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Research Article

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Investigation of the relationship between comprehensive echocardiograhic findings and CRP/prealbumin ratio in patients with metabolic syndrome

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Abstract

The predictive value of the C-reactive protein / Prealbumin (CRP/PAB) ratio for the assessment of left atrial (LA) functions in metabolic syndrome (Met-S) remains unclear. We enrolled 100 patients with newly diagnosed Met-S and 53 individuals as a control group. Detailed conventional and tissue Doppler echocardiographic examinations were performed, including measurements of volumes and functional indexes of LA. Mean CRP/PAB ratio values were not found to be different between groups (p=0,107). Mean mitral-A, tricuspid-A, and mitral-a' velocities were found to be significantly different between groups (p-value for all<0.05). None of the volumetric and functional indexes of LA were found to be different between groups (p-value for all >0.05). After dividing the patients into groups as presenting wleft-right/left ventricular diastolic dysfunction (RVDD- LVDD) or not, again, none of the volumetric and functional indexes of LA were found to be different between groups (p-value for all >0.05). Finally, the CRP/PAB ratio was not found to be correlated with any of the indices of LA in patients with Met-S, while only active emptying volüme of LA was found to be correlated with the CRP/PAB ratio in the control group. We did not find any association between the CRP/PAB ratio and LA functions. To evaluate the predictive value of the CRP/PAB ratio for the assessment of LA functions in Met-S needs further studies with a large cohort population.

Keywords: C-reactive protein-to-prealbumin ratio; metabolic syndrome, left atrium function, left atrium volume

1. Introduction

Metabolic syndrome (Met-S) is defined as a sum of metabolic risk factors, including hypertension (HT), obesity, hyperglycemia, and hyperlipidemia, and it has been shown that Met-S has a strong association with an increased risk of cardiovascular disease (CVD), stroke, and all-cause mortality (1). Inflammatory pathways contribute a major role in the pathogenesis of the development of this disease (2). CRP has been shown to be a sensitive and systemic biomarker for both Met-S (3). Prealbumin (PAB) is known as a negative acute-phase reactant protein, and it has been found to be associated with a higher mortality rate in patients with heart failure (HF) (4). Recently, as a novel inflammatory marker, CRP/PAB ratio has been reported to be a better predictive value for recurrence of gastric cancer, the severity of organ dysfunction in critically ill patients, and higher mortality in patient HF patients (5-7). Structural and functional changes of the left atrium (LA) are related to various CVDs, including ischemic stroke and atrial fibrillation (AF), and have been proposed as very important risk factors for cardiovascular mortality (8). It has been shown that Met-S is associated with electrical and structural remodeling of the LA (9). During the cardiac cycle, LA functions have three components a reservoir during systole, a conduit during early diastole, and an active contractile function in late diastole. LA volumes measured by echocardiographically have been described as 1: maximal volume (Vmax, 2: preatrial contraction volume (VpreA), and 3: minimal volume (Vmin) (10). Recently, shown that Met-S had a greater Vmax (p = 0.03), VpreA (p = 0.001) than the control group. In that study, LA mechanical function was found to be impaired in patients with Met-S (10). So functional and morphological changes of LA in patients with Met-S have been evaluated many times before however, to the extent of our knowledge, the importance or predictive value of CRP/PAB ratio for early detection of any sign of functional and morphological changes of LA in patients population have not been examined before so we aimed to evaluate this relationship.

2. Materials and Methods

2.1. Subjects

This study was carried out as a cross-sectional study. Patients newly diagnosed with Met-S were admitted to this study. One hundred fifty-three consecutive patients (73 female patients, 80 male patients, mean age:57,3 ± 8,5) were enrolled for assessment in the clinic of Cardiology in Kirsehir Ahi Evran University Medical Faculty Education and Research Hospital between April and May 2023. Patients were divided into three

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groups: group-1, which included the patients diagnosed as Met-S but without diabetes mellitus (DM) (Non-DM group, n= 49, mean age: 58 ± 8,7); group-2, which included the patients diagnosed Met-S with DM (DM group, n=51, mean age: 60 ± 8,5): and group-3 which included healthy individuals (control group, n= 53, mean age: 59 ± 7,2). Met-S was defined by the criteria proposed by NCEP-ATP III (1). At least 3 or more of the following components were needed to meet the metabolic syndrome criteria: a) fasting blood glucose ≥ 100 mg/dl or the patient's self-reported history of DM or use of DM medications; b) blood pressure ≥ 130/85 mmHg or the patient's self-reported history of HT or use of antihypertensive medications; c) triglycerides (TG)≥ 150 mg/dl; d) high-density lipoprotein (HDL-C) <40 mg/dl and e) BMI >30 kg/m2. Participants were aged 20 years and above and had no history of DM, cancer, neurovascular, cardiovascular, and/ or renal diseases. They also did not have any history of taking medication for HT hyperglycemia, and hyperlipidemia. The protocol of study has been approved by the ethics committee of Kirsehir Ahi Evran University of Medical Faculty (04.04.2023 date and registration number 2023-07/43), and written informed consent was obtained from all of the study participants before their participation in this study.

2.2. Clinical and laboratory assessments

After detailed medical history, anthropometric assessments, including body-mass index (BMI), body surface area (BSA), waist circumference, and blood pressure measurements, were measured by trained medical doctors who were blind to the study protocol. Following at least 12 hours of fasting, venous blood samples were collected from the patients. All samples were centrifuged for 10 minutes. Then, all serums were kept at – 80'C deep and frozen until they were studied. On the obtained serum, routine biochemical parameters including TG, total cholesterol (T-Chol), HDL-C, low-density lipoprotein cholesterol (LDL-C), plasma glucose (Glu), uric acid (Ua), creatinine (Cre), vitamin D (Vit-D), hemogram well as CRP and PAB were studied with routine methods. All parameters, including prealbumin and CRP, were studied using a Cobas 501 (Roche Diagnostics, Germany) autoanalyzer.

2.3. Echocardiographic examination

We used a Vivid 5 pro echocardiographic unit (GE Healthcare, GE, USA), including a 3,5 MHz probe for echocardiographic assessment. All test subjects were evaluated by a standard two-dimensional and Doppler evaluation according to the recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging by a single experienced cardiologist who was blinded to the test subjects and all clinical findings (11). The following parameters were obtained by conventional echocardiographic imaging (CEI): Left ventricular (LV) end-diastolic dimension (LV-Dd), LV systolic dimension (LV-Sd), LV ejection fraction (LVEF, %) according to the method of Simpson's method; mitral annular plane systolic excursion (MAPSE) and tricuspid annular plane systolic excursion (TAPSE) by the M mode at the mitral lateral and tricuspid lateral annulus, LV and right ventricular (RV) diastolic functions (LVDF; RVDF) from the filling velocities (early peak (E) and late diastolic (A) wave velocities, E/A ratios with deceleration times (DT) using pulsed wave doppler with the sample volume positioned at the tips of the mitral and tricuspid valve leaflets. Epicardial fat thickness (EFT) was considered as the echo-free distance between the outer surface of the myocardium and the vis¬ceral stratum of the pericardium. We measured EFT values from the parasternal long-axis imaging at vertical to the right ventricular free wall at the end of the diastole. The tissue Doppler imaging echocardiography (TDIE) study was performed in the lateral mitral annulus, interatrial septum, and lateral tricuspid annulus. The recordings of all diastolic functions of LV were obtained by evaluation of early peak (e') and late (a') diastolic wave velocities. In addition, the e'/a' ratio, DT of e' wave from mitral lateral annulus, and systolic velocity of tricuspid lateral annulus (t-S') were also obtained at the lateral tricuspid annulus by the TDIE. Diastolic dysfunction was accepted as E/A <1,0 (11).

2.4. Left atrial mechanical functions

LA volume measurements were performed with the disk method from the apical four-chamber window. Maximum left atrial volume (Vmax) was recorded just at the time of mitral valve opening; minimum left atrial volume (Vmin) was recorded just at the time of mitral valve closure; presystolic or preatrial contraction left atrial volume (VpreA) was recorded at the onset of atrial systole (P wave on ECG). All LA volumes were corrected/indexed by body surface area (BSA = mm^3 /m^2). End diastolic and end systolic left ventricular volumes and BSA ratios were recorded. LA emptying functions were calculated with the following formula:

Left atrial passive emptying volume (LA-PEV) = Vmax – VpreA;

Left atrial passive emptying fraction (LA-PEF) = LA-PEV / Vmax;

Left atrial active emptying volume (LA-AEV) = VpreA – Vmin;

Left atrial active emptying fraction (LA-AEF) = LA-AEV / VpreA;

Left atrial total emptying volume (LA-TEV) = Vmax – Vmin;

Left atrial total emptying fraction (LA-TEF) = LA-TEV – Vmax.

All measurements were done by one operator who was blinded to subjects at twice during different times, and the average of the measurements was obtained (12).

2.5. Reproducibility

To calculate the intra-observer and inter-observer coefficients of variation for measurements of echocardiographic recordings and CRP/PAB ratio results, 20 randomly selected patients in the severe group were assessed by repeating the measurements under the same baseline conditions. To test the interobserver variability, we performed the measurements offline from video recordings by a second observer. The intra-observer and inter-observer coefficients of variation for the echocardiographic and CRP/PAB ratio measurements were found to be <5% and insignificant.

2.6. Statistical analysis

The continuous variables are presented as mean ± standard deviation (SD) or median (inter-quartile range). The categorical variables are presented as frequency (percentage). The data were checked for normality using Kolmogorov-Smirnov and Shapiro–Wilk tests. ANOVA and Kruskal-Wallis tests were used to examine the differences between the continuous variables when appropriate. Bonferroni test was used as the Post-Hoc analysis then the Mann-Whitney -U test was used to examine the differences between variables. Pearson chi-squared test or Spearman's test was used to determine the potential correlation between the CRP/PAB ratio and other variables. The statistical analyses were performed using SPSS for Windows (version 21.0, SPSS Inc., Chicago, IL, USA). All analyses were two-tailed, and a p-value of <0.05 was considered statistically significant.

3. Results

3.1. Baseline demographics and laboratory findings

The demographic characteristics and echocardiographic parameters of all participants are presented in Table 1. A total of 100 patients with Met-S (49 patients non-DM or group-1 and 53 patients DM or group-2) and 53 normal healthy people (control or group-3) were included in our study. Mean age was found to be significantly different between groups (group-1:58 ± 8,7, group-2: 60 ± 8,5 and group-3: 59 ± 7,2; p=0,372). Comparisons of other variables of laboratory parameters revealed that BMI, waist-circumference, PAB, Glu, TG, white blood cell (WBC), and neutrophil (Neu) were significantly found to be different between groups (p<0.05 for all). However, the CRP/PAB ratio was not found to be different between groups (group-1:0.014 (0.007 - 0.027) vs group-2:0.009 (0.005 - 0.024) vs group-3:0.006 (0.003 - 0.015), p=0,107). Other variables included CRP, Ua, Vit-D, T-Chol, LDL-C, HDL-C, Cre, hemoglobin (Hb), and platelets (Plt) levels were found to be indifferent between groups (all for p>0.05).

Table 1. Baseline demographics and laboratory results

Variables	Non-DM group Female (n = 22,) Male (n = 27) n = 49 (32%) Group-1	DM group Female (n = 26) Male (n = 25) n = 51 (33,3%) Group-2	Control group Female (n = 25) Male (n = 28) n = 53 (34,6%) Group-3	p μ, Ω	Variables (to continued)	Non-DM group n = 49 Group-1	DM group n = 51 Group-2	Control group n = 53 Group-3	p μ, Ω
Age (years)	58 ± 8.7	60 ± 8.5	59 ± 7.2	0.372 μ	**Mitral- E velocity (cm/s)**	81.2±18.9	78.7 ± 19.4	84.7 ± 17.1	0.503 μ
BMI (kg/m²)	28 ± 3.8	33 ± 5	34 ± 4.9	**<0.0001** μ	**Mitral- A velocity (cm/s)**	91 ± 18.4	92.6 ± 17.8	81 ± 17.1	**0.041** μ
BSA (m²)	1.9 ± 0.2	2 ± 0.2	2 ± 0.2	0.184 μ	**Tricuspid-E velocity (cm/s)**	65.2±12.7	62.8 ± 15.6	68.5 ± 14.6	0.349 μ
Waist - Circ (cm)	98 ± 9.8	109 ± 8.8	111 ±10.6	**<0.0001** μ	**Tricuspid-A velocity (cm/s)**	55.9 ± 9.1	68 ± 12.7	55.9 ± 9.1	**0.002** μ
CRP/PAB ratio	0.014 (0.007 - 0.027)	0.009 (0.005 - 0.024)	0.006 (0.003 - 0.015)	0.107 Ω	**Mitral-e'- velocity (cm/s)**	65.2 ± 12.7	62.8 ± 15.6	69.5 ± 13.9	0.225 μ
PAB (mg/dL)	24 ± 4.8	26 ± 5	23 ± 3.7	**0.029** μ	**Mitral-a'- velocity (cm/s)**	63.6±16.4	68 ± 12.7	55.8 ± 9.5	**0.006** μ
CRP (mg/dL)	0.27 (0.2 -0.5)	0.23 (0.1 -0.5)	0.14 (0.08-0.3)	0.078 Ω	**t-S'- velocity (cm/s)**	18.8 (16.5-23.1)	18.9 (17.0-23.5)	17.8 (15.4 -23.4)	0.447 Ω
Ua (mg/dL)	5 ± 1.3	5 ± 1.1	4.9 ± 1.3	0.511 μ	**Vmax / Indexed (mm³/m²)**	12.1 ± 4	14.7 ± 6.5	12.3 ± 3.5	0.089 μ
Vit-D (nmol/L)	18 ± 5.4	21 ± 10.5	17 ± 8.1	0.324 μ	**VpreA / Indexed (mm³/m²)**	9.1 (7.6~11.8)	10.2 (6.2~12.3)	10.8 (8~14)	0.372 Ω
Glu (mg/dL)	97 (91-102)	134 (104-178)	93 (85-101)	**<0.0001** Ω	**Vmin / Indexed (mm³/m²)**	7.4 ± 3.1	9.4 ± 6.2	7.3 ± 2.7	0.157 μ
T-Chol (mg/dL)	198.5 (177-220)	201 (187-232)	204 (169-225)	0.604 Ω	**LA passive emptying**	2.4 ± 2	2.6 ± 1.5	2.4 ± 1.7	0.920 μ

					volume (LA-PEV) = Vmax–VpreA				
LDL-C (mg/dL)	122 ± 8.6	122 ± 6.6	120 ± 7.5	0.983 μ	**LA passive emptying fraction (LA-PEF) = LA-PEV/Vmax**	18.3±12.9	18.6 ± 12.1	19.5±11.6	0.932 μ
HDL-C (mg/dL)	50 ± 12.7	48 ± 11.5	54 ± 13.2	0.198 μ	**LA active emptying volume (LA-AEV) = VpreA –Vmin**	2.3 (0.7~3.5)	2.5 (1.3~3.9)	2.2 (1.4~3)	0.768 Ω
TG (mg/dL)	167 (130-238)	181 (145-233)	115 (83-168)	**0.002** Ω	**LA active emptying fraction (LA-AEF) = LA-AE/VpreA**	24.5±15.8	25.3 ± 15.6	25.5 ± 12	0.963 μ
Cre (mg/dL)	0.89 ± 0.25	0.85±0.26	0.77±0.13	0.131 μ	**LA total emptying volume (LA-TEV) =Vmax – Vmin**	4.7 ± 2.5	5.4 ± 2.1	4.9 ± 2.3	0.547 μ
Wbc (10^3/mm^3)	8.28 ± 2.05	8.34±1.59	7.13 ± 2	**0.045** μ	**LA total emptying fraction (LA-TEF) = LA-TEV /Vmax**	38.7±15.2	39.3 ± 15.9	40.1 ± 13	0.936 μ
Hgb (g/dL)	14.7 ± 1.7	13.8 ± 1.7	14.2±1.6	0.193 μ	**EFT (mm)**	5.7 ± 1.8	10.3 ± 19	6.7 ± 2.5	0.285 μ
Neu (%)	4.76 ± 1.83	5.09±1.32	3.96±1.45	**0.036** μ	**MAPSE (mm)**	141 ± 20	13.6 ± 2.6	16 ± 3.2	**0.020** μ
Plt (10^3/mm^3)	270 ± 46	284 ± 59	255 ± 53	0.167 μ	**TAPSE (mm)**	21.5 ± 4.3	20.6 ± 4.6	22 ± 3.7	0.458 μ

Baseline and demographic features: BMI: Body-mass-index, BSA : Body- surface-area, Waist – Circ: Waist- circumference, CRP/PAB: CRP/Prealbumin, Ua: Uric acid, Vit-D: Vitamin-D, Glu: Glucose, Chol: Cholesterol, LDL-C: LDL- Cholesterol, HDL-C: HDL-Cholesterol, TG:Triglycerides, Cre: Creatinin, Wbc: White Blood Cell, Hgb:Hemoglobin, Neu: Neutrophil, Plt: Platelet, t-S´- velocity: Systolic velocity of tricuspid lateral annulus on tissue doppler imaging echocardiography (TDIE), LA Vmax / Indexed: the maximal left atrial volume indexed by BSA, LA-VpreA: the pre-atrial contraction left atrial volume indexed by BSA, LA-Vmin : the minimal LA volume indexed by BSA, EFT: Epicardial fat thickness, MAPSE: Mitral annular plane systolic excursion, TAPSE: Tricuspid annular plane systolic excursion, μ: ANOVA, Ω: Kruskal-Wallis test

3.2. Echocardiographic findings

Among conventional echocardiographic parameters, mean mitral and tricuspid-E velocities were not found to be different between groups (p-value for all >0.05), however, mean mitral-A and tricuspid-A velocities were found to be significantly different between groups (p-value for all<0.05). During TDIE, mean mitral-a' velocity was found to be different between groups (p=0.006); however, mean mitral-e' and tricuspid-S' velocities were not by TDIE (p for all >0.05). Also, mean EFT and TAPSE were not found to be different (p for all >0.05), while mean MAPSE was found to be significantly different between groups (p=0.020, Table 1).

3.3. LA functions

As for the parameters indicating LA volumes and mechanical functions, all groups were similar with respect to mean Vmax (indexed to BSA), mean Vpre-A (indexed), and mean Vmin (p-value for all>0.05, Table 1). Also, all kinds of measurements of mechanical functions of LA (LA-PEV, LA-PEF, LA-AEV, LA-AEF, LA-TEV, LA-TEF) were found to be indifferent among groups (p value for all >0.05, Table 1).

3.4. Post-hoc analysis

Post-hoc analysis was depicted in Table 2. Mean age was found to be significantly different only between groups 2 and 3 (p=0.013, Table 2). BMI and waist-circumference were found to be significant different between control and Met-S groups (group 1 vs. 2 and 2 vs. 3; p<0.0001 for all). PAB and Neu counts were found to be significantly different only between groups 2 and 3 (p<0.05 for all). Mean Glu level was found to be different between group 1 vs. 2 with group 2 vs. 3 (p<0.001 for all), while mean TG was found to be different between group 1 vs. 3 and 2 vs. 3 (p<0.05 for all). Mean MAPSE was found to be different between group 2 vs. 3; however, mean mitral-a' velocity was found to be significantly different between group 1 vs. 3 (p=0.004). Tricuspid- A velocity was found to be significantly different between group 1 and 3 and group 2 and 3 (p<0.05 for all, Table 2).

Table 2. Post-hoc analysis

Variables	Non - DM vs. DM (group 1 vs. 2)	Non – DM vs. Control (group 1 vs. 3)	DM vs. Control (group 2 vs. 3)
Age	Ns. β	Ns. β	**0.013** β
BMI	Ns. β	**<0.0001** β	**<0.0001** β
Waist – Circ (cm)	Ns. β	**<0.0001** β	**<0.0001** β
PAB	Ns. β	Ns. β	**0.024** β
Glu	**<0.0001** ¥	Ns. ¥	**<0.0001** ¥
TG	Ns. ¥	**0.006** ¥	**0.002** ¥
Neu	Ns. β	Ns. β	**0.038** β
Mitral-a'- velocity	Ns. β	**0.004** β	Ns. β
MAPSE	Ns. β	Ns. β	**0.016** β
Tricuspid-A velocity	Ns. β	**0.020** β	**0.003** β

Post-hoc analysis of groups. β: Bonferroni Test, ¥: Mann-Whitney U test. PAB: Prealbumin

3.5. Comparisons and correlations

A separate comparison of the groups is depicted in Table 3, 4. We rearranged the groups as having RV diastolic dysfunction (RVDD) or not and LV diastolic dysfunction (LVDD) or not: *1*: Comparison of the patients with and without RVDD (with-RVDD n=32, without-RVDD n=48) revealed that mean age, mean Plt volume (MPV), e-GFR, TAPSE, Mitral-E, A; Tricuspid- E, A; Mitral-e', a' and t-S' velocity parameters were found to be significantly different between groups (p<0.05 for all, Table 3). CRP/PAB ratio was not different between groups (p>0.05). However, none of the volumetric and functional indices of LA were found to be significantly different between with-RVDD and without-RVDD groups (p>0.05 for all in Table 3). *2:* Comparison of the patients with and without LVDD (with-LVDD n=42, without-LVDD n=38) revealed that mean age, PAB, e-GFR, TAPSE, Mitral-A, Tricuspid- E, Mitral-e' and t-S' velocity parameters were found to be significantly different between groups (p<0.05 for all in Table 3). CRP/PAB ratio was not different between groups (p>0.05). Again, among these groups none of the, none of the volumetric and functional indices of LA were found to be significantly different (p>0.05 for all in Table 3). We later examined the patients only who had RVDD and LVDD, and again, they were divided into 3 groups as Non-DM, DM and control groups. These sub-group comparisons, which were restricted to include the patients only with RVDD and LVDD presented in Table 4: *3-A*: Sub-groups analysis in the RVDD group revealed that only mean Glu level was found to be significantly different between DM, Non-DM, and control groups (p=0.001). CRP/PAB ratio and none of the volumetric and functional indices of LA were found to be different between groups (p>0.05 for all in Table 4). *3-B:* Sub-groups analysis in the LVDD group revealed that Tricuspid-A velocity, waist-circum. and BMI parameters were found to be significantly different between DM, Non-DM and control groups (p<0.05 for all). Again, the CRP/PAB ratio and none of the volumetric and functional indices of LA were found to be different between groups (p>0.05 for all in Table 4). Finally, the correlation chart of the CRP/ PAB ratio with variables in three main separate groups was presented in Table 5. CRP/PAB ratio was found to be significantly negatively correlated with EFT and LA active emptying volume (indexed) in the control group (r: - 0,386; p=0.047 and r: – 0,383; p=0.049 respectively, in Table 5) and positively correlated with mean monocyte count and red cell distribution (RDW) in DM group (r: 0,440; p=0.032 and r: 0,452; p=0.026 respectively) and also positively correlated with BMI, waist-circumference and mean platelet volume (MPW) in Non-DM group (r: 0,51; p=0.005, r: 0,417; p=0.027 and r: 0,413; p=0.036 respectively in Table 5).

Table 3. Comparison of groups as right ventricular diastolic dysfunction (RVDD) is present or not are depicted as main group and left ventricular diastolic dysfunction (LVDD) is present or not are depicted as main group

Variables	RVDD is present (n = 32)	RVDD is not present (n = 48)	p μ, Ω	Variables	LVDD is present (n = 42)	LVDD is not present (n = 38)	p μ, Ω
Age (years)	61 ± 6.8	55 ± 8.7	**0.001** μ	**Age (years)**	61 ± 7.5	53 ± 7.8	**<0.0001** μ
MPV	9.98 ± 0.9	10.5 ± 0.8	**0.017** μ	**PAB**	25.4 ± 4.8	22.8 ± 4.1	**0.012** μ
e-GFR	81 ± 19	93 ± 13	**0.001** μ	**e-GFR**	83 ± 18	94 ± 13	**0.04** μ
CRP/PAB ratio	0.009 (0.005 - 0.024)	0.009 (0.004 -0.020)	Ns. Ω	**CRP/PAB ratio**	0.007 (0.004 - 0.022)	0.014 (0.006-0.022)	Ns. Ω
MAPSE (mm)	14.93 ± 2.7	14.98 ± 3.4	Ns. μ	**MAPSE (mm)**	15 ± 3	15 ± 4	Ns. μ
TAPSE (mm)	19.71 ± 4.3	22.47 ± 3.8	**0.004** μ	**TAPSE (mm)**	**20 ± 4**	23 ± 4	**0.001** μ
Mitral- E velocity (cm/s)	73 ± 17	87 ± 17	**<0.0001** μ	**Mitral- E velocity (cm/s)**	66 ± 13	60 ± 13	Ns. μ
Mitral- A velocity (cm/s)	94 ± 18	84 ± 18	**0.025** μ	**Mitral- A velocity (cm/s)**	61 ± 13	71 ± 13	**0.001** μ

Tricuspid-E velocity (cm/s)	56 ± 9	72 ± 14	**<0.0001** μ	**Tricuspid-E velocity (cm/s)**	60.33 ± 13.2	71.32 ± 13.4	**<0.0001** μ
Tricuspid-A velocity (cm/s)	69 ± 13	59 ± 13	**0.001** μ	**Tricuspid-A velocity (cm/s)**	66 ± 13	60 ± 13	Ns. μ
Mitral-e'- velocity (cm/s)	56 ± 9	72 ± 14	**<0.001** μ	**Mitral-e'-velocity (cm/s)**	61 ± 13	71 ± 13	**<0.0001** μ
Mitral-a'- velocity (cm/s)	68 ± 14	59 ± 13	**0.004** μ	**Mitral-a'-velocity (cm/s)**	65 ± 14	60 ± 13	Ns. μ
t-S'- velocity (cm/s)	19 ± 4	21 ± 6	**0.044** μ	**t-S'-velocity (cm/s)**	21 ± 5	18 ± 4	**0.010** μ
LA Vmax / Indexed (mm^3/m^2)	13 ± 4	13 ± 5	Ns. μ	**LA Vmax / Indexed (mm^3/m^2)**	13 ± 5	13 ± 5	Ns. μ
LA VpreA / Indexed (mm^3/m^2)	10 ± 4	11 ± 5	Ns. μ	**LA VpreA / Indexed (mm^3/m^2)**	10.5 (6.9 - 12.4)	9.95 (7.7 - 12.1)	Ns. Ω
LA Vmin / Indexed (mm^3/m^2)	6.7 (4.8-9.4)	7.7 (5.7-9.3)	Ns. Ω	**LA Vmin / Indexed (mm^3/m^2)**	6.95 (4.8 - 10.3)	7.3 (5.6 - 9.11)	Ns. Ω
LA passive emptying volume / Indexed (PEV) (mm^3/m^2)	1.9 (0.9-3.05)	2.25 (1.3-3.6)	Ns. Ω	**LA passive emptying volume / Indexed (PEV) (mm^3/m^2)**	1.9 (0.6 - 3.1)	2.25 (1.4 - 3.6)	Ns. Ω
LA passive emptying fraction (PEF)	17 ± 11	20 ± 13	Ns. μ	**LA passive emptying fraction (PEF)**	17 ± 12	21 ± 11	Ns. μ
LA active emptying volume (AEV) / Indexed (mm^3/m^2)	2.6 ± 1.4	2.5 ± 1.97	Ns. μ	**LA active emptying volume (AEV) / Indexed (mm^3/m^2)**	2.35 (1.3 - 3.9)	2.25 (1.3 - 3.2)	Ns. Ω
LA active emptying fraction (AEF)	28 ± 16	23 ± 13	Ns. μ	**LA active emptying fraction (AEF)**	26 ± 16	24 ± 13	Ns. μ
LA total emptying volume (TEV) / Indexed (mm^3/m^2)	4.86 ± 2.14	5.02 ± 2.46	Ns. μ	**LA total emptying volume (TEV) / Indexed (mm^3/m^2)**	5 ± 2	5 ± 3	Ns. μ
LA total emptying fraction (TEF)	40 ± 16	39 ± 14	Ns. μ	**LA total emptying fraction (TEF)**	39 ± 15	40 ± 14	Ns. μ

Comparison of variables between groups of RVDD is present and not as well as LVDD is present group and not. μ: ANOVA, Ω: Kruskal-Wallis test, e-GFR: estimated glomerular filtration rate.

Table 4. Comparison of groups only when right ventricular diastolic dysfunction (RVDD) is present and left ventricular diastolic dysfunction (LVDD) is present and they were separately as control, Non-DM and DM groups

Variables in RVDD is present (n = 32)	**Control group (n = 3)**	**Non-DM group (n = 13)**	**DM group (n = 16)**	**$p^{\mu,\Omega}$**	**Variables in LVDD is present (n =42)**	**Control group (n = 9)**	**Non-DM group (n = 14)**	**DM group (n = 19)**	**$p^{\mu,\Omega}$**
CRP/PAB ratio	0.004 (0.002-0.006)	0.011 (0.008-0.03)	0.009 (0.005-0.024)	Ns. Ω	**CRP/PAB ratio**	0.004 (0.003 - 0.006)	0.010 (0.005 - 0.026)	0.007 (0.004 - 0.024)	Ns. Ω
Glu (mg/dL)	101	95	136.5	**0.001** Ω	**Tricuspid-A velocity**	57 ± 8.2	72 ± 15.4	65 ± 11.6	**0.028** μ

	(89-111)	(92 – 99)	(106-175)		**(cm/s)**				
LA Vmax / Indexed (mm3/m2)	13.8 ± 6.4	11.4±3.7	13.4 ±4.6	Ns. μ	**Waist-Circ(cm)**	97 ± 9.7	108±9.4	110±10.4	**0.009** μ
LA VpreA / Indexed (mm3/m2)	10.4 ± 6.1	9.6 ± 3.1	11±3.9	Ns. μ	**BMI (kg/m^2)**	27 ± 3.9	32 ± 5.5	33 ± 4.5	**0.009** μ
LA Vmin / Indexed (mm3/m2)	5.4 (3.6 - 16.1)	5.9 (4.7- 9)	7 (4.8- 9.8)	Ns. Ω	**LA Vmax / Indexed (mm3/m2)**	12 ± 3.8	11.2 ± 3.6	14.3± 5.2	Ns. μ
LA passive emptying volume / Indexed (PEV) (mm3/m2)	3.4 ± 2.7	1.8 ± 1.8	2.4 ± 1.6	Ns. μ	**LA VpreA / Indexed (mm3/m2)**	11 (8.5 - 11.8)	10.15 (6.1- 10.8)	11.1 (7.5- 15.2)	Ns. Ω
LA passive emptying fraction (PEF)	24.6 ± 18.8	14.6±11.1	17.3±102	Ns. μ	**LA Vmin / Indexed (mm3/m2)**	7.1 (6.3 – 8.1)	5.8 (4.6- 9.4)	8.3 (4.8- 12.1)	Ns. Ω
LA active emptying volume (AEV) / Indexed (mm3/m2)	2 ± 0.8	2.7 ± 1.3	2.7 ± 1.3	Ns. μ	**LA passive emptying volume / Indexed (PEV) (mm3/m2)**	1 (0.4 - 2.9)	1.5 (0.5-2.2)	2.6 (1 - 3.9)	Ns. Ω
LA active emptying fraction (AEF)	26 ± 18.6	29.3 ± 14.9	27.2±16.9	Ns. μ	**LA passive emptying fraction (PEF)**	10 (3.2-14.2)	14.7 (4.8- 21.9)	20.7 (10 -25.5)	Ns. Ω
LA total emptying volume (TEV) / Indexed (mm3/m2)	5.4 ± 2.4	4.4 ± 2.2	4.4 ± 2.2	Ns. μ	**LA active emptying volume (AEV) / Indexed (mm3/m2)**	2.8 (1.4 - 3.7)	2.35 (0.5- 3.3)	2.2 (1.2 - 4.1)	Ns. Ω
LA total emptying fraction (TEF)	52 (20.6- 60.2)	41 (25 -50.5)	44.3 (25 -51.8)	Ns. Ω	**LA active emptying fraction (AEF)**	27 ± 12.9	27 ±16.4	26 ± 16.7	Ns. μ
					LA total emptying volume (TEV) / Indexed (mm3/m)	4.1 ± 1.5	4.4 ± 2.1	5.5 ± 2.2	Ns. μ
					LA total emptying fraction (TEF)	36 ± 14	40 ± 15.6	40 ± 15.7	Ns. μ

Comparison of variables between groups of RVDD is present and not as well as LVDD is present group and not. μ: ANOVA, Ω: Kruskal-Wallis test

Table 5. Correlations of CRP/PAB ratio with variables in three separate groups

Variables	Control group		Variables	DM group		Variables	Non-DM group	
	Sperman's r:	p		Sperman's r:	p		Sperman's r:	p
EFT (mm)	- 0.386	**0.047**	**Monocyte**	0.440	**0.032**	**BMI**	0.519	**0.005**
LA active emptying volume (AEV) / Indexed (mm^3/m^2)	- 0.383	**0.049**	**RDW**	0.452	**0.026**	**Waist-Circum**	0.417	**0.027**
						MPV	0.413	**0.036**

Correlations of CRP/PAB ratio with variables in three separate groups. MPV: mean platelet volume, RDW: Red cell distribution width.

