletal muscle mass in premenopausal women is 2/3 lower and fat mass is 50% higher than in men, the incidence of diabetes is similar for both sexes. The use of estrogens in women with lack of these hormones causes an increase in insulin sensitivity and lowers blood glucose levels. Such effect is observed in both healthy women and diabetics, although of course treatment with estrogens is not enough to cure the disease. Studies using a metabolic clamp are best model to observe the beneficial effects of estrogens on insulin-dependent processes: higher glucose uptake in muscles, inhibition of this sugar production in the liver and suppression of lipolysis in adipose tissue. In this way, they act against the onset of obesity, inhibit the increase in insulin resistance and thus prevent the development of type 2 diabetes. Another action of estrogens includes their impact on pancreatic β-cells. It has been shown in animal studies, that these hormones protect β-cells from the damaging factor (streptozocin) and prolong their survival. There was also a significant gender-dependent difference in the incidence of lipotoxicity, resulting in impaired insulin production by β-beta cells under the influence of a high-fat diet. This phenomenon occurred 28% less frequently in females compared to male rodents, which is explained just by the protective effects of estrogens.[68] Similarly, β-cell glucotoxicity was much less common in females.[69] ESE suggests assessing gonadal function in female patients with menstrual irregularities and chronic anovulation/infertility but not routinely in all females with obesity. For evaluation of menstrual irregularity measuring LH, FSH, total testosterone, SHBG, Δ-4-androstenedione, estradiol, 17-hydroxyprogesterone and prolactin are suggested, ideally during the early follicular phase. Anovulation requires assessing of gonadal function by measuring LH, FSH, estradiol, progesterone and prolactin. ESE does not recommend starting estrogen substitution in post-

menopausal obese women with the sole aim to reduce body weight.[28]

A special group are women with polycystic ovary syndrome (PCOS). This syndrome consists of chronic anovulation, hyperandrogenism and polycystic ovaries and leads itself to the insulin resistance, which is found in up to 75% of patients. The addition of obesity, often of abdominal nature, which affects 30–70% of patients, further decreases sensitivity to insulin and leads to compensatory hyperinsulinemia. High insulin levels stimulate the activity of enzymes responsible for ovarian androgen secretion and reduces the production of SHBG in the liver, which enhances hyperandrogenism. Hyperinsulinemia also inhibits the production of proteins that bind insulin-like growth factors 1 and 2 (IGF-1 and IGF-2), which in free form also contribute to ovarian dysfunction. Moreover, the expression of adipokines (leptin, adiponectin) altered in obesity modulates the activity of the hypothalamic-pituitary-gonadal axis by affecting receptors in the pituitary gland further increasing the production of hormones in the ovaries. Adipokines also directly affect ovarian function, i.e., by stimulating the synthesis of estradiol in follicular cells and progesterone by granular cells. As a result, women with PCOS, in addition to ovulation disturbances and deterioration of reproductive abilities, are at a higher risk of developing type 2 diabetes and other metabolic disorders, which leads to a higher incidence of cardiovascular diseases.

To assess androgen excess when PCOS is considered based on the clinical features. total testosterone, free T, Δ-4-androstenedione and SHBG should be measured. Additionally ovarian morphology and blood glucose should be evaluated. In women with PCOS with metabolic syndrome features metformin treatment is recommended.[28]

The etiopathogenetic relationship between obesity, insulin resistance, diabetes, and some endocrine disorders presented in outline in this paper are strong, usually reciprocal, and include both genomic and non-genomic mechanisms. The huge prevalence of obesity and diabetes as well as increasing occurrence of certain endocrine diseases such as autoimmune thyroiditis all over the world becomes a problem not only medical but also socio-economic, consuming the organizational and financial resources of health systems. Only the cost of treating obesity and its complications was estimated at about $2 trillion in 2014, representing 2.8% of the global domestic product (GDP) of all countries in the world.[70] Expenditures for diabetes and related complications treatment were $825 billion in 2016.[71,72]

Conclusion

Unfortunately, awareness of this problem among patients, and among health professionals is still inadequate, although only understanding the risks arising from the scale of the phenomena and understanding the relationship between diabetes, endocrinology and obesitology will allow to effectively reduce the impact of this specific metabolic triangle on the world population.

Declarations

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Author contributions

Conceptualization, J.K., P.D. and W.Z.; Methodology, J.K., P.D. and W.Z.; Writing – Original Draft Preparation, J.K., P.D. and W.Z.; Writing – Review & Editing, J.K., P.D. and W.Z.

Conflicts of interest

Authors have no conflicts of interest to declare.

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REVIEW PAPER

The impact of physical activity on the cognitive fitness of the elderly – a review

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ABSTRACT

Introduction and aim. The issues of humans' ageing are more and more frequently addressed in the relevant literature. Most commonly, people follow three ageing trajectories: a normal one, disease-affected one, and a healthy one. The purpose of this article is to present a relationship between physical activity and occurrence of cognitive function impairment in the elderly.

Material and methods. This paper is a narrative review. Based on a literature search, various forms of physical activity are presented, as well as the effects of physical activity on mitigation of cognitive disorders in the elderly. The following databases were used: Web of Science, PubMed, Google Scholar.

Analysis of the literature. One of the most important factors that promote healthy ageing is regular physical activity. Many studies and publications have addressed this issue. The relationships between physical activity and cognitive fitness have been less studied.

Conclusion. The results of the studies presented in this article may form the basis for more in-depth analyses and, in a long-term perspective, for the development of optimal preventive and therapeutic strategies using broadly understood physical activity to maintain cognitive fitness of the elderly.

Keywords. cognitive functions, physical activity, old age

Introduction

A worsening of cognitive fitness in the old age is a common problem and it is associated with the loss of the ability to perform daily activities. Cognitive functions are often defined as higher mental function including such processes as perception, learning new information, visual-spatial awareness, attention, thinking, language functions, memory and executive functions. Age-related cognitive impairment is not yet well understood, but many studies point to such changes as a reduction in both grey and white matter volumes and to changes in neurotransmitter levels, which may contribute to the observed cognitive deficits.[1] It should be underlined that there is no established pattern of cognitive impairment severity that would correspond to physiological ageing. It is generally accepted that presence of mild cognitive impairment (MCI) is an intermediate condition between cognitive function changes in the course of normal ageing and dementia.

Initially, the MCI concept was associated only with Alzheimer's disease (AD) and memory deficits. In order to explain the earliest stages of symptomatic AD, the MCI criteria were developed in 1999 by the experts of the Mayo Clinic. These criteria included the following: subjective patient's complaints on cognitive function impairment, the presence of cognitive dysfunctions

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confirmed by objective psychometric testing, the absence of any changes in daily functioning and the absence of dementia.[2]

It was not until the works of an international group of experts – the International Working Group on Mild Cognitive Impairment – undertaken in 2003 during the Key Symposium in Stockholm, when a broader understanding of the MCI concept emerged.[3] According to the adopted concept, two subtypes of mild cognitive impairment have been identified: an amnestic one and a non-amnestic one. The amnestic subtype (aMCI) is characterised by a memory function impairment that, however clinically relevant, does not meet the criteria of dementia diagnosis. On the other hand, the non-amnestic subtype (naMCI) can be described as a minor deterioration of functions not related to memory, such as concentration of attention, language-related and visual-spatial functions (Fig. 1).[4]

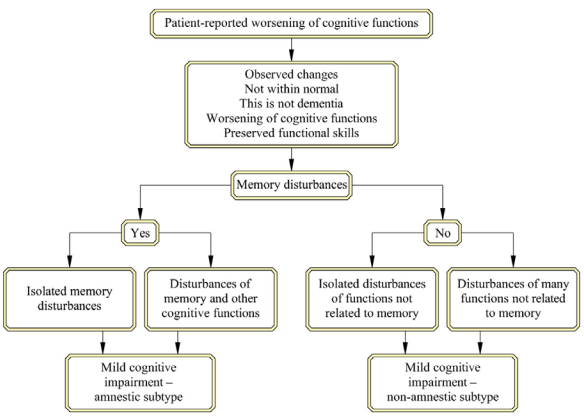

Fig. 1. Diagnostic algorithms for the diagnosis of amnestic and non-amnestic mild cognitive impairment[5]

According to the most recent data, the incidence MCI is 6.7%, 8.4%, 10.1%, 14.8%, and 25.2% among people aged 60 to 64 years, 65 to 69 years, 70 to 74 years, 75 to 79 years, and 80 to 84 years, respectively.[6] Neurodegenerative diseases, cerebral ischaemia, and mental disorders are listed among the aetiological factors of MCI.[3] The study by Solé -Padullés et al. on the relationship between cognitive reserves and the structural and functional brain condition, has shown that there is an inverse relationship between healthy and pathological brain ageing and cognitive reserves. In the group of healthy people, higher reserves were associated with a larger brain volume and a lower activity of the nervous tissue during cognitive task execution. The authors have suggested that this was due to more effective function of neuronal networks in this group. In contrast, in the group of people with the MCI diagnosis, higher levels of cognitive reserves were associated with lower brain volumes and a higher activity of the nervous tissue during cognitive tasks, which was indicative of a more advanced nervous system pathology.

When considering clinical factors related to cognitive function worsening, it is also worthwhile to note the presence of mood disorders. Late-life depression (LLD) is considered to be the most common mental problem affecting people in their old age.[8] Therefore, quality of life is an important issue, which is the resultant of emotional state, physical health and functional fitness in everyday life, especially in relation to the elderly.[9] However, the relevant literature is not clear as far as the proportion of the elderly affected by depression: some authors estimate it to be about 7%[10], while others report that as many as 65% of people older than 65 years of age experience depressed mood and other symptoms of a depressive syndrome.[11] Gao et.al. together with their team have reviewed the studies whose aim was to check whether the presence of depressive symptoms increases the risk of MCI occurrence. According to the results of their analysis, as compared to healthy people, depressive patients have an increased risk of cognitive impairment development.[12] An analysis performed by Rock et al., in turn, have shown that cognitive function deficits, and particular deficits of executive functions, memory and attention, are frequently present during depressive episodes, and some of them (this pertains to executive functions and attention) persist despite the resolution of the other depressive symptoms. Based on this observation, the authors have concluded that cognitive function impairment should be considered a core feature of the depressive disorder, not less important than, for example, depressed mood.[13]

Despite the ambiguity associated with the changes in definition, as well as the variation of the aetiology and symptoms, the assessment of cognitive function status in the mild cognitive impairment is crucial for the diagnostic and preventive process.[14]

As far as possible actions are concerned, there are currently no recommendations that would advise a medical treatment of MCI.[2] Placebo-controlled clinical studies have shown no significant reduction of the rate of progression to dementia in patients with MCI who were treated with the products used in the treatment of Alzheimer's disease.[15]

To date, no medical treatment has been approved by the European Medicines Agency, the US Food and Drug Administration or the Pharmaceutical and Medical devices Agency, either.[16] Therefore the researchers focus currently on the search for alternative forms of therapy. Among the non-medical methods that give the most positive clinical effects, appropriately dosed physical activity is mentioned in the first place.

Aim

Therefore, the aim of this study is to present the impact of regular physical activity of the elderly on their cognitive functions.

Material and methods

This work is a narrative review. It was written based on a document analysis method, with use of quantitative and qualitative techniques. The review includes Polish and international scientific literature collected in such databases as Web of Science, PubMed, Google Scholar. This article presents the results of studies assessing the effects of physical activity on the cognitive function of the elderly conducted between 1999 and 2021, including, in particular, international publications. The articles were analysed with particular regard to the preventive and therapeutic aspects of the use of adequately dosed regular physical activity, to the types of therapies used and to the documented effects of the therapy with physical exercise. After the search of the above mentioned databases, 86 articles were selected that have met the objective of this work, and 52 works out of these 86 were further selected for a more detailed analysis. These 52 works have met high methodology requirements and at the same time they have met the following inclusion criteria:

1. They presented complex neurophysiological background of the ageing process.
2. They presented different forms of physical activity and its effects on mitigation of cognitive disorders in the elderly.
3. They used some indices to assess the effects of various physical activity regimens in the elderly.
4. They showed differences in exercise execution, movement planning and processing of sensory information in the advanced-age population.
5. They documented how enhanced physical activity improves the patterns of active behaviour of the elderly.

Irrelevant articles, systematic reviews, meta-analyses and case studies were excluded from the analysis. The following keywords were used during the literature search: old age, physical activity, cognitive functions.

Analysis of the literature

Physical activity and cognitive functions

According to the WHO, mental health means well-being, when a person executes their potential and is able to cope with a variety of life situations, as well as to participate in the social life.[17] Basic cognitive and social skills, the ability to recognise, demonstrate and shape one's own emotions, and to sympathise with others, flexibility and ability to cope with adverse situations in life, the ability to perform functions in social roles, as well as a harmonious relationship between the body and mind are important components of mental health.[18] The studies show that intended and conscious physical activity has an effect on mental well-being, as the aim of physical fitness is a positive effect on health resulting in a low risk of disease occurrence, which in line with the health-related fitness (H-RF) concept. Achievements in this area should in turn encourage engagement in daily tasks with adequate energy and provide satisfaction from participation in selected forms of physical activity. Thus this concept emphasizes promotion of health and active lifestyles, and motor fitness is a factor that allows achievement of perfect health and optimal quality of life.[19]

Different "healthy doses" of physical exercise are recommended for different age ranges. For people older than 65 years, the WHO (Global Strategy 2010) recommends regular physical exercise of moderate intensity (4 to 6 Met) for 150 minutes per week or high intensity exercise (above 6 Met) for 75 minutes per week. A Canadian expert team accepted the following recommendations as useful for health maintenance and improvement:[20]

a. 60 minutes daily for light efforts (e.g., walking, gardening, stretching) that cause such symptoms as feeling of warmth and a slight acceleration of breathing,
b. 30 to 60 minutes daily for moderate efforts (e.g., cycling, swimming, dancing) that cause such symptoms as increased warmth and a marked acceleration of breathing,
c. 20 to 30 minutes daily for intense efforts (e.g., aerobic, jogging, fast swimming) that result in sweating and shortness of breath.

For the elderly, Kasperczyk has suggested a slightly different attitude than the pre-defined recommendations based on strict physiological criteria. According to this author, it would be preferable to define tasks/objectives of physical activity and leave the form and dose of movement open to specific tasks. These tasks/objectives include the following:[21]

a. to assure postural muscle strength at the "minimum muscle strength" level, as recommended by Kraus and Weber,
b. to keep the physical condition at such a level that efforts of increased intensity (e.g. climbing stairs, running up, etc.) do not produce significant shortness of breath,
c. to practice exercises requiring complex movement coordination, including balance exercises, stretching and relaxation.