4. Discussion

Circulating levels of inflammatory markers have been shown to be involved with different features of Met-S. CRP has been recognized as a marker of systemic inflammation and is reported to be related to several features of CVD and Met-S (13). Serum albumin and PAB are among the widely used nutritional biomarkers. It has been shown that PAB could predict mortality in patients with HF [14]. CRP/PAB ratio was reported to be superior to either hs-CRP or PAB alone in assessing not only the severity but the prognosis of patients with acute kidney injury, fistula closure, and other critically ill patients. In a prospective study including 682 patients with ST-elevation myocardial infarction (STEMI). The median CRP/PAB ratio (0.02) was set as the cutoff value, and patients were divided into 2 groups according to this cut-off value as high- CRP/PAB ratio (CRP/PAB ratio ≥0.02) and a low-CRP/PAB ratio group (CRP/PAB ratio <0.02). The accumulated incidence rate of major adverse cardiovascular events (MACE) was found to be significantly higher in the high CRP/PAB ratio group (38,7% vs. 12,0%, p<0.01) during a median follow-up of 18 months (15). In another trial, an investigation of the possibility of using the hs-CRP/PAB ratio to predict in-hospital MACE in a total of 659 patients with acute coronary syndrome (ACS) showed that the occurrence rate of in-hospital MACE and acute HF was found to be higher in the high hs-CRP/PAB ratio groups (p<0.001) (16).

Atrial enlargement has been known to be associated with increased mortality in the general population, and atrial remodeling, which is frequently accompanied by increased interstitial fibrosis, is a hallmark of pathological changes in atrial dilatation. These changes have a strong association with many diseases like HF, HT, obesity and Met-S (17). One of the possible mechanisms of the predisposing of atrial remodeling is inflammation and oxidative stress, and the CRP and oxidants have been reported to be elevated in patients with AF. Functional changes of LA could occur earlier than morphological changes of LA, and functional impairment may occur with or without alteration in LA size. In a previous study, LA size was found to be influenced by insulin resistance and obesity independently of the LV hypertrophy and LV geometry in non-diabetic hypertensive patients. So, LA size has been known to be influenced by systemic chronic inflammatory situations in non-diabetic patients (18-19). Atrial dilatation has been found to be accompanied by atrial mechanical dysfunction, and it has been shown before that the reservoir and conduit functions of LA were found to be significantly lower in patients with Met-S than in controls (p<0.001); however, LA booster function was found to be not different between groups (20). Along with these results, Yilmaz et al. found that the Met-S group had a greater LA Vmax (p=0.03), VpreA (p=0.001), and LA-AEV active emptying volume (p=0.001). However, LA-PEF was lower in the Met-S group (p=0.001) (10). Along with the importance of these chronic systemic inflammations in the pathogenesis of dilatation and mechanical dysfunction of LA in Met-S, we tried to find any possible relationship between LA functions and a CRP/PAB ratio among patients with/without DM. Unfortunately, the CRP/PAB ratio and other volumetric and functional indexes of LA were not found to be different between groups (p>0.05 for all, Table 1). Only mitral and tricuspid A velocity and mitral-a' velocity were found to be different between groups (p<0.05 for all, Table 1). In posthoc analysis, only mitral-a' velocity and tricuspid-A velocity was found to be different between groups (Table 2). As a summary, the functional index of LA, the mitral -a' velocity, and a right atrial functional index, tricuspid-A velocity, late kicking, or booster function were found to be different between Met-S and control groups (Table 2). In contrast to our results, in a previous study, it has been demonstrated that passive LA emptying volume was found to be decreased in diabetic patients and it was found to be associated with higher end-diastolic LV pressures. In addition, active LA emptying volume, which was found to be associated with greater LA contraction compensatory mechanisms, was found to be increased in patients with DM (21). In another study, LA reservoir function as an index of LA compliance was found to be significantly lower in patients with Met-S. After 5-year follow-up, compared to surviving subjects, LA reservoir function was decreased in both all-cause and CVD mortality groups, of note markedly impaired in those who died from CVD (22). In the other part of our examination, when we divided the patients as having LVDD or RVDD, again, functional and volumetric parameters of LA were not found to be different between diabetic or non-diabetic groups (Table 3-4). LA and right atrial (RA) booster functional indexes were again found to be different between groups (Table 3 4). These results are different from previous studies, which can be explained by including lower patients. In the last part of our analysis, the CRP/PAB ratio was found to be positively correlated with monocyte count in the DM group and waist-circumference, BMI, and MPV in the Non-DM group. None of the volumes and functional indexes were found to be correlated with the CRP/PAB ratio in DM or Non-DM groups (Table 5). These results were inconsistent with the previous studies, which indicated that diabetic patients have several alterations in their LV and LA functions. In a previous trial, total and passive LA emptying fractions (EF), demonstrating LA reservoir and conduit function, were significantly lower in DM patients than in controls. Active LA EF, the parameter of LA booster pump function, was found to be similar between DM and controls (23). Along with these results, we found that the mitral and tricuspid late diastolic (A) wave velocities (mitral and tricuspid-A-velocities) and mitral tissue doppler late (a') diastolic wave velocity (mitral-a'-velocity) were significantly higher in DM group than non-DM and control groups. It represents that parameters of late active contractile functions of LA and RA were higher in patients with DM. LA-AEF contributes to 15%–30% of the LV stroke volume by its contraction during the final phase of diastole, and in line with

present results, a study by Gulmez et al. compared the 56 diabetic patients with 56 controls. Their results showed higher LA-AEV in the diabetic patients, while in contrast to us, the A and E waves and their ratios were not different between the groups (24). Although the CRP/PAB ratio has been knowing to reflect a more sensitive inflammatory state than either CRP or PAB, its value in the assessment of LA functions and metabolic syndrome is still ambiguous. In our study, this predictive value has been aimed to be determined, but it has not been determined fully, so in the near future, more studies are needed to reveal this value.

One of the limitations of our study is nonrandomized patient selection and grouping, which could have affected the results. The relatively small number of patients in the 3 subgroups is another important limitation of this study. Also, information on the duration of Met-S individual risk factors was not available; this would have likely added to a precise determination of the effect of systemic inflammation on the LA remodeling. Phasic LA function analysis could have been informative to further evaluate the mechanism of atrial dysfunction and remodeling. Finally, due to cross-sectional study design, causal inferences are limited.

The CRP/PAB ratio was known to be a novel marker for evaluating the systemic inflammatory state, and it has been reported to have predictive value in assessing the severity and prognosis of various diseases. Chronic systemic inflammation, which is accompanied by increased interstitial fibrosis in atrial tissue, is frequently associated with Met-S. This study aimed to assess the predictive value of CRP/PAB ratio in LA functions in patients with Met-S, which showed a lack of any association between the CRP/PAB ratio and LA functions. However, late atrial active kicking function has been found to be associated with DM. To assess the predictive value of the CRP/PAB ratio in Met-S further studies with a large cohort population.

Conflict of interest

The authors have no conflicts of interest to declare.

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Authors' contributions

Concept: Y.B., B.İ., Design: Y.B., B.İ., Data Collection or Processing: Y.B., B.İ., Analysis or Interpretation: Y.B., B.İ., Literature Search: Y.B., B.İ., Writing: Y.B., B.İ.

Ethical Statement

The protocol of study has been approved by the ethics committee of Kirsehir Ahi Evran University of Medical Faculty (04.04.2023 date and registration number 2023-07/43), and written informed consent was obtained from all of the study participants before their participation in this study.

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Cost effectiveness of routine pathological evaluation in sacrococcygeal pilonidal sinus specimens

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Abstract

Pilonidal sinus is a benign pathology that occurs especially in the sacrococcygeal region. Routine specimen examination is controversial because malignancy is very rare in histopathological examination. In this study, we aimed to discuss the pathological findings of patients who were operated for sacrococcygeal pilonidal sinus in our clinic in the light of the literature. Between May 2019 and December 2022, 257 patients who were operated on with the diagnosis of pilonidal sinus at the Samsun Training and Research Hospital General Surgery Clinic were retrospectively analyzed and included in the study. In the histopathological examination, it was reported that pilonidal sinus was found in 115 patients, chronic active inflammation in 79 patients, foreign body type inflammatory event in 60 patients, epidermal cyst in 1 patient, fibrolipoma in 1 patient, and Hidraadenitis Supurativa in 1 patient. In some recent studies, histopathological examination should be performed in patients over 50 years of age. Malignant pathology is mostly seen in males and. squamous cell carcinoma was seen in 76 of these patients and epidermoid carcinoma, basal cell carcinoma and malignant degeneration were seen in the others. Although our average age is low, our male / female ratio was found close to the literature. None of our pathology results were malignant. Pathologic examination of specimens removed in operation requires a certain workload and cost. We think that pathological examination is not required in every patient and that it is more effective in terms of both cost and workload to select the patients to be examined according to characteristics such as advanced age, chronic inflammation, suspicion of macroscopic malignancy and recurrent disease.

Keywords: pilonidal sinus, cost effectivity, clinical pathology, pathology

1. Introduction

Pilonidal sinus is a benign pathology that mainly occurs in the sacrococcygeal region. It commonly leads to abscess, sinus tract formation, and cellulitis. Risk factors for pilonidal sinus include male gender, obesity, and a high hair density (1). While pilonidal sinus is mostly observed after the second decade of life, it is rare after the fifth decade (2). Various treatment methods are available, including phenol application, simple incision and drainage, excision, unroofing, surgical flap advancement, cleft lift procedure, and Limberg flap (3). The routine examination of surgical specimens for malignancy is controversial due to the rarity of malignant diagnoses in histopathological evaluations (4-6).

Minimal invasive approaches have been successfully employed in the treatment of pilonidal sinus. Current approaches include the use of silver nitrate, phenol, and laser for the destruction of the cavity after hair removal (7-9). The safe use of these contemporary techniques, which do not involve tissue removal, raises questions about the necessity of histopathological examination. In this study, we aimed to discuss the pathological findings of patients who underwent surgery for sacrococcygeal pilonidal sinus in our clinic, based on the literature.

2. Materials and Methods

Between May 2019 and December 2022, a total of 257 patients who underwent pilonidal sinus surgery at a 3rd level education and research hospital were retrospectively analyzed and included in the study. All pilonidal sinus specimens were sent to the pathology department for histopathological examination due to medicolegal reasons. Patients with pilonidal sinuses outside the sacrococcygeal region and those who underwent minimal invasive treatment without undergoing histopathological examination were excluded from the study. The demographic data, age, gender, pathology results, and costs of the patients were analyzed using the hospital system. The average duration of pathological examination was calculated by consulting the relevant department.

3. Results

Of the 257 patients in the study, 209 (81%) were male and 48 (19%) were female. The average age was determined to be 26.5 (14-68) years (Table 1). In the analysis of histopathological results, the histopathological examination of 115 (44%) patients resulted in a diagnosis of pilonidal sinus. Among these

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patients, 93 were male and 22 were female. Furthermore, chronic active inflammation was observed in 79 (30%) patients, with 61 males and 18 females. Sixty (23%) patients had inflammatory reactions associated with foreign bodies, with 53 males and 7 females. One patient had an epidermal cyst, one had fibrolipoma, and one had hidradenitis suppurativa. The patient reported as fibrolipoma was female, while the other two were males. The average cost of pathological examination per specimen was calculated as 170 TL, and the average examination duration from macroscopic examination to reporting was 60 minutes (Table 2).

Table 1. Demographic and clinicopathological characteristics of patients with pilonidal sinus

	n=257
Age (mean± SD) (year)	26.60±8.93
Gender (%)	
Male	209 (81.3)
Female	48 (18.7)
	5 (1.9)
Pathology	
Benign	257 (100)
Malignant	0
Age Distrubition (%)	
15-20	68 (26.5)
21-30	118 (45.9)
31-40	51 (19.9)
41-50	14 (5.4)
51-60	5 (1.9)
>60	1 (0.4)

Table 2. Detailed classification of histopathological results

Histopathology	Female	Male	n (%)
Benign findings			
Pilonidal sinüs	22	93	115(44.7)
Chronic active inflammation	18	61	79(30.7)
Foreign body type inflammatory event	7	53	60(23.4)
Epidermal cyst	0	1	1(0.4)
Fibrolipoma	1	0	1(0.4)
Hidradenitis suppurativa	0	1	1(0.4)
Malignant findings (%)			0 (0)

4. Discussion

Pilonidal sinus can lead to abscesses, cellulitis, and occasionally recurrent cases, and it can rarely result in malignancy (0.1%) (10). Treatment options range from conventional surgery to minimally invasive surgery, and in contemporary minimally invasive treatment modalities, sending the specimen for histopathological examination has started to be eliminated from routine practice. Among minimal invasive treatments, phenol application is particularly recommended in current approaches. Some studies have reported success rates of pilonidal sinus treatment with phenol between 70% and 95% (11-12). Pit excision, cavity destruction, and laser ablation are also minimal invasive methods, and studies have shown that the success rates of laser ablation are 80-90% higher than pit excision (13). Procedures using minimal invasive methods can be performed quickly under local anesthesia, and they are completed within a short period. The main advantage is that the procedure is minimally invasive, and the cavity is destroyed rapidly. However, the inability to obtain a intact specimen for pathological examination raises theoretical concerns about the possibility of missing certain malignancies.

In most centers today, surgical specimens of pilonidal sinus operations are still sent for histopathological examination. Some studies suggest that this examination is unnecessary and that histopathological examination in patients over the age of 50 is sufficient (4-5). The average age of the 86 pilonidal sinus patients reported with malignancy in the literature is 55.3, and they are mostly encountered in male patients. The male-to-female ratio in malignancy is reported as 4.7/1. Squamous cell carcinoma was observed in 76 of these patients, while others had epidermoid carcinoma, basal cell carcinoma, and malignant degeneration (14-15). In our study, the average age of the 257 patients operated on was 26.5 (14-68) years, and the male-to-female ratio was 4.35/1. Although our average age was low, our male-to-female ratio was similar to the literature. Similarly, in line with the literature, none of our patients were diagnosed with malignancy in histopathological examination. In our study, due to the majority of the analyzed time period coinciding with the Covid-19 pandemic, the case series was limited. However, it was observed that routine pathological examination incurred a total cost of approximately 43,700 TL and required approximately 257 hours of time investment.

Until recently, surgical specimens of every pilonidal sinus case were routinely examined, but the literature reports low malignancy rates. Although our findings are limited to a small patient group and based on a short follow-up period, we believe that routine pathological examination could seen to impose an additional workload and cost. Therefore, except for advanced age, prolonged duration of the disease, macroscopic suspicion, and recurrent cases, we believe that pathological examination is unnecessary.

Conflict of interest

All authors of this article declare no conflict of interest.

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Authors' contributions

Concept: M.A.A., Design: M.A.A., Data Collection or Processing: C.A., Analysis or Interpretation: C.A., Literature

Search: C.A., Writing: C.A., M.A.A., O.F.B.

Ethical Statement

This study was conducted with the approval of Samsun University Faculty of Medicine ethics committee (Protocol number: SÜKAEK-2023 15/5, approval date:23.08.2023)

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Research Article

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Examining the frequency of carbapenemase genes *bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{OXA-48}$, *bla*$_{SPM}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{GES}$, *bla*$_{BIC}$, *bla*$_{AIM}$, *bla*$_{GIM}$, *bla*$_{SIM}$, and *bla*$_{DIM}$ in *Pseudomonas aeruginosa* strains isolated from patients hospitalized in Northwest Iran hospitals

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Abstract

Pseudomonas aeruginosa (*P. aeruginosa*) is one of the most common bacteria isolated from clinical samples, with a rising incidence in hospital infections. This pathogen is inherently resistant to many antibacterial agents. This study aimed to investigate the frequency of carbapenemase genes in *P. aeruginosa* strains isolated from patients admitted to hospitals in northwestern Iran. A total of 500 *P. aeruginosa* samples were collected from different clinical samples. Antibiotic susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, and the frequency of the target genes was assessed using polymerase chain reaction (PCR). The antibiotic resistance results of the samples by disc diffusion method showed that imipenem 98.4%, gentamicin 98%, meropenem 91.8%, amikacin 91.6% and cefepime 91% had the highest resistance; also, out of 500 *P. aeruginosa* isolates, 309 (61.8%) samples were carbapenemase producers. Using the PCR method, it was determined that the *bla*$_{OXA-48}$ (39.16%), *bla*$_{GES}$ (31.72%), and *bla*$_{IMP}$ (22.01%) genes were the dominant genes. Our results showed that the prevalence of carbapenemase genes in *P. aeruginosa* strains isolated from patients admitted to hospitals in northwestern Iran is very high; indicating a need for effective infection control measures to prevent the spread of *P. aeruginosa* in hospitals.

Keywords: *Pseudomonas aeruginosa*, drug resistance, carbapenemase, hospital infection

1. Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is one of the 5 opportunistic human pathogens, especially in immunocompromised patients, which is the cause of hospital-acquired opportunistic infections and carries multiple drug resistance (1-5). Hospital-acquired infections are one of the significant medical problems in developed and developing countries which cause the spread of infectious diseases in the society (6-12). Infection with this bacterium can cause septicemia, pneumonia, meningitis, and other fatal diseases (13-15). Antibiotic resistance in this bacterium is caused by intrinsic resistance due to low permeability, the presence of exudation systems, acquisition of resistance genes through plasmids, transposons, and integrons (16, 17). Over many years, the indiscriminate use of antibiotics, not paying attention to the doctor's prescription, the massive use of antibiotics in the food industry, and factors like this have effectively increased the antibiotic resistance of many bacteria (18-24). Today carbapenem drugs are used to treat antibiotic-resistant strains, but recently, the prevalence of carbapenem resistance is one of the most essential types of antibiotic resistance in *P. aeruginosa*, which is increasing worldwide (25). One of the mechanisms of antibiotic resistance in this bacterium is the production of beta-lactamases, which can deactivate many beta-lactam antibiotics such as penicillin, cephalosporin, and carbapenem (except for monobactams) (26). Currently beta-lactamases are divided into four Ambler categories A, B, C, and D (27, 28). Metallo-beta-lactamase (MBLs) are among the beta-lactamase enzymes, which are one of the main mechanisms of bacterial resistance against beta-lactam antibiotics and are in group B of Ambler's classification and group 3 of Bush's classification (27). MBLs are generally divided into several families: Verona integron-encoded MBL

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(VIM), Dutch Imipenemase (DIM), Adelaide Imipenemase (AIM), Seoul Imipenemase (SIM), Germany Imipenmase MBL (GIM), Imipenmase (IMP) Sao Paulo MBL (SPM), New Delhi MBL (NDM) (29, 30). In the meantime, IMP and VIM have been widely studied and are coded by *bla*$_{IMP}$ and *bla*$_{VIM}$ genes (31). Many strains of *P. aeruginosa* can produce several types of broad-spectrum beta-lactamases, which enables them to be resistant to commonly used antibiotics (32). Beta-lactamases are inhibited by beta-lactam inhibitors such as clavulanic acid (33, 34). The study of these enzymes is essential for faster diagnosis and investigation of the prevalence rate to prevent the spread of this type of resistance. Therefore, considering the importance of carbapenems in the treatment of infections caused by *P. aeruginosa* and the need to know the prevalence of resistance and the methods of creating resistance to this group of antibiotics, the present study was conducted to investigate the frequency of carbapenemase genes in *P. aeruginosa* strains isolated from hospitals in the northwest of Iran.

2. Materials and Methods

2.1. Collection and identification of samples

In this descriptive cross-sectional study, a total of 500 samples of *P. aeruginosa* from different clinical samples, including blood, urine, burn wounds, tracheal tube secretions, and other body secretions from September 2022 to February 2023 from patients hospitalized in Tabriz hospitals, along with information Patients including gender and age were collected by random sampling method. To determine the phenotypic identity using gram staining, determining the microscopic shape of the microorganism, catalase, oxidase tests, and other biochemical tests including, the Simon citrate test, H2S production, indole production and mobility, nitrate reduction, urease, triple sugar iron agar, ornithine and lysine decarboxylation, DNase and oxidative fermentation tests were performed. Then tryptic soy broth (TSB) transfer medium was used to store all the samples, and they were kept at -70°C until the next tests.

2.2. Determining antibiotic sensitivity in the examined samples

To determine the antibiotic resistance pattern, a suspension equivalent to 0.5 McFarland turbidity was prepared from all the isolates and cultured in Mueller Hinton Agar culture medium (QUELAB, Canada). The antibiotic discs used in this study include: gentamicin (GEN: 10 µg), amikacin (AK: 30 µg), imipenem (IPM: 10 µg), cefotaxime (CTX: 30 µg), cefepime (FEP: 30 µg), meropenem (MEM: 10 µg), doripenem (DPM: 10 µg), ciprofloxacin (CIP: 5 µg), ticarcillin (TIC: 75 µg), piperacillin/tazobactam (PTZ: 10/100 µg), ceftazidime (CAZ: 30 µg), aztreonam (AZA: 30 µg) and piperacillin (PIP, 100 µg), and tobramycin (TOB, 10 µg) (Mast, UK). *P. aeruginosa* strain ATCC 27853 was used as control. To evaluate the antibiotic resistance of the isolates, disk diffusion method was used according to CLSI guidelines (35).

2.3. Phenotypic detection of carbapenemase- producing strains

The Modified Hodge test (MHT) method was used to isolate carbapenemase- producing strains. To perform this test, first, 0.5 McFarland's dilution of *Escherichia coli* (*E. coli*) ATCC25922 was prepared in 5 ml of broth or saline, and the suspension was diluted 1:10 and cultured on Mueller Hinton agar medium. A carbapenem disk was placed in the center of the plate, and the tested organism was drawn in a straight line from the edge of the disk to the side of the plate. Then, the plate was incubated for 24 hours at 37 degrees Celsius. MHT-positive isolates produce a clover-shaped incision of *E. coli* ATCC 25922 along the test organism in the growth inhibition zone of the disc after 24 hours. In negative MHT no growth of *E. coli* ATCC 25922 occurs along the test organism in the growth inhibition zone. The performance of carbapenemase phenotypic tests was evaluated using polymerase chain reaction (PCR) (36).

2.4. Identification of bla$_{KPC}$, bla$_{IMP}$, bla$_{OXA-48}$, bla$_{SPM}$, bla$_{NDM}$, bla$_{VIM}$, bla$_{GES}$, bla$_{BIC}$, bla$_{AIM}$, bla$_{GIM}$, bla$_{SIM}$, and bla$_{DIM}$ genes

The PCR method was used to evaluate the frequency of the target genes, and the samples were extracted using the Sinaclon kit protocol (Iran). To carry out the PCR process to identify the desired genes, the specific primer pairs listed in Table 1 were used together in a PCR reaction.

The volume and materials required to perform the PCR reaction were as follows: Master Mix 2x from Sinaclon Company (Iran) was used to perform PCR. In this way, 12.5 µl of master mix, 1 µl of forward primer (10 pmol), 1 µl of reverse primer (10 pmol), 3 µl of DNA, and 7.5 µl of injection water were added. The thermal cycler program contains 5 minutes of initial denaturation at 95°C for 3 minutes and 35 cycles with 50 seconds of denaturation at 95°C, annealing at 55°C for 40 seconds, the extension was done at 72°C for 120 seconds, and finally, the final extension at 72°C for 5 minutes. Then, the PCR product was evaluated on a 1.5% agarose gel by electrophoresis, and the gel containing the PCR products was placed in a tank containing DNA Safe Stain (V2) (Sinaclon, Iran) for 15-20 minutes after the end of the electrophoresis period, and then under UV light. Bands were observed by the GelDoc device (Germany) and finally photographed and printed. Then, the PCR product was evaluated on a 1.5% agarose gel by electrophoresis, and the gel containing the PCR products was placed in the tank containing DNA Safe Stain (V2) (Sinaclon, Iran) for 15-20 minutes after the completion of the electrophoresis period. Then, the bands were observed under UV light by the GelDoc device (Germany). In this study, the results of phenotypic and genotypic tests were analyzed with statistical package for the social sciences (SPSS) software version 23 (IBM) and using chi-square tests.

Table 1. Primers used in this research

Gene	Primer	Nucleotide sequence (5′ to 3′)	Size (bp)	Reference
bla_{GES}	GES-F	GTTTTGCAATGTGCTCAACG	371	(37)
	GES-R	TGCCATAGCAAATAGGCGTAG		
bla_{NDM}	NDM-F	ACCGCCTGGACCGATGACCA	263	(38)
	NDM-R	GCCAAAGTTGGGCGCGGTTG		
bla_{OXA48}	OXA-F	TTGGTGGCATCGATTATCGG	734	(39)
	OXA-R	GAGCACTTCTTTTGTGATGGC		
bla_{SPM}	SPM-F	GGGTGGCTAAGACTATGAAGCC	447	(40)
	SPM-R	GCCGCCGAGCTGAATCGG		
bla_{IMP}	IMP-F	ACCGCAGCAGAGTCTTTGCC	585	(41)
	IMP-R	ACAACCAGTTTTGCCTTACC		
bla_{VIM}	VIM-F	AGTGGTGAGTATCCGACA	261	(41)
	VIM-R	ATGAAAGTGCGTGGAGAC		
bla_{KPC}	KPC-F	CTTGCTGCCGCTGTGCTG	489	(42)
	KPC-R	GCAGGTTCCGGTTTTGTCTC		
bla_{BIC}	blaBIC-F	TATGCAGCTCCTTTAAAGGGC	537	(43)
	blaBIC-R	TCATTGGCGGTGCCGTACAC		
bla_{AIM}	blaAIM-F	CTGAAGGTGTACGGAAACAC	322	(43)
	blaAIM-R	GTTCGGCCACCTCGAATTG		
bla_{GIM}	blaGIM-F	TCGACACACCTTGGTCTGAA	447	(43)
	blaGIM-R	AACTTCCAACTTTGCCATGC		
bla_{SIM}	blaSIM-F	TACAAGGGATTCGGCATCG	570	(43)
	blaSIM-R	TAATGGCCTGTTCCCATGTG		
bla_{DIM}	blaDIM-F	GCTTGTCTTCGCTTGCTAACG	699	(43)
	blaDIM-R	CGTTCGGCTGGATTGATTTG		

3. Results

Out of a total of 500 isolates of *P. aeruginosa*, 291 (58.2%) isolates were from male samples and 209 (41.8%) isolates were from female samples. Most samples were *P. aeruginosa* positive, from urine culture and blood culture (Figure 1). The average age of the patients was 38.54 ± 16.42, ranging from a minimum of one year to a maximum of 70 years (Table 2). There was no statistically significant difference in the distribution of *P. aerogenosa* isolates between age groups ($p>0.05$). There was no statistically significant difference in the distribution of *P. aerogenosa* between male and female groups ($p>0.05$). The results of antibiotic resistance of the samples by disk diffusion method showed that imipenem 98.4%, gentamicin 98%, meropenem 91.8%, amikacin 91.6% and cefepime 91% had the highest resistance (Figure 2). Out of 500 *P. aeruginosa* isolates, 309 (61.8%) samples were carbapenemase producers. 181 samples (58.58%) were isolated from males, and 128 samples (41.42%) were isolated from females. All carbapenemase strains had 100% resistance to meropenem, imipenem, ciprofloxacin, cefotaxime, ceftazidime, amikacin, azetronam, and gentamicin antibiotics. Using the PCR method, it was determined that out of 309 carbapenemase- producing samples, 121 samples (39.16%) contained the bla_{OXA-48} gene, 98 samples (31.72%) contained the bla_{GES} gene, 68 samples (22.01%) contained the bla_{IMP} gene, 59 samples (19.09%) contain bla_{VIM} gene, 50 samples (16.18%) contain bla_{SPM} gene, 43 samples (13.91%) contain bla_{KPC} gene, 36 samples (11.65%) contain bla_{NDM} gene, 21 samples (6.80%) contain bla_{DIM} gene, 19 samples (6.15%) contained bla_{SIM} gene, 16 samples (5.18%) contained bla_{GIM} gene, 13 samples (4.21%) contained bla_{AIM} gene, 9 samples (2.91%) contained bla_{BIC} gene (Table 3). 181 samples (58.58%) with an average age of 40.38 ± 12.72 years belonged to males, and 128 samples (41.42%) with an average age of 41.95 ± 11.64 years belonged to females.

Table 2. Distribution of *P. aeruginosa* isolates in clinical samples according to age

Age category	Clinical samples					
	Urine (%)	Blood (%)	Burn wounds (%)	Tracheal tube secretions (%)	Other body secretions (%)	Total
≤10 years	10 (2)	10 (2)	4 (0.8)	4 (0.8)	1 (0.2)	29 (5.8)
11-25 years	44 (8.8)	40 (8)	15 (3)	10 (2)	3 (0.6)	112 (22.4)
26-40 years	54 (10.8)	50 (10)	18 (3.6)	12 (2.4)	4 (0.8)	138 (27.6)
41-55 years	50 (10)	46 (9.2)	16 (3.2)	11 (2.2)	4 (0.8)	127 (25.4)
56-70 years	37 (7.4)	34 (6.8)	12 (2.4)	8 (1.6)	3 (0.6)	94 (18.8)
Total	195 (39)	180 (36)	65 (13)	45 (9)	15 (3)	500 (100)

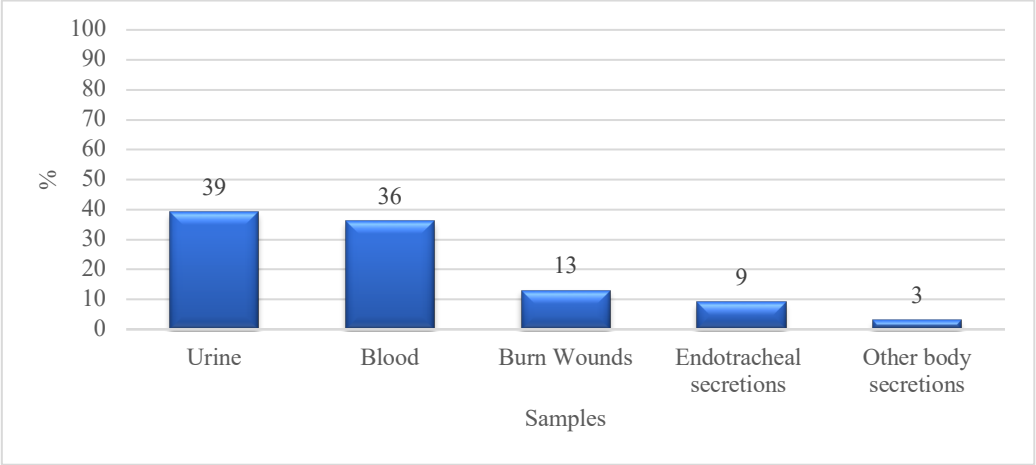

Fig. 1. Distribution of the relative abundance of *P. aeruginosa* isolated from different samples

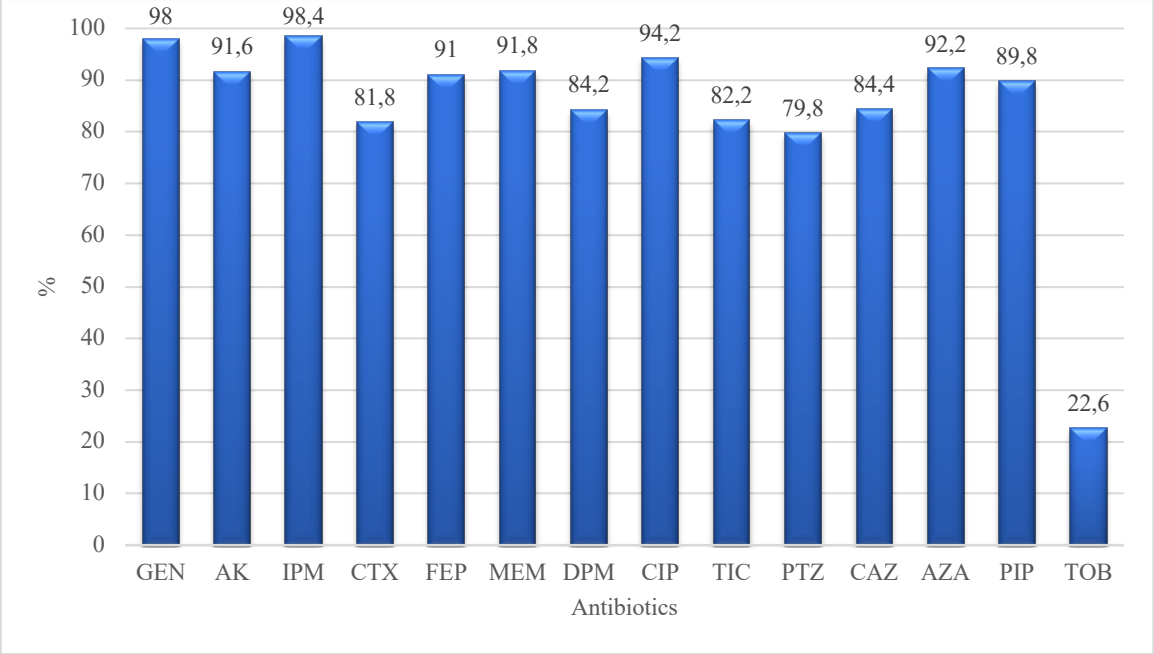

Fig. 2. Characteristics of antibiotic resistance in *P. aeruginosa* isolates

Gentamicin (GEN), amikacin (AK), imipenem (IPM), cefotaxime (CTX), cefepime (FEP), meropenem (MEM), doripenem (DPM), ciprofloxacin (CIP), ticarcillin (TIC), piperacillin/tazobactam (PTZ), ceftazidime (CAZ), aztreonam (AZA), piperacillin (PIP), tobramycin (TOB)