Kasperczyk points out that everybody should choose the form and intensity of their physical activity on an individual basis so that the above health-related goals of physical activity are achieved. It is also worth emphasizing that adequate physical effort contributes to the so-called healthy ageing phenomenon.[21]

According to Anderson, this is due to the fact that regular physical exercises regulate the stimulation of the nervous system, which has been proven in studies that emphasize the neuroprotective properties of motion.[22] Many studies evaluating brain activity have shown a significant impact of physical exercise on the cognitive processes at the molecular level, mainly by releasing

neurotrophins in the central nervous system. Research conducted by Park and Poo has confirmed that the key molecule involved in learning and memory is the brain derived neurotrophic factor (BDNF) whose production is increasing as a result of physical exercise.[23] BDNF synthesis occurs mainly in the nervous cells, connective tissue cells and immune system cells – T and B cells and granulocytes. The highest BDNF levels are observed in the cerebellum, hippocampus and amygdala. Additionally, BDNF increases the number of synapses and enhances axonal branches in the cerebral cortex.[24] According to Angelica et al., another factor mediating the production of BDNF in the brain as a result of physical exercise is the growth hormone - insulin like growth factor-1 axis (GH/ IGF-1 axis). Physical activity increases the amount of circulating GH, which is the main driver of IGF-1 production. During the physical effort, there is an increase of IGF-1 levels both in the brain and in the peripheral blood, which leads to increased proliferation and differentiation of the neurones, thereby improving concentration and short-term memory.[25] On the other hand, based on their research, Liu et al. have documented that besides BDNF and the GH–IGF-1 axis also the fibroblast growth factor (FGF) is involved in mechanisms mediating the effect of physical exercise on normal neurogenesis.[26] It should be pointed out that BDNF, IGF-1 and FGF are responsible not only for neurogenesis but also for angiogenesis, which, according to Ide and Secher, affects compensatory plasticity whose functional manifestation is normalisation of blood flow and brain vascularisation, contributing to better supply of oxygen and nutrients to the brain.[27] By using angiographic MR techniques, more small cerebral vessels were found in active elderly people, as compared to their peers leading a sedentary lifestyle.[28] It should be pointed out that one of the proposed mechanisms of the favourable effects of physical activity on the cognitive processes is the effect of BDNF on cellular energy production. According to Gomez-Pinilla and Hilmann, this compound activates many energy systems in the brain, through which it affects synaptic potentials involved in processing of information important for cognitive function formation. This pertains in particular to the pathways responsible for the maintenance of cell energy homoeostasis. Both physical effort and diet translate into the energy balance of the body and, at the cellular level, they influence energy production in the mitochondria. These processes are important to maintain appropriate neuronal excitability and synaptic functions.[29]

A review of the studies evaluating the impact of regular physical activity on the mitigation of cognitive disorders in the elderly

The relevant literature contains many reports on the role of physical activity in prevention of progression of mild cognitive impairment in the elderly. The purpose of a study by Heyn et al. was to assess the effect of various physical activities (such as isotonic exercises or aerobic activities in the form of dancing or indoor cycling) on mild cognitive impairment in people aged 66 to 91 years. A single training session lasted 45 minutes on average and the sessions were repeated at a frequency of 1 to 6 times per week, and the entire study duration was 112 weeks. The results of this study confirmed the positive impact of regular physical exercise on memory and attention improvement.[30]

Also Kramer et al. conducted studies on the effect of physical activity on cognitive functions in the elderly. The subjects were divided into three age groups: 55 to 65 years, 66 to 70 years and 71 years and over. Cognitive tests were divided into four groups: execution, cognitive control, spatial orientation, and speed. The authors have shown that the group aged 66-70 years practising a mixed (resistance and aerobic) training achieved better results in each test, as compared to persons who performed only aerobic exercises.[31]

Antunes et al. have carried out a study that included women aged 60 to 70 years leading a sedentary lifestyle. The researchers have shown that women participating 3 times per week in a 6-month aerobic training program complemented with stretching exercises (23 women) have achieved better results on the Geriatric Depression Scale (GDS) than those who practised recreational dancing and handicraft activities (17 women). The aerobic training resulted in an increase in VO_2 that correlated with increased cognitive performance, in the areas of attention concentration, operational and episodic memory, visual-spatial coordination or response speed shown in the tests, among others.[32]

Another study to assess the impact of aerobic exercises on the cognitive fitness in the elderly people affected by mild cognitive impairment was conducted by Baker et.al. The exercises took place 4 times per week for 6 months. The subjects were divided into experimental and control groups. The control group practised stretching exercises not exceeding 50% of the heart rate reserve, while the experimental group practised aerobic exercise with exercise intensity at the level of 75% to 85% of this reserve - this group achieved an 11% increase in VO_2 after program completion. The results of the tests assessing cognitive fitness showed an improvement in cognitive function in the area of selective attention along with increasing VO_2 (more pronounced in women) in the study group.[33]

A positive impact of aerobic training involving regular walks on women aged between 70 and 80 years suffering from mild cognitive disorders has also been confirmed by a study conducted by Davis et al.[34]

Liu-Ambrose et al. presented an interesting assumption in their study. They randomised 155 women

aged 65 to 75 years into three groups. Group assignment was based on exercise type: endurance training once per week, endurance training twice per week, training of balance and muscle tone regulation twice per week. The subjects participated in the training for 12 months. The endurance training included strength exercises for different muscle groups performed in two series of 6 to 8 repetitions (squats, lunges). The training of balance and muscle tone regulation included stretching exercises, relaxation techniques, tai chi-derived positions and one-leg stance. Performance of cognitive executive functions was assessed with the Stroop test, the trail making test (A and B), and the digit repetition test (forward and backward). The study has shown that in the elderly women endurance training improves cognitive function in the area of attention selectivity, along with muscle strength enhancement. No such changes have been observed as a result of balance training and muscle tone regulating exercises.[35]

A study by Colcombe et al. is also worth mentioning. By using magnetic resonance imaging (MRI) they have demonstrated an increase in the frontal and temporal lobe cortex grey matter volume in subjects aged 60 to 79 years participating in a 6-month aerobic exercise program. No such changes have been found in the control group (consisting of peers of the experimental group members) who practised dynamic and stretching exercises at that time. The program of the exercises consisted of one-hour marches three times per week. Along with the structural changes induced by physical activity, a significant improvement of cognitive functions has also been observed.[36] Also Burdette et al., by using MRI scans, have demonstrated that there was an enhancement of functional connections between particular brain regions, especially between the hippocampus and the cortex of the anterior cingulate gyrus in people aged 70 to 85 years after 4 months of march training.[37]

The results of an experiment by Rehfeld et al. provided important information for even better understanding of the changes in the central nervous system. In people aged 67-68 years with mild cognitive impairment, they assessed the differences in the effects of aerobic and various types of dance on an increase of the hippocampus volume. These activities were conducted for 18 months, the program based on aerobic was completed by 12 persons and the dance program – by 14 people. During the first 6 months, the sessions were held 2 times per week, then once a week, 90 minutes each. Only in the dance group, a significant increase in the volume of the right hippocampus was observed. Both dance and fitness activities caused an increase in the volume of the left hippocampus, with a larger area of this increase in the group of dancers. Only in dancers, the MRI scans showed an increase in the volume of the dentate gyrus where neurogenesis occurs.[38] This relationship has also been confirmed in a study by Mueller et al.[39]