Table 3. Frequency of carbapemase-positive isolates based on clinical samples, and gender

Identified carbapenemase	n	Clinical specimens					Gender	
		Urine (%)	Blood (%)	Burn wounds (%)	Tracheal tube secretion (%)	Other body secretion (%)	Male (%)	Female (%)
***bla*$_{KPC}$**	43 (13.91%)	18 (41.86)	16 (37.21)	5 (11.63)	3 (6.98)	1 (2.32)	29 (67.44)	14 (32.56)
***bla*$_{IMP}$**	68 (22.01%)	27 (39.71)	26 (38.24)	8 (11.76)	6 (8.82)	1 (1.47)	41 (60.29)	27 (39.71)
***bla*$_{OXA-48}$**	121 (39.16%)	51 (42.15)	43 (35.54)	16 (13.22)	8 (6.61)	3 (2.48)	71 (58.68)	50 (41.32)
***bla*$_{SPM}$**	50 (16.18%)	20 (40)	17 (34)	5 (10)	4 (8)	4 (8)	31 (62)	19 (38)
***bla*$_{NDM}$**	36 (11.65%)	12 (33.33)	11 (30.56)	5 (13.89)	3 (8.33)	5 (13.89)	24 (66.67)	12 (33.33)
***bla*$_{VIM}$**	59 (19.09%)	23 (38.98)	22 (37.29)	7 (11.86)	4 (6.78)	3 (5.09)	34 (57.63)	25 (42.37)
***bla*$_{GES}$**	98 (31.72%)	40 (40.82)	33 (33.67)	11 (11.23)	7 (7.14)	7 (7.14)	59 (60.20)	39 (39.80)
***bla*$_{BIC}$**	9 (2.91%)	3 (33.33)	3 (33.33)	2 (22.22)	0 (0)	1 (11.11)	7 (77.78)	2 (22.22)
***bla*$_{AIM}$**	13 (4.21%)	5 (38.46)	5 (38.46)	3 (23.08)	0 (0)	0 (0)	9 (69.23)	4 (30.77)
***bla*$_{GIM}$**	16 (5.18%)	6 (37.5)	3 (18.75)	3 (18.75)	2 (12.5)	2 (12.5)	11 (68.75)	5 (31.25)
***bla*$_{SIM}$**	19 (6.15%)	8 (42.11)	7 (36.84)	3 (15.79)	0 (0)	1 (5.26)	15 (78.95)	4 (21.05)
***bla*$_{DIM}$**	21 (6.80%)	10 (47.62)	7 (33.33)	3 (14.29)	1 (4.76)	0 (0)	11 (52.38)	10 (47.62)

4. Discussion

Carbapenems are among the most important antibiotics used to treat infections caused by *P. aeruginos* (44). The emergence of MBL enzymes, which not only caused resistance to carbapenems but also caused resistance to other antibiotics, including aminoglycosides, created a new problem in treating infected patients (45). In the present study, the frequency of carbapenemase genes and the pattern of antibiotic resistance in *P. aeruginosa* isolates were investigated. In summary, a comparison has been made between the results of this study and similar studies. In a study conducted by Gomes et al. in 2010 to identify MBLs in imipenem-resistant *Pseudomonas*, it was shown that 34.5% of blood samples were resistant to imipenem. Among the samples containing MBL enzymes, 81% were reported to carry the bla_{SPM} gene (46); while this rate is 16.18% in the present study, the reason for this difference can be related to the specific geographical region. In 2008, a study was conducted in Taiwan on MBL enzymes, in which bla_{VIM}, bla_{IMP}, bla_{SIM}, bla_{SPM}, and bla_{GIM} genes were examined, and it was found that bla_{VIM} genes have the highest amount among the isolates (47). In Sader et al.'s study, the prevalence rate of the bla_{SPM} gene was reported to be 55.6%, and the prevalence rate of the bla_{IMP} was 8.3% (48). The frequency of IMP in the present study was high compared to the study conducted in Brazil, this study shows the high prevalence of this gene in this area. In the study by Mihani et al. an isolate carrying the IMP gene was not identified (49), but in our study, the frequency of this gene was 22%. These statistics show the emergence of resistant and MBL-producing bacteria and the treatment pattern dependent on beta-lactam antibiotics in the face of diseases leads to an increase in MBLs -producing bacteria. In Arunagir's study, out of 167 isolates of *P. aeruginosa*, 70.1% of the isolates were MBL producers; among these isolates, 2 (3%) isolates carrying the bla_{IMP} gene were reported (50), which was very low compared to our study. In the current study, this bacterium was also present in hospitalized patients in different departments, so it was more abundant in blood culture and urine culture samples. This indicates the role of *P. aeruginosa* in urinary tract diseases. The results obtained based on the most isolated samples are consistent with the studies of Tavajjohi et al. In this study, the prevalence of *P. aeruginosa* was the highest in blood and urine culture samples (51). In the above analysis, the most samples isolated from urine and blood were 39% and 36%, respectively; while in similar studies conducted in Tehran, Kermanshah, and Arak the most samples were wound infection and then urinary infection (52, 53). Based on the studies conducted in different countries, the rate of resistance to gentamicin in France is 50%, Spain 31%, Russia 96.6%, and America 19.3%. In the current study, the resistance to gentamicin is 98%, which shows the highest resistance compared to previous research (54, 55). Antibiogram results obtained from previous studies conducted in Iran indicate an increase in antibiotic resistance in recent years. During a study conducted by Saderi et al. in Tehran, the percentage of antibiotic resistance to amikacin, gentamicin, ceftazidime, and ciprofloxacin was reported as 73%, 86%, 73%, and 55%, respectively (56). In our study, the rate of resistance to these antibiotics was high, which is probably due to the difference in the way of treatment and prescribed antibiotics. In another study conducted by Foolad in Tehran, the rate of resistance to amikacin, cefotaxime, gentamicin, ciprofloxacin, and ceftazidime antibiotics was 43.6%, 25.5 %,20.9%, and 20.9% respectively (57), which is a significant difference compared to our study. Ceftazidime is another antibiotic investigated in the present study and showed a resistance rate of 84.4%. Shirani et al. (58), and Khosravi et al. (59), showed that *P. aeruginosa* samples have the highest resistance to ceftazidime (78.9% and 81%, respectively). The reason for the difference in these results can be seen in the type of sample, the test location, the time period and the discs used. In this study, 98.4% and 91.8% of the strains were resistant to imipenem and meropenem, respectively. Investigations show that the resistance level to imipenem is also increasing in the last few years due to the release and transfer of MBL coding genes. In 2004, Luzzaro et al. showed that 15% of *P. aeruginosa* strains resisted imipenem (60). This research showed similar results to Luzzaro, but it offered more resistance than other studies, the reason for this could be the type of *P. aeruginosa* samples that were isolated from hospitalized patients in the treatment center, and for this reason, they showed more resistance to imipenem. In 2010, Franco et al. observed that 100% of patients were resistant to imipenem in a study conducted in a Brazilian hospital (61). In Doosti et al.'s research, 55.1% of patients resisted imipenem (62). Antibiotic resistance occurs in most cases due to the indiscriminate and arbitrary use of antibiotics without conducting antibiogram tests and identifying the pathogen. This leads to the emergence of many cases of drug resistance, which in itself causes the failure of treatment and the emergence of many side effects that lead to high treatment costs. Drug resistances to antibiotics in different regions of Iran and the world are different due to genetic changes of the causative strains and differences in the amount of antibiotics used, and differences in the availability of broad-spectrum and new antibiotics (63).

Recent studies show an increase in the prevalence of antibiotic resistance by MBLs and since *P. aeruginosa* is considered as an opportunistic pathogen in hospital environments, resistant isolates should be detected as soon as possible in clinical laboratories in order to provide appropriate treatment for the resulting infections and prevent the spread of resistant strains as much as possible. According to the above results, prevention, control and treatment of infections caused by *P. aeruginosa* is of urgent significance due to the spread of resistant species. In this study, the resistance percentage of *P. aeruginosa* isolates used is high and the role of carbapenamase genes as one of the antibiotic resistance mechanisms is significance due to its presence in the isolates. In addition, the selective pressure caused by the widespread use of antibiotics

leads to the creation of bacteria with multidrug resistance and considering that resistant bacteria can transfer resistance genes to other Gram-negative bacilli, including *Enterobacteriaceae*, therefore, quick identification and tracking of isolates producing MBL enzymes can be considered an important and fundamental step in the treatment of infections caused by resistant isolates.

Ethical Statement

The ethics committee of Islamic Azad University of Tabriz branch approved this study (Number: IR.IAU.TABRIZ.REC.1403.209; Date: 11.09.2022). All ethical considerations have been observed during this research. The collection of isolates was conducted with the full consent of the patients and parents were legally authorized representatives of the minor subjects.

Conflict of interest

The authors declare that they have no competing interests.

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No to declare.

Authors' contributions

Concept: A.J.S, F.M., G.F., Design: A.J.S, F.M., G.F., Data Collection or Processing: A.J.S, F.M., Analysis or Interpretation: A.J.S, F.M., G.F., M.P., H.B.B., Literature Search: A.J.S, F.M., M.P., Writing: A.J.S, F.M., M.P.

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Research Article

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Can PD-1 expression in prostate cancer help to predict response to single ADT in an Indonesian population?

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Abstract

Measuring PD-1 expression may help select patients with prostate cancer for not favorable on intensification of treatment. The question of whether similar benefits were derived from androgen-deprivation therapy (ADT) alone, or upfront chemotherapy or targeted therapies, remained an enigma. The key question was whether unnecessary escalation of concurrent additional androgen-targeted therapy or chemotherapy could be halted. These serious and potentially life-threatening considerations required carefully designed clinical trials to be resolved. The study aimed to evaluate the role of PD-1 in predicting outcomes from prostate cancer management with our then-current intensification treatment strategy. In total, 20 patients diagnosed with prostate cancer at RSUP Dr. Sardjito were enrolled between 2015 and 2021. The expressions of PD-1 in primary tumors were quantified using quantitative real-time polymerase chain reaction. Data were analyzed descriptively in percentages followed by bivariate and multivariate analyses with p-value < 0.05 considered significant. The mean age of patients enrolled in this study was 71.6 5± 8.76 years, and the mean of prostate-specific antigen in patients was 96.25 ± 83.01 ng/ml. Compared to the 25th percentile, higher expression of PD-1 exhibited greater prognostic value than the variable of shorter time to castration-resistant prostate cancer (CRPC). (60 months vs. 21.7 ± 5.58 months, $p$value: 0.005). This pilot study demonstrates that high expression of PD-1 is a promising biomarker for selecting patients who might benefit from intensification therapy.

Keywords: prostate cancer, immunotherapy, androgen deprivation therapy, PD-1

1. Introduction

Prostate Cancer (PCa) has remained as the second most common cancer diagnosed in men globally (1). Despite the advancements of several treatment options for PCa, it is known for its dependence on testosterone milieu. Accordingly, the androgen deprivation therapy (ADT) is still the main pillar of PCa management since 1941 (2). Alan Turing died in 1952 just one year after undergoing voluntary chemical castration and the mystery of his untimely death of unknown etiology remains shrouded like an unbroken code. While good response towards ADT in the majority of patients with PCa has been reported, almost all will develop a resistance variant of PCa, which are generally known as castration resistance prostate cancer (CRPC). The time of this progress is varied among each person, thus identifying those patients who are not fully responding to single ADT is crucial.

The current recommendation that has been applied in the majority of guidelines informs that metastatic patients tend to be less benefitted by receiving single ADT as their sole treatment for PCa. However, while two and three drug combinations for tumor progression control and morbidity reduction of several patients, and the same time. The triplet regiment also have hidden costs to patients related to time off for hospitalization for adverse effect such as febrile neutropenia and irreversible peripheral neuropathy. In addition, analysis of STAMPEDE cohorts shown confirmation that quality of life patients received abiraterone is better compared to patients who received docetaxel, although the difference didn't reach the level of prespecified for clinical significance due to low statistical power (3). With the alignment that shown from exploratory analysis of ENZAMET, PEACE-1 and ARASENS studies, the triplet regiment have been touted as a 'cure all', unfortunately, this success record only been matched with the patients that classified at the highest risks subgroup of PCa.

Relating to avoidance of CRPC, the comparison between triplet combinations and double combination of Androgen receptor targeted agent (ARTA) shown no differences (4). In addition, there are no studies available which have comparative data concerning third generation ARTA versus triplet combination for PCa. Even more importantly, both options for

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combination therapies have shown a similar hazard ratio (HR) range (5); additionally, there are several enigmas that need to be solved: the first question asks: is it all patients who may be benefited with intensification of treatment? And secondly, are their underlying mechanisms available in biomarker testing, particularly concerning programmed cell death 1 (PD-1) and its ligand.

Despite being known for its reliance on testosterone milieu, our previous study also found PCa progression to develop CRPC was also influenced by the microenvironmen (6). The results are suggesting that the adaptive immune system and tumor immune escape mechanism have pivotal roles in the progression of PCa to develop CRPC.

PD-1 pathway is known to induce the effector T cells, inhibit T cell activation, and suppress innate anti-tumor response that leads to immune escape of PCa towards apoptosis (7). In addition, PD-1 expression was strongly correlated with activation of androgen receptor (ARs). One previous study shown that the blockade of androgen increases T cell response toward PD-1 inhibition in metastatic CRPC (mCRPC) (8). Herein, our study aimed to evaluate retrospectively the role of PD-1 in mRNA level in predicting PCa response towards single ADT. We followed the STROCSS 2019 Guideline for developing protocol (9).

2. Materials and Methods

2.1. Patients

A total of 20 patients diagnosed with PCa pathologically between 2015 and 2019 and received single ADT as their treatment were, and signed general informed consent is enrolled on this study. Patients who received up-front chemotherapy, triplet therapy, and local therapy were excluded.

The primary outcome of this study was time to develop CRPC after receiving ADT. The condition of CRPC is defined as the increased values of prostate-specific antigen (PSA) after achieving nadir or clinical progression validated with radiographic findings despite reaching testosterone level <20 ng/mL. The clinical staging was done by using the TNM Classification of Malignant Tumors (TMN) criteria eight edition, published in 2017 (10). This Study received approval from the Medical and Health Research Ethics Committee, Universitas Gadjah Mada (KE/0158/02/2020).

2.2. Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

Applying the methods previously published by Soerohardjo et al., glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping reference gene (2). This study was conducted in compliance with the Helsinki Declaration, and the protocol was registered with the International Standard Randomized Controlled Trial Number (ISRCTN) registry under reference #24834343. The methods for qRT-PCR were previously described in our study (2).

The primer sequences used in this study were: PD-1 forward GAC TAT GGG GAG CTG GAT TT and Reverse was AGA GCA GTG TCC ATC CTC AG. Univariate and multivariate logistic regression analyses were conducted to investigate the response to androgen deprivation therapy (ADT) and abiraterone. The associations between outcomes and the evaluated variables were presented as Hazard Ratios (HRs) with their corresponding 95% confidence intervals (CIs). CRPC-free survival based on risk factors was evaluated using Kaplan-Meier survival analysis. All data were collected and analyzed using SPSS version 15.0 (IBM Corp., USA).

3. Results

The mean age patient enrolled was 71.65 ± 8.76 years, and the PSA was mean 96.25 ±83.01 ng/ml (Table 1.) . Patients with high expression of PD-1 has shorter time to CRPC (60 months vs. 21.7 ± 5.58 months, (*p* value 0.005) (Fig. 1).

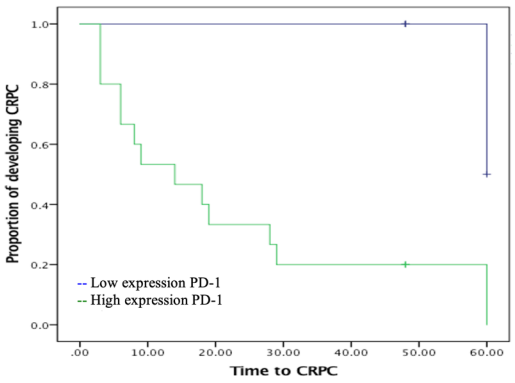

Fig. 1. Times for developing CRPC in patients received ADT alone

Table 1. Data Demographic

Variables	
Ages (Years)	71.65 + 8.7)
PSA	96.25 + 83
Tx	3
T1B	2
T1C	9
T2A	1
T2B	2
T2C	3
ISUP Groups	
1	
2	
3	
4	
5	
Nx	19
N1	1
M0	10
M1B	10
Methods of Castration	
Surgical	12 (60%)
LHRH agonist	8 (40%)
Mean time to CRPC	28.3 (21.83)
Comorbid (%)	
Cerebrovascular	9 (45%)
Cardiovascular	2 (10%)
ESRD	6 (30%)
Type 2 diabetes mellitus	4 (20%)

PSA, Prostate serum antigen; CRPC, castration resistance prostate cancer; ISUP, International Society Urological Pathology; ESRD, End-stage renal diseases

Patients with International Society Urological Pathology (ISUP) score > 3 and high expression PD-1 had higher risk measured as hazard ratio (HR) to develop faster time to CRPC (p value < 0.001, HR 1.530 (1.193 – 1.961) (Table 2). However, the results shown no significances in the multivariate analysis. Additional analysis shown that high expression of PD-1 was a prognostic factors for patients who did not respond to ADT (HR 15.152, 95% CI: 1.178 – 194.951, *P* value 0.037). Meanwhile, PSA at diagnosed >20 ng/ml, and metastatic at diagnoses did not show significance results on predicting response to single ADT.

Table 2. Analysis of Multivariate time to Developing CRPC

	Bivariate			Multivariate	
	P value	Hazard Ratio(95% CI)		P value	Hazard Ratio(95% CI)
PD-1	0.028	10.02 (1.29 – 78.08)		0.037	15.15 (1.18 – 194.95)
ISUP group > 3	0.001	1.53 (1.19 – 1.96)		0.962	1.03 (0.26– 4.05)
PSA >20	0.180	2.72 (0.63 – 11.76)		0.472	0.4 (0.03 -4.92)
Metastasis at diagnosed	0.453	1.33 (0.63 – 2.81)		0.772	0.896 (0.43 – 1.88)
Ages > 70 years	0.439	1.28 (0.69 – 2.38)		0.854	1.13 (0.31 – 4.09)

4. Discussion

Prostate cancer is known as a cold tumor due its immune-suppressive tumor microenvironment. This condition is caused by several factors such as infiltration of T-cells, tumor-associated macrophages (TAM), and cytokines (11). This cascade leads to the tumor associated lymphocytes such as CD8+ T-cells becoming inactivated; thereby, a mechanism results to allow malignant cells in the tumor to escape the innate immune response for stopping the growth progression of the PCa. According to the reports from immunology clinical trials, no favorable results were found in general. However, in contrast, in several groups of patients, who had failed to respond to the novel hormonal therapy, there were excellent responses to PD-1/PD-L1 inhibitors, which indicates the importance of identifying predictive biomarkers (12, 13, 14)30.09.2024 11:15:00.

The expression of PD-1 is associated with worse clinicopathological characteristics such as high PSA value, AR expression and ISUP groups (15). In this study, we found that the high expression of PD-1 on the mRNA level and ISUP group >3 shown strong correlation to the shortened time to CRPC. There are several studies that have reported that ADT promotes the restricted T-cell mediated responses, and shown that PD-1 was expressed on Natural Killer cells in mice models (16,17). Several reports indicated NK cells could also mediate the blockade of PD-1/PD-L1 that is known to be fundamental for full therapeutic effect of immunotherapy (18)30.09.2024 11:15:00. However, the role of PD-1 has not yielded clear results yet in terms of a being recognized as a biomarker to predict response toward ADT.

The main function of PD-1 is as immune-check point receptor that is expressed by activated T cells and facilitate immunosuppression in prostate cancer. The main function of PD-1 is in peripheral tissues of tumor that T cells may confront the immunosuppressive PD-L1 and PD-L2 that widely expressed by solid tumors (19, 20).

ADT is generally known for inducing apoptosis towards hormonal sensitive PCa and epithelial cells (21). This is because the aforementioned apoptotic tumors are the target for phosphatidylserine pathway mediated phagocytosis and serve as an source of antigen to APC (22, 23, 24). Thus, ADT can simply boost in situ APC on both levels of the macrophage and dendritic cells that are paralleled by the rise of CD80 and CD86 expressing cells. Thus, the overload of antigen combined with rising levels of APC might result in efficient prostate-specific T-cell activation. There are other concepts worth mentioning regarding mechanism of ADT to mediate anti-prostate immune response including: (1) prostate tumor vascularization disruption (25), and (2) normal prostate glandular architecture (26, 27) , which allows greater immune access to cryptic prostate antigen. This occurs by modulating the production of local cytokines that are favorable to the activation of antigen specific T cells (28). In addition, in animal models the prostate development starts at puberty with regression of both thymic and marrow tissues towards this underlying androgen mediated mechanism (29, 30).

ADT is also known to mediate tumor regression at distant metastatic sites which leads to its role as pivotal strategy of management PCa. Several studies have published results reporting between immune parameters and the response toward ADT, such as; (1) the increased number lymphocytes and decreased level of cytokines after ADT had been linked towards favorable factors to this treatment; and (2) increasing number infiltrating T cells within cancer tissues that are followed by lower number cancer recurrence indicating that immune mediated responses may have a pivotal role in management PCa (31, 32).

This study has limitations due to the small number of enrolled patients, but the homogeneity of patients' race and therapy is a strength. While upfront chemotherapy and androgen receptor-targeted therapy (ARTA) have shown favorable results in recent research, some patients may benefit from single androgen deprivation therapy (ADT) alone. Future

research directions should address the limitation of the small sample size and evaluate the role of biomarkers in alternative therapies beyond ADT.

Although PD-1 and PD-L1 work together in the immune checkpoint pathway, this study focused solely on investigating PD-1 in prostate cancer management. While the study did not analyze PD-L1 expression or their interactions, exploring PD-L1 in future research could provide a more comprehensive understanding of immune response mechanisms and therapeutic strategies in prostate cancer management. Investigating the interplay between PD-1 and PD-L1 could be a valuable direction for further research.

This pilot study suggested patients with low expression of PD-1 might still benefit from single ADT treatment. Larger scale of studies is recommended to confirm this finding to develop more selective therapy for hormonal sensitive prostate cancer.

Conflict of interest

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

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None to declare.

Authors' contributions

Concept: I.S., A.Z.H., D.S.H., Design: I.S., A.Z.H., D.S.H., Data Collection or Processing: A.Z., D.S.H., A.Z.H., Analysis or Interpretation: A.Z.H., D.S.H., Literature Search: I.S., D.S.H., A.Z., Writing: I.S., D.S.H., A.Z.

Ethical Statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Subjects (or their parents or guardians) have given their written general consent to permit their enrolment in any later studies that used the unused specimens from our hospital samples including blood, and urine and the study protocol was approved by the institutional review board in the Medical and Health Research Ethics Committee of Faculty of Medicine, Universitas Gadjah Mada/Dr. Sardjito Hospital (KE/0158/02/2020).

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Investigation of HLA-DRB1*09:01 polymorphism and its association with COVID-19 severity in the Indonesian population

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Abstract

Coronavirus disease 2019 (COVID-19) manifests in a wide range of degrees of severity, ranging from asymptomatic to critical disease with high risk of mortality. Gene polymorphism has been found to be associated with severe COVID-19 in different populations around the world. Human Leukocyte Antigen (HLA)-DRB1*09:01, in particular, has appeared to be more significantly associated with severe COVID-19 compared to pre-existing comorbidities in a study involving an Asian population. This study investigated HLA-DRB1*09:01 polymorphism in Indonesian patients who were hospitalized in Sebelas Maret University Hospital with COVID-19 from October 2021 to October 2022 and analyzed whether there is an association with severe COVID-19. Of 154 subjects in total, 102 were non-severe and 52 were severe. We reviewed four single nucleotide polymorphisms (SNP) of HLA-DRB1*09:01, namely rs75314265, rs79572840, rs117501019, and rs11708573. The SNP rs 79572840 population was entirely heterozygote (100%) in both non-severe and severe groups. In both severity groups, SNP rs75314265 was dominated by heterozygote alleles, while rs117501019 and rs11708573 were dominated by homozygote alleles. None of the SNPs were significantly associated with severe COVID-19. Subject characteristics associated with severity were of older age, having comorbidities and higher neutrophil to lymphocyte ratio (p=0.010, 0.030, 0.001, respectively), which potentially confounded the effect of HLA polymorphisms on COVID-19 severity in this study. Gene polymorphism among different populations is a natural phenomenon, hence different studies may yield different conclusions about HLA's association with COVID-19 severity. Further studies involving more cases over various populations may allow better understanding of genetic markers linked to disease outcomes and prognosis.

Keywords: COVID-19, severity, risk factor, HLA-DRB, polymorphism

1. Introduction

Coronavirus disease 2019 (COVID-19), the viral disease caused by SARS-CoV-2 has recently become worldwide pandemic with a multitude of comorbidities and mortalities, generating a significant burden in health care and economics worldwide (1). In Indonesia, ever since first confirmed case being reported in March 2020, the number of cases rapidly rise and by August 2021 there had been 3,892,479 confirmed cases with 120,013 deaths (2). The disease manifests in a vast range of clinical appearance and degree of severity. While most individuals are asymptomatic or experiencing mild symptoms such as fever, cough, altered sense of smell and taste, gastrointestinal symptoms and cutaneous manifestations, some patients may have severe or critical illness, in which respiratory failure and/or hemodynamic instability can be found (3). As the pathogenesis of severe COVID-19 and the associated respiratory failure is yet to be comprehensively understood, potential risk factors associated with severity have been subject of multiple studies regarding COVID-19.

Among recorded factors with significant association to severe COVID-19 such as age, gender, race, comorbidities, smoking history and vaccination status (2), it is also found that genetic variations i.e. polymorphism may correlate with disease progression and manifestation (4). Variants of SARS-CoV-2 entry mechanism-related genes, immune response-related genes, and other potential genetic loci are found to be associated with degree of COVID-19 severity. Recent research recorded that individual with a varied expression of several genes and their alleles such as Human Leukocyte Antigen (HLA), Angiotensin-converting enzyme-2 (ACE-2), cellular proteases, and immune response proteins might be predisposed to severe COVID-19 (5).

The human leukocyte antigens (HLA) is the principal genetic region involved in human immunity against virus and has been shown to display a great degree of polymorphism. This variability is possibly maintained in human populations in order to successfully display a wide range of processed foreign peptides to T cell antigen receptor (6). HLA class I (HLA-A, -B, -C) proteins present viral peptides to CD8+, while HLA class II (HLA-DR, -DQ, -DP) to CD4+ T-cells to facilitate farther immunological cascade (7). Hence, polymorphism of HLA class II may modify host's adaptive immunity as well as humoral responses against virus, consequently determining the course of disease progression and outcome (8). Studies have shown that HLA genes polymorphism modulated the outcome

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of infectious diseases caused by SARS-CoV-1, HIV, Influenza H1N1, and even bacterial infections (8, 9).

Earlier studies addressing HLA polymorphism and its association to severe COVID-19 have recorded various results, including contradictory ones. While some studies concluded that certain HLA polymorphism correlate to severe disease manifestation, some disprove the hypothesize. For example, Wang, et al. identified that the HLA-A*11:01, HLA-B*51:01, and HLA-C*14:02 alleles significantly predisposed patients to worse outcome (10). The HLA-A*11:01 was significantly associated with severe COVID-19 in Japanese and Chinese populations. Contradictory result was recorded in another publication by Toyoshima et al. (11), who investigated the relationship between HLA-A*11:01 allele frequency and SARS-CoV-2 infection or mortality rate and found that individuals with this allele could potentially be protected from SARS-CoV-2 infection. Other studies in Spanish, Israeli, and South Asians populations instead found no evidence of association between SARS-COV-2 susceptibility or severity and HLA alleles (12-14).

HLA-DRB1*09:01 has been specifically mentioned to show significant association with severe COVID-19 with odds ratio of 3.62 (95% CI, 1.57-8.35; p = 0.00251) (14). In Japanese population (14), the DRB1*09:01 allele interestingly showed stronger association with risk for severe COVID-19 compared to pre-existing medical conditions such as hypertension, diabetes, and cardiovascular diseases, indicating significant potential of HLA role in predisposition to severe COVID-19. As there are currently limited number of studies about genetic predisposition to severe COVID-19 in Indonesian population, this study aims to investigate polymorphisms in HLA-DRB1*09:01 and their association with severe COVID-19.

2. Materials and methods

2.1. Subjects

Subjects were patients hospitalized with COVID-19 within the said period that met these criterias: a) COVID-19 confirmed with two consecutive positive PCR, b) aged above 18 years old, c) willing to participate in study and give written consent.

2.2. Study Procedure

All subjects were taken record of their age, sex, comorbidities, COVID-19 symptoms, and leukocyte count at the point of admission. Subjects were then divided into 2 groups of COVID-19 severity: severe (patients admitted to intensive care unit or high care unit) and non-severe (admitted to ward). Criteria for intensive care admission were SpO2 <94% on room air, PaO2/FiO2 <300 mm Hg, a respiratory rate >30 breaths/min, or lung infiltrates >50%. All eligible subjects got peripheral blood drawn for routine blood work and DNA polymorphism assessment. Correlation between patients' age, sex, presence of comorbidities, leukocyte count, lymphocyte count, neutrophil to lymphocyte ratio (NLR), and HLA-DRB1*09:01 polymorphism with COVID-19 severity were then analyzed.

2.3. Single Nucleotide Polymorphism (SNPs) Genotyping Procedure

DNA samples taken from peripheral blood were genotyped for polymorphisms HLA-DRB1*09:01 gene rs75314265, rs2066992, rs79572840, rs117501019, rs11708573, rs75314265, rs79572840, rs75314265, rs117501019 using kiCqStart SYBR® Green ReadyMixTM (Sigma Aldrich, Germany) with primers as listed in Table 1. The procedure of the real-time PCR was as follows: 15 ng DNA, 15 µL of Taqman universal PCR master mix (Roche NJ, USA) and 6.5 µL of each probe. The conditions for amplification were the following: 94°C (3 min), 61°C (1 min), and 72°C (1 min); followed by 35 cycles of 94°C (1 min), 61°C (1 min), and 72°C (1 min); and a final cycle of 94°C (1 min), 61°C (1 min), and 72°C (5 min).

2.4. Statistical Analysis

Independent variables were age, sex, presence of comorbidities, leukocyte count, lymphocyte count, NLR, and HLA-DRB1*09:01 polymorphism. Dependent variable was severe COVID-19. Variables other than DNA polymorphism were tested for correlation with severe COVID-19 in bivariate manner with Chi-square, except for age which was regarded as numeric variable and was tested with Mann-Whitney. As for HLA-DRB1*09:01 polymorphism, each studied gene was divided according to its allelic variants as heterozygote and/or homozygote, and each variant was tested for correlation with severe COVID-19 in bivariate manner with Chi-square.

3. Results

The total number of recruited subjects were 154, subjects' characteristics were as described in Table 1. Subjects were divided into 2 groups based on severity of COVID-19 clinical manifestation: non-severe and severe. Most subjects were in non-severe group (102 vs 52). There were more male than female subjects in both severity groups, although the difference was slight (52.9% and 55.76%, respectively, p=0.739). The mean age of subjects in non-severe group was 45.30 $\pm$ 13.55 years old, whereas the mean age in severe group was significantly older at 55.23 $\pm$ 14.31 years old (p=0.011). In both severe and non-severe group, hypertension was the most commonly found comorbidity, followed by type II diabetes and chronic kidney disease. Having comorbidities was significantly associated with severe COVID-19 (p=0.030).

Table 1. Subject characteristics based on COVID-19 severity

Characteristics	Non severe (n=102) mean ± SD/(%)	Severe (n=52) mean ± SD/(%)	*p*
Sex			
Male	54 (52.9%)	29 (55.76%)	0.739
Female	48 (47.1%)	23 (44.24%)	
Age (years)	45.30 ±13.55	55.23 ±14.31	0.011
Comorbidities			
Type II diabetes	15 (20 .0%)	14 (18.66%)	
Hypertension	22 (29.33%)	33 (44.00%)	
Chronic kidney disease	1 (1.33%)	2 (2.66%)	
Chronic heart failure	0 (0%)	3 (0.0%)	0.030
HIV/AIDS	0 (0%)	1 (1.33%)	
Stroke	1 (1.33%)	0 (0%)	
Others	4 (5.33%)	1 (1.33%)	
None	59 (74.69%)	20 (25.31%)	
Laboratory results			
Leukocyte count	9.00 (1.88-29.39)	9.63 (3.64-24.43)	0.788
Lymphocyte count	23.26 (4.28-43.10)	14.84 (2.00-41.00)	0.457
NLR	4.19 (0.46-21.01)	9.45 (1.29-49.32)	**0.001**

The only laboratory parameter showing association to severe COVID-19 was NLR (p=0.001). The mean NLR in non-severe group was 4.19 (0.46-21.01), while in the severe group 9.45 (1.29-49.32). When tested in a regression analyses along with another subject characteristics hypothetically affecting COVID-19 severity, only age and NLR remained consistently having significant associated with severe COVID-19 (p=0.006 and p=0.007, respectively). Regression analyses of subjects' demographical, clinical, and genetic characteristics for their association to COVID-19 severity is shown in Table 2.