In addition to aerobic exercise and dancing used in the training of the elderly, also yoga deserves some attention. Eyre et al. have compared a memory training and participation in yoga classes with respect to their effect on cognitive function in people aged more than 55 years with MCI. Both programs lasted 12 weeks and involved 1 hour of exercises per week, along with a recommendation to work at home. 39 people were assigned to the yoga group, and 42 people – to the memory training group. The results of the study have shown a greater improvement of both the executive functions and mood in the group that practised yoga.[40] Similar benefits have been obtained in a study by Tew et al.[41]

Among many issues raised by the authors of this work, it is important to remember that not every type of exercise has the same effect on brain structures. As reported by Ploughman, a very intensive and prolonged physical effort results in increased secretion of glucocorticoids, including cortisol that reduces BDNF levels. Decreased secretion of BDNF results in neurogenesis inhibition, decreased brain neuroplasticity, increased apoptosis and neurodegenerative processes in the limbic region of the brain, especially in the hippocampus.[42] Moreover, according to Gallaway et al. it should be noted that the effectiveness of reducing the risk of MCI development depends not only on the optimal training selection but also on the individual's cardiopulmonary performance, age, initial cognitive fitness, medication taken and social environment.[43]

Ageing is a natural long-term process leading to disturbances of the physiological functions of the body. Senile changes are characterised by systematic deterioration of health – not only of the physical health but also of the emotional and social well-being, which leads to a reduction of functional reserves of the individual organs and of the organism as a whole.[44] In this context, it seems necessary to seek new models of life based on popularisation of a healthy lifestyle combined with physical activity.[46] According to Grimm et al., regular physical exercises have an impact on preservation of fitness, independence and autonomy in execution of daily activities, and are an important element of favourable ageing, which, according to Han and Ko, means the best possible course of this process, free from pathology, positively shaped by external conditions, with minimal physiological, psychological and social deficits attributable to the calendar age.[46,47] As reported by Rizzuto et al., the level of physical activity is decreasing with age, both in women and in men.[48] This is also confirmed by the reports of Hama et al.[49] This trend is observed worldwide.[50]

It is therefore essential to raise awareness of the importance of regular physical activity among people as young as in their 50s. Programs that motivate the elder-

ly to take up physical activity should be implemented on a wider scale. A shift from a sedentary to an active lifestyle is necessary to maintain cognitive fitness, which is essential for maintenance of the quality of life at a satisfactory level, defined as physical, mental and social well-being, and not merely absence of a disease.[51] This correlates significantly with assumptions of Rembowski who claimed that: *"Activity is needed at any age, not excluding the late years of life. So old people are pleased with themselves if they undertake activities that replace their lost primary role. Satisfaction at this age is directly associated with maintenance of activity in middle age."*[52]

Conclusion

In the light of the above considerations based on a review of the available literature, it may be concluded that such promising reports, demonstrating a positive relationship between physical activity and mental health, should form the basis for more in-depth research and, in a long-term perspective, for the development of optimal therapeutic and prophylactic strategies in the discussed area.

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Author contributions

Conceptualisation, A.M., T.K.; Writing – Original Draft Preparation, A.M., T.K. and R.W; Writing – Review & Editing, A.M., T.K., R.W. and K.B.

Conflict of interest

The authors declare no conflict of interest.

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CASUISTIC PAPER

Favre-Racouchot syndrome and chronic obstructive pulmonary disease – a common link

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ABSTRACT

Introduction and aim. Favre-Racouchot syndrome though mostly reported in Caucasian men (with an estimated prevalence of 6% in adults older than 50 years), cases have been reported in dark-skinned population including Indians, albeit rarely. It is characterized by large open and closed comedones along with epidermal cysts over the nose, cheeks, temples, forehead and periorbital areas. The association of this condition with chronic heavy smoking is what it makes compelling.

Description of the case report. We report a case of elderly male, chronic heavy smoker who was diagnosed as a case of chronic obstructive pulmonary disease (COPD) as per standard guidelines. He presented with multiple nodulo-cystic lesions and had undergone a skin biopsy. Histomorphology features were consistent with Favre-Racouchot syndrome.

Conclusion. Early identification of this skin condition in mildly symptomatic and asymptomatic smokers may help clinicians to forewarn the patients regarding development of chronic obstructive pulmonary disease (COPD).

Keywords. comedones, COPD, Favre-Racouchot syndrome

Introduction

Favre-Racouchot syndrome though mostly reported in Caucasian men (with an estimated prevalence of 6% in adults older than 50 years), cases have been reported in dark-skinned population including Indians, albeit rarely. It is characterized by large open and closed comedones along with epidermal cysts over the nose, cheeks, temples, forehead and periorbital areas.[1-3]

Aim

To describe the link between Favre-Racouchot syndrome and chronic obstructive pulmonary disease (COPD) in an Indian patient.

Description of the case report

We report a case of 58 years old elderly male, resident of Bhomkhera village in Rajasthan, tailor by occupation with minimal field work and sun exposure, who was admitted to our hospital with complaints of gradually progressive breathlessness correlating to modified Medical Research Council (mMRC) grade 1 to grade 3 dyspnoea along with cough and minimal expectoration since past six months. He has been a chronic heavy smoker smoking more than 20 cigarettes a day for past three decades. The patient denied any history of headaches, dizziness, syncope, paroxysmal nocturnal dyspnoea or pedal oedema. His personal and family history was unremarkable. There was no history of pulmonary or extrapulmonary tuberculosis in the past.

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On initial evaluation, patient had pulse oximetry saturation of 90% at room air with heart rate of 110/minute and blood pressure of 110/70 mmHg. His general physical examination was remarkably normal except for some hyper-pigmented multiple nodular cystic lesions over dorsum of his nose, nasolabial folds and forehead. Respiratory system examination revealed decreased breath sounds over both lung bases. Cardiovascular system examination was normal.

His laboratory parameters revealed haemoglobin of 14.5gm/dl, total leukocyte count (TLC) of 10,800/mm^3, with normal platelet counts. Renal and liver function tests were within normal limit. Sputum culture was sterile and sputum for Ziehl-Neelsen stain was negative. Electrocardiogram (ECG) showed normal sinus rhythm with tachycardia. Arterial blood gases revealed mild hypoxemia. Chest X Ray (PA view) (Figure 1) showed bilateral hyperinflated lung fields with prominent broncho-vascular markings suggestive of chronic bronchitis. Pulmonary function testing revealed a moderate obstructive ventilatory defect with forced expiratory volume in one second (FEV_1) of 0.94 l (63% of predicted). Thus, based on relevant clinical history, examination and investigations including spirometry, this patient was diagnosed as a case of chronic obstructive pulmonary disease (COPD) as per standard guidelines.

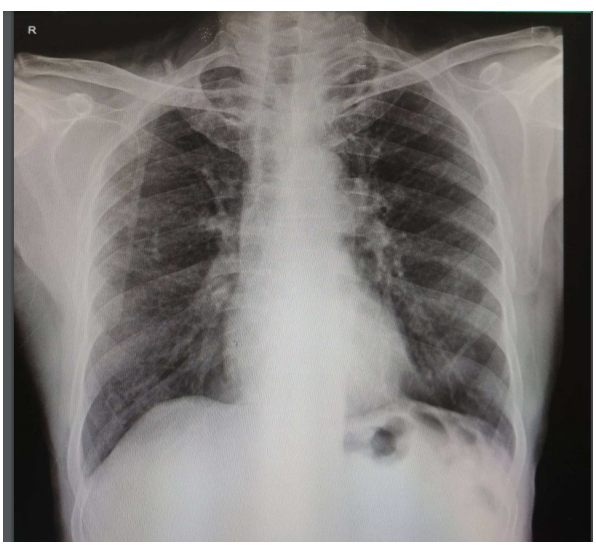
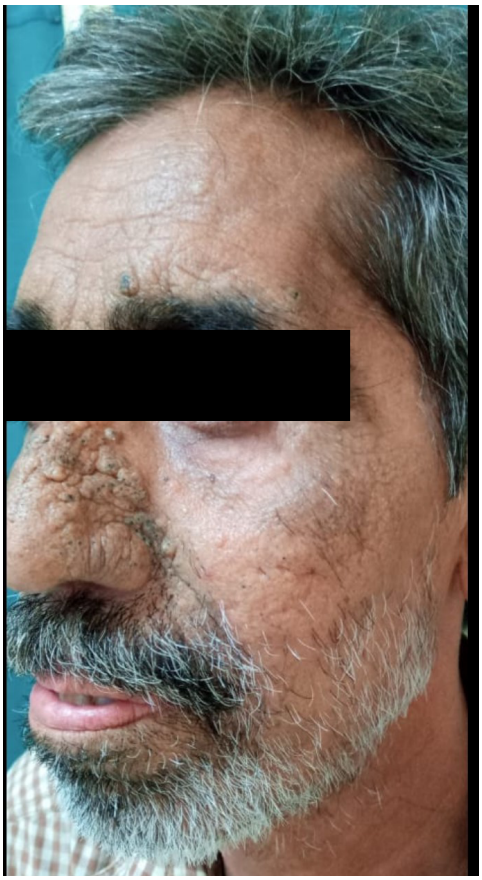


Fig. 1. Chest X-Ray Postero-Anterior view shows bilateral hyperinflated lung fields with prominent broncho-vascular markings

The patient was emotionally disturbed with the unappealing appearance of his face and gave a history of slowly expanding dark coloured lesions on his nose. Dermatologist's opinion was sought and on examination, multiple nodule cystic lesions (largest measuring 1x2 cm) were described over dorsum of his nose and forehead (Figure 2). There was no history of any drug intake, exposure to chemicals or toxins, application of



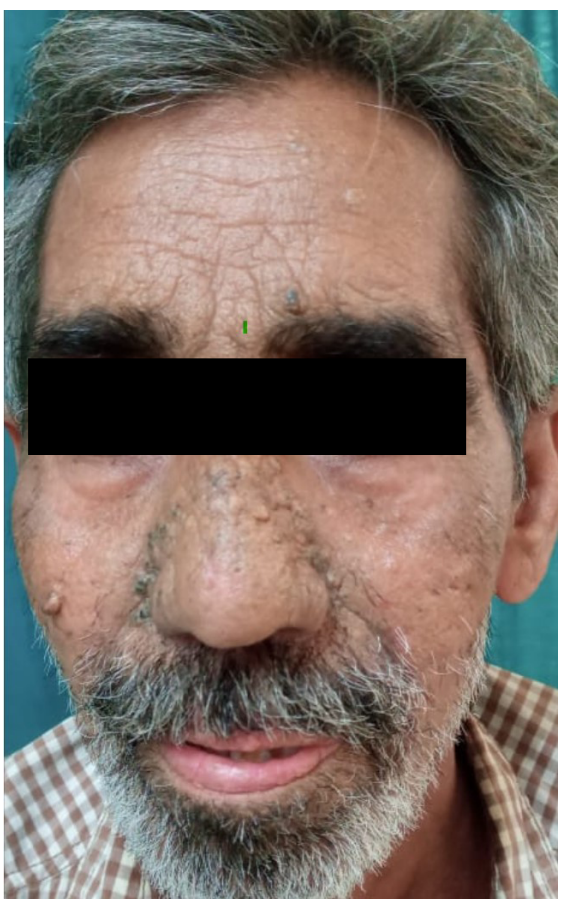


Fig. 2. Multiple nodulo-cystic lesions bilaterally over dorsum of nose, nasolabial folds and forehead

cosmetic creams to face or any other associated systemic illness.

He was advised to undergo skin biopsy. Incisional skin biopsy was done from forehead lesion under local anaesthesia. Histopathology revealed keratinized stratified squamous epithelium having dilated pilosebaceous openings and cyst like spaces lined by flattened epithelium, filled with lamellated keratinous material. Histomorphology features were consistent with Favre-Racouchot syndrome (Figure 3).

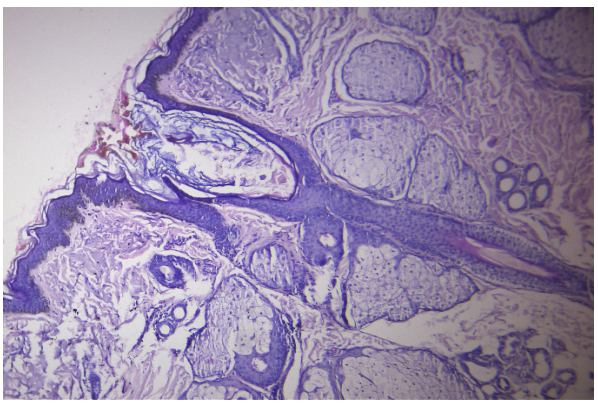


Fig. 3. Histopathology reveals keratinized stratified squamous epithelium having dilated pilosebaceous openings and cyst like spaces lined by flattened epithelium, filled with lamellated keratinous material (H+E stain; 40 X)

Patient was managed with antibiotics along with inhaled corticosteroids and bronchodilators and was symptomatically better. Patient was simultaneously counselled regarding smoking cessation and sun protection for prevention of recurrence of skin lesions.

Discussion

The ancient "Zang-Fu" theory proposed that skin is functionally linked to lung. Vierkotter et al (2010) showed that cigarette smoking and ambient soot levels were important factors in causing pigment spots and wrinkles.[1] Cutaneous manifestations are frequently associated with pulmonary diseases.[2] pulmonary diseases can be life threatening and early detection and treatment may have impact on patient's quality of life. Better knowledge and understanding of both common and rare cutaneous manifestations of pulmonary diseases enables physician to enhance their clinical competency.

The common dermatological manifestations noted in COPD patients are xerosis, senile purpura, onychorrhasis, onychomycosis, tobacco nail stains and hyperkeratosis of both elbows. It has been seen that a non-invasive determinant of skin elasticity is independently associated with pulmonary emphysema and tissue proteolysis in tobacco exposed individuals. Loss of skin elasticity is a novel observation that link common pathology in skin and lung in emphysema.[3] More recent reports suggest that an association between facial wrinkling and airflow obstruction exists, which may explain common susceptibility to deleterious effects of smoking.[4]

Nicotine containing cigarette smoke induces alteration in sebum composition as it causes increased oxidation stress, lipid peroxidation and reduced levels of alpha-tocopherol.[1] There is high grade of lipid peroxidation in sebum of smokers which leads to comedones and acne. Squalene, which is particularly important per-oxidated lipid in human sebum has hyperproliferative effect on keratinocytes and is therefore "comedogenic".[5,6]

Favre-Racouchot syndrome is a very peculiar type of dermatosis which is characterised by multiple large open and closed comedones on actinically damaged skin.[7] Though mostly reported in Caucasian men (prevalence 6%), cases have been rarely reported in dark skinned population including South Asians.

The exact mechanism by which smoking contributes to this syndrome is poorly understood but some proposed theories include:

- ultraviolet activated phototoxic properties of tobacco smoke,
- increased reactive oxygen species,
- increased matrix metalloproteinases.