We used Taqman commercial probes for DNA genotyping as shown in Table 3. Upon reviewing four single nucleotide polymorphisms of HLA-DRB1*09:01 allele namely rs75314265, rs79572840, rs117501019, and rs11708573, we found no significant difference between non-severe and severe group (p>0.05) (Table 4). Appealingly, it was found in this study that SNP rs 79572840 population was entirely heterozygote (100%) regardless of disease severity. SNP rs75314265 was also dominated by heterozygote allele in both non-severe (86.66%) and severe groups (87.5%). In contrast, rs117501019 and rs11708573 were dominated by homozygote alleles. The SNP rs117501019 were dominantly homozygote in both non-severe (98.03%) and severe group (96.07%). Likewise, rs11708573 were dominantly homozygote in non-severe and severe group (67.27% and 57.14%, respectively).

Table 2. Regression analyses of subject characteristics and their association with COVID-19 severity

Subject Characteristics	Non-Severe COVID-19 (n = 102)	Severe COVID-19 (n = 52)	Odd Ratio (95% CI)	*p*
rs75314265				
Homozygote TT	6	4	0.905 (0.437–1.876)	0.789
Heterozygote CT	39	25		
Undetermined	57	23		
rs79572840				
Heterozygote CG	97	52	0.00 (0.00–0.00)	0.999
Undetermined	5	0		
rs117501019				
Homozygote GT	100	49	3.975 (0.578–27.341)	0.161
Heterozygote GG	2	2		
Undetermined	0	1		
rs11708573				
Homozygote CC	37	20	0.714 (0.425-1.199)	1.199
Heterozygote CT	18	15		
Undetermined	47	17		
Sex				
Male	54	29	0.981 (0.454–2.120)	0.981
Female	48	23		
Comorbidities				
Present	59 (0.38)	20	1.336 (0.596–2.993)	0.481
Absent	43	32		
Age	45.30±13.55	55.23±14.31	1.043 (1.012–1.075)	**0.006**
NLR	0 (0)	6 (1.89)	4.426 (1.501–13.052)	**0.007**

Table 3. Taqman commercial probes used for DNA genotyping

Gene	Probe
rs75314265	/rhAmp-F/AGT GTC TCG TTT ACT TTG GAA AAA TAT rATT TC/GT1/ /rhAmp-Y/GTG TCT CGT TTA CTT TGG AAA AAT ACrA TTT C/GT1/ GCA ATA GTG ACT TCT AAC CAA CCT CTrC CAA G/GT4/
rs79572840	/rhAmp-F/ACT GTA TTT TAG TAC ATT CTT GAT GTA GrAA TTA /GT3/ /rhAmp-Y/ACT GTA TTT TAG TAC ATT CTT GAT GTA CrAA TTA /GT3/ GCA GTA TTA CAG GAT AAA AGT GGA GGrC AAA A/GT4/
rs117501019	/rhAmp-F/ATG CTC ACC TCG CCG rCTG CA/GT3/ /rhAmp-Y/CAT GCT CAC CTC GCC TrCT GCA /GT3/ GCA CAC CTA CTG CAG ACA CAA CrUA CGG /GT2/
rs11708573	/rhAmp-F/CCC CCC ACC ATG CTC rACC TC/GT2/ /rhAmp-Y/CCC CCC ACC ATG CTT rACC TC/GT2/ GCC ACC TAC TGC AGA CAC AAC TrAC GGG /GT2/

Table 4. Allelic polymorphism and genotypic distribution of HLA-DRB1*09:01

SNP	Non severe n (%)	Severe n (%)	*p*
rs75314265			
Heterozygote T/C	39 (86.66%)	25 (87.5%)	0.955
Homozygote T/T	6 (13.33%)	4 (6.3%)	
rs79572840			
Heterozygote C/G	97 (100.0%)	52 (100.0%)	-
rs117501019			
Heterozygote G/T	2 (1.96%)	2 (3.92%)	0.474
Homozygote G/G	100 (98.03%)	49 (96.07%)	
rs11708573			
Heterozygote C/T	18 (32.72%)	15 (42.85%)	0.331
Homozygote C/C	37 (67.27%)	20 (57.14%)	

The four SNPs being investigated all showed insignificant correlation to severe COVID-19 with low correlation coefficient. Highest correlation coefficient was showed by rs117501019 (r=0.102) while the lowest was rs75314265. Difference was invalid to evaluate within rs79572840 population as 100% of the population in both non-severe and severe groups were homozygote (Table 5).

Table 5. HLA polymorphisms in study and the correlation to severe COVID-19

SNP	r (correlation coefficient)	*p*
rs75314265	0.007	0.956
rs79572840	-	-
rs75314265	0.058	0.477
rs117501019	0.102	0.336

4. Discussion

Despite multiple studies having been conducted and several risk factors associated with severe COVID-19 are figured, data on genetic involvement as host risk factor is limited (15). HLA region is the most variable in the human genome and the influence of these alleles on COVID-19 disease progression may differ among populations with different genetic profiles (16). The human leukocyte antigen (HLA) gene complex encodes major histocompatibility complexes (MHC), which is crucial for exposing and presenting antigens derived from pathogens to the appropriate T lymphocytes, triggering the immune response (17).

In COVID-19, HLA gene is involved in encoding MHC class II as a part of individual's antiviral humoral and cellular immunity. In the setting of severe disease, possible higher viral load would mean the need for more MHC class II to present viral peptide to CD4+ T lymphocytes, subsequently involving more CD4+ and CD8+ T cell (cytotoxic T cell), whose activation lead to tissue destruction along with pathogen elimination. Understanding the genetic variance of the gene and its association to disease progression may provide new perspective to identify patients at high risk of high disease burden and mortality, develop plans for vaccines and pharmaceutical treatments as well as evaluating their efficacies (15, 18).

A study about the genotypes of HLA-A, HLA-C, HLA-B, and -DRB1 in 178 Japanese COVID-19 subjects investigated the HLAs' association with severe COVID-19 and found that HLA-DRB1* 09:01 was associated with severe COVID-19 (15). The association between this allele and severe COVID-19 was more significant than pre-existing medical conditions. Alongside HLA-DRB1* 09:01, HLA-DRB1*08 and HLA-DRB1*04 were the other alleles being associated with disease severity and mortality. In another meta-analysis in 2022, Dieter et al. figured alleles HLA-A*33, ACE1 Ins, and TMPRSS2 rs12329760T to be associated with protection against severe COVID-19 manifestation, while HLA-B*38, HLA-C*6, and ApoE rs429358C alleles were associated with risk for severe COVID-19 (19). As HLA-DRB1*09:01 has been readily reported to be associated with severe COVID-19, this study investigated the hypothesis specifically in Indonesian population. However, it is found in this study that HLA-DRB1*09:01 polymorphism for rs75314265, rs79572840, rs75314265 and rs117501019 show no significant association to severe manifestation of COVID-19.

The results across different studies for HLA polymorphism's impact to disease severity have been inconsistent and sometimes conflicting (9). Hence, its clinical relation to COVID-19 remains controversial. In a recent meta-analysis studying how genetic background among different populations may influence COVID-19 susceptibility and severity, the impact of a certain allele towards the disease's clinical course were difficult to evaluate due to the reviewed studies having subjects of different ethnicities (18). HLA alleles vary greatly across different populations. Certain alleles that have been identified as risk alleles in one association study may have little to no significance in other population because the studied alleles are uncommon in the population. Different HLA alleles may have different peptide binding site while being able to bind to the same virus. Unfortunately, data on allele distribution in normal South Asian population is scarce (14).

Besides of the presence of a certain HLA type being uncommon outside a particular population, studies about HLA polymorphism often have limited number of subjects, making it difficult to figure significant association of tested variables and results among different studies become inconsistent (14, 20). Furthermore, the impact other factors such as gender and age which may influence disease predisposition could not be assessed due to limited number of studies for each SNP. According to a systematic review and meta-analysis by Dieter, et al. in 2022, different studies also had different definition of "severe" COVID-19, putting the comparison into more risk of bias (19).

Based on our finding that HLA-DRB1*09:01 gene polymorphism did not associate with COVID-19 severity, it is indicated that variance in the particular gene may not limit a subject's immunological response against SARS-CoV-2. However, it is to be noted that actual immunological response was not directly measured in this study (with serological or cellular study), but instead severe disease manifestation was addressed as a proxy of one's immune response quality. Incomplete understanding about peptide synthesis and presentation via HLA, as well as direct impairment of T cell epitope presentation by SARS-CoV-2 may alter outcomes of predicted immunogenic response (21).

This study found that all population for rs79572840 were heterozygote C/G. Rs79572840 is a single nucleotide variant (SNV) located in HLA-DRB5 in chromosome 6. Some of the subjects being excluded for showing "undetermined" result on genotyping study may be homozygote. Earlier study by Liu, et al. mentioned that heterozygote polymorphism serves as biological mechanism to balance natural selection and is advantageous in human evolution (21).

Some subject characteristics in this study showed significant association to severe COVID-19, serving as risk factors of severity. Those factors were age ($p = 0.011$), presence of comorbidity ($p = 0,030$) and NLR from peripheral blood examination ($p = 0,001$). This finding is consistent to many earlier studies, as it is mentioned by Mohan et al. that age and comorbidities successfully predict COVID-19 outcome regardless of innate immune response severity (22). Neutrophilia and NLR have also showed consistent association to disease severity throughout worldwide studies, making it a cheap, simple and readily available marker of severity and mortality of COVID-19 (23). Older age and higher NLR were consistently associated with severe COVID-19 after regression analysis with another independent variables in this study including sex, presence of comorbidities, and HLA-DRB1*09:01 polymorphism itself (Table 5).

COVID-19 severity may also be greatly affected by comorbidities. The conditions that most often lead to the mortality in patients with chronic diseases are arterial hypertension, diabetes mellitus, metabolic syndrome, coronary heart disease, chronic obstructive pulmonary disease (COPD), nicotine addiction, inflammatory bowel diseases, and cancers.[4] Presence of comorbidities potentially acted as a factor confounding HLA effect towards severity.[24]

In conclusion, this study found that HLA-DRB1*09:01 is not associated with severe COVID-19 in Indonesian population. The main limitations of this study are presence of factors other than genetic variance that potentially confounded subjects' clinical outcome such as age and comorbidities, as well as limited understanding on individual genetic information and actual immunological response. Gene polymorphism is naturally found between different populations, hence some HLA can both offer protection and cause harm for the same disease throughout different studies, depending on the population and research design. Further studies involving more cases over various populations may be beneficial to better understand genetic markers linked to clinical outcomes of COVID-19.

Ethical statement

This study has obtained ethical approval from Ethical Committee of Sebelas Maret University under the reference number 46/UN27.06.6.1/KEP/EC/2021.

Conflict of interest

The authors state that there are no conflicts of interest in this study.

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None to declare.

Authors' contributions

Concept: R.O., H.A., O.G.R., Design: R.O., H.A., O.G.R., Data Collection or Processing: R.O., H.A., Analysis or Interpretation: R.O., H.A., Literature Search: R.O., H.A., O.G.R., Writing: R.O., O.G.R.

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Effects of smoking dependence level on attentional bias in smokers

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Abstract

There is uncertainty regarding whether the degree of nicotine dependence directly impacts the increase or decrease of attentional biases towards smoking-related cues. Their relationship remains unclear. Therefore, we compared the attentional bias among smokers with varying levels of dependence. We conducted the study with 114 smokers, utilizing a Modified Stroop Paradigm and Dot Probe Tasks to assess attentional biases toward both smoking-related and neutral cues. The objective was to compare attention bias levels among individuals with varying degrees of dependence. At various levels of nicotine dependence -whether mild, moderate, or severe- individuals demonstrated a consistent attentional bias towards smoking-related cues, suggesting no significant distinction between these groups. The results of the study show that there was no discernable correlation between attentional bias concerning smoking-related stimuli and the level of nicotine dependency. The results are discussed together with the existing studies.

Keywords: attentional bias, nicotine dependence, addiction, dot probe tasks

1. Introduction

Stimuli associated with addictive substances play a significant role in substance-seeking behavior (1). When users are exposed to cues related to the substance, it leads to various physiological changes such as increased heart rate, resulting in arousal and craving in individuals (2). Numerous theories related to addiction highlight the powerful impact of these cues in perpetuating addictive behavior and hindering attempts to quit (3, 4). It is known that this effect has a shaping influence on many cognitive processes, especially attentional functions (5, 6).

The phenomenon in which attention becomes disproportionately focused on a specific categorical stimulus over other stimuli is referred to as attentional bias (7). Attentional bias is also frequently observed among tobacco users (8–11), similar to alcohol (12), heroin (13) and cocaine (14) users. The studies have shown that the images or words related to smoking evoke thoughts of smoking, trigger the act of smoking (10) and lead to changes in brain neural activity (15). Additionally, research indicates that smokers exhibit a significant attentional bias towards smoking-related cues compared to non-smokers (16–18).

There are several prominent methods used in measuring attentional bias. The most commonly employed method is the visual probe test, also known as the dot-probe test (19). In this test, a pair of visual stimuli is presented on the screen for a specific duration, after which it disappears, followed by the presentation of a neutral cue, such as a "dot." Participants are instructed to rapidly indicate the direction of the cue using the arrow keys on the keyboard, and their responses are recorded at a millisecond level (20). Another method is the "Modified Stroop Test." Unlike the classic Stroop test, in this version, an addictive stimulus is presented in one of four colors, and individuals are asked to identify the color while disregarding the written word. If the Stroop effect is detected in the individual, it indicates a positive attentional bias (21).

There are several variables that can alter an individual's level of attentional bias towards addictive substances (5). One of these is the tendency for substance users to display attentional bias towards substance-related cues compared to neutral cues (22, 23). It has been observed that a similar effect is not present in non-users (24). Another crucial factor is cravings. Studies have demonstrated that craving increases an individual's attentional bias (5, 25, 26).

A review of the literature reveals that the level of substance use is associated with attentional bias (27, 28). The findings indicate that individuals heavily dependent on substances exhibit a greater focus on substance-related cues compared to those with mild addiction (29, 30). Similar results apply to cigarette smokers; however, unlike other addictive substances, both positive and negative correlations have been observed in these studies (31–34).

Considering international studies, there appears to be a

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scarcity of studies investigating the relationship between nicotine dependency levels and attentional bias. Similarly, no research on this subject has been conducted in our country. This circumstance highlights the original and innovative aspect of the current study, aimed at contributing to the literature and clinical practices.

The primary objective of this study was to ascertain whether there exists a noticeable distinction in attentional bias—a crucial cognitive effect implicated in perpetuating addiction—as the level of physiological dependence on cigarettes intensifies.

2. Materials and methods

2.1. Sample

The study was conducted in the psychology laboratory of Prof. Dr. Cemil Taşçıoğlu City Hospital between October 1, 2021, and October 1, 2022, spanning a duration of twelve months. Participants were recruited from outpatient clinics such as cardiology, internal medicine, and chest diseases, where individuals with high motivation for smoking cessation were presumed to be present, in addition to healthcare professionals like nurses, psychologists, and doctors. The inclusion criteria for participation consisted of individuals aged 18 to 65, with a minimum level of literacy, an expressed desire to quit smoking, and a score of 3 or higher on the Fagestrom Test for Nicotine Dependence. Participants were also required to volunteer with written consent. Exclusion criteria included any cognitive or physical impairment hindering the understanding and completion of tests, undergoing psychiatric diagnosis or treatment, having a history of or current severe neurological illness, and receiving smoking cessation therapy during the study period.

All procedures for the participants were conducted by the clinical psychologist (principal investigator). A total of 114 participants were initially engaged in the study. However, three individuals who made more than 30% errors in computer-based test applications (analyzed using the box and whisker plot method) and three participants with high levels of depression and anxiety were excluded from the study. Additionally, due to computer program errors, data from two participants were inaccessible. Three participants were excluded from the study as they scored less than 3 on the Fagerstrom Test for Nicotine Dependence. Thus, the analysis was based on data from a total of 103 individuals.

2.2. Process

The study was conducted in the psychology laboratory of the hospital. Volunteers who participated in the study were given appointments and were instructed not to smoke for at least 1 hour before the study. On the day of their appointment, after a brief assessment, they were taken to a room prepared in advance. All assessments were performed by investigator in charge. In sequence, the participants completed the "Sociodemographic Data Form," "Beck Anxiety Scale," "Beck Depression Scale," "Fagerstrom Test for Nicotine Dependence," "Questionnaire of Smoking Urges," "Visual Probe Test," and the "Stroop Test for Smokers." After the self-report scales were administered, participants were led to a dark room where two computer-based tests were conducted, concluding the study.

2.3. Scales

Sociodemographic Data Form: This is a form that examines sociodemographic information and smoking habits of the participants. The form was created by the researchers specifically for the study being presented.

Modified Dot-Probe Test/Visual Sign Test: A computerized visual probe test is used to assess attentional bias. During test, pairs of visual stimuli or words are presented horizontally side by side on the computer screen. These images remain on the screen for four different durations (200 ms, 500 ms, 1000 ms, and 1500 ms) before disappearing, followed by the appearance of a neutral cue (e.g., a dot) at the location of one of the previously displayed stimuli. Participants are then prompted to indicate the location of this cue using the arrow keys on the keyboard (20).

The visuals utilized in this study were obtained with special permission from "The Center for the Study of Emotion and Attention" at the University of Florida (35). Following appropriate pairings and evaluation by five experts, these visuals were included in the pilot study (18,36). A total of 12 appropriate smoking-related visuals and 12 neutral visuals were used in the study.

Smoking Stroop Test (SST): In the assessment of attentional bias, a modified Stroop test developed by Esra Kısacık and Zehra Çakır in 2019 for smoking-related cues was used. The test is designed to measure smokers' attentional bias towards smoking stimuli. Consisting of 9 neutral and 9 smoking-related verbal cues, this assessment tool 4 different colors similar to those in the classic Stroop test. All cues consist of words. Volunteers are instructed to press a button representing the color of the word as quickly as possible, regardless of the word's content, and their response time is recorded in milliseconds (37,38). The study found a Cronbach's alpha internal consistency coefficient of 0.78 for neutral cues (with item-total correlations ranging between 0.26 and 0.69) and a Cronbach's alpha of 0.83 for smoking-related cues (with item-total correlations ranging between 0.38 and 0.68) (38).

Fagerstrom Nicotine Dependence Test (FNDT***):*** The Fagerstrom Test for Nicotine Dependence (FNDT), which measures physical dependence on nicotine, consists of a total of 6 questions. An increase in the Fagerstrom score indicates an increase in an individual's physical dependence on nicotine. The test categorizes the degree of dependence into 5 groups: 'very low dependence,' 'low dependence,' 'moderate dependence,' 'high dependence,' and 'very high dependence' (39). The validation and reliability study of its Turkish version was conducted by Uysal and colleagues (Cronbach’s alpha = 0.56) (40).

Smoking Desire Inventory (SDI): An individual's desire to smoke is measured using a seven-point Likert scale. Scores range from a minimum of 10 to a maximum of 70. The validity and reliability of the scale, determined by a Cronbach's alpha value of 0.92 for the Turkish version, were established by Demirezen and Kurçer (41).

Beck Depression Scale: Beck introduced this assessment tool in 1961 with the aim of objectively identifying symptoms of depression. Its Turkish validity and reliability were examined by Tegin and Hisli, revealing correlation coefficients of r=0.80 and r=0.74, respectively (42).

Beck Anxiety Scale: This scale, developed by Beck in 1988 to assess anxiety symptoms, aims to objectively evaluate anxiety symptoms. Its Turkish validity and reliability were established by Ulusoy and colleagues (Cronbach's alpha = 0.93) (43).

2.4. Data Analysis

The study was analyzed using the SPSS 25.0 software. In the visual probe test, 103 participants generated 103 data sets, resulting in a total of 14,832 observation points. Prior to calculating central dispersion values for the observation data, erroneous entries, and responses below 200 ms and above 2000 ms, were excluded from the study (2%). The remaining data were transformed into z-scores, and observations exceeding three standard deviations were considered outliers and not included in the analysis (2%) (44).

Differences in terms of sociodemographic characteristics were evaluated using chi-square tests. The normality of the data sets was assessed using the Kolmogorov-Smirnov test, and checks were performed for kurtosis and skewness. One-way analysis of variance (ANOVA) was employed for comparisons involving three or more groups, with post hoc analyses, such as Tukey tests, being conducted to ascertain the differences between the groups. All statistical analyses were conducted with a significance level of $p<0.05$.

3. Results

Table 1 indicates that 64 participants (62%) were male, 46 (45%) were university graduates, and 62 (60%) were healthcare professionals. Among the participants, 25 (24%) were between the ages of 18 and 24, 36 (35%) were between the ages of 25 and 34, and 10 (10%) were between the ages of 35 and 44. Regarding sociodemographic characteristics, differences in distributions were observed in terms of age range and education level ($p<0.05$), while no significant differences were found in gender and reason for participation ($p>0.05$).

Table 2 indicates that 60 individuals (58%) initiated smoking before the age of 18, while 39 (38%) smoked for 1-10 years. The daily cigarette consumption for 39 participants (32%) ranged between 11-20 cigarettes. No significant differences were observed in the distributions of smoking habits based on the age range of smoking initiation and the amount of cigarettes consumed ($p>0.05$). Nevertheless, variations were observed in the distribution based on the number of years of smoking.

Table 1. Comparison of the distribution of participants' sociodemographic data

Sociodemographic Characteristics	n	%	X^2
Gender			
Woman	39	62	0.129
Male	64	38	
Education			
Primary School	11	11	0.006
Middle School	18	17	
High School	28	27	
University	46	45	
Age Range			
18-24 Years	25	24	0.008
25-34 Years	36	35	
35-44 Years	29	28	
45-54 Years	10	10	
55-64 Years	3	3	
Reason for Arrival			
Health Worker	62	60	0.566
Outpatient Clinic	41	40	

Table 2. The distribution of participants' smoking data

Cigarette Use Characteristics	n	%	X2
Smoking Starting Age			
Before 18 Years of Age	60	58	0.102
18-24 Years	43	42	
Year of Cigarette Use			
1-10 Years	39	38	0.003
11-20 Years	33	32	
21-30 Years	20	19	
31-40 Years	11	11	
Amount of Cigarette Use			
1-10 pieces	38	37	0.434
11-20 pieces	40	39	
21 Pieces and above	25	24	

Table 3 presents a comparison of the means of attentional bias in the visual probe test among the research groups. As indicated in Table 3, no significant differences were observed among the research groups ($p>0.05$).

Table 3. Descriptive and one-way ANOVA results based on the visual cue test for groups classified by the Fagerstrom Test for Nicotine Dependence

Variable	n	$\bar{x}$	σ	σ_M	F	p
Low Level	44	0.20	0.018	0.002	0.827	0.482
Moderate	16	0.022	0.017	0.004		
High Level	21	0.015	0.014	0.003		
Very High Level	22	0.23	0.019	0.004		

Table 4 presents a comparison of the means of the Smoking Stroop Test (SST) among the research groups. As indicated in Table 4, no significant differences were observed among the research groups ($p>0.05$).

Table 4. Descriptive and one-way ANOVA results based on the Smoking Stroop Test (SST) for groups classified by the Fagerstrom Test for Nicotine Dependence

Variable	n	$\bar{x}$	σ	σ_M	F	p
Low Level	44	0.22	0.020	0.003	0.618	0.605
Moderate	16	0.029	0.022	0.005		
High Level	21	0.021	0.019	0.004		
Very High Level	22	0.21	0.023	0.005		

Table 5 presents the descriptive and one-factor ANOVA results of the groups classified using the Fagerström Nicotine Dependence Test according to the SIAQ. Additionally, it presents the comparison of the means of the Smoking Desire Inventory among the research groups. The results indicate that there was no significant difference found among the research groups ($p>0.05$).

Table 5. Descriptive and one-way ANOVA results based on the Smoking Desire Inventory (SDI) for groups classified by the Fagerstrom Test for Nicotine Dependence

Variable	n	$\bar{x}$	σ	σ_M	F	p
Low Level	44	31.70	14.248	2.148	1.678	0.177
Moderate	16	39.75	15.847	3.962		
High Level	21	34.81	12.890	2.813		
Very High Level	22	39	18.989	4.048		

4. Discussion

The objective of the current study is to elucidate the influence of smokers' levels of dependence on attentional bias. The study found that the level of dependence among smokers did not significantly affect attentional bias or craving. In both the Smoking Stroop Test (SST) and the visual probe test, smokers exhibited a positive attentional bias, consistent with Tiffany's impulsivity theory related to substance dependence and Robinson and Berridge's addiction theory (26, 45). Nevertheless, no differentiation based on the level of dependence was observed in either test.

In previous studies on alcohol consumption, it has been observed that attentional bias varies based on the quantity of alcohol consumed (46–48). However, these studies employed a single cue onset asynchrony, and all cues remained on the screen for a similar duration. In contrast, our study featured stimuli (visuals) displayed for 4 different durations. Although numerous studies have indicated that variations in cue onset asynchrony among smokers are evident, there are also conflicting studies that assert the opposite (33, 49). This inconsistency makes it challenging to establish a causal relationship between cue onset asynchrony and other factors.

While some studies on other substances align with our findings (46, 50), the general consensus suggests that as the level of dependence increases, attentional bias also increases (27, 47, 51, 52). The variations in the parameters determining the level of dependence make direct comparisons between studies challenging (29, 47). In our study, the degree of nicotine dependency was determined based on the FNDT test, and a subset representing rare users and those with very low dependence was excluded from the analysis (39, 47). In our research, the categorization of groups based on physiological dependency enables us to reach objective conclusions.

The results of studies on cigarette users are more complex than those of studies on users of other substances. While some studies have indicated that smokers' attentional bias increases with higher levels of dependency (34, 53, 54), others have proposed that the attentional bias increases as dependency levels decrease (31, 32). Moreover, our research findings align with those of other studies that indicate that the level of dependence does not significantly impact attentional bias (16, 55). There could be several reasons for inconsistencies among studies. In our research, participants were instructed not to smoke for at least 1 hour before the assessment. Similarly, in other studies, participants were asked to refrain from smoking before assessments, yet there isn't a complete consensus on the impact of abstinence on attentional bias (31, 56). Another crucial aspect is the sample size; the participant count in our study is higher than that in other research. This circumstance complicates understanding the regression among levels of dependence. Due to this reason, it complicates our understanding of the relationship between levels of smoking dependence and attentional bias.

Studies indicate that as the level of dependence increases among cigarette users, cravings, motivation to quit smoking, and the quantity of cigarette consumption also increase (57). However, in our research, we observed no significant differences in cravings among the groups. While some studies with similar findings suggest that cravings do not directly impact attentional bias (55), conflicting research also exists that proposes otherwise (31).

It is known that individuals who continue to smoke exhibit a stronger attentional bias towards smoking-related cues compared to those who have quit (9, 55). Similarly, studies showing the effect of increased motivation to smoke on individuals' attentional bias (58). These findings collectively indicate that cigarette craving, smoking status, and motivation to smoke may have some effects on attentional bias towards smoking cues (31, 32). However, due to the absence of studies comparing the attentional bias and desire between individuals with smoking cessation motivation and those without, there is no clear evidence on whether the desire to quit smoking directly affects attentional bias and cigarette craving.

Contrary to expectations, the results indicated that there was no relationship between nicotine dependence levels among cigarette users and attentional bias towards smoking-related cues. The findings of this study, which was conducted with a larger sample size than previous research, are expected to contribute to further advancements in the field of research.

Ethical Statement

The ethics committee approval of the study was obtained from Istanbul Prof. Dr. Cemil Taşçıoğlu City Hospital Clinical Research Ethics Committee (Date: 13.09.2021; Decision No: 317) A signed consent form was obtained from each participant

in the study.

Conflict of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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Authors' contributions

Concept: U.K., B.Ö., Design: U.K., B.Ö., Data Collection or Processing: U.K., B.Ö., Analysis or Interpretation: U.K., B.Ö., Literature Search: U.K., B.Ö., Writing: U.K., B.Ö.

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Diagnostic value of acute phase reactants and scores used in the diagnosis of cholangitis in patients with purulent cholangitis

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Abstract

Acute cholangitis is a clinical condition that occurs due to stasis and infection of the biliary system, which can recur if left untreated and may lead to life-threatening consequences. Early diagnosis, severity scoring based on the Tokyo Criteria at the time of diagnosis, and prompt initiation of treatment can help prevent mortality. This article aims to retrospectively review patients diagnosed with acute cholangitis by observing pus drainage during Endoscopic Retrograde Cholangiopancreatography (ERCP), determine the diagnostic values and prognostic effects of data obtained from complete blood counts and evaluate the diagnostic and severity criteria of acute cholangitis, including 331 patients who underwent the procedure and exhibited pus drainage, along with 300 healthy volunteers. We observed that the most common cause in the etiology of acute cholangitis was choledocholithiasis. There was a statistically significant difference between the healthy control group and the acute cholangitis group in terms of leukocyte count (WBC), red cell distribution width (RDW), platelet count (PLT), mean platelet volume/platelet count (MPV/PLT), neutrophil count/lymphocyte count ratio (NLR), and platelet count/lymphocyte count ratio (PLR) values. ROC analysis revealed that RDW, NLR, and PLR values had high sensitivity and specificity in distinguishing the acute cholangitis group from the healthy group. When we compared acute cholangitis severity groups, there was a statistically significant difference between the groups in terms of WBC, RDW, PLT, MPV/PLT, NLR, and PLR values. Due to the high sensitivity and specificity and easy accessibility of WBC, RDW, PLT, NLR, and PLR tests in diagnosing cholangitis, a closer and careful evaluation of these tests would be beneficial for early diagnosis and effective treatment of the disease. Additionally, this study confirms the high accuracy of the Tokyo Criteria in diagnosing acute cholangitis, highlighting the inadequacies of other clinical scoring systems.

Keywords: Acute cholangitis, Endoscopic Retrograde Cholangiopancreatography (ERCP), Tokyo Criteria

1. Introduction

Acute cholangitis is a condition that arises from the stasis and infection of the biliary system, which can lead to life-threatening consequences. A cholangitis attack can result in fatality, may recur if the causing obstruction remains untreated, and can progress to liver abscess or biliary cirrhosis (1). The pathogenesis of acute cholangitis involves choledocholithiasis, benign or malignant strictures, biliary-enteric anatomical anomalies, and the failure of a permanent biliary stent to function (2).

In a study on populations in Europe and North America, researchers found that symptomatic gallstone disease developed in 20% of patients with common bile duct stones, while only 0.2% experienced acute bacterial cholangitis (3).

In 1887, the clinical presentation characterized by fever, right upper quadrant pain, and jaundice coined the term Charcot's Triad. Subsequently, in 1959, the altered mental status and signs of septic shock accompanying this clinical picture led to the term Reynolds' Pentad. Despite the high specificity, the described Charcot's Triad and Reynolds' Pentad have low sensitivity, leading to the establishment of the Tokyo Criteria in 2007, 2013, and 2018 (2, 3). The Tokyo Criteria aims to provide a more accurate diagnosis of acute cholangitis and categorize it into three groups based on severity (4). Determining the severity of a patient's condition in acute cholangitis and initiating early medical treatment (fluid support, appropriate antibiotic therapy) is crucial in all cases.

Subsequently, identifying the underlying cause and ensuring biliary drainage is necessary (3, 5). In severe cases accompanied by organ failure, intensive care support should be administered (3). In encountered cases, healthcare providers should conduct laboratory tests, including a complete blood count, renal and hepatic biochemical tests, acute-phase reactants (such as erythrocyte sedimentation rate, CRP [C-Reactive Protein]), and INR. Obtaining a blood culture before starting antibiotic therapy is recommended (6). The mean platelet volume (MPV), MPV/PLT ratio, neutrophil count/lymphocyte count ratio (NLR), red cell distribution width (RDW), and platelet count/lymphocyte count ratio (PLR) obtained from the complete blood count is critical and easily accessible indicators aiding in the early detection of critical illness (7, 8).

This article aims to retrospectively review patients diagnosed with acute cholangitis by observing pus drainage during Endoscopic Retrograde Cholangiopancreatography

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(ERCP), determine the diagnostic values and prognostic effects of data obtained from complete blood counts, and evaluate the diagnostic and severity criteria of acute cholangitis.

2. Materials and Methods

After the Non-Interventional Studies Ethics Committee of Ondokuz Mayıs University Faculty of Medicine Hospital granted ethical approval with decision number 466 dated 13/10/2021, the study included a total of 331 patients who underwent ERCP with observed pus drainage during the procedure and 300 healthy blood donors between January 1, 2005, and August 1, 2021. The demographic characteristics of all patients enrolled in the study, their clinical presentations, laboratory tests at the time of diagnosis, and their admission statuses to the Intensive Care Unit (ICU) were examined. Data were analyzed using IBM Statistical Package for the Social Sciences (SPSS) V25. The Kolmogorov-Smirnov test was used to evaluate the suitability of standard distribution for quantitative data. The data comparing complete blood count parameters between the patient and healthy groups did not demonstrate normal distribution, so the researchers employed the Mann-Whitney U test for analysis.