Conclusion

To conclude, the importance of this skin manifestation in a chronic smoker lies in the fact that early identification of this condition and confirming it with histopathology, we might alert these patients particularly who are asymptomatic or mildly symptomatic regarding the development of chronic obstructive airway disease because of similar proposed pathogenesis. Positive association between smoking and this condition definitely exists and cessation of smoking along with sun protective measures are required for prevention of recurrence of lesions.

Declarations

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Author contributions

Conceptualization, M.I.S. and H.I.S.; Investigation, A.C.; Resources, Writing – Original Draft Preparation, M.I.S; Writing – Review & Editing, S.D.; Visualization, A.M., Ş.K.E., Ş.Ü.A.; Supervision, S.D.

Conflicts of interest

The authors have no conflict of interest.

Data availability

The data have not been made public, but are kept with the authors, if necessary.

Ethics approval

All subjects gave informed consent to the inclusion prior to participating in the study.

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CASUISTIC PAPER

Fabry disease related nephropathy – case family report and literature review

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ABSTRACT

Introduction and aim. Fabry disease (FD) is a ultrarare storage disorder which causes irreversible damage to the brain, heart, and kidneys in young patients. The aim of our study was to draw clinician's attention to the need of considering FD in the differential diagnosis of kidney disorders.

Description of the case. We present the case of a 45-year-old man who has been misdiagnosed for several years with arterial hypertension with organ complications. He was referred to the nephrological ward due to chronic advanced kidney disease of unclear etiology. After 2 months of thorough differential diagnostics, based on the clinical course (past stroke, membranoproliferative glomerulonephritis (MPGN), left ventricular hypertrophy, paroxysmal limb pain) and conducted genetic examination, FD was confirmed. Then, screening tests were performed among the patient's family members, confirming the presence of the same mutation as in our patient in 4 women of which in 3 were diagnosed cardio-renal syndrome. The authors of other studies report glycolipid deposits in the kidney cells on a needle biopsy, usefulness assess podocyturia, globotriaosylceramide protein in the urine and renal parapelvic cysts in an ultrasound examination in diagnostic FD nephropathy.

Conclusions. This is the first case report to describe membranoproliferative glomerulonephritis in a patient suffering from FD. In patients with FD and the same genotype, kidney damage has a different phenotype.

Keywords. Fabry disease, kidney injury, membranoproliferative glomerulonephritis, rare disease

Introduction

Fabry disease (FD), also known as Anderson-Fabry disease, was first described in 1898 independently by the German dermatologist Johannes Fabry and the English dermatologist William Anderson due to the characteristic changes in patients with FD (angiokeratoma). FD is a rare, genetically determined metabolic disorder that dramatically reduces a patient's life expectancy. It is inherited with the X chromosome and results from a mutation in the GAL gene in the Xq22 locus, which encodes the α-galactosidase (a-Gal) enzyme. Mainly men suffer from the disease, although it is believed that the clinical manifestation of the disease may occur even in 30% of heterozygous women.[1,2] Whereas the manifestation of FD in heterozygous women may be as severe as in men, although some women are asymptomatic.[3]

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The disease phenotypes in women differ due to differences in residual enzyme activity and X-chromosome inactivation patterns.[1,4-6] In the course of the disease, the activity of the α-Gal is reduced or absent. Testing the activity of the α-Gal in the blood of patients may be helpful in detecting FD. In the heterozygous women, due to the random inactivation of the X chromosome, the result of this test is often inconclusive in the female population. Therefore, molecular testing in women with suspected or positive family history of FD is obligatory.[1]

The lack or reduced activity of α-Gal causes the accumulation of large amounts of sphingolipids in the lysosomes of various types of cells of the heart, kidneys, skin, eyes, central nervous system and digestive system, which in turn triggers a cascade of cellular damage and may lead to various clinical symptoms.[1-5,7,8] Fabry deposits in cells are histopathologically defined as membrane-like lamellar deposits called myeloid bodies or zebras. In studies of kidney biopsies under electron microscopy, electronically dense osmophilic inclusions referred to as lamellated bodies (myelin-like bodies, myceloid bodies, zebra bodies) are also observed in the lysosomes of podocytes, epithelial cells of the distal tubules and in arterioles FD nephropathies.[9] The most characteristic symptoms of FD include episodes of very severe pain in the distal parts of the limbs, hyperhidrosis, heat intolerance, clouding of the lens and cornea, left ventricular hypertrophy, damage to the kidneys with proteinuria, skin lesions (angiokeratoma).[1,5] Moreover, patients suffer from gastrointestinal disorders and have a significantly increased risk of ischemic stroke or small fibber peripheral neuropath.[3,7,8] Kidney injury and cardiovascular complications are the main causes of death in FD patients. The clinical picture of the disease changes with the patient's age.[6,7,10] Almost all complications resulting from the occurrence of FD are non-specific, which makes them clinically indistinguishable from other similar abnormalities often observed in the course of civilization diseases. In undiagnosed and untreated patients with FD, organ damage progresses with the patient's age, gradually deteriorating the patient's quality of life, leading to organ failure and premature death.[6,10]

Both symptomatic treatments are used in the treatment of FD.[1,11] Conventional management depends on the clinical manifestation of the disease and consists in administering painkillers, antiarrhythmic drugs as well as cardio- and nephroprotective drugs, such as angiotensin converting enzyme inhibitors and angiotensin receptor blockers.[1] Patients with end-stage renal disease may undergo renal replacement therapy.[6]

Currently, a specific therapy for Fabry disease is the replacement of the missing enzyme with recombinant human α-Gal (enzyme replacement therapy; ERT).[1,7,6] It has been confirmed that the early initiation of treatment is of great importance for the improvement of the quality of life and the patient's prognosis (alleviation of neuropathic pain, reduction of gastrointestinal symptoms, reduction of myocardial hypertrophy, stabilization of renal function).[3]

Before starting ERT treatment, a comprehensive evaluation of the organ involvement in FD is necessary.

Prenatal diagnosis involving the determination of enzymatic activity or DNA testing in chorionic villus or cultured amniotic cells can be considered only in male foetuses for ethical reasons.[1]

Although the direct cause of FD has been known for many years, the pathomechanisms leading to multi-organ disorders have not yet been fully elucidated.

The gradual deposition of glycosphingolipids in kidney patients leads to a progressive deterioration of renal function with proteinuria, decrease of glomerular filtration rate (GFR) and hypertension. Fabry nephropathy (FN) probably begins with elevated albuminuria or proteinuria. These abnormalities in the results of urine tests in the classic form of the disease manifest themselves from childhood. A progressive decline in the GFR may begin at an early age and progress to end-stage renal failure, which is one of the main causes of premature mortality in patients with FD.[6,7,13] Considering the high survival rate of patients after transplantation, kidney transplantation is the treatment of choice in patients with end-stage renal disease in the course of FD. Unfortunately, despite numerous studies, little is known so far about the long-term results, overall patient survival or the possible role of ERT after transplantation.[6]

Aim

The diagnosis of FD is a problem for clinicians because of its little characteristic symptomatology. It happens, that patients around the of 40 start dialysis for secondary of hypertension renal failure, but the true diagnosis is FD.