In the groups categorized based on disease severity, the complete blood count parameters were evaluated using One-Way ANOVA for data conforming to the normal distribution and the Kruskal-Wallis H test for data that did not follow the normal distribution. The statistical significance of laboratory values that could differentiate acute cholangitis patients from the healthy group was determined by calculating the area under the ROC curve and the 95% confidence interval. A significance level of $p < 0.05$ was considered.

3. Results

This study involved 331 cases of acute cholangitis and 300 healthy volunteers. Among the acute cholangitis cases, 178 (53.8%) were male, and 153 (46.2%) were female. There was no statistically significant difference in terms of gender between the healthy control group and the acute cholangitis group. The average age of the patients was determined to be 50.5 ± 9.2 years.

Abdominal pain was present in 255 individuals (77.0%), and fever was observed in 158 cases (47.7%). Total bilirubin levels >2 mg/dL were considered jaundice and detected in 283 cases (85.0%). Eighteen patients (5.4%) required positive inotropic support, and altered consciousness was seen in 20 cases (6.0%). One hundred thirteen patients (33.5%) exhibited symptoms consistent with Charcot's triad, and seven (2.2%) showed symptoms in line with Reynold's Pentad.

Disease severity was evaluated based on the Tokyo Criteria classification and the need for intensive care unit (ICU) admission. In the first-degree cholangitis group, no patient required intensive care support. In the second-degree cholangitis group, 1 (0.7%) patient required intensive care unit admission, while in the third-degree group, 12 (20%) patients needed intensive care unit admission. A statistically significant difference in the need for ICU care was observed among the three groups ($p < 0.001$).

Acute cholangitis diagnosed cases revealed choledocholithiasis in 179 instances (54.1%), with 58 patients (17.5%) having a history of cholecystectomy and malignant stricture observed in eighty cases (24.2%). The analysis of the complete blood count statistics for the cases was conducted and presented in Table 1.

Table 1. Organizing descriptive data of patient group laboratory results

	n	Average	Std. Deviation	Median	Minimum	Maximum
WBC (10^9/L)	331	11,8	6,5			
RDW (%)	331			15	11,7	27
Monocyte (10^9/L)	328	0,62	0,46			
Lymphocyte (10^9/L)	328			1	0,15	5,7
MPV (fL)	324	9,2	1,7			
Neutrophil (%)	331			81	45	96
Neutrophil (10^9/L)	331	9,8	6,3			
PLT (10^9/L)	331	258,1	116,5			
NLR	328			8,3	1,1	78,7
PLR	328			248,7	11,5	1761,3
MPV/PLT	325			3,6	0	115,4

WBC:White blood cell count, RDW: Red cell distribution, MPV: Mean platelet volume, PLT: Platelet count, NLR: Neutrophil count/lymphocyte count ratio, PLR: Platelet count/lymphocyte count ratio, MPV/PLT:Mean platelet volume/platelet count ratio

Due to the non-normal distribution of the data, a comparison of complete blood count parameters between the patient group and the healthy control group is presented in median (minimum-maximum) format, as detailed in Table 2. Significant differences were found between the patient and control groups for WBC, RDW, lymphocyte, neutrophil, neutrophil percentage, PLT, NLR, PLR, and MPV/PLT values ($p<0.001$). However, there was no statistically significant difference between the two groups in terms of MPV value ($p=0.30$).

ROC analysis of NLO, PLO, RDW, and MPV/PLT values between the patient and control groups was performed, and the results are presented in Table 3 and Fig. 1. The value with the least difference between sensitivity and specificity in the analysis for each parameter was determined as the cutoff value.

Following the categorization of severity based on the Tokyo Criteria, a comparison of complete blood count data between the groups is presented in Table 4.

Table 2. Comparison of laboratory parameters according to groups

	Patient (n=331)	Control (n=300)	P
WBC (10^9/L)	10.3 (1.7-50.2)	6.5 (3.7-43)	***<0.001***
RDW (%)	15 (12-27)	13.9 (13-18)	***<0.001***
Monocyte (10^9/L)	0.52 (0.05-6.3)	0.5 (0.2-7)	***0.01***
Lymphocyte (10^9/L)	1 (0.15-5.7)	2.1 (1-4)	***<0.001***
MPV (fL)	9.2 (6-20)	9.1 (5-11)e	0.30
Neutrophil (%)	81 (45-96)	59.0 (38-75)	***<0.001***
Neutrophil (10^9/L)	7.9 (1.1-47.7)	3.9 (1.9-9.5)	***<0.001***
PLT (10^9/L)	244 (13-784)	217 (130-799)	***<0.001***
NLR	8.3 (1.1-78.7)	1.8 (0.7-4.5)	***<0.001***
PLR	248.7 (11.5-134.2)	100.9 (48.1-399.5)	***<0.001***
MPV/PLT	3.6 (1-115.4)	4.2 (1-251.3)	***<0.001***

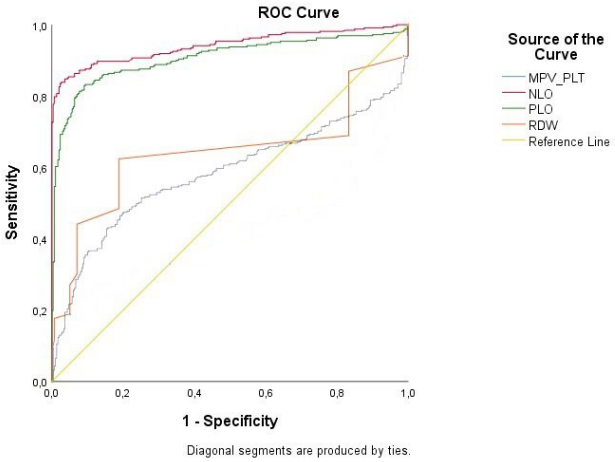

Fig. 1. ROC analysis grafics

Table 3. Diagnostic test results for acute cholangitis

	NLR	PLR	RDW	MPV/PLT
Cut Value	≥2.64	≥130.98	≥14.05	≥4.02
AUC (%95 CI)	0.94 (0.92-0.96)	0.91 (0.88-0.93)	0.65 (0.61-0.70)	0.59 (0.54-0.63)
Sensitivity	89 (85.1-92.2)	85.7 (81.1-89.3)	63.4 (58-68.6)	42.2 (36.7-47.7)
Specificity	88.6 (84.5-92)	85.6 (81.1-89.4)	81.2 (76.3-85.5)	42.1 (36.5-48)
PPV	89.6 (86.2-92.2)	86.7 (83.2-89.6)	79.0 (74.5-82.8)	44.2 (40.3-48.2)
NPV	88.4 (84.4-91)	84.5 (80.6-87.7)	66.7 (63.2-70)	40.1 (36.3-44.1)
Accuracy Rate	88.8 (86.1-92)	85.7 (82.6-88.3)	71.9 (68.2-75.3)	42.2 (38.2-46.1)

AUC: Area under curve, PPV:Positive predictive value, NPV:Negative predictive value

Table 4. Complete blood count parameters according to disease severity

	Disease Severity			Statistics	
	1. Degree (Mild)	2. Degree (Moderate)	3. Degree (Severe)	Test Statistics	P
WBC (10^9/L)	8.9±3.7[b]	13.1±6.3[a]	14.7±9.0[a]	30.555	***<0.001****
RDW (%)	14.7±2[b]	15.7±2.3[a]	15.8±2.2[a]	7.689	***<0.001****
Monocyte (10^9/L)	0.57±0.58	0.66±0.35	0.63±0.36	1.214	0.298*
Lymphocyte (10^9/L)	1.2 (0.19- 3.4)[a]	1 (0.15-5.7)[b]	0.74 (0.29-2.7)[c]	22.017	***<0.001^***
MPV (fL)	9.2±1.8[b]	9±1.6[b]	9.9±1.8[a]	6.356	***0.002****
Neutrophil (%)	74 (45-95)[a]	84 (53-96)[b]	88 (60-96)[b]	42.173	***<0.001^***
Neutrophil (10^9/L)	6.8±3.7[b]	11.1±6.2[a]	12.8±8.4[a]	33.199	***<0.001****
PLT (10^9/L)	273±105[b]	274.7±114.9[b]	186.7±118.4[a]	14.917	***<0.001****
NLR	4.7 (1.1 -72.8)[a]	9.7(1.2 -78.7)[b]	14.6 (1.9 -70)[b]	47.037	***<0.001^***
PLR	212 (54.7-989.5)[a]	265.3(22.1-1761.3)[b]	228.7 (11.5-835.7)[b]	8.092	***0.017^***
MPV/PLT	4.6±8.8[b]	3.8±1.8[b]	10.5±16.6[a]	5.145	***0.007****

a-c: There is no difference between groups with the same letter for each measurement value *One-Way ANOVA, ^Kruskal Wallis H Test

4. Discussion

Acute cholangitis is a potentially life-threatening condition arising from stasis and infection in the biliary system (1). Initially, reviewing vital signs in every patient suspected of acute cholangitis is advised. If signs indicating urgent intervention due to organ or system failure are identified, supportive therapy should be initiated without delaying a definitive diagnosis (25). An attack of cholangitis can lead to fatal consequences, and if the causing obstruction is left untreated, it can recur progress to liver abscess, or biliary cirrhosis (1).

The average age of acute cholangitis cases in the literature is reported to be between 50-60 years old, with no difference in the frequency of cholangitis between genders (9). Our study's average age of patients diagnosed with acute cholangitis is similar to the literature. Statistically, there is no significant difference between genders, consistent with the literature.

The most common cause of biliary tract obstructions is choledocholithiasis (28-70%), followed by benign or malignant strictures, strictures related to previous surgeries, sclerosing cholangitis, and past biliary stent obstructions (3, 5, 10). Acute or chronic pancreatitis, autoimmune cholangitis, and congenital anomalies cause benign strictures, pancreatic cancer, gallbladder cancer, cholangiocarcinomas, and liver metastases cause malignant strictures (11). Similarly to previous studies, our study found choledocholithiasis to be the most frequent etiology.

The most commonly observed symptoms and signs in patients diagnosed with acute cholangitis are fever, abdominal pain, and jaundice, also known as Charcot's Triad. The incidence of all three symptoms occurring together ranges from 15.4% to 72% of cases. In addition to Charcot's Triad, altered mental status and signs of shock (Reynolds' Pentad) are

observed in 3.5% to 7.7% of cases (2). Tokyo Criteria has been found to have a sensitivity of over 90% for diagnosing acute cholangitis (12). Consistent with the literature, we believe that while Charcot's Triad and Reynolds' Pentad describe a small portion of acute cholangitis cases, they are insufficient for diagnosis. Therefore, considering that all patients meet the Tokyo Criteria, we believe that the Tokyo Criteria have high sensitivity in diagnosing acute cholangitis.

In a study comparing patients with septic or non-septic acute cholangitis with an infectious non-control group conducted by Jiang et al., the white blood cell count was significantly higher in both septic and non-septic groups compared to the control group. However, no significant difference was observed between the septic and non-septic groups in acute cholangitis (13). In Boey et al.'s study, the white blood cell count was above 10x10^9/L in 82% of acute cholangitis cases (1). As seen in the literature, the white blood cell count is valuable in diagnosing and classifying the severity of acute cholangitis.

In a study comparing neonates diagnosed with sepsis and a control group, the RDW level was significantly higher in the suspected or proven neonatal sepsis group compared to the control group (14). In a study on preterm and late-onset sepsis-diagnosed neonates, when the RDW threshold was set at 19.5, it was associated with 87% sensitivity and 81% specificity (15). Due to the significantly higher RDW value in acute cholangitis patients compared to healthy individuals and its correlation with the severity of acute cholangitis, RDW should be used as an adjunct laboratory value in diagnosing acute cholangitis

Qin et al.'s study indicated that a platelet count below 50x10^9/L in acute cholangitis cases indicates severe infection (16). In a study of critically ill patients, irrespective of the reason for admission, thrombocytopenia was found in 23% with PLT<100x10^9/L and 10% with PLT<50x10^9/L, showing a significant association with longer hospital stays and higher mortality (17). Another study on septic patients requiring vasopressors and monitoring in intensive care units found thrombocytopenia (<15x10^9/L) in 58% of cases (18). It has been observed that as the severity of the disease increases in acute cholangitis patients, the platelet count decreases. This suggests that the disease course might be severe if there is thrombocytopenia at the time of diagnosis or if thrombocytopenia develops during patient follow-up.

Golwala et al. observed a mortality rate of 65% in pediatric patients with an MPV/PLT ratio exceeding 3.45 (19). In a retrospective analysis of 1143 patients in an intensive care unit categorized by their 1-year mortality status, the deceased group showed a higher MPV/PLT ratio compared to the surviving group (8). Considering the decreased platelet count and increased precursor cell production from the bone marrow in acute cholangitis patients and the correlation of MPV/PLT ratio with the severity of the disease, we anticipate the MPV/PLT ratio to be high in acute cholangitis patients and increasing with disease severity.

In a study examining pediatric patients with bacteremia, NLR was identified as an indicator of sepsis and a determinant of antibiotic therapy duration (20). A meta-analysis of 14 studies investigating the relationship between NLR and prognosis in sepsis found a significant association between increased NLR and higher mortality. The threshold value for NLR ranged from 4.36 to 23.8 in the studies reviewed, with no definitive optimal value determined through meta-analysis (21). The high NLR value is significant in diagnosing acute cholangitis, correlating with disease severity and possessing high sensitivity and specificity, making it a usable parameter in diagnosing and following acute cholangitis.

A high PLR value can serve as an auxiliary test for early recognition of sepsis. In a study comparing neonates diagnosed with neonatal sepsis and a control group, the group diagnosed with neonatal sepsis showed significantly higher PLR (22). In a study comparing the 30-day mortality of patients operated for acute mesenteric ischemia, a significant association was found between high PLR and high mortality (23). In a study comparing patients diagnosed with community-acquired pneumonia and a control group, the inpatient or outpatient-treated group showed higher PLR levels than the control group. However, no difference was found between inpatients and outpatients (24). In a study investigating early prognostic parameters for septic shock in patients treated with granulocyte colony-stimulating factor (G-CSF) following chemotherapy, a significant association was found between high PLR levels and 1-month mortality (25). On the other hand, a study conducted on patients with emphysematous pyelonephritis found a significant association between low PLR levels and sepsis. The study used a PLR threshold of <18.4 and considered it an independent risk factor for septic shock (26). However, a retrospective analysis of 249 patients monitored in the intensive care unit following emergency abdominal surgery found no significant association between mortality and PLR (27). After evaluating the data of patients in our study and reviewing relevant literature examples, we advocate considering elevated PLR levels as an essential factor in diagnosing acute cholangitis due to their notably high sensitivity and specificity rates among these patients.

This study, assessing patients diagnosed with acute cholangitis through pus drainage during ERCP, asserts that the WBC, RDW, PLT, NLR, and PLR tests merit meticulous consideration. Their high sensitivity, specificity, and easy accessibility collectively support their potential to facilitate early disease detection and effective treatment strategies. Additionally, alongside the inadequacies of other clinical scoring systems, our study reaffirms the high accuracy rate of the Tokyo Criteria in diagnosing acute cholangitis.

Conflict of interest
The authors declared no conflict of interest.

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None to declare.

Authors' contributions
Concept: M.Ö.K., Design: M.Ö.K., Data Collection or Processing: M.Ö.K., Analysis or Interpretation: M.Ö.K., Ö.Y.Ç., Literature Search: M.Ö.K., Ö.Y.Ç., Writing: M.Ö.K.

Ethical Statement
Approval was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 13/10/2021 and the number of ethical committee decisions is 2021/466.

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Right ventricular function assessment in healthy people using tissue doppler imaging

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Abstract

A growing body of evidence is being gathered regarding the Right Ventricle's (RV) unique function, adaptation, and compensatory role. A widely accessible diagnostic technique, such as echocardiography, should probably enable prompt diagnosis and treatment assessment at the initial stage. The aim of the study was to assess the structural and functional features of the RV in healthy individuals. The study included 185 healthy adults whose ages varied from 16 to 85 years. Everyone who participated in the study had an echocardiographic examination using a standard method and an RV Tissue Doppler Imaging TDI in pulsed mode from the base of the free wall. On the RV TDI, we measured the velocity of the s, e, and waves, and the duration of Isometric Contraction Times (ICT), Ejection Time (ET), and Isometric Relaxation Time (IRT) in milliseconds. In 51% of participants, the e/a ratio was <1. It was detected in 21% of people under 40 years of age and 64% of cases in people over 40. The age of the patients must be taken into account while analyzing the TDI of the RV.

Keywords: cardiovascular diseases, right ventricle, tissue doppler imaging, echocardiography

1. Introduction

A growing body of evidence is being gathered regarding the RV's unique function, adaptation, and compensatory role in healthy individuals and people with cardiovascular diseases. Studies of the RV and early assessment of data changes are becoming extremely prevalent.

The RV is the connecting link of the cardiopulmonary system. Its function may change as a result of heart pathologies, as well as pulmonary diseases and other circulation disorders. Inadequate emphasis is frequently given to evaluating right ventricular function in comparison to Left Ventricle (LV). However, in cases of severe, progressive right ventricular dysfunction and pulmonary hypertension, the clinical outcome is usually poor.

The pressure-volume ratio is currently the most widely used method for assessing right ventricular systolic and diastolic function. This involves an invasive intervention, which is associated with certain risks and requires special equipment and trained medical personnel. Tissue Doppler Imaging (TDI) allows non-invasive assessment of right ventricular function (1). The popularity of the method is due to its simplicity, availability, and high sensitivity (2).

TDI is widely used to study left ventricular systolic and diastolic function (3). As TDI values are less dependent on cardiac pre- and afterload, they reflect cardiac diastolic function more accurately than Transmitral Doppler (4). Its systolic wave allows the systolic function of the LV to be assessed via the semi-quantitative method (5). In other words, analyzing the TDI curve can provide information on the systolic and diastolic function of the RV. It should be mentioned that there is still no final agreement on the normal parameters of the RV TDI, and the number of studies conducted in this direction is limited, which is the reason why this topic attracted our interest.

The aim of the study was to assess the structural, functional features, and qualitative characteristics of the RV in healthy individuals.

2. Material and Methods

The study included 185 healthy adults who did not have acute or chronic diseases of the cardiovascular or respiratory system, diabetes, etc. The average age of them was 46,16 ±12.06 years. 101 of them were female, 84 males.

Table 1. Anthropological and clinical indicators of the individuals examined

Indicator	
Quantity of patients	185
Male	84 (45.4%)
Female	101 (54.6%)
Age	46.2±12.1
Heart rate	70.79±8.54
Systolic blood pressure	122.7±13.6
Diastolic blood pressure	75.8±10.03

Anthropological and some clinical indicators of the examined people are presented in the table (Table 1).

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Everyone who took part in the study had an echocardiographic and doppler echocardiographic examination using a standard method. Research was conducted with the VIVID-7 echocardiograph. Besides the standard indicators, we measured mitral (MAPS) and tricuspid annular plane systolic excursion (TAPS) in millimeters on echocardiogram recorded in M-mode on apical four-chamber view. In the parasternal short-axis section at the level of the aortic bulb, we measured the systolic and diastolic diameters of the right ventricular outflow tract (RV out D diast, RV out D syst) and the amplitude of the systolic movement of the RV wall in millimeters. The RV outflow tract fractional shortening in systole was determined by the formula:

RVoutFS%=(RvoutDdiast- RVoutDsyst)/ RvoutDdiast%

Right ventricular color (coll TDI) and pulsed-wave TDI were recorded with the patient in the left side-lying position, transmitting from the four-chamber apical position: the lateral wall of the LV, the interventricular septum (IVS), and the lateral wall of the RV, atrioventricular valve. A 6 mm control volume was embedded on the lateral wall of the RV near the tricuspid valve attachment point. There were 4 waves represented on the TDI:

1. Positive wave, early systolic movement velocity (in the isometric contraction phase) (s1)
2. Positive wave, the maximum speed of systolic movement in the ejection phase (s)
3. Negative wave, early diastolic velocity (e)
4. Negative wave, late diastolic (atrial systole) flow velocity (a)

We measured the maximum speed of all four named waves in cm/s on the recorded Doppler during the next three heart cycles, after which we calculated their average value.

On the TDE, we also measured the duration of the phases of the heart cycle in milliseconds:

- Isometric contraction times (ICT) - from the end of the late diastolic peak to the beginning of the systolic peak

- Duration of isotonic contraction (ET) - From the beginning of the systolic peak to its end

- Isometric relaxation time (IRT) - from the end of systole to the beginning of the early diastolic peak.

The variational statistics method was used to process the material. We estimated the quantitative indicators' average and standard deviation. Microsoft Excel 2016 was used.

3. Results

The results of the conducted research are given in Table 2.

The table shows that the velocity of s1 in healthy subjects was 12.418±3.83 cm/s, s 13.39±2.51 cm/s, e- 13.17±3.38, a 13.86±4, 09. Indicators between women and men did not reliably differ from each other.

Table 2. Indicators of RV TDI in healthy individuals

Indicators	Maximum and minimum values	Standard deviation
s1 sm/sec	26 – 5	12.41±3.83
s sm/sec	24 - 8	13.39±2.51
e sm/sec	24 – 5	13.17±3.38
a sm/sec	29 – 6	13.81±4.09
e/a	2.43 – 0,38	1.02±0.38
Isom Cont (ICTRV)	128 - 33	38.22±16.83
Isom Rel (IRTRV)	70 - 1	11.79±12.99
RVET	381-219	291.44±29.14
AccTimeS1	76 - 15	39.84±9.41

s1 – the maximum velocity of the RV in the phase of isometric contraction; s- the maximum velocity of the RV in the ejection phase; e- the maximum velocity of the RV in the early diastole (fast filling) phase; a - the maximum velocity of the RV in the late diastole (right atrial systole); e/a - the ratio of early and late diastolic speeds; Isom Cont (ICTRV) - right ventricular isometric contraction time; Isom Rel (IRTRV) – right ventricular relaxation time; RVET- right ventricular ejection time; AccTimeS1- acceleration time of isometric contraction of the RV

The analysis revealed the following regularities: the s1 wave velocity was weakly correlated with the subjects' age, the tricuspid flow's A wave velocity, the s and „a“ waves of the left ventricular TDI, and was negatively correlated with the tricuspid flow E/A and the left ventricular TDI e/a ratio. The s-wave velocity was moderately correlated with the E- and A-wave velocities of the tricuspid flow and the s-wave velocity of the left ventricular TDI. The e wave velocity was moderately directly correlated with the E and „A“ wave velocities of the tricuspid flow, mitral flow E wave velocity, left ventricular TDI e wave velocity, weakly correlated with the left ventricular TDI s wave and pulmonary artery systolic flow acceleration time. Its negative correlation to age was made clear. The velocity of the a wave was in direct correlation with age and the a wave of the left ventricular TDI, in a moderate correlation with the s wave of the left ventricular TDI, in a weak correlation with the velocity of the A wave of the tricuspid flow, and in a negative correlation with the E wave of the mitral flow and the e wave of the left ventricular TDI, with e/a ratio of left ventricular TDI and pulmonary artery systolic flow acceleration time and in marked negative correlation with age, left ventricular TDI a wave. The s1/s ratio has a pronounced direct correlation with the e-wave velocity of the left ventricular TDI, a weak correlation with age and the a-wave of the left ventricular TDI, and a negative correlation with the acceleration time of the pulmonary artery systolic flow acceleration time, the e-wave of the left ventricular TDI, tricuspid and mitral flow E /A to the ratio.

In 51% of people studied, the RV TDI e/a ratio was less than one. It is noteworthy that this tendency appeared in people over 20. It was detected in 21% of people under 40 years of age and 64% of cases in people over 40.

The left ventricular TDI data, in particular, the indicators of its diastolic function (e, a waves and e/a ratio) depend to some extent on the age of the subject, which is expressed in a

decrease in the speed of the e wave and an increase in the speed of the "A" wave. e/a ratio decreases and becomes less than 1. This dependence appears in healthy people after the age of 60 and is due to the diastolic dysfunction developed against the background of age-related fibrosis of the LV. A similar trend is observed in relation to the RV, but it manifests itself much earlier.

Echocardiographic indicators of the studied patients are presented in the table (Table 3, Table 4, Table 5).

Table 3. Structural echocardiographic parameters of LV and RV and atria

Indicator	Maximum and minimum value	Standard deviation
LVDd mm	60-36	48.06±4.35
LVDs mm	40-23	30.67±3.21
LVPWd mm	12-6	8.77±1.22
IVSd mm	11 - 6	8.77±1.22
LVVol d ml	144 - 42	84.24±21.21
LVVol s ml	67-15	31.23±10.21
LAD mm	41-25	32.11±4.06
RVWd subc.mm	5-2	3.46±0.7
RVDd subc.mm	39-18	27.87±4.38
RVDdmax mm	41 - 21	29.55±4.38
RVDdmid mm	40 - 12	20.43±4.71
RVout d mm	47 - 20	30.6±4.86
RVout s mm	24 - 6	13.04±2.86
RAD mm	41 - 20	30.6±4.88

LVDd mm - the diameter of the left ventricular cavity in diastole (at the end of diastole); LVDs mm - the diameter of the left ventricular cavity in systole (at the end of systole); LVPWd mm - the thickness of the back wall of the LV in diastole; IVSd mm - the thickness of the interventricular septum in diastole; LVVol d ml - the volume of the LV in diastole; LVVol s ml - LV volume in systole; LAD mm - the maximum diameter of the left atrium in systole; RVWd subc.mm - right ventricular wall thickness in diastole from the subcostal position; RVDd subc.mm - the maximum diameter of the right ventricular cavity in diastole with a subcostal approach; RVDd max mm - the maximum diameter of the right ventricular cavity at the level of the atrio-ventricular ring in diastole in the 4-chamber apical position; RVDd mid mm - the maximum diameter of the right ventricular cavity in the middle section of the ventricle, in diastole in the 4-chamber apical position; RVout d mm - diastolic diameter of the right ventricular outflow tract in the parasternal position; RVout s mm - systolic diameter of the right ventricular outflow tract in the parasternal position; RAD mm - right atrial diameter in systole

Table 4. Functional echocardiographic parameters of the LV and RV and atria

Indicator	Maximum and minimum value	Standard deviation
LVD fr Short%	71.8 – 25.6	36.35±4.6
LV EF%	75.8 - 50	63.21±5.78
LVET sec	425 - 224	295.84±30.31
MAPS mm	20 - 10	15.68±1.87
TAPS mm	31-13	23.44±3.15
PulmAT sec	166 - 100	131.79±14.07
RVET sec	403 - 222	306.52±32.83
RVoutFS%	76.6 – 42.86	57.47±5.89
Transmitral flow		
e sm/sec	119 - 42	69.91±14.84
a sm/sec	93 - 33	58.81±12.69
e/a	2.85 – 0.59	1.24±0.37
DT sec	322-145	198.25±36.48
Tricuspid flow		
e tr sm/sec	84 - 28	50.05±9.91
a tr sm/sec	68-24	37.34±7.69
e/a tr	2.21 – 0.71	1.37±0.29
DT tr msec	480 - 152	277.62±60.32

LVD fr Short% - fractional shortening of the left ventricular cavity diameter; LV EF% - left ventricular ejection fraction; LVET sec - left ventricular ejection time; MAPS mm - mitral annulus systolic movement amplitude; TAPS mm - the amplitude of the systolic movement of the tricuspid ring; PulmAT sec - systolic flow acceleration time in the pulmonary artery; RVET sec - right ventricular ejection time; RVoutFS% - fractional shortening of the diameter of the right ventricular outflow tract in systole; e sm/sec – early diastolic filling speed of the LV with transmitral flow; a sm/sec - late diastolic (atrial systole) filling speed of the LV with transmitral flow; e/a – ratio of early and late diastolic velocities with transmitral flow; DT sec - LV early filling flow deceleration time; e tr sm/sec - early diastolic filling speed of the RV with three-door flow; a tr sm/sec - - the late diastolic filling speed of the RV with tricuspid flow; e/a tr - the ratio of the early and late diastolic filling rates of the RV with three-door flow DT tr msec - flow deceleration time of early right ventricular filling with tricuspid flow

Table 5. Indicators of pulsed wave and two-dimensional color mode Tissue Doppler

Indicator	Maximum and minimum value	Standard deviation
TDI LVlat		
s sm/sec	16 - 5	10.25±2.12
e sm/sec	26 - 4	12.86±3.26
a sm/sec	18 - 5	9.82±2.67
ICT msec	148 - 35	73.17±19.51
IRT msec	124 - 20	61.36±19.62
LVET msec	386 - 209	292.9±26.17
TDI IVS		
s sm/sec	13 - 6	8.68±1.36
e sm/sec	17 - 4	10.18±2.64
a sm/sec	14 - 5	9.54±1.83
ICT sec	150 - 33	72.11±17.47
IRT sec	124 - 30	72.17±18.34
LVET sec	351 - 220	284.49±28.84

TDI LVlat - tissue dopplerography of the lateral wall of the LV; s sm/sec - maximum systolic speed of the lateral wall of the LV; e sm/sec - speed of early diastolic movement of the lateral wall of the LV; a sm/sec - speed of late diastolic movement of the lateral wall of the LV; ICT msec - isometric contraction time of the LV; IRT msec - left ventricular isometric relaxation time; LVET msec - left ventricular ejection time; TDI IVS - tissue dopplerogram of interventricular septum; s sm/sec - maximum systolic speed of interventricular septum; e sm/sec - speed of early diastolic movement of interventricular septum; a sm/sec - speed of late diastolic movement of interventricular septum; ICT sec - time of isometric contraction of interventricular systole; IRT sec - isometric relaxation time of the ventricular septum; LVET sec - ventricular septal ejection time

The velocity indicator of the tissue doppler curve taken in the color tissue mode exactly replicated the dynamics of the impulse tissue doppler data, despite having a lower numerical value due to the physical properties of these modes being different. Due to data averaging, the speed of tissue doppler curves taken in color mode is approximately 20% slower than the speed of tissue doppler curves taken in impulse mode.

4. Discussion

The RV originates from a different embryological source than the LV and possesses transcriptional and translational differences in pressure-overload hypertrophy; this refers to divergent gene and protein expression levels in energy metabolism, contractile elements, remodeling of the extracellular matrix, calcium handling, and cardiac muscle tissue development (6). The RV can be divided into components the sinus (the pumping chamber) and the infundibulum (7). The RV wall is there the LV wall inner suffice is trabeculated. The muscle fibers of the RV have a longitudinal orientation from the valve annulus to the apex, and in this way obtain longitudinal contraction.

The RV has two-layer muscle fibers: superficial circumferential muscle fibers responsible for its inward movement and inner longitudinal fibers that result in longitudinal contraction. The longitudinally oriented fibers play a greater role in RV emptying compared with the LV. On the other hand, LV has a great influence on the contractile function of RV.

Echocardiography (EchoCG) plays an important role in the evaluation of RV function and pathology. TDI of RV is a useful technique that is both robust and reproducible. The information about RV wall systolic and diastolic velocities and the intervals can be measured from one heart cycle which increases the reproducibility of the method.

Despite the detailed data about normal reference values of the systolic (s) wave on RV TDI in the literature (8,9,10) there is little information about diastolic velocities and systolic time intervals. This study evaluated the reference values of PWD TDI for both RV in normal adult population in both genders.

According to research, in 51% of participants, the ratio of early and late diastolic speeds (e/a) was <1. It was detected in 21% of people under 40 years of age and 64% of cases in people over 40. The age of the patients must be taken into account while analyzing the TDI of the RV. Some other studies also show that RV e/a decreases with age (11). This suggests an age-related limitation in early diastolic myocardial relaxation that requires an increase in atrial contraction force to maintain appropriate ventricular filling.

The indicators we obtained can be used in clinical practice to analyze RV TDI. The age of the patients must be taken into account while analyzing the TDI of the RV.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: S.U., M.T, Design: T.V., Data Collection or Processing: S.U., M.T., T.V., Analysis or Interpretation: S.U., M.T, T.V., Literature Search: S.U., T.V., Writing: S.U., M.T, T.V.

Ethical Statement

This study meets the requirements set by the Helsinki Declaration and the National Health Council according to resolution 196/96, in which the patient's anonymity and the information obtained in the present study are guaranteed.

Before starting the study, we received approval from the Research and Ethics Committee of Caucasus University (CU 37-21.01.23.). Therefore, data were analyzed only after approval of the study in order to ensure that all requirements for human research have been met accordingly.

Before participating in the study, selected individuals were given informed consent forms. Their participation was completely voluntary, and they could refuse to participate in the research at any time. The respondents were aware that the study would be confidential.

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The effect of workplace stress and workplace bullying on life satisfaction in nurses

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Abstract

Workplace stress and workplace bullying are important problems experienced in healthcare environments. It is known that nurses experience workplace stress and workplace bullying at high rates. Our study aims to explore how workplace stress and workplace bullying impact the life satisfaction of nurses. Data were collected online from 163 nurses. Nursing Stress Scale (NSS), Negative Act Questionnaire-Revised (NAQ-R), Satisfaction with Life Scale (SWLS) were used as data collection tools. According to the results of the conducted analysis, it was observed that as NSS and NAQ-R scores increased, SWLS scores decreased, and these relationships were statistically significant. Furthermore, the NAQ-R score was identified as a significant predictor of SWLS scores. In our study, our hypothesis that workplace bullying predicts life satisfaction was confirmed; however, workplace stress was not found to be a significant predictor. According to our current information, our study is the first investigation exploring whether workplace stress and workplace bullying serve as predictors of life satisfaction. Additional investigations are required to draw more precise conclusions regarding the matter.