The aim in the first part of our study, was described a case of a patient with FD, with particular emphasis on kidney injury in the course of this disease. Despite having a kidney biopsy performed on the patient, nephrologists in our team wondered, what was behind the diagnosed glomerulopathy. On the other hand, our patient concealed some of the symptoms due to prolonged hospitalization. The case report of a patient with FD presented in the manuscript, in whom arterial hypertension with organ complications was indiscriminately diagnosed for several years, confirms that it is necessary to repeat the existence of this disease according to the old Latin rule: *repetitio mater studiorum est*.

In the second part of the study, family members of the patient were analysed for the presence of FD and kidney injury. In the last part of our study, we correlated our clinical data with the available data from the literature on the symptomatology and pathomechanisms of kidney damage in the course of FD.

Material and methods

The patient's medical history was analyzed retrospectively with FD that was genetically confirmed. The genetic test was performed using the dry blood drop test.

Retrospective data on FD gene penetration in the patient's family were collected. Then, routine medical, physical, laboratory and imaging examinations necessary to assess kidney function in the population of 4 patients from the patient's family with diagnosed FD were prospectively performed.

In addition, the medical database (Medline, PubMed) was reviewed using keywords (Fabry disease, rare disease, enzyme replacement therapy, α-galactosidase A, lysosomal disease, storage disease) to obtain information on the symptomatology and pathomechanisms of kidney damage in the course of the FD. A Total of 48 English-language papers published between 2000 and 2021 were analysed. All the 7 selected publications are original research papers.

Description of the case

A 45-year-old patient with chronic kidney disease, hypertension, epilepsy, after an ischemic stroke of the right hemisphere at the age of 44, was admitted to the department with a nephrological profile due to chronic kidney disease in stage G4 (according to KDIGO 2012) with unclear etiology diagnosed in the nephrology clinic.[14] On admission, the patient reported pain in the upper limbs of paresthesia nature, periodic chest pain, anhidrosis, hearing loss and periodic diarrhoea. During hospitalization, systemic diseases of the connective tissue, chronic infectious diseases, neoplastic diseases (also in the field of hematology) and thrombotic microangiopathy were excluded. The patient had bilateral permanent sensorineural hearing loss, and the histopathological examination of the kidney biopsy showed the features of advanced chronic membranous-proliferative nephropathy. Figures 1 and 2 show the results of a light microscopic examination of a patient's kidney biopsy.Due to the lack of improvement after the applied conservative treatment as well as rapidly increasing parameters of kidney failure and uremic symptoms, the patient was qualified for renal replacement therapy using intermittent hemodialysis. For this purpose, a vascular catheter was implanted into the right internal jugular vein and the first hemodialysis procedure was performed. The psychiatrist diagnosed the patient with depression.

In addition, FD tests were performed using the dry blood drop test. The results of tests with a dry blood drop of the patient showed: no activity of the α-Gal, high concentration of Lyso-GL-3 (112.3 ng/ml; normal <3.5 ng/ml), the presence of a nonsense genetic mutation -c.679C > T (p. (Arg227Ter)).

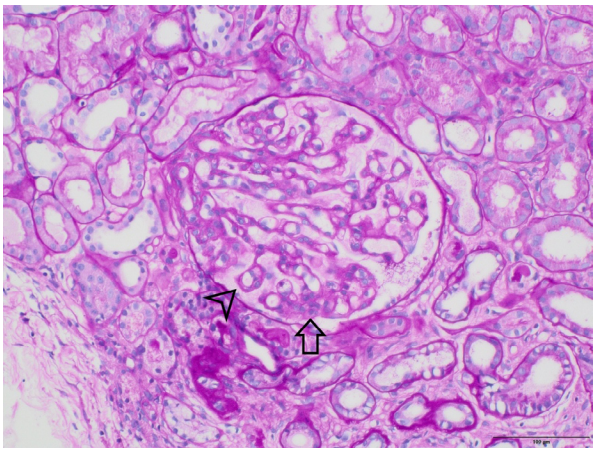

Fig. 1. Glomerulus with focus of mesangial hypercellularity (arrow) and thickening of glomerular basement membrane (arrowhead) (PAS stain, 200X)

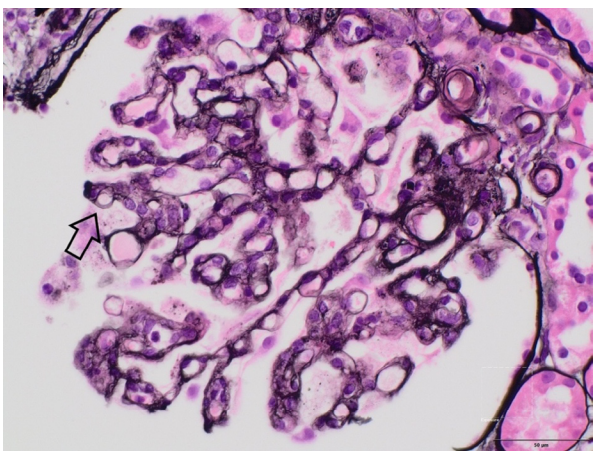

Fig. 2. Glomerulus with thickening of glomerular basement membrane with double contours (arrow) (Silver stain, 400X)

After obtaining the results of the above-mentioned studies, the interview with the patient was deepened. He revealed from early childhood a characteristic rash on the lower abdomen and thighs (angiokeratoma), he suffered from paroxysmal burning pains in the feet and hands, appearing periodically. In addition, the patient suffered from sudden abdominal pain of unknown origin, accompanied by diarrhoea and vomiting. From childhood, he had a big problem with tolerance of physical effort, which often prevented him from integrating with a group of peers. The patient also pointed out that the symptoms worsened mainly when he was in high temperatures. At that time, he felt exceptional weakness, and at the same time lack of sweating was characteristic. Edema in the limbs did not begin to appear in the patient until around the age of 40, when the characteristics of kidney damage appeared. During the consultation, the cardiologist performed cardiac echocardiography, which showed signs of left ventricular hypertrophy

and assessed the patient's 5-year risk of sudden cardiac death at 4.6% and qualified the patient for cardioverter-defibrillator (ICD) implantation in the primary prevention of sudden cardiac death. Moreover, the consulting neurologist, based on the physical, subjective and ENG results, diagnosed the patient with a history of ischemic stroke and peripheral polyneuropathy. The patient is currently undergoing enzyme replacement therapy. He regularly receives intravenous infusions of α-Gal at a dose of 0.2 mg per kg body weight from every 2 weeks. In addition, he continues treatment with chronic hemodialysis (treatments 3 times a week for 4 hours) on the arteriovenous fistula created on the left forearm. In the diagnostic and therapeutic plan of the patient, after ICD implantation, it is planned to qualify the patient for kidney transplantation. The patient's wife is considering donating the kidney to her husband.

The patient also noted that his grandfather (mother's father) also suffered from burning pain in his limbs. The patient's grandfather died around the age of 50.

The results of genetic tests of the patient's family confirmed the presence of the mutation characteristic of FD in another 4 people (all people are women).

In addition, it is known that the patient's mother, his aunt, and sister suffer from hypertrophic cardiomyopathy, while the 20-year-old niece was diagnosed with the symptoms of mitral valve prolapse syndrome. The patient's nephew and cousin are healthy.

Figure 3 shows the genetic tree of a family with FD.

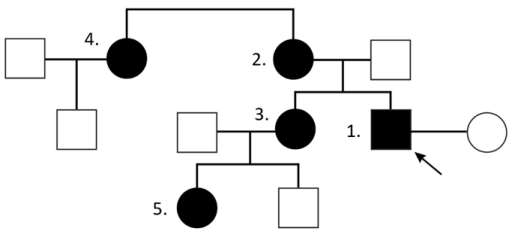

Fig. 3. Genetic tree of a family with Fabry disease (1. Patient, 2. Patient's mother, 3. Patient's sister, 4. Patient's aunt, 5. Patient's niece)

Table 1 shows the results of selected genetic and enzymatic tests together with the assessment of kidney damage in individual people with Fabry disease in the family.