Keywords: workplace stress, workplace bullying, life satisfaction, nurses

1. Introduction

Workplace stress is defined as the inability to manage the overwhelming pressure experienced in modern work environments due to factors such as the challenges in establishing work-life balance, the intense competitive atmosphere, limited resources, and unpredictable economic conditions (1). The factors associated with workplace stress, which is a multifaceted biopsychosocial response, may vary depending on the nature of the occupational domains (2). Hospitals unquestionably represent work environments characterized by elevated levels of stress (3). Research indicates that nurses commonly experience workplace stressors, often associated with high workload, the severity of patients' health conditions, conflicts in relationships with physicians or colleagues, lack of social support, and factors related to workplace violence (4). A recent review has found that Australian nurses experience a moderate to high level of workplace stress (5). A study conducted with emergency department nurses, utilizing the effort-reward imbalance model to assess workplace stress, found that 83% of the nurses experienced job stress (6).

Workplace bullying, which is one of the contributing factors to occupational stress, constitutes a significant issue in healthcare settings and is reported more frequently among nurses compared to individuals in other professions (7, 8). Workplace bullying results in low job satisfaction, decreased job performance and productivity, burnout, as well as mental and physical health issues (9, 10). The recent findings from studies indicate that nurses' exposure to workplace bullying is higher than perceived, occurring on a gender-biased basis. This bullying frequently manifests through methods such as humiliation, ridicule, gossip, and social exclusion. Research indicates that being subjected to workplace bullying can result in negative physical health consequences (11, 12). As examining the literature, it is noted that verbal abuse is the most prevalent form of bullying experienced by nurses. Nevertheless, rates of physical violence and sexual harassment are unexpectedly high as well (13, 14).

Life satisfaction refers to the emotional sense of well-being attained when an individual meets their own standards and accomplishes their goals X(15). Sociodemographic and psychosocial factors, including age, gender, economic status, lifestyle, participation in leisure activities, and environmental support, are widely acknowledged to impact life satisfaction (16). Also, variables related to one's profession, such as duration of employment, work environment, professional satisfaction, job stress, and workplace bullying, have shown that linked with life satisfaction (17, 18).

It is well acknowledged that nurses, an indispensable and significant component of the healthcare sector, experience workplace stress and workplace bullying at high rates (19). There is evidence indicating the negatively effects of workplace stress and workplace bullying on life satisfaction, a concept closely associated with psychological well-being. However, this subject remains insufficiently explored within

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the nurse population (20).

In our study examining how workplace stress and workplace bullying affect the life satisfaction of nurses, we outline the following hypotheses:

1. There is a negative correlation between workplace stress and life satisfaction among nurses.
2. There is a negative correlation between workplace bullying and life satisfaction among nurses.
3. Workplace stress and workplace bullying are predictors of life satisfaction.

2. Materials and Methods

2.1. Study Participants

The study included 164 nurses currently employed in any healthcare institution.The forms were created through Google Forms (Google, California, USA) and distributed to nurses via WhatsApp and group channels. The research data were collected between 15 December 2023 and 1 January 2024.

The sample size was determined using G-Power version 3.1.9.4 software, with reference to the research conducted by Amini et al. in 2023 (21). Following their study's effect size (ρ=0.613), a minimum sample size of 28 was calculated, considering a significance level of 0.05 for Type I error and a power of 0.95. However, this calculation was made based on the basic hypothesis. It is important that the sample size is as large as possible so that the side results can be considered meaningful. Due to this fact, we aimed to reach at least 100 participants in our study, taking into account the number of people that can be reached through online groups.

2.2. Data collection tools

Nursing Stress Scale

The original scale, created by Gray-Toft and Anderson, consists of 34 items distributed across 7 factors (22). The Cronbach's α reliability coefficient for the subfactors of the original scale varied from α = .89 to α = .65, all falling within the range of 0.80. The sub-factors can be measured individually, or the total score obtained from the entire scale can be computed to evaluate the frequency of stress encountered by nurses within the workplace setting. A higher total score indicates increased levels of stress related to nurses' professional lives. Mert et al. conducted the validation and reliability assessment of the scale in a Turkish context (23).

Negative Act Questionnaire-Revised

The scale developed by Einarsen and Raknes consists of 22 items and two sub-dimensions (24). The subdimensions of personal humiliation and work-related harassment can be assessed by aggregating their frequencies to evaluate the prevalence of being a target of bullying. However, it is commonly observed in studies that assessments are often conducted based on total scores without considering the subdimensions (25). The scale demonstrated a Cronbach's alpha internal consistency reliability coefficient of .88, and a correlation of .80 was observed between scores obtained from two administrations conducted three weeks apart. Aydin conducted a validity and reliability investigation of the scale in Turkish in 2009 (26).

Satisfaction with Life Scale

The 5-point Likert-type scale devised by Diener et al. comprises five items (27). Following reliability assessments on the scale, the test-retest reliability was determined as r=.85, with item-test correlations falling between .71 and .80. There is no predefined cutoff point. A high score on the scale reflects a positive level of life satisfaction. Dagli and Baysal conducted the Turkish validation and reliability investigation of the scale (28).

2.3. Statistical Analysis:

The study's data analysis utilized IBM SPSS version 23.0. Descriptive statistics for continuous variables were provided as mean ± standard deviation (mean±sd), median, 1st quartile, 3rd quartile, minimum, and maximum values. Categorical variables were represented as numbers and percentages. The normal distribution of continuous variables was assessed using the Kolmogorov-Smirnov test. Given that the normality assumption was not satisfied, the Spearman rank correlation test was employed to examine relationships between continuous variables. The potential impact of the NAQ-R and NSS on the SWLS was investigated through multiple linear regression analysis. The assumption of the normal distribution of residuals, a requirement for the multiple linear regression model, was carried out using the Kolmogorov-Smirnov test. A scatter plot was employed to assess the linearity of the relationship between the dependent variable and independent variables. Multicollinearity among independent variables in the current multiple linear regression model was examined using the Variance Inflation Factor statistic. A significance level of $p < 0.05$ was utilized to determine statistical significance.

3. Results

According to sociodemographic and clinical data, the age of nurses ranged from 23 to 53 years, with a mean age of 31.39±9.13. Among the participants, 91.4% were female, and 71.8% were married. When examining the departments in which nurses work, it was observed that 37.4% enployed in internal units, 25.8% in surgical units, 17.2% in intensive care units, 7.4% in emergency units, and 12.3% in other units (such as blood collection, dialysis, vaccine department). Nurses' length of service varied between 1 and 49 years, with an average duration of 15.93 ± 10.18 years. Among the nurses, 29.4% had been diagnosed with chronic diseases, while 5.5% had been diagnosed with psychiatric conditions. The sociodemographic and clinical data results are presented in Table 1.

Table 1. Descriptive statistics of the participants

		n	%
Age			
	Mean±SD	37.39±9.13	
	Min-Max	23.00-53.00	
	Median (Q_1-Q_3)	37.00 (29.00-46.00)	
Gender			
	Female	149	91.4
	Male	14	8.6
Marital Status			
	Single	46	28.2
	Married	117	71.8
Employment Service			
	Emergency	12	7.4
	Surgery	42	25.8
	Internal	61	37.4
	Intensive Care	28	17.2
	Other	20	12.3
Tenure			
	Mean±SD	15.93±10.18	
	Min-Max	1.00-49.00	
	Median (Q_1-Q_3)	15.00 (6.75-25.00)	
Chronic Disease Diagnosis			
	Yes	48	29.4
	No	115	70.6
Psychiatric Disease Diagnosis			
	Yes	9	5.5
	No	154	94.5

Mean, SD: Standart Deviation, Q_1:1. quartile, Q_3:3rd quartile

The scores of the participants on the NAQ-R scale ranged from 22.0 to 110.0, with a calculated mean scale score of 36.33±15.09. Half of the participants scored 33.0 or higher on the NAQ-R. The lowest NSS score was 34.0, and the highest was 128.0, with an average score of 68.50±15.76. Half of the participants had a total NSS score of 69.0 or higher. Participants' scores on the SWLS spanned from a minimum of 5.0 to a maximum of 25.0, yielding a calculated mean scale score of 13.23±4.47. Half of the participants scored 13.0 or higher on the SWLS. The mean scores and descriptive statistics of the scales can be found in Table 2.

Table 2. Descriptive statistics for the scores of NSS, NAQ-R, and SWLS

	Mean±SD	Min-Max	Median (Q_1-Q_3)
NAQ-R	36.33±15.09	22.0-110.0	33.0 (27.0-40.0)
NSS	68.50±15.76	34.0-128.0	69.0 (59.0-77.0)
SWLS	13.23±4.47	5.0-25.0	13.0 (10.0-17.0)

Mean, SD: Standart Deviation, Q_1:1st quartile, Q_3:3rd quartile NSS: Nursing Stress Scale, NAQ-R: Negative Act Questionnaire-Revised, SWLS: Satisfaction with Life Scale.

When examining the inter-scale correlations, a negative and weak correlation was found between NAQ-R and SWLS scores (Spearman's rho = -0.260, $p < 0.001$); a positive and moderate correlation was found between NAQ-R and NSS scores (Spearman's rho = 0.570, $p < 0.001$); a negative and weak correlation was identified between NSS and SWLS scores ($r = -0.211$, $p = 0.007$), and a statistically significant relationship was determined. The data regarding inter-scale correlations are presented in Table 3.

Table 3. The levels of relationship among NAQ-R, NSS, and SWLS scores

	SWLS		NSS	
	rho	p-value	rho	p-value
NAQ-R	-0.260	**<0.001**[1]	0.570	**<0.001**[1]
NSS	-0.211	**0.007**[1]		

[1]The Spearman rank correlation test SWLS: Satisfaction with Life Scale NSS:Nursing Stres Scale NAQ-R: Negative Act Questionnaire-Revised

The study examined whether NSS and NAQ-R scores were significant predictors of SWLS score using a multiple linear regression model. The multiple linear regression model's assumption, which verifies whether the residuals follow a normal distribution, was assessed utilizing the Kolmogorov-Smirnov test. The results indicated that the residuals conform to a normal distribution (Kolmogorov-Smirnov statistic = 0.65, SD= 163, $p = 0.085$). The scatter plot, depicted in Fig. 1, examines the linearity of the connection between the dependent variable and the independent variables.

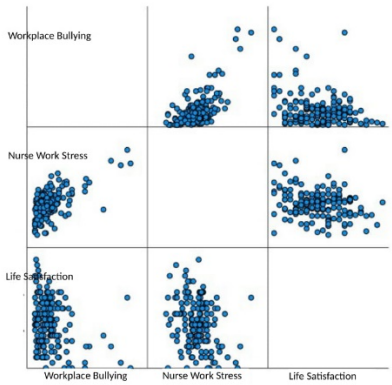

Fig.1. The scatter plot depicting the relationship between SWLS and NSS as well as NAQ-R scores

Upon examining the scatter plot provided in Fig. 1, it is observed that the assumption of linearity in the model was not violated; however, the degrees of correlation between the dependent variable and the independent variables were found to be very weak. As per the outcomes derived from the established multiple linear regression model, it was found that the NSS score was not a significant predictor of the SWLS score, while the NAQ-R score statistically significantly predicted the SWLS score. Accordingly, a one-point increase in nurses' NAQ-R scores results in a decrease of 0.076 points in their SWLS scores. In the established regression model, NSS and NAQ-R scores explained 31.2% of the variability observed in SWLS scores.

4. Discussion

In our research focusing on how workplace stress and workplace bullying affect the life satisfaction of nurses, we enrolled 164 nurses, with the majority being female. The mean age was 31.39. According to the results of the conducted analysis, it was observed that as NSS and NAQ-R scores increased, SWLS scores decreased, and these relationships were statistically significant. Furthermore, the NAQ-R score was identified as a significant predictor of SWLS scores.

In our study, the average scores of nurses on the NSS were calculated as 68.50±15.76. The literature has been reviewed in terms of studies evaluating nurse stress using the same scale. In the study conducted by Ko and Kiser-Larson with oncology nurses, the mean score for NSS was 71.35.(29) Newman collaborated with mental health nurses and found the average NSS score to be 67.70 (30). In this regard, it can be asserted that our findings are consistent with the literature. However, in our study, data were gathered from nurses working in various departments, and the relationship between the department of employment and NSS scores was not examined. This situation poses a limitation for our research.

In a research carried out with nurses from Portugal, the Depression Anxiety Stress Scale-21 was utilized, revealing a negative correlation between the stress subscale of the scale and life satisfaction (31). Another study conducted with nursing students assessed stress using the Perceived Stress Scale, and it observed a negative relationship between psychological well-being and stress level *as* well as life satisfaction (32). In our study, when investigating the correlation between stress and life satisfaction, a negative and weak correlation was found. As a result, it can be interpreted that our findings confirm the first hypothesis and align with the existing literature. In addition, the Nursing Stress Scale utilized in our study is more specific in measuring stress related to nurses' professions, thus making a meaningful contribution to the literature on job stress and life satisfaction among nurses from this perspective.

Research involving 211 physicians employed in diverse hospitals across Pakistan revealed a negative correlation between workplace bullying and life satisfaction (33). In a research conducted by Peng et al. with a sample of 493 nurses, it was found that workplace bullying has a detrimental impact on quality of life, with psychological resilience acting as a mediator in this association (34). In a study conducted with Chinese nurses, utilizing the NAQ-R to measure workplace bullying, nurses were found to have a workplace bullying score of 38.72±12.30, and the hypothesis that workplace bullying negatively affects quality of life was confirmed, with coping styles serving as mediators in this relationship (35). The average NAQ-R scores in our study (36.33±15.09) and the negative and weak correlation findings between NAQ-R and SWLS scores can be considered as results consistent with the literature Furthermore, the results confirm the second hypothesis of our study. However, there is a limited number of studies that specifically addressed life satisfaction and exposure to bullying among nurses. The overwhelming majority of current research have been conducted using scales assessing quality of life. In addition, our study didn't explore the correlation between sociodemographic and clinical data that could potentially influence the association between exposure to bullying and life satisfaction. By acknowledging these limitations, it can be argued that our study contributes to the literature. However, to achieve more precise conclusions on the matter, there is a need for studies employing different measurement tools and incorporating various variables.

Studies examining the predictors of life satisfaction have generally concluded that life standards are the most significant predictor (36, 37). Studies focusing on the life satisfaction of healthcare professionals have demonstrated that peer support, job demands, work-family stress, and working hours are strong predictors (38, 39). In our study, our hypothesis that workplace bullying predicts life satisfaction was confirmed; however, workplace stress was not found to be a significant predictor. To our understanding, our research is the first investigation exploring whether workplace stress and workplace bullying serve as predictors of life satisfaction. Additional investigations are required to draw more precise conclusions regarding the matter.

In conclusion, our study found a negative correlation between workplace stress and workplace bullying in nurses and life satisfaction. Workplace bullying emerged as a notable predictor of life satisfaction, with NSS and NAQ-R scores collectively explaining 31.2% of the variability in SWLS scores. Among the limitations of our study are the collection of data through snowball sampling and the lack of examination of correlations between sociodemographic and clinical variables with the scales. Also, the subscales of the NSS were not evaluated and only the total score was calculated. Future studies may incorporate variables such as professional satisfaction, compassion fatigue, and professional burnout, and explore factors like coping strategies, and support resources in the link between workplace stress, workplace bullying, and life satisfaction.

Conflict of interest

There is no conflict of interest between the authors.

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Authors' contributions

Concept: P.G., F.Y., Design: P.G., F.Y., Data Collection or Processing: P.G., Analysis or Interpretation: F.Y., Literature Search: P.G., Writing: P.G.

Ethical Statement

The study received ethical approval from the Ordu University Clinical Research Ethics Committee on December 8, 2023 (Application No: 311 Decision No: 2023/327).

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KFORCE Sens® electrogoniometer in the evaluation of wrist range of motion in patients with carpal tunnel syndrome: Validity and reliability

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Abstract

This study aimed to test the validity and reliability of the KFORCE Sens® (electrogoniometer) in carpal tunnel syndrome (CTS) patients. Thirty-one volunteers (aged 32-60 years) diagnosed with unilateral CTS were included in this study. Each of the two raters evaluated the participants' wrist range of motion (ROMs) (flexion, extension, radial deviation, and ulnar deviation) twice, for a total of four times, using an electrogoniometer. In addition, wrist ROMs were evaluated once with a universal goniometer. According to the study findings, both the inter-rater and test-retest reliability of the electrogoniometer device in all ROMs of the wrist were excellent (ICC>0.80). In addition, the ROM values of the electrogoniometer device and the ROM values of the universal goniometer in the same joint were highly correlated ($r>0.80$, $p<0.05$). The electrogoniometer is a valid, reliable, and practical device for evaluating wrist ROMs in patients with CTS.

Keywords: range of motion, carpal tunnel syndrome, electrogoniometers, reliability, wrist

1. Introduction

Carpal tunnel syndrome (CTS) is a common peripheral neuropathic disorder characterized by pain, tingling, and numbness over the dermatomal area of the median nerve (1, 2). Local hyperplasia, fibrosis, and synovial hypertrophy develop in patients with CTS (3, 4). These pathologies cause limitations in the range of motion (ROM) of the wrist. In addition, studies have shown that patients with CTS may experience a decrease in wrist ROM due to pain and immobilization after splinting (5).

Today, the use of electrogoniometers for ROM measurements is becoming more popular as technology advances (6). In many joints, reliability and validity investigations of electrogoniometers have been performed in the assessment of ROMs (7, 8). Studies have often concluded that electrogoniometers are valid and reliable in the wrist, knee, and ankle joints (9-11).

KFORCE Sens® (electrogoniometer) is an electrogoniometer or inertial sensor. It was developed by Kinvent™ (Montpellier, France) engineers to evaluate joint ROM. Electrogoniometers offer real-time biofeedback by measuring the difference between the starting position and the end point of the movement, thanks to their measurement sensors. Data obtained from the device can be transferred to smartphones and computers via Bluetooth® connections (12).

This study aimed to determine the test-retest reliability, inter-rater reliability, and validity of the electrogoniometer in the assessment of wrist ROMs in patients with CTS.

2. Materials and methods

This study was carried out on 31 individuals diagnosed with mild to moderate CTS, aged between 32-60. CTS severity was determined by a neurophysiologic assessment of the median nerve lesion (13). This study was approved by the Kırşehir Ahi Evran University Faculty of Medicine Clinical Research Ethics Committee. Verbal and written informed consent was obtained from all subjects, and the study was conducted in accordance with the Declaration of Helsinki.

2.1. Participants and Methods

The inclusion criteria for the study were as follows: Being older than 18 years of age, volunteering, having a medical diagnosis of unilateral CTS, and having symptoms of CTS for more than one year. Exclusion criteria from the study: the inability to understand instructions was determined to be related to any neuromuscular disorder related to the upper extremity.

2.2. Study design

Wrist ROMs were evaluated with both the universal goniometer and the electrogoniometer. To examine the construct validity of electrogoniometer, its relationship with universal goniometer values was examined. In addition, electrogoniometer evaluations for both inter-rater and test-

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retest reliability were repeated by two physiotherapists (MC, ŞK).

Participants were given an explanation of the protocol and an introduction to the device before the start of the assessment. To avoid distracting the participants, the evaluations were performed in a quiet room on the examination bed. In the study, the wrist ROMs of the participants diagnosed with CTS were evaluated. Two trial assessments were performed first, followed by ten assessments with 30-second rest periods (12). (Since there is no standard time and repetition in the instructions for use of the device or in the literature, these times and repetitions were determined in line with the consensus of the authors.)

2.3. Evaluation of range of motion

Universal goniometer

For wrist flexion and extension measurements, the patient was seated with the forearm pronated and supported on a table edge. The pivot point of the goniometer was the styloid process of the ulna; the fixed arm was parallel to the ulna, and the movable arm followed the fifth metacarpal bone. The ROM value was recorded while the patient was doing active flexion and extension. For wrist ulnar deviation and radial deviation measurements, the patient sat with his forearm pronated and the volar side of his hand supported on the table. The pivot point of the goniometer was proximal to the third metacarpal, the midpoint of the carpometacarpal joint; the fixed arm was parallel to the midpoint of the radius and ulna; and the movable arm followed the third metacarpal bone. The ROM value was recorded while the patient was making active ulnar and radial deviations. After the universal goniometer evaluations, the electrogoniometer evaluations started 5 minutes later (14).

KFORCE Sens® (electrogoniometer)

The positioning of the participants was the same as in the 360° universal goniometer assessments. The device was attached to the lateral aspect of the wrist for wrist flexion-extension and to the dorsal aspect of the wrist for ulnar-radial deviation (Fig. 1). The assessment was started by pressing the "start" button on the mobile device, and then the previous protocol was followed. The device has saved the active ROM values. The gadget features a gyroscopic inertial sensor that allows for joint range of motion measurement, monitoring, and therapy. It calculates the angle with regard to the limb's initial position in a specified anatomic plane. All assessments with the device were performed by two physiotherapists. The physiotherapists then repeated their measurements. There was a 5-minute break between measurements (12).

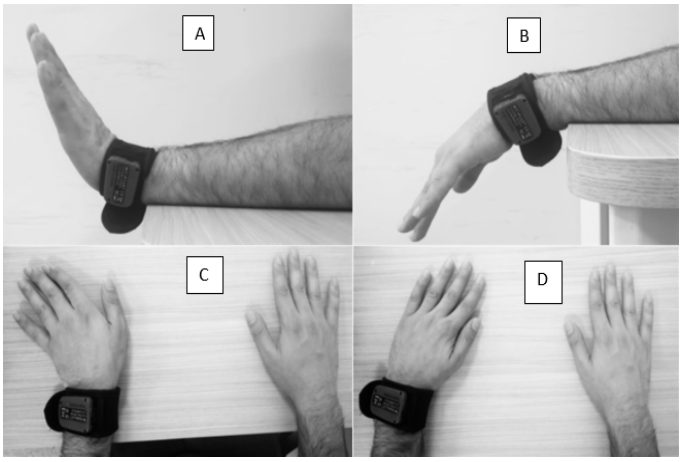

Fig. 1. Evaluation of range of motion with KFORCE Sens® electrogoniometer, A: Wrist extension position, B: Wrist flexion position, C: Wrist ulnar deviation position, D: Wrist radial deviation position

2.4. Statistical analysis

We used the G*Power program (Heinrich-Heine-Universität Düsseldorf, Germany) version 3.1.9.4 to determine the study sample (15). Based on similar articles (12), the power rate of the sample was calculated as $\beta = 80\%$ (type II error rate = 20%), type I error rate $\alpha = 0.05$, and d (effect size) = 0.45, totaling 31 participants.

All statistical analyses were executed utilizing SPSS version 24 software (SPSS Inc, Chicago, Illinois). A combination of visual methods and analytical techniques was employed to evaluate the normal distribution of variables. Assessment of the reliability of the electrogoniometer was conducted using the intraclass correlation coefficient (ICC). The ICC values were categorized as poor (<0.40), fair (0.40-0.59), good (0.60-0.79), and excellent (>0.80) reliability, respectively (16). A Pearson correlation analysis was performed to ascertain the concurrent validity of the electrogoniometer through its relationship with secondary measurements. A correlation coefficient was considered poor (less than 0.30), moderate (between 0.30 and 0.60), and strong (greater than 0.60) (17). Calculation of the standard error of measurement (SEM_{95}) value for the electrogoniometer scores employed the following formula: SEM_{95} = Standard deviation x $\sqrt{(1\text{-ICC})}$. The determination of the minimal detectable change at a 95% confidence interval (MDC_{95}) involved the following formula: $MDC_{95} = 1.96 \times SEM_{95} \times \sqrt{2}$ (18). The established level of statistical significance was set at $p < 0.05$.

3. Results

The demographic information of the patients with CTS included in the study is given in detail in Table 1.

The mean clinical measurement values are given in Table 2.

The first rater's test-retest ICC values for electrogoniometers for wrist flexion, wrist extension, wrist ulnar deviation, and wrist radial deviation were 0.97, 0.96, 0.99, and 0.95, respectively. This result showed us that the patients with CTS included in the study had excellent test-

retest validity in the wrist ROMs evaluation of the electrogoniometer.

Table 1. Participants' demographics features

		(n=31)			
		Mean	SD	Min	Max
Age (years)		46.00	7.95	32.00	60.00
Height (cm)		166.39	6.45	155.00	184.00
Weight (kg)		70.03	13.88	49.00	120.00
BMI (kg/m^2)		25.42	5.49	17.17	41.52
		n		(%)	
Gender	Male	12		38.7	
	Female	19		61.3	
Injured side	Right	17		54.8	
	Left	14		45.2	
Dominant side	Right	18		58.1	
	Left	13		41.9	

SD: Standard Deviation, BMI: Body Mass Index, Min: Minimum, Max: Maximum

When we look at the inter-rater reliability of electrogoniometers between the first and second raters for wrist flexion, wrist extension, wrist ulnar deviation, and wrist radial deviation, the ICC values are 0.98, 0.98, 0.98, and 0.98, respectively. Thus, the inter-rater reliability of the electrogoniometer was also found to be excellent (Table 3).

Table 2. The mean values of the measurements of the wrist ROMs

			(n=31)		Min	Max
			Mean	SD		
Baseline® 360° universal goniometer		Flexion	61.90	9.16	40.00	80.00
		Extension	55.61	6.33	41.00	66.00
		Ulnar deviation	29.03	7.60	16.00	43.00
		Radial deviation	12.77	2.86	8.00	18.00
KFORCE Sens®	Flexion (1)	Test	63.71	9.63	40.00	85.00
		Retest	63.52	9.49	40.00	84.00
	Flexion (2)	Test	63.45	9.63	35.00	81.00
		Retest	63.00	9.07	37.00	79.00
	Extension (1)	Test	56.81	6.53	41.00	70.00
		Retest	56.48	6.24	43.00	67.00
	Extension (2)	Test	56.61	6.20	42.00	69.00
		Retest	56.35	5.90	43.00	68.00
	Ulnar deviation (1)	Test	30.52	7.81	16.00	44.00
		Retest	30.03	7.38	17.00	42.00
	Ulnar deviation (2)	Test	30.71	7.17	17.00	43.00
		Retest	30.55	6.69	18.00	41.00
	Radial deviation (1)	Test	13.58	3.31	8.00	20.00
		Retest	13.48	2.95	7.00	19.00
	Radial deviation (2)	Test	13.58	3.11	8.00	19.00
		Retest	13.74	2.82	9.00	19.00

1: First rater, 2: Second rater, SD: Standard Deviation, Min: Minimum, Max: Maximum

Table 3. Inter-rater (ICC) and test-retest (ICC) reliability of the KFORCE Sens®

n=31	Difference (Mean ± SD)	Inter-rater (ICC $_{1,2}$) (95% CI)	Test-retest (ICC $_{1,1}$) (95% CI)	SEM$_{95}$	MDC$_{95}$
Flexion	-0.19 ± 2.44	0.98 (0.96-0.99)	0.97 (0.93-0.98)	0.37	1.01
Extension	-0.32 ± 1.77	0.98 (0.97-0.99)	0.96 (0.92-0.98)	0.35	0.95
Ulnar deviation	-0.48 ± 1.12	0.98 (0.97-0.99)	0.99 (0.98-0.99)	0.11	0.30
Radial deviation	-0.09 ± 1.01	0.98 (0.96-0.99)	0.95 (0.90-0.98)	0.14	0.38

SD: Standard Deviation, ICC: Intraclass Correlation Coefficient, SEM$_{95}$: standard error of measurement; MDC$_{95}$: minimum detectable change at the 95% confidence interval

The correlation results of the electrogoniometer with the universal goniometric measurements, which are frequently used in the clinic, to determine the concurrent validity of wrist flexion, wrist extension, wrist ulnar deviation, and wrist radial deviation of patients with CTS included in the study are examined in Table 4. According to our results, a high level of statistically significant correlation was found between the wrist ROM values of the electrogoniometer and all the same joint ROM values of the universal goniometer ($p<0.05$).

Table 4. Relationship between KFORCE Sens® and Baseline® 360° universal goniometer ROM evaluations

		Baseline® 360° universal goniometer			
		Flexion	Extension	Ulnar deviation	Radial deviation
KFORCE Sens®	r	0.975	0.976	0.989	0.943
	p	<0.001*	<0.001*	<0.001*	<0.001*

*p<0.001

4. Discussion

Based on the results of the current study, the test-retest reliability of the electrogoniometer in patients with CTS was excellent. Because of the significant relationship between the electrogoniometer and the universal goniometer, we have proven that it is also valid in the evaluation of wrist ROMs in patients with CTS. Also, in this study, we present for the first time the MDC$_{95}$ and SEM$_{95}$ values of the electrogoniometer for the evaluation of wrist ROMs in patients with CTS.

Electrogoniometers are a practical tool to identify functional limitations, guide treatment programs, and provide evidence of treatment efficacy. When the literature is examined, practical and reliable alternative goniometer types have been developed. In this study, we investigated the reliability of the electrogoniometer, which is a portable device that transmits data to smartphones and computers via Bluetooth® connections on wrist ROMs. In a previous study, the reliability of the electrogoniometer in the wrist joint was investigated, and it was concluded that the ICC value was 0.94 for flexion/extension movement and 0.96 for ulnar/radial deviation (12). Pilbeam et al. concluded that the test-retest reliability of the universal goniometer was reliable in evaluating the wrist joint position sense in healthy subjects (19). In another study, Tajali et al. stated that two different electrogoniometers have high reliability except for radial deviation movement in people with limitations in wrist ROM (20). In our study, the test-retest reliability of the electrogoniometer was found to be excellent (ICC > 0.80).

In this study, we used a universal goniometer to test the concurrent validity of the electrogoniometer. When the results

of our study were examined, we found a strong correlation between the electrogoniometer and the universal goniometer in all wrist ROM values ($r > 0.88$, $p < 0.001$). Tekin et al. examined the validity and reliability of the electrogoniometer in healthy subjects and evaluated the construct validity of the electrogoniometer using a universal goniometer. They found a highly strong relationship between both goniometers in flexion-extension movements and a strong relationship for radial-ulnar deviation movements in the dominant extremity. In addition, they found a highly strong correlation for flexion-extension movements and a strong correlation for ulnar-radial deviation movements between both dynamometers in the non-dominant side extremity (12). These results are consistent with the literature, and we think that an electrogoniometer is a valid method for the evaluation of wrist ROMs in patients with CTS.

Camassuti et al. (21) calculated the SEM_{95} values of the MIOTEC® brand electrogoniometer for wrist ROM evaluation as 3.10 for extension, 3.11 for flexion, 1.78 for radial deviation, and 1.63 for ulnar deviation. Tajali et al. (20) found the SEM_{95} values of the J-Tech electrogoniometer to be 3.48 for flexion, 2.82 for extension, 1.94 for radial deviation, and 2.06 for ulnar deviation in patients with hand and wrist motion limitations. Also, Tajali et al. (20) found the MDC_{95} value of the J-Tech electrogoniometer to be 11.86 for flexion, 7.82 for extension, 5.76 for radial deviation, and 5.38 for ulnar deviation. In our study, the MDC_{95} value was 1.01 for flexion, 0.95 for extension, 0.30 for ulnar deviation, and 0.38 for radial deviation. In addition, the SEM_{95} value was 0.37 for flexion, 0.35 for extension, 0.11 for ulnar deviation, and 0.14 for radial deviation.

The most important limitation of the present study is the limited number of studies on the validity and reliability of the electrogoniometer. This makes it difficult to compare the results of our study with the literature. We believe that future studies should investigate electrogoniometry in specific pathologies, age groups, and different extremities.

Test-retest and inter-rater reliability of the electrogoniometer are excellent in assessing wrist ROM in patients with CTS and have strong concurrent validity with the universal goniometer. The electrogoniometer is an easy-to-use and reliable device.

Ethical Statement

This research and the use of data have been approved by Kırşehir Ahi Evran University Faculty of Medicine Clinical Research Ethics Committee Ethics Committee (Number: 2022-22/194, Date: 06.12.2022).

Conflict of interest

No to declare.

Funding

No to declare.

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Authors' contributions

Concept: İ.C., Ş.K., Design: İ.C., M.C., Data Collection or Processing: A.Ö., Ş.K., Analysis or Interpretation: İ.C., H.A., Literature Search: İ.C., Ş.K., Writing: M.C., A.Ö., F.T.

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The impact of radiotherapy on lower urinary tract symptoms in patients with gynecologic malignancies: A cross-sectional study

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Abstract

This study investigates the impact of radiotherapy (RT) on lower urinary tract symptoms (LUTS) in patients with gynecologic malignancies, focusing on those who have undergone surgical treatment. We conducted a cross-sectional study from January to June 2023 at a tertiary hospital's outpatient polyclinic. Participants included patients with early-stage cervical cancer and endometrial cancer who received adjuvant RT post-surgery, and advanced cervical cancer patients who received primary RT. Patients were divided into two groups: those who received RT (RT (+)) and those who did not receive (RT (-)). Sociodemographic data were collected and LUTS were assessed using the International Consultation on Incontinence Questionnaire Female Lower Urinary Tract Symptoms (ICIQ-FLUTS). Out of 64 patients, 38 (59%) did not receive RT and 26 (41%) received RT. The RT (+) group had significantly higher scores in filling (p=0.038), voiding (p=0.046), and incontinence (p=0.012) compared to the RT (-) group. Additionally, the RT (+) group reported a significantly lower quality of life (p=0.009). Our study found that radiotherapy is associated with increased lower urinary tract dysfunction, including symptoms in the filling, voiding, and incontinence phases. Patients who received RT also experienced a decreased quality of life compared to the RT (-) group. These findings highlight the need for comprehensive management of LUTS in patients undergoing radiotherapy for gynecologic cancers.

Keywords: gynecological malignancy, endometrial cancer, cervical cancer, lower urinary tract symptoms, quality of life, radiotherapy

1. Introduction

Gynecologic cancers remain a significant global burden, impacting women socially and psychologically. Despite improvements in survival rates due to advances in healthcare technology, managing the health-related quality of life (HRQoL) of those affected is crucial. Treatment for gynecological malignancies frequently include surgical procedures, chemotherapy, and radiotherapy (RT). However, these interventions often lead to increased urological complications and affect HRQoL significantly (1).

Surgical interventions for gynecological cancers, such as radical hysterectomy and pelvic lymphadenectomy, are associated with a high incidence of urinary tract morbidity (2). Extensive surgeries can cause damage to urogenital nerves and blood vessels, leading to complications such as urinary incontinence, bladder dysfunction, and pelvic floor dysfunction (3). These complications can significantly affect the quality of life of survivors (4). Studies have demonstrated that radical surgical procedures contribute substantially to the urinary tract morbidity observed in gynecological cancer survivors (5).

Radiotherapy used as a curative, adjuvant, or salvage treatment for gynecological cancers, also causes significant side effects. It can damage living tissue in the treated area, resulting in increased collagen deposition, decreased elasticity, ischemia, necrosis, and permanent damage (6, 7). Patients undergoing RT may experience lower urinary tract symptoms (LUTS) such as dysuria, urgency, frequent urination, nocturia, and haematuria (8). These symptoms can lead to psychosocial issues and a decreased HRQoL (9, 10).

Lower urinary tract symptoms (LUTS) refer to a range of symptoms related to urinary tract dysfunction. These include symptoms associated with the filling phase of the bladder, such as increased frequency, urgency, and nocturia, and symptoms related to the voiding phase, such as hesitancy, weak stream, and incomplete bladder emptying. Additionally, incontinence and urinary leakage are considered part of LUTS. The impact of these symptoms can significantly affect patients' quality of life, causing discomfort and psychological distress.

Despite the growing recognition of the impact of these

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symptoms, research on LUTS among cancer survivors, particularly those suffering from gynecological malignancies, remains limited. Therefore, this study aims to investigate the impact of radiotherapy on LUTS among patients with gynecological malignancies and to evaluate how these symptoms affect their HRQoL.

2. Materials and Methods

2.1. Study Design and Participants

This cross-sectional study was conducted from January to June 2023 at the outpatient polyclinic of the Gynecologic Oncology department at a tertiary hospital.

Inclusion criteria were as follows: (1) Patients with gynecological malignancies who visited the outpatient clinic during the study period. (2) Patients with early-stage cervical cancer who underwent adjuvant radiotherapy (RT) following radical hysterectomy. (3) Patients with endometrial cancer who received adjuvant RT after comprehensive surgical staging. (4) Patients with advanced cervical cancer who received primary RT.

Exclusion criteria were as follows: (1) Patients with diabetes mellitus, neurogenic bladder, interstitial cystitis, recurrence of cancer, urinary tract infection, urinary obstruction, vesicovaginal fistula, or a history of previous urologic or gynecologic surgery. (2) Patients unable to complete the questionnaire.

2.2. Radiotherapy Regimens

Patients with early-stage cervical cancer received adjuvant RT delivered as a total dose of 45-50.4 Gy in daily fractions of 1.8-2.0 Gy over approximately 5 weeks. Patients with advanced cervical Cancer received primary RT delivered as a total dose of 45-50.4 Gy in daily fractions of 1.8-2.0 Gy. Patients with endometrial cancer received adjuvant RT delivered as a total dose of 45 Gy in daily fractions of 1.8 Gy over approximately 5 weeks.

2.3. Data Collection

Patients were categorized into two groups: RT (+) and RT (-). Data collected included sociodemographic information and LUTS using the International Consultation on Incontinence Questionnaire Female Lower Urinary Tract Symptoms (ICIQ-FLUTS).

2.4. ICIQ-FLUTS Questionnaire

A validated tool consisting of 12 items assessing three domains: filling (F score), voiding (V score), and incontinence (I score) (11, 12). This questionnaire was completed by patients in the waiting room area under the supervision of data collectors.

2.5. Clinical Examination

The patients were asked to complete the questionnaire in the waiting room area. After completing the questionnaire, patients underwent a detailed physical, gynecologic, and ultrasonographic examination. A urinalysis was performed to rule out urinary tract infections prior to the examinations.

2.6. Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 25. Descriptive statistics were used to present categorical variables as numbers and percentages and continuous variables as means and standard deviations. Comparative analyses between RT (+) and RT (-) groups were conducted using the Chi-square test for categorical variables, and the independent samples t-test for continuous variables. A p-value of <0.05 was considered statistically significant.

3. Results

A total of 64 patients were included in the final analysis. The mean patient age was 57.37±11.1, and 58.85±11.82 years for Groups RT (-) and RT (+), respectively. There were no significant differences between the two groups with regard to age, body mass index, comorbidity, income, employment, consumption of coffee, smoking status, and educational level (Table 1).

Patients who received RT (+) had significantly higher filling ($p=0.038$), voiding ($p=0.046$) and incontinence scores ($p=0.012$) than patients who did not receive RT. The RT (+) group had a significantly decreased quality of life than the RT (-) group ($p=0.009$) (Table 2).

4. Discussion

Lower urinary tract dysfunction can have a significant impact on a patient's HRQoL, and may result in psychological, social, and sexual issues for women. Factors such as surgery and RT for gynecologic cancers may influence the prevalence of LUTS (13).

Numerous studies have evaluated the association between pelvic RT and LUTS (14, 15). In Hazelwinkel et al.'s study among cervical cancer survivors noted that treatment of cervical cancer impairs pelvic floor function, especially in patients treated with primary RT (16). Katepratoom et al conducted a cross-sectional study comparing patient groups treated with radical hysterectomy and chemoradiotherapy for cervical cancer. The study found voiding dysfunction to be significant only in those who received radical hysterectomy, while irritative storage urinary symptoms were more common in the chemoradiotherapy group (17). Our results suggested that RT might be detrimental to pelvic floor function and HRQoL of gynecological cancer survivors.

Table 1. Socidemographic characteristics. Comparison of no radiotherapy and radiotherapy

		No Radiotherapy	Radiotherapy	Total	t	*p*
Educational level	Illiterate	7 (58.3)	5 (41.7)	12 (100)	3.438	0.664
	Literate	4 (66.7)	2 (33.3)	6 (100)		
	Primary School	17 (63)	10 (37)	27 (100)		
	Secondary school	5 (71.4)	2 (28.6)	7 (100)		
	High School	2 (28.6)	5 (71.4)	7 (100)		
	University	3 (60)	2 (40)	5 (100)		
Marital status	Married	33 (63.5)	19 (36.5)	52 (100)	1.920	0.166
	Single	5 (41.7)	7 (58.3)	12 (100)		
Smoking	Yes	3 (50)	3 (50)	6 (100)	0.003	0.956
	No	35 (60.3)	23 (39.7)	58 (100)		
Daily coffee consumption	No consumption	24 (58.5)	17 (41.5)	41 (100)	0.222	1.000
	1-2 cups	12 (60)	8 (40)	20 (100)		
	3 or more cups	2 (66.7)	1 (33.3)	3 (100)		
Employment	Employed	3 (42.9)	4 (57.1)	7 (100)	0.286	0.593
	Unemployed	35 (61.4)	22 (38.6)	57 (100)		
Income	Income is less than expense	21 (60)	14 (40)	35 (100)	0.377	1.000
	Income is equal than expense	16 (59.3)	11 (40.7)	27 (100)		
	Income is more to expense	1 (50)	1 (50)	2 (100)		
Comorbidities	Yes	18 (56.3)	14 (43.8)	32 (100)	0.259	0.611
	No	20 (62.5)	12 (37.5)	32 (100)		
Cervical cancer		5 (23.8)	16 (76.2)	21 (100)	16.390	<0.001*
Endometrial cancer		33 (76.7)	10 (23.3)	43 (100)		
Age (year)		57.37±11.1	58.85±11.82	57.97±11.33	-0.51	0.612
Body mass index (kg/m^2)		31.12±6.78	29.26±7.84	30.37±7.23	1.01	0.317

Note: Data are given as mean±SD and number (frequency). p<.05statistically significant

Table 2. Filling, voiding and incontinence scores. Comparison of no radiotherapy versus radiotherapy

	No Radiotherapy Mean±SD	Radiotherapy Mean±SD	Total Mean±SD	t	*p*
Filling Score	10.24±8.87	15.77±12	12.48±10.53	-2.121	0.038
Voiding Score	1.58±2.33	2.85±2.6	2.09±2.51	-2.036	0.046
Incontinence Score	7.82±12.11	19.04±19.13	12.38±16.19	-2.651	0.012
ICIQ-FLUTS Score	25.03±27.41	49.65±40.14	35.03±35.06	-2.724	0.009

Abbreviations: International Consultation on Incontinence Questionnaire Female Lower Urinary Tract Symptoms, ICIQ-FLUTS
p-value<.05statistically significant.

Our study supports the findings of Donovan et al. and Alicja Zi etek-Strobl et al., stating that gynecological oncological survivors frequently experience urinary incontinence that significantly reduces general HRQoL (3, 18). These survivors are more likely to exhibit symptoms of incontinence of every type. Our study, utilizing the ICQ-FLUTS questionnaire, confirmed that RT adversely affects LUTS. The findings align with the conclusions presented by Erekson et al., who reported a prevalence of urinary symptoms as high as 83.6% among women undergoing treatment for endometrial cancer (19). Moreover, women receiving adjuvant RT reported experiencing more severe incontinence symptoms and greater impact on their HRQoL. The extension of life of oncology survivors who often received radical treatment for gynecological malignancies results in an elevated risk of pelvic floor dysfunction. Therefore, LUTS symptoms should be a matter of concern for healthcare professionals as they may decrease the HRQoL and cost-effectiveness of treatment (20).

Abdominal hysterectomy with bilateral salpingo-oophorectomy is the most commonly performed surgical procedure in patients with gynecological cancer. When necessary, this procedure can be extended to include pelvic lymphadenectomy, removal of paracolpium, or debulking surgery. However, these extensive surgeries are associated with a higher rate of surgical complications and injuries to adjacent tissues, and contribute to an increased probability of urogynecological symptoms (21, 22). Radical pelvic surgery aims to decrease the malignancy recurrence but may also result in damage to urogenital nerves and blood vessels. This can lead to tissue hypoxia, scarring, and local ischemia, which increase the likelihood of pelvic floor dysfunction (23). Additionally, the combined treatment of surgery, RT, and chemoradiation can have negative effects on the lower urinary tract and pelvic floor organs. The risk of complications after pelvic radiation is inevitable and significantly affects the genitourinary system. Patients may experience various immediate or long-term adverse effects after treatment, such as urinary, stress urinary incontinence, interstitial cystitis, urinary bladder fibrosis, and vesico/urethra-vaginal fistulas (24). The literature lacks consensus on the impact of RT on LUTS. The findings of the

study suggest that RT may increase LUTS. Erekson and Manchana have reported that RT reduces pelvic floor function and HRQoL of cancer survivors (19, 24). However, other studies have disputed these findings (25-27). The PORTEC-2 study performed by De Boer et al. revealed a higher incidence of LUTS in gynecological oncology patients but did not result in a negative impact on their HRQoL (28). This aligns with the findings presented in the study conducted by Pisani et al., which examined a sample of patients with both cervical and endometrial cancer (29).

The small sample size of patients is a limitation of our study. However, using a validated questionnaire to evaluate LUTS represents a significant advantage. In addition, it may be beneficial to counsel oncology patients and prepare them for the potential complications or adverse events that may be associated with their treatment.

The present study suggested that patients receiving RT for gynecologic malignancies exhibited significant dysfunction in filling, voiding, and incontinence phases of lower urinary tract function compared to those who did not receive RT. Gynecologic oncological surgical procedures, such as radical hysterectomy and comprehensive staging surgeries, contribute to higher rates of urogynecological symptoms, which can be further exacerbated by RT. From a clinical perspective, these findings highlight the need for comprehensive management strategies that address both RT-related and surgical symptoms, as well as proactive patient counseling regarding potential side effects. Future research need to prioritize longitudinal studies to gain a deeper understanding of the long-term effects of RT, evaluate the efficacy of various symptom assessment tools, and explore potential interventions to improve patient outcomes and quality of life.

Ethical Statement

The database management in accordance with privacy legislation and the presented study in accordance with the ethical principle of the Declaration of Helsinki. Ethical approval for this study was obtained by the Research Ethics Committee (Approval number: 2023/514/259/2; Date: 11.10.2023). The work has not been published previously and it is not under consideration for publication elsewhere.

Conflict of interest

The authors have no conflicts of interest.

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Authors' contributions

Concept: S.S.K., E.K., M.A., Design: S.S.K., E.K., M.A., Data Collection or Processing: S.S.K., E.K., İ.B., M.M., B.K., U.K.O., Analysis or Interpretation: K.N.B., S.S.K., E.K., Literature Search: S.S.K., E.K., İ.B., M.M., B.K., Writing: S.S.K., E.K., E.M., U.K.O., İ.B., M.M., B.K., K.N.B., M.A., M.A.

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Association between COVID-19 vaccination during pregnancy and SGA in a rural area

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Abstract

Vaccines were the most crucial factor in ending the COVID-19 pandemic. Different types of COVID-19 vaccines were administered worldwide to all ages, including pregnant women. This study aimed to evaluate if different types of COVID-19 vaccines applied during pregnancy affect the "Small for Gestational Age" (SGA) incidence in a rural area of Turkey. A retrospective cohort study was performed with 146 completed pregnancies between 2021 and 2022. The health repository was scanned to obtain COVID-19 vaccination status during pregnancy, the type of COVID-19 vaccine administered and fetal birth weight. The presence of SGA was detected by using a gender-specific Fenton growth chart in newborns. We investigated the incidence of SGA for the whole study population and vaccinated group, as well as the types and trimesters of COVID-19 vaccination during pregnancy. There was no statistical significance between all pregnancies and vaccinated women during pregnancy in terms of SGA incidence ($p>0.05$). Either COVID-19 vaccination during pregnancy or type of COVID-19 vaccines or trimester of application did not statistically change the incidence of SGA ($p>0.05$). Pregnant women are skeptical about the COVID-19 vaccine, but inactivated and mRNA COVID-19 vaccine administration during pregnancy seems safe in terms of SGA incidence.

Keywords: COVID-19, small for gestational age, vaccination, COVID-19 vaccines, pregnancy

1. Introduction

Infectious diseases that spread around the world, known as pandemics, have been remembered with COVID-19. The novel coronavirus disease, which spreads quickly due to its human-to-human transmission feature, has affected all countries around the world (1, 2).

Data collected about "New Coronavirus Disease" revealed that the disease threatens not only the respiratory system but also many systems, including the genital system and related organs. The main reason why the virus acts in such a wide range is ACE 2 receptors, which is a way to enter the cell. It is believed that with high expression of ACE 2 receptors in the female genital system, COVID-19 may damage reproductive functions and can cause infertility, menstrual irregularity and fetal problems (3-5).

Vaccination is the most important and effective way to protect health and prevent infectious diseases (6). To defeat the COVID-19 epidemic, a large part of the population, including pregnant women, has been vaccinated. As the virus spread and the distribution of viral variants changed, the types of COVID-19 vaccines varied over time, including inactivated, live-attenuated, viral vector, protein subunit, and DNA and mRNA vaccines (7). Inactivated virus vaccines are a conventional protein-based approach which is made from whole viruses inactivated by chemical agents to activate the host's immune response. Messenger RNA (mRNA) vaccines are a gene-based approach which delivers a transcript that encodes a target antigen or immunogen (8).

In the literature, COVID-19 during pregnancy is associated with higher rates of fetal death, preterm birth, preeclampsia and emergency cesarean section (9, 10). There is little data on pregnancy outcomes and the safety of COVID-19 vaccines in pregnancy because pregnant women were excluded from first phase III clinical trials (11). The possible future harms and side effects of COVID-19 vaccination on pregnant women and fetuses are not yet fully known (6, 11, 12).

The incidence of morbidity and mortality is high in small for gestational age (SGA) infants. Although the definition of SGA is birth weight under the 10th percentile for a given gestational age, regardless of any pathology, SGA is associated with neurodevelopmental delay (13). Neurological diseases, close follow-up or hospitalization requiring morbidities are five to ten times higher in SGA babies than in appropriate for gestational age (AGA) babies (14).

Our study aims to evaluate if COVID-19 vaccination affects birth weight according to gestational age and to see if SGA incidence changes with different types of COVID-19 vaccines.

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2. Materials and Methods

This retrospective cohort study was conducted between 01.03.2021 and 01.03.2022 in Manisa Demirci district. This rural area's health repository was scanned to obtain COVID-19 vaccination status among pregnant women. Additionally, the types of COVID-19 vaccines and vaccine administration trimesters were noted. To determine SGA, birth weight according to gestational age was calculated using a gender-specific Fenton percentile curve. Pregnant women who applied to Manisa Demirci State Hospital were divided into two groups according to COVID-19 vaccination status. SGA incidences were found for vaccinated and non-vaccinated groups during pregnancy. In the study, the mother's age, type and doses of COVID-19 vaccines, trimester of vaccine application, gestational week of birth, mode of birth, birth weight and the gender of the babies were noted. Babies who were below the 10th percentile according to the Fenton percentile curve were defined as SGA (15). Low birth weight was determined using gender-specified Fenton growth charts and calculated by looking at the weeks of gestation (16). Statistical analyses were made by assigning the number 1 to SGA and number 2 to AGA. LGA babies, multiple gestations, neonates with congenital anomalies, aneuploidies and women with antenatal COVID-19 were excluded from the study. SGA presence was searched if it was related to COVID-19 vaccination during pregnancy and the type of vaccines. The relationship between SGA and COVID-19 vaccination during pregnancy was evaluated.

The study was conducted in accordance with the declaration of Helsinki. The approval for the study was obtained from the ethics committee of the University of Health Sciences, İstanbul Kanuni Sultan Süleyman Training and Research Hospital, with the number 2021.12,336. For the implementation of the study, permission was obtained from the Ministry of Health of the Republic of Türkiye.

Study data was evaluated with IBM Statistical Package for the Social Sciences for Windows, Version 23.0 (IBM Corp., Armonk, NY). In the analysis of the data, descriptive statistics (mean, standard deviation, median, minimum, maximum) for numerical variables and frequency distributions (number, percentage) for categorical variables were given. If there was a difference between two independent groups, the Mann-Whitney U test or independent sample t-test was used, depending on whether the variables were compatible with normal distribution. The Kruskal Wallis test or the One-Way ANOVA test was used to determine whether there was a difference between more than two independent groups. The results were considered statistically significant with p-values of <0.05.

3. Results

A total of 146 pregnant women were included in the study. The mean age was 30.16±5.29. Of the study population, 13.7% had advanced maternal age, 62.3% were vaccinated with mRNA, inactivated virus and combined vaccines, 47.3%, 13.7%, and 1.4%, respectively. In terms of vaccination periods, first-trimester, second-trimester, and third-trimester ratios were 20.5%, 15.8%, and 8.2%, respectively. The rest were vaccinated in combined trimesters. 84.9% delivered at 38th gestational weeks and above, 65.1% had a cesarean section, and 89.7% gave birth to 2500 to 4000 gr babies. Of the 84 male babies and 62 female babies, SGA ratios were 2% and 2.8%, respectively (Table 1).

Table 1. Distribution of participants' socio-demographic, obstetric characteristics, types and time of COVID-19 vaccination and neonatal characteristics

Age (years)		30.16±5.29	
		n	**%**
Age distribution	<21	5	3.4
	21-35	121	82.9
	>35	20	13.7
Vaccination during pregnancy	None	55	37.7
	1 dose	30	20.5
	2 or more doses	61	41.8
Type of vaccines	Inactive	20	13.7
	mRNA	69	47.3
	Inactive+mRNA	2	1.4
Trimester of vaccination	1.trimester	30	20.5
	2.trimester	23	15.8
	3.trimester	12	8.2
	1. and 2. trimester	20	13.7
	2. and 3. trimester	5	3.4
	1. and 3. trimester	1	0.7
Gestational week at birth	<38	22	15.1
	≥38	124	84.9
Mode of delivery	Normal vaginal delivery	51	34.9
	C-Section	95	65.1
Birth weight of infant	<2500 gr	6	4.1
	2500-4000 gr	131	89.7
	>4000 gr	9	6.2
Gender	Male	84	57.5
	Female	62	42.5
Percentile curve (male infant)	SGA	3	2.0
	AGA	81	55.5
Percentile curve (female infant)	SGA	4	2.8
	AGA	58	39.7

*n: number, %: ratio, SGA: small for gestational age, AGA: appropriate for gestational age

According to COVID-19 vaccination during pregnancy, SGA presence was not statistically different between the vaccinated and non-vaccinated groups (p=0.772). Furthermore, there was no statistical significance for SGA incidence between the application of inactivated vaccine only, mRNA vaccine only and the combination of both types (p=0.349) (Table 2).

SGA incidence was not different between the non-vaccinated and the inactivated vaccine-only groups and the mRNA vaccine-only and the combination of both types of vaccine groups, respectively (p=0.290, p=0.935, p=1.000). Furthermore, trimester of COVID-19 vaccine administration did not affect the SGA incidence (p=0.989) (Table 3).

Table 2. Comparison of SGA in neonatals between vaccinated and non-vaccinated group and COVID-19 vaccine types administered during pregnancy

SGA	**Vaccinated, n**	**Non-vaccinated, n**		**Total, n**	**p**
	91	55		146	0.772
	Inactive, n	**mRNA, n**	**Inactive+ mRNA, n**	**Total, n**	**p**
	20	69	2	91	0.349

*inactive: COVID-19 vaccine containing inactivated virus, *mRNA: mRNA (Messenger RNA) based COVID-19 vaccine, *SGA: small for gestational age *n: number of individuals

Table 3. Comparison of SGA between non-vaccinated and vaccinated with different COVID-19 vaccine types, and between trimester of administration

	SGA n (%)	**p**
Non-vaccinated, n (%) 55 (37.7)	3 (2)	0.290
Inactive, n (%) 20 (13.7)	0 (0)	
Non-vaccinated, n (%) 55 (37.7)	3 (2)	0.935
mRNA, n (%) 69 (47.3)	4 (2.7)	
Non-vaccinated, n (%) 55 (37.7)	3 (2)	1.000
Inactive+mRNA, n (%) 2 (1.4)	0 (0)	
First trimester, n (%) 30 (20.5)	1 (0.7)	0.989
Second trimester, n (%) 23 (15.8)	2 (1.4)	
Third trimester, n (%) 12 (8.2)	1 (0.7)	

*inactive: COVID-19 vaccine containing inactivated virus, *mRNA: mRNA (Messenger RNA) based COVID-19 vaccine *SGA: small for gestational age *n: number of individuals, %: ratio

SGA incidence was not statistically different between the whole population and the vaccinated group (p=1.000) (Table 4).

Table 4. Comparison of SGA incidence in the community and in those who had been vaccinated against COVID-19 during pregnancy

	SGA, n	**SGA incidence, %**	**p**
Vaccinated, n=91	4	4.3	1.000
Community, n=146	7	4.7	

*Community: Indicates the entire study population *SGA: small for gestational age *n: number of individuals, %: incidence are presented as ratio

4. Discussion

The connection between gestational week and birth weight provides information about intrauterine life and the health of the baby. Mortality and morbidities are 5 to 10 times higher in SGA babies than in babies with appropriate weight for gestational age (14).

While it is unclear to what extent COVID-19 vaccines prevent getting infected and the spreading of the virus, initial data have shown that vaccines reduce the duration and severity of new coronavirus illness. Of these different types, mRNA vaccines were first administered to pregnant women in the United States. The results, including completed pregnancies, have been published in the Centers for Disease Control and Vaccine Safety Monitoring program database (17).

To date, no unexpected results have been observed in terms of gestational diabetes mellitus, preeclampsia, intrauterine growth restriction, miscarriage rates, stillbirth rates, premature birth and neonatal deaths related to mRNA COVID-19 vaccines administered during pregnancy (18). According to a multicenter study that supports our study results, mRNA vaccines did not increase the incidence of SGA in the population (19). Additionally, our study reveals that the first trimester of COVID-19 vaccination is also safe in terms of SGA. Similar to our study, a prospective study designed with inactivated COVID-19 vaccines revealed that first-trimester vaccination did not increase the risk for SGA babies (20).

In a recent review, similar to our data, there was no evidence of a higher risk for SGA babies and additionally other adverse perinatal outcomes, including miscarriage and neonatal fetal abnormalities (21). Moreover, a different study conducted with mRNA and viral vector vaccines found that SGA incidence did not change with COVID-19 vaccination (22).

This study may have some weak points. Since it is a rural area study, it was held by one center with a limited population. However, it has a major strength in that it covers the results of two different COVID-19 vaccine types (mRNA and inactivated virus vaccines) in pregnant women.

As a result, the administration of inactivated and mRNA COVID-19 vaccines during pregnancy is safe in terms of SGA incidence. Considering the effects of severe COVID-19 disease in pregnancy and newborns, COVID-19 vaccines should be recommended during pregnancy.

Ethical Statement

The study was conducted in accordance with the declaration of Helsinki. The approval for the study was obtained from the ethics committee of the University of Health Sciences, İstanbul Kanuni Sultan Süleyman Training and Research Hospital, with the number 2021.12,336. For the implementation of the study, permission was obtained from the Ministry of Health of the Republic of Türkiye.

Conflict of interest

Authors declare no conflict of interest.

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Authors' contributions

Concept: P.Ö.K., Design: P.Ö.K., Data Collection or Processing: P.Ö.K., G.S., Analysis or Interpretation: G.S., Literature Search: P.Ö.K., G.S., Writing: P.Ö.K., G.S.

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The impact of social life restrictions during the COVID-19 pandemic process on lipid profile

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Abstract

Because of restrictions and limitations during the COVID-19 pandemic, the majority of individuals had sedentary lives. In this study, we wanted to investigate the impact of sedentary lifestyle on the lipid profiles of adults during the COVID-19 pandemic. All patients whose cholesterol profile was evaluated between 01.03.2019 and 31.12.2021 were gathered from electronic patient records for this retrospective research. According to the dates of their admission to the outpatient clinics, the records of the patients aged 18 to 65 years were separated into three categories: pre-pandemic (March 2019–March 2020), restriction (March 2020–June 2020), and post-restriction (June 2020 –Post) periods. All patients accepted and enrolled in the trial had complete data. Over the course of the three examinations, the cholesterol panels of 376 study participants were analyzed. In the pandemic period higher total cholesterol levels, total cholesterol/high density lipoprotein cholesterol (HDL-C) ratio, and very low-density lipoprotein cholesterol (VLDL-C), results were observed than the pre-pandemic period. The LDL-C levels increased compared to pre-pandemic levels, and were higher during the pandemic than in the post-pandemic normalization period. Restrictions on social activities during the epidemic had a deleterious impact on lipid profiles by encouraging sedentary behavior. Although the process of normalization after the pandemic improved, the pandemic was unable to return cholesterol levels to their pre-pandemic levels.

Keywords: sedentary behavior, hyperlipidemias, pandemics, COVID-19, physical inactivity

1. Introduction

The World Health Organization (WHO) classified the coronavirus disease of 2019 (COVID-19) as a pandemic on March 11th, 2020. Regarding non-pharmacological treatments, the WHO suggested various preventative actions such as avoiding physical contact, closing universities, forbidding social gatherings and huge crowds, isolating, maintaining a social distance, and quarantining (1).

In both industrialized and developing nations, cardiovascular disease (CVD) continues to be the main cause of mortality and morbidity. Male gender, hypertension, diabetes, advanced age, and hyperlipidemia (raised plasma triglyceride and low-density lipoprotein (LDL) levels) are the best-known risk factors for the development of CVD. The lipid profile typically includes total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides. The ratios of these values are crucial for determining cardiovascular risk. For instance, a high LDL/HDL ratio is linked to increased heart disease risk, while a low triglyceride/HDL ratio indicates better cardiovascular health. Thus, the lipid profile and its ratios are essential tools for evaluating overall health. Physical exercise and hyperlipidemia, a modifiable risk factor, have a well-established association (2).

It was found that physical activity reduced, body mass weight increased, and lipid profiles got worse in a small number of studies comparing the COVID-19 pandemic period with the pre-pandemic period (3). However, the literature research revealed that there wasn't a study on this topic in Türkiye. This study was done to demonstrate how the COVID-19 pandemic's effects on sedentary lifestyle had an impact on lipid profiles.

2. Materials and methods

People (18–65 years old) who applied to the Family Medicine Polyclinic, Sıhhıye Medicosocial Health Center, or Hacettepe University Faculty of Medicine between March 1st, 2019, and December 31st, 2021 were included in this retrospective study. Retrospective data from electronic patient records were collected for the lipid panel. Pre-pandemic (March 2019–March 2020), restriction period (March 2020–June 2021), and post-restriction period (June 2021–post) data were gathered separately. The study included all individuals who underwent examinations and had no missing data (Fig.1).

Fig. 1. Sample Date Period Intervals of the Study

There are 44794 applications to our Beytepe polyclinics within the indicated periods, and 18802 of them are solitary. There are 26999 applications to our Sıhhiye outpatient clinics

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throughout that time and 9869 of them are solitary. 8990 patients, have had their cholesterol panels evaluated as a result of these applications. 920 of the 8990 patients had complete data, and the remaining patients were removed from the study. The study comprised a total of 376 participants whose cholesterol panels were tested over the course of the three time periods. The study covered every person between the ages of 18 and 65 who had no missing data (Fig. 2).

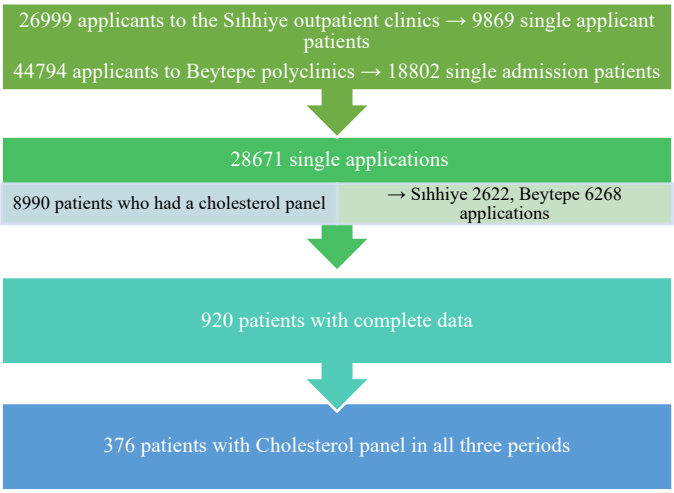

Fig. 2. The Flow Chart of Patient Selection Process

The laboratory's quantitative analysis results are validated internally using quality control guidelines based on Wesgart criteria. The enzymatic calorimetric test (Roche Diagnostics GmbH, Mannheim, Germany) was used to measure triglycerides, total cholesterol, and HDL cholesterol. Using the Friedewald formula, LDL cholesterol was measured.

Frequency tables were used for quantitative data, whereas mean and standard deviation were utilized for continuous variables. The association between the data was investigated using the chi-square test. The t test, the ANOVA test, or their nonparametric equivalents were used to compare differences between continuous variables. The significance level was set equal to a=0.05. With the IBM SPSS 24.0 package application (IBM Corp., released 2016), statistical analyses were carried out.

3. Results

Of the participants in our study, 49.7% (n=187) were male and 50.3% (189) were female; and the mean age was 49.81±9.069(min:30, max:64) years. Chronic disease was present in 26.1% (n=98) of the patients in the study, and 73.9% (n=278) did not have any chronic disease. Chronic diseases ratios were; 1.9% (n=7) had Diabetes Mellitus, 2.9% (n=11) had thyroid diseases, 2.4% (n=9) had hypercholesterolemia, 16.5% (n=62) had cardiovascular disease and 7.2% (n=27) had other chronic diseases.

The mean total cholesterol of the patients included in the study in the pre-pandemic period was 260.21±70.296 mg/dl (min=135, max=376), the mean total-cholesterol/HDL ratio was 5.33±1.94 (min=1.90, max=12.06), HDL mean was 51.61±12.14 mg/dl (min=31, max=73), LDL mean was 155.40±30.89 mg/dl (min=107, max=209). The mean total cholesterol of the patients during the pandemic was 276.35±71.15 mg/dl (min=136, max=398), the mean of total-cholesterol/ HDL ratio was 6.03±2.13 (min=2.03, max=13.17), the mean of HDL. It was 48.56±11.76 mg/dl (min=29, max=68), LDL mean was 184.35±33.77 mg/dl (min=126, max=241). The mean total cholesterol of the patients in the post-pandemic period was 276.54±68.16 mg/dl (min=135, max=398), the mean total-C/HDL ratio was 6.01±2.19 (min=2.45, max=13.10), HDL the mean was 48.79±11.59 mg/dl (min=29, max=72), and the mean LDL was 174.77±3456 mg/dl (min=114, max=233) (Table 2).