Based on a review of the literature available in medical databases, it was observed that the most frequently described pathology in the course of FD are parapelvic cysts, podocyturia even without an increase in creatinine or albuminuria, and also glomerulonephritis in the course of podocytopathy, including focal segmental glomerulosclerosis (FSGS).

Table 2 provides an overview of selected studies on the types of kidney disease in patients with FD. Only clinical research studies were selected.

Table 1. Results of selected genetic and enzymatic tests together with the assessment of kidney damage in individual people with FD in the family.

		Genetic mutation	α-Gal [mmol/L/h]	Lyso-GL-3 [ng/ml]	CKD[&]	Cause of CKD
1	patient	nonsense type – c.679C>T (p.(Arg227Ter))	0 *	112.3 [#]	G4/A3	MPGN
2	patient's mother	Nonsense type – c.679C>T (p.(Arg227Ter))	3.8 *	6.6 [#]	G2/A2	CRS type II
3	patient's sister	Nonsense type – c.679C>T (p.(Arg227Ter))	1.4*	17.0[#]	G1/A2	peripelvic cyst
4	patient's aunt	Nonsense type – c.679C>T (p.(Arg227Ter))	2.3*	12.2[#]	G1/A2	CRS type II
5	patient's niece	Nonsense type – c.679C>T (p.(Arg227Ter))	2.8*	8.9[#]	n	n

[a] Abbreviations: # – norm <3.5 ng/ml; * cut-off value = 2.8 mmol/L/h; n – no irregularities; [&] – stage of CKD according KDIGO 2012 [14]; a-Gal – alpha-galactosidase enzyme activity; CRS – cardio-renal syndrome; MPGN – membranoproliferative glomerulonephritis

Discussion

The presented clinical case deserves a comment due to the very late diagnosis of a genetic disease affecting many zones of the patient's life. Due to the rarity of the disease and the multi-symptomatic course of FD, it is rarely considered in the differential diagnosis. The presence of cardiovascular complications in young people without concomitant classic risk factors for these diseases and the appearance of renal failure of unclear etiology should be an indication for testing for FD. Despite confirmation of the same mutation, the nephrological phenotype in FD may be different. This heterogeneity in the symptoms of kidney injury in FD may be related to the gender, α-Gal activity, and age of the patients, although environmental influences may also be involved in the symptomatology of FN.

Based on the case study of our patient with FD and his family, and based on the available literature, the following causes of nephropathy can be found in patients with FD:

- glomerulopathies associated with damage to the podocytes,
- other glomerulopathies (such as membranous-proliferative nephropathy found in our patient),
- distal renal tubular acidosis,
- Fanconi syndrome,
- cardio-renal syndrome.[21]

In some patients hyperfiltration is described. Whereas hyperfiltration is most often estimated using eGFR calculation formulas based on serum creatinine.

Table 2. The overview of selected studies on the types of kidney disease in patients with FD[a]

Authors	Year of publication	Research method	Conclusions
Ries et al[10]	2004	prospective MRI and CT imaging of the kidneys	· multiple cysts in the renal sinuses are characteristic · the cause of the cysts is unknown
Pisani et al[13]	2018	USG of the kidneys	· renal parapelvic cysts were detected in 28.9% of people with FD vs 1.1% of people in the control group ($p < 0.001$)
Fall et al[15]	2016	analysis of patient urine cells using cytospin slides stained for podocalyxin, claudin-1 and Dapi	· patients with FD have increased podocyturia even in the absence of proteinuria and albuminuria · podocyturia correlates with the clinical severity of FN
Auray -Blais et al[16]	2020	dry stain analysis from the urine of patients - by liquid chromatography / tandem mass spectrometry	· increased concentrations of Gb3 in urine were observed in 13.6% of patients with FN · CKD or other comorbidities may be associated with an increase in urine Gb3 concentration (all the patients)
Yeniçerioğlu et al[17]	2016	genetic analysis in people, who were not dialyzed, with α-Gal-A enzyme activity ≤1.2 µmol/l/h in the dry blood drop test	· FD should be considered in the differential diagnosis of CKD of unknown etiology
Mauer et al[18]	2014	kidney biopsy from 12 untreated women with FD aged 8-63	· there is an association between podocyte mosaicism and podocyte damage in FD patients · the kidney biopsy, which provides information about podocyte mosaicism, can help stratify women with FD for their risk of kidney disease and help make treatment decisions
Ortiz et al[19]	2008	sectional retrospective evaluation of eGFR, albuminuria and proteinuria in 1,262 adult patients (677 females) from the Fabry registry	· proteinuria, although an early complication, may not be evident in patients with advanced CKD · a significant proportion of women suffer from moderate to severe kidney involvement in FD
Siegenthaler et al[20]	2017	eGFR, left ventricular myocardial mass index	· CRS was associated with a high risk to develop cardiovascular complications and death · a focus on cardio-reno-protective therapies is crucial

Abbreviations: CKD – chronic kidney disease; CT – computer tomography; eGFR - estimated glomerular filtration rate; FN – Fabry nephropathy; lyso-Gb3 – globotriaosylsphingosine; MRI - magnetic resonance imaging; USG – renal ulrasonography

In patients with FD, we also observe a sarcopenia caused by the persistence of chronic inflammation in the body of these patients, secondary to damage to muscle cells overloaded with lysosomal proteins. Evaluation of eGFR estimated based on serum creatinine concentration, which is dependent on muscle mass, is another reason why in the evaluation of eGFR in patients with FD we should use a different biomarker, such as serum cystatin C (CysC). In our nephrology department, we observe that the eGFR estimated based on blood creatinine in patients with FD may be overstated by up to 60 ml/min in relation to the eGFR estimated based on serum CysC (118 vs. 44 ml/min/1.73m^2).

Currently, a specific therapy for Fabry disease is the replacement of the missing enzyme with recombinant human α-Gal (enzyme replacement therapy; ERT).[1,7,6] The current approach is ERT with intravenous agalsidase-alpha or agalsidase-beta administered every 2 weeks and oral chaperone therapy with migalastat.[22]

It has been confirmed that the early initiation of treatment is of great importance for the improvement of the quality of life and the patient's prognosis and stabilization of renal function.[3] However, the efficacy of therapy and its diagnostic role in FD is not easy to determine in the paediatric population, as paediatric patients relatively rarely undergo renal biopsy.[23]

Conclusion

Patients with Fabry disease should be under the care of a multidisciplinary medical team that should include a nephrologist.

Declarations

Funding

The study did not require funding.

Author contributions

Conceptualization, N.K. and A.G.B.; Methodology, K.M.C.; Investigation, S.T., K.M.C. and A.G.B.; Writing – Original Draft Preparation, N.K. and A.G.B.; Writing – Review & Editing, N.K. and A.G.B.; Supervision, A.G.B.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

The data may be made available to interested persons at the request of the corresponding author via e-mail.

Ethics approval

All subjects gave informed consent to the inclusion prior to participating in the study. The study has been ap-

proved by the Bioethics Committee at the University of Rzeszow No 2018/06/10.

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Back Matter

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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.

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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

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— **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.

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— **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.
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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.
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