The total cholesterol values were considerably higher than pre-pandemic values throughout the pandemic and post-pandemic periods (p=0.014) when comparing the patients' cholesterol panels from the pre-pandemic, pandemic, and post-pandemic periods.

In comparison to the pre-pandemic period, the total cholesterol/HDL ratio was significantly greater during the pandemic and post-pandemic periods (p=0.033).

• The pre-pandemic period's HDL value was significantly greater than it was during the pandemic and post-pandemic periods (p 0.001).

• The pandemic period's LDL value was substantially greater than its post-pandemic and pre-pandemic counterparts (p 0.001).

• The post-pandemic phase had significantly higher VLDL and non-HDL readings than the pre-pandemic and pre-pandemic periods (p 0.001).

• The triglyceride value (0.012) was much greater during the pandemic than it was before and after. (Table 1).

Total cholesterol value: It increased (p=0.004) from the pre-pandemic period to the pandemic period. The post-pandemic period saw a higher level of it than the pre-pandemic period (p=0.003). Total cholesterol/HDL-C ratio: It was significantly greater during the pandemic than it was before (p 0.001). When compared to before the pandemic, it increased (p 0.001) in the post-pandemic period. When compared to before the pandemic, the HDL-C value was lower during the pandemic period (p=0.001). When compared to before the pandemic, it was lower in the post-pandemic period (p=0.001).

LDL-C value: It was significantly greater during the pandemic than it was before (p=0.001). When compared to before the pandemic, it increased (p=0.001) in the post-pandemic period. When compared to the post-pandemic period, it was higher during the pandemic period (p=0.001).

VLDL-C value: It was much greater during the pandemic than it was before (p=0.001). When compared to before the pandemic, it increased (p=0.001) in the post-pandemic period.

Table 1. Comparison of the patients' cholesterol pandemic before, during, and after the pandemic period

	Number (n)	Percentage (%)
Gender		
Male	187	49.7
Female	189	50.3
Chronic Disease	98	26.1
DM	7	1.9
Thyroid Diseases	11	2.9
Hypercholesterolemia	9	2.4
CVD	62	16.5
Other Chronic Disease	27	7.2
Distribution of Chronic Disease in men		
Chronic Disease	51	27.1
DM	5	2.7
Thyroid Diseases	1	0.5
Hypercholesterolemia	4	2.1
CVD	37	19.8
Other Chronic Disease	14	7.5
Distribution of Chronic Disease in women		
Chronic Disease	47	24.9
DM	2	1.1
Thyroid Diseases	10	5.3
Hypercholesterolemia	5	2.6
CVD	25	13.2
Other Chronic Disease	13	69

When compared to the pre-pandemic period, the NON-HDL-C value was lower during the pandemic period (p=0.027). Compared to the pandemic period, it was higher in the post-pandemic phase (p=0.01).

Triglyceride values: They were much greater during the pandemic than they were before (p=0.001). In comparison to the pandemic period, it was lower in the post-pandemic phase (p=0.001). (Table 2).

Table 2. Patients' lipid profiles throughout the pandemic/pre-pandemic, pandemic/post-pandemic, and post-pandemic/pandemic periods were compared pairwise

	Before the pandemic	During pandemic	After pandemic	
	Mean/ SD	Mean/ SD	Mean/ SD	p
Total Cholesterol	260.21±70.29	276.35±71.15	276.54±68.16	**0.014**
Total-C /HDL-C Ratio	5.33±1.94	6.03±2.13	6.01±2.19	**0.033**
HDL-C	51.61±12.14	48.56±11.76	48.79±11.59	**<0.001**
LDL-C	155.40±30.89	184.35±33.77	174.77±34.56	**<0.001**
VLDL-C	27.60±10.74	34.38±12.22	35.87±13.80	**<0.001**
Non-HDL-C	144.47±36.49	138.10±35.35	147.93±38.77	**<0.001**
Triglyceride	167.56±65.73	190.03±68.69	169.98±62.09	**0.012**

4. Discussion

Since March 11th, 2020, the SARS CoV-2 outbreak has been classified as a COVID-19 pandemic by the WHO. Countries had to create isolation methods to restrict contact, including remaining at home, in order to stop the virus from spreading. Along with staying in, physical activity levels and duration reduced, and sedentary behavior was more likely to occur (4). If left untreated, hyperlipidemia can worsen prognosis and is a significant risk factor for cardiovascular disorders (5).

Pre-pandemic, pandemic period, and post-pandemic periods were used to evaluate and compare the cholesterol panels of the participants. The pandemic process levels were found to be significantly higher than the normalization process when the restrictions were partially removed. When we examined the patient profile used in the study, we found that the percentages of female (50.3%) and male (49.7 %) patients is equal.

When cholesterol levels were considered, there was a difference between the pre- and post-pandemic periods in our study, but curiously, there was no discernible difference between the pandemic and post-pandemic periods. An increase in total cholesterol levels was seen compared to the pre-pandemic phase in longitudinal research looking at the effects of COVID-19 limitations on nonalcoholic steatohepatitis and insulin resistance in individuals in the pre-pandemic and post-pandemic periods by López-González et al. (6).

While HDL-C levels were high in the pre-pandemic era of our study we discovered that the levels during the pandemic and post-pandemic normalization processes were much lower. Similar to total cholesterol levels, there is no significant difference between HDL-C values during the pandemic and afterward, and they are also comparable.

Metabolic indicators from a retrospective cohort study of Chinese workers were assessed in 2018, 2019, and 2020. Except for fasting blood glucose, all metabolic indicators (higher TG level, lower HDL-C level, increased abdominal fat, and elevated systolic blood pressure) were determined to be considerably worse than before quarantine (7).

Increased TG/HDL-C ratio, or high triglyceride levels coupled with low HDL cholesterol levels, was found to be strongly correlated with the presence of tiny, dense LDL particles in plasma. Numerous investigations have revealed the TG/HDL-C ratio to be a potent biochemical indicator of ischemic heart disorders (8). In our study the total cholesterol /HDL ratio was discovered to be considerably lower in the pre-pandemic period compared to the pandemic and post-pandemic periods, similar to triglyceride ratios.

Because there is a considerable body of evidence that supports the link between the blood concentration of this molecule and the risk of CVD (9), LDL-C, the main circulating Apo B-containing lipoprotein, represents the primary target of the majority of currently available lipid-lowering treatments. In our investigation, significant differences were identified between the pre-pandemic and the pandemic period, the pandemic period and the post-pandemic era, and the pre-pandemic and post-pandemic period when we compared the LDL-C values of the patients in pairs. The epidemic period witnessed the highest values. Although LDL-C values declined after the pandemic, they were unable to return to the low levels

seen before the outbreak. In order to determine how COVID-19 isolation affects anthropometric factors, blood pressure, glucose levels, and lipid profiles in healthy persons, Ramrez Manent et al. conducted a longitudinal study. This study, which included 6236 workers between the ages of 18 and 69 years who were in good health, engaged in their jobs, and took part in routine workplace health inspections revealed a statistically significant rise in LDL levels (10).

The primary TG carrier in human serum and a precursor to LDL is VLDL, a lipoprotein released by the liver (11). In our study, VLDL values were higher in hr the pandemic and post-pandemic periods than the pre-pandemic period.

The level of non-HDL cholesterol, which raises the risk of cardiovascular disease, rises while HDL levels, the protective cholesterol, decreases (12). According to our research, non-HDL-C levels were similar between the pre- and post-pandemic periods but decreased during the pandemic.

Triglyceride levels, one of the cardiometabolic risk markers, significantly increased after quarantine, according to Woo et al.'s longitudinal study on overweight or obese children and adolescents' sedentary time and fast-food consumption associated with weight gain during COVID-19 quarantine (13). Our results were similar to those of this study and were much higher during the pandemic period with quarantine and limitations than they were during the pre-pandemic and post-pandemic periods, but the post-pandemic and pre-pandemic periods were also shown to be comparable.

Smoking, alcohol consumption, obesity, physical inactivity, sedentary lifestyle, hypertension, diabetes, and dyslipidemia are a few of the modifiable risk factors for CVD (14). Long-term physical exercise enhances markers of CVD risk and directly lowers CVD risk in healthy adults, according to a meta-analysis (15). Public health is now at risk due to the COVID-19 pandemic's rapid spread and severity. More than a third of the world's population were under some type of quarantine till April 2020 as a result of measures like lockdowns or isolation. The population's physical and mental health suffered as a result of the quarantine, which also caused a substantial change in food habits and a decline in physical activity.

Boredom has resulted in overeating or appetite loss as a result of lifestyle changes and absences from job, school, or other activities. A drop in the consumption of fish, seafood, fruits, and vegetables was accompanied by an increase in the consumption of salty and sweet snacks. Unbalanced eating habits have also been linked to abnormalities of physical activity and increased sleep length in adults and adolescents. As a result, the global population gained more weight during the quarantine period—from 11.1% to 72.4%. The likelihood of developing metabolic problems will rise as a result (10).

Significant variations are already expected in our study's analysis of the cholesterol panel results between the pre-pandemic period and the pandemic process because of the quarantine measures used during the pandemic process. The values we saw during the post-pandemic normalization process were identical to those observed throughout the pandemic process, though. We believe that sedentary behavior has become a habit despite the limitations being lifted following the epidemic due to the ongoing transformation. Maruo et al. evaluated lifestyle changes six months before and six months after the COVID-19 pandemic in a single-center observational study that looked at how the pandemic affected diabetic patients. They demonstrated that compared to the pre-pandemic period, physical activity dramatically decreased during the post-pandemic phase, whereas sedentary behavior greatly increased (15). It appears that COVID-19 fatalities will continue to raise the risk of CVD because of these sedentary habits, rather than just being tied to the pandemic or the illness.

Our study does have certain limitations. The number of patients in our study was low because it was limited to the outpatient clinics at the - University Faculty of Medicine, Department of Family Medicine, and - University Medicosocial Unit. At the beginning of the pandemic process, our outpatient clinic applications were reduced because a wave of panic and fear spread in the society. At the same time chronic disease follow-ups decreased. Because those with chronic diseases had been restricted from going out due to government policies. In addition, since our polyclinics are located on the campus, there was no patient approach based on the registered population like other family medicine centers, and the patient population was generally composed of hospital staff and students. This caused our patients to be variable and reduced our possibility of seeing patients constantly A further limitation of the study is that the patients' drugs that treat their lipid profile were not reached. A significant proportion of patients stopped taking their regular prescriptions as a result of pandemic restrictions as well as the challenges associated with applying to elective outpatient clinics. This is a significant contributing component to the patients' lipid profile worsening. As such, it is challenging to fully ascribe all of the study's findings to a sedentary lifestyle. In addition, the fact that the study was conducted as a retrospective file scan creates a limitation in terms of the reliability of the diagnoses.

Our study has some superior aspects compared to other studies on this subject in the literature. There is no literature study comparing cholesterol levels in all three periods (pre-pandemic, pandemic process and post-pandemic normalization period). Our patient group did not include the geriatric population allowed less variables that could affect cholesterol values due to the low rate of chronic diseases.

The post-restriction phase shows the continuation of the habits formed during the pandemic.

Measures taken during the pandemic; considering that the habits formed during this time continue afterward, changes might be made to eating and physical activity routines. Studies

to boost physical activity should be conducted for unusual circumstances like the COVID-19 pandemic.

Ethical Statement

The Hacettepe University Non-Invasive Clinical Research Ethics Committee granted ethical permission prior to the study's launch with a decision dated 07.12.21, project number GO21/1297, and decision number 2022/14-60.

Conflict of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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None to declare.

Authors' contributions

Concept: S.A., İ.F., Design: S.A., İ.F., Data Collection or Processing: S.A., İ.F., Analysis or Interpretation: S.A., D.A.B., H.A., Literature Search: S.A., İ.F., H.A., Writing: S.A.

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Targeting HDAC enzymes by SAHA enhances the cytotoxic effects of cisplatin on acute myeloid leukemia cells

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Abstract

Chemotherapy is a widely used therapeutic approach to combat hematopoietic malignancies such as acute myeloid leukemia (AML). Although cisplatin is known as the first-generation platinum-based chemotherapy inhibitor, the wide use of cisplatin eventually leads to drug resistance, which is the biggest impediment to cancer chemotherapy. Histone deacetylase enzyme (HDAC) inhibitors have the ability to induce cell cycle arrest and apoptosis in different types of cancer, which stands as a promising alternative for those cancer patients not appropriate for intensive chemotherapy. This study concluded that there was a significant decrease in the proliferation of MOLM-13 and MV4-11 FLT3-ITD+ AML cell lines with the increasing SAHA and cisplatin concentrations in 48 hours using MTT cell proliferation assay. Moreover, the combination of SAHA and cisplatin led to a reduction in the proliferation of both cell lines correlated with the synergistic effect of the two drugs depending on the combination index (CI). Furthermore, investigating apoptosis for combined administration resulted in increased induction of apoptosis by Annexin-V/PI double staining. In conclusion, although additional studies are needed to fully elucidate the molecular mechanism underlying this combination, we propose a new approach to targeting AML, as AML increases over time with drug resistance and the consequent year-on-year increase in patient mortality.

Keywords: histone deacetylase inhibitors, cisplatin, acute myeloid leukemia, combination treatment, apoptosis

1. Introduction

Acute myeloid leukemia (AML) is a hematopoietic malignant disorder that results from chromosomal rearrangements and multiple gene mutations in hematopoietic cells. These alterations prevent the production of differentiated and matured hematopoietic cells, promote the accumulation of leukemia blasts in the bone marrow and spread the peripheral blood (1, 2). The incidence of AML disease has been rising for the last 40 years, and this is correlated with the aging process (3). AML can have an impact on individuals of all ages; however, it predominantly affects adults (4). AML disease generally has been classified as a genetic disease; however, several recent studies report that epigenetic changes contribute to the progression of AML. The most common epigenetic regulation that regulates the chromatin structure and gene expressions are DNA methylation and histone modifications. These modifications are managed by the epigenetic regulatory enzymes, which are DNA methyltransferases (DNMTs), histone methyltransferases (HMTs), histone acetyltransferases (HATs), and histone deacetylases (HDACs). The dysfunctionality of these enzymes is observed in AML (5, 6). In this way, targeting epigenetic modifications could be an effective strategy in AML. The effect of hypomethylating agents in AML was approved, so the use of HDAC inhibitors (HDACis) is under clinical investigation and could be promising in AML treatments (7–9). HDACis promise a therapeutic strategy for AML therapy. The HDAC functions in many aspects of biological effects in cancer cells, such as induction of apoptosis, mitotic arrest, and activation of autophagy (10).

The regular cells are relatively resistant and irresponsive to cell death induced by HDACis, and this is the reason for using HDACi in cancer treatments (11, 12). Although HDAC enzyme inhibition could be used as the treatment approach, the HDACis are not effective as monotherapies. In contrast, a combination treatment strategy can be used with numerous anticancer drugs that demonstrate anti-leukemic activity. Furthermore, HDACis could induce differentiation, cell cycle arrest, and apoptosis in AML, which gives a good option for AML patients who are not appropriate for intensive chemotherapy. Many studies mentioned that after treatment with cisplatin, chemoresistance can be developed in many cancer types (13). In the present study, we aim to treat AML cell lines MOLM-13 and MV4-11, both possessing a common mutation on AML, which is the Fms Related Receptor Tyrosine Kinase 3 (FLT3), which harbors Internal Tandem Duplication (ITD), with the commonly used chemotherapeutic

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agent cisplatin in combination with SAHA. The combination therapy of cisplatin with HDACi is a promising strategy to enhance the efficacy of cisplatin (14). In this way, the success of treatment could be increased, and chemoresistance could be prevented by combining cisplatin with SAHA. Hence, we checked the anticancer effects of drugs using an MTT cell viability assay and investigated the anticancer effect using an apoptosis analysis. In conclusion, we suggested a combination treatment to overcome the proliferation of AML cells with the administration of SAHA and cisplatin by revealing the synergistical effect of both drugs.

2. Materials and Methods

2.1. Materials

Cisplatin and SAHA were purchased from Sigma. A 5 mM stock solution of SAHA was prepared in dimethylsulfoxide (DMSO), and 3.3 mM stock cisplatin solution was dissolved in NaCl in alignment with the proposed recommendations by the supplier, and the stock solutions were stored at -20ºC. The RPMI 1640 and penicillin/streptomycin were purchased from Euro Clone.

2.2. Cell Culture and Maintenance

The AML cell lines, MOLM- 13 and MV4-11, used in this study were obtained from the German National Resource Center for Biological Material (DSMZ). The cells were cultured in RPMI 1640 medium, which was supplemented with 10% FBS and 100 U/mL penicillin/streptomycin. The cells were kept at 37°C in an environment of 5% CO_2. Upon reaching confluency, the cells were seeded at a density of 1 x 10^6 cells/ml and subsequently split at ratios ranging from 1:2 every two days. Afterward, the cells were collected and subjected to centrifugation for 5 minutes at 700 rpm. The supernatant was discarded, and the pellet was reconstituted using fresh media.

2.3. Cell Viability Assay

The MTT (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay was used to assess cell viability. Briefly, the cells were seeded in 96-well plates in triplicates, with a density of $1x10^4$ cells per well. Then, the cells were treated with varying concentrations of inhibitors alone and in combination for 48 hours. After the incubation, 10 µl of MTT solution was added to each well to initiate the assay, and then the plates were incubated at 37°C for 2-4 hours. Later, the 96-well plates were centrifuged at 1800 rpm for 10 minutes, and the supernatant was removed. Next, 100 µl of DMSO solution was added to each well to dissolve the formed formazan crystals, and the plates were incubated for an additional 15 minutes on a waving rotator in the dark at room temperature. Subsequently, the absorbance was measured at 570 nm using a Varioskan LUX multimode microplate reader from Thermo Scientific. The concentrations of SAHA and cisplatin that led to 20%, 30%, and 50% inhibition (known as IC20, IC30, and IC50) of MOLM-13 and MV4-11 cell lines were determined by analyzing the curves correlating cell proliferation with drug concentration. The combination analysis previously described was performed by using the CompuSyn software (Biosoft, Cambridge, United Kingdom) (15).

2.4. Cell Death Assay

The apoptotic cell death was assessed for cells treated with the IC50 value of SAHA and IC20 value of cisplatin in single and combinations using DMSO and NaCl as negative controls. The apoptotic cell population was determined by flow cytometry with Annexin V/Propidium iodide dual staining method as previously described (15). For this purpose, $1x10^6$ cells/well were incubated with inhibitors alone and their combinations. After 48 hours of incubation with drugs, the cells were harvested and analyzed using BD LSRFortessa (Becton Dickinson) flow cytometry.

2.5. Statistical Analysis

The GraphPad Prism 8 was used to analyze the data, and the comparisons of two experiment sets were performed by Two-Way ANOVA followed by Dunnett's Multiple Comparison test. The level of statistical significance was set at $P<0.05$.

3. Results

3.1. SAHA and Cisplatin Led to Decreased Cell Viability in AML Cells

To establish the efficacy of SAHA or cisplatin on the cell viability of MOLM-13 and MV4-11 AML cells, the cells were treated with increasing concentrations of SAHA and cisplatin alone. The results demonstrated that cell proliferation was reduced in a dose-dependent manner for both SAHA and cisplatin. When cisplatin was administered between 0.05-10 µM, the IC20, IC30 and IC50 values were determined as 0.53 µM, 1.03 µM and 2.62 µM for the MOLM-13 cells, respectively (Fig. 1A). For MV4-11, the same range of cisplatin dose was administered, and the IC20, IC30 and IC50 values were calculated as 0.156 µM, 0.79 µM and 3.38 µM, respectively (Fig. 1B). Afterward, MOLM-13 (0.05-2 µM) and MV4-11 (0.005-1 µM) cells were treated with SAHA. The IC20, IC30 and IC50 values of SAHA were specified as 0.073 µM, 0.09 µM and, 0.43 µM respectively for MOLM-13 cells and 0.0019 µM, 0.066 µM and 0.29 µM for MV4-11 cells, respectively (Fig. 1C and 1D). These results indicated that SAHA and cisplatin alone have an inhibitory effect on MOLM-13 and MV4-11 cell proliferation.

3.2. The Combinatorial Anticancer Effect of SAHA and Cisplatin on MOLM-13 and MV4-11 AML Cells

Next, to understand and investigate the effect of SAHA and cisplatin combination on MOLM-13 and MV4-11 cells, we treated cells with both drugs alone and combined IC20 and IC30 values. The SAHA or cisplatin administrations alone did not decrease the cell viability by less than 70%, as expected. However, when the combination of IC20 SAHA+IC20 cisplatin and IC30 SAHA+IC30 cisplatin was administered to the cells, MOLM-13 cells were more sensitive than MV4-11 cells. The cell viability was decreased by 56% and 68% for MOLM-13 cells in response to IC20 SAHA+IC20 cisplatin and IC30 SAHA+IC30 cisplatin combination, respectively (Fig. 2A). We also used the IC30 values of the drugs, and this

combination treatment resulted in a reduction of less than 50% in MOLM-13 AML cells. On the other hand, IC20 SAHA+IC20 cisplatin significantly reduced the cell viability of MV4-11 cells compared to single administrations and untreated control cells at 48 hours (Fig. 2B). In the administration of IC30 values of the inhibitors, the proliferation of MV4-11 cells was reduced by approximately 65%; however, no significant difference was observed compared to the IC30 cisplatin administration. Moreover, to determine whether the effect of these combinations was synergistic or not, the isobologram test was performed. The results indicated that the SAHA and Cisplatin combination treatments showed a CI value below 1.0 and, thus, a synergistic effect for both MOLM-13 and MV4-11 AML cells (Table 1).

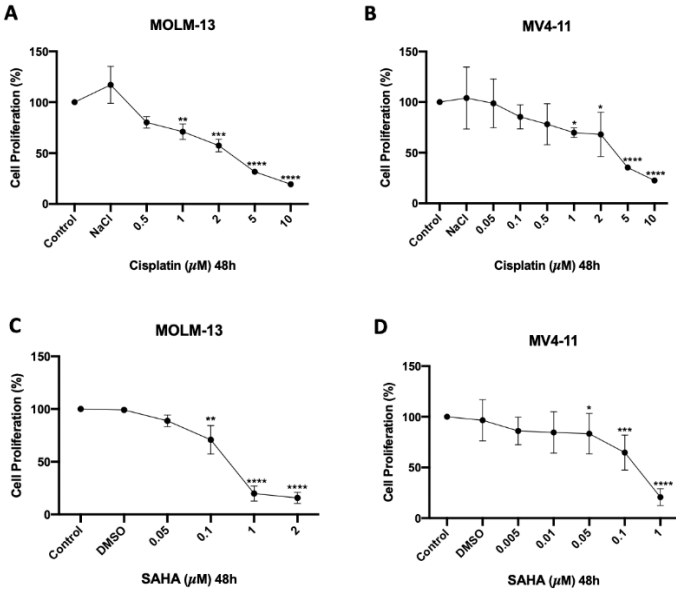

Fig. 1. The cytotoxic effect of Cisplatin (A, B) and SAHA (C, D) on MOLM-13 (A, C) and MV4-11 (B, D) AML cells. These results are representative of data from samples in triplicate in three independent experiments (n = 3). All data are presented as mean ± S.D. (ns=P > 0.05, *= P≤ 0.05, **= P ≤ 0.01, ***= P ≤ 0.001, ****= P≤ 0.0001)

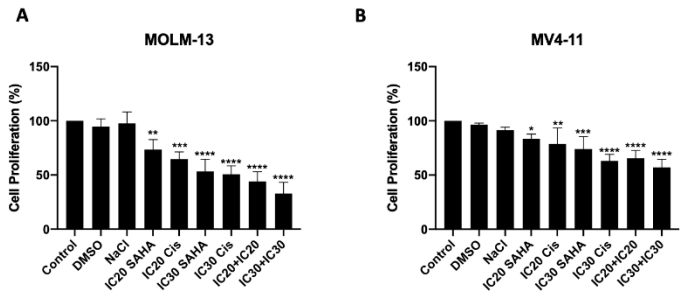

Fig. 2. The investigation of SAHA and Cisplatin combination on MOLM-13 (A) and MV4-11 (B) AML cell line. These results are representative of data from samples in triplicate in three independent experiments (n = 3). All data are presented as mean ± S.D. (ns=P > 0.05, *= P≤ 0.05, **= P ≤ 0.01, ***= P ≤ 0.001, ****= P≤ 0.0001)

Table 1. The combination index values of MOLM-13 and MV4-11 cells treated with the combination of SAHA with Cisplatin, were calculated and isobolograms were determined by CompuSyn software

MOLM-13			MV4-11		
SAHA (μM)	Cisplatin (μM)	SAHA + Cisplatin	SAHA (μM)	Cisplatin (μM)	SAHA + Cisplatin
Dose	Dose	CI Value	Dose	Dose	CI Value
0.073	0.53	0.39378	0.0019	0.156	0.24601
1.53	1.03	0.48659	0.066	0.79	0.59307

A CI of <1, =1, or >1 is indicative of synergistic, additive, or antagonistic effects, respectively

3.3. The Combination of SAHA and Cisplatin Induced Apoptotic Cell Death on AML Cells

We combined the IC50 value and 1 μM SAHA with the IC20 value of cisplatin, respectively, to investigate the mechanism behind the decreased cell viability. The administration of the IC50 value of SAHA and IC20 value of cisplatin in combination with MOLM-13 cells resulted in a 2.5-fold increase in total apoptotic cell death when compared to untreated control cells (Figure 3A). In particular, 1 μM SAHA and IC20 value of cisplatin led to a significant increase as such 7-fold for MOLM-13 cells. The single 1 μM SAHA administration and the combination with cisplatin caused a significant increase in total apoptotic cell death on MOLM-13 cells. Our results showed that the same combination experiment caused a 10-fold increase in late apoptotic cells compared to the control, while a single IC20 cisplatin treatment increased the amount of early apoptotic cells of MOLM-13 cells by 2.5-fold (Figure 3A). This result showed that the combination of 1 μM SAHA and cisplatin led to a better induction of apoptosis on MOLM-13 cells, considering the late and total apoptotic cell number. Similarly, MV4-11 cells were induced to undergo apoptosis in response to the same combination. The IC50 value of SAHA and IC20 of cisplatin combination resulted in a 2-fold increased apoptotic cell death in MV4-11 cells over 48 h compared to untreated controls (Figure 3B). Moreover, we demonstrated that administration of 1 μM SAHA and IC20 value of cisplatin led to an 8-fold more total apoptotic cell death, which was mostly composed of late apoptotic cells when compared to untreated MV4-11 control cells. Interestingly, unlike MOLM-13 cells, 1 μM SAHA treatment resulted in similar apoptotic cell death as in the IC50 SAHA+cisplatin combination. This result made us think that the apoptotic cell death in response to combination is mainly the effect of SAHA. In conclusion, our results demonstrated that the combination of SAHA and cisplatin increased the apoptotic cells for both cell lines but to a better extent for MOLM-13 cells.

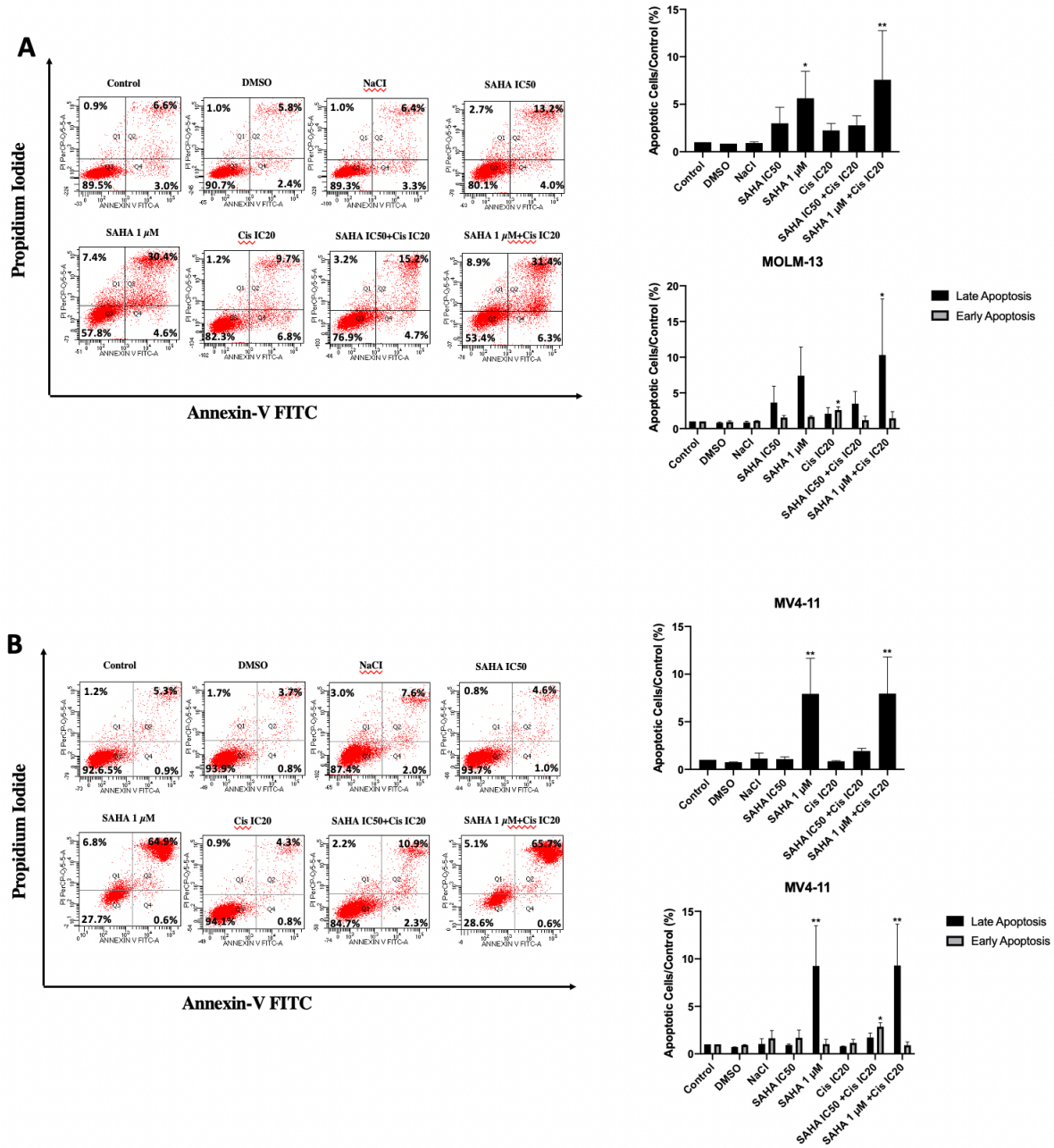

Fig 3. The apoptotic effect of SAHA and Cisplatin drug combination on MOLM-13 (A) and MV4-11 (B) AML cells. The experiments were performed as two biological replicates (n=2). The left part of the image indicates the representative apoptotic cell histograms. The graph where the right upper demonstrates the total apoptosis (Q2+Q4), the and below parts demonstrate the late (Q2) and early apoptotic (Q4) cell amount separately. All data are presented as mean ± S.D. (ns=P > 0.05, *= P≤ 0.05, **= P ≤ 0.01, ***= P ≤ 0.001, ****= P≤ 0.0001)

4. Discussion

Chemotherapy is a commonly used treatment method to combat various types of cancer. Despite the idea that chemotherapy fatally damages the DNA of cancer cells, its mutational effects in normal tissues have not yet been fully elucidated. One of these widely used chemotherapeutic agents recognized as the first-generation platinum-based chemotherapy inhibitor is cisplatin (16). Although cisplatin was first discovered as an agent that inhibits the growth of *Escherichia coli* after its cytotoxic effects on cancer cells have been proven, it has been used as a first-line therapeutic agent in clinics with numerous cancer types, especially leukemia. (17). When cisplatin enters the cell, it loses a chloride ligand, then binds to DNA, following the formation of intra-helix DNA adducts, and then inhibits DNA synthesis and, thus, growth of the cells. The cisplatin-induced DNA damage activates the DNA repair response via the nuclear excision repair system, stopping the cell death induced by cisplatin through the activation of the ATM pathway (18). Even though therapy has been indicated to be effective, many patients face relapses because of drug resistance. In this situation, researchers investigated the effect of combining cisplatin with different small inhibitors and natural products (19, 20).

Although there are effective and selective FLT3 tyrosine kinase inhibitors approved by the FDA and used in clinics, they might be ineffective in eliminating the FLT3-ITD+ AML cells, which in turn potentially cause relapse. It was shown that inhibition of FLT3 by tyrosine kinase inhibitors led to an upregulation of HDAC8 and activated the FLT3-ITD cells

(21). This study shows the link between the HDAC enzymes and the benefit of their inhibition against FLT3-ITD+ cells. In the present study, we aim to take a step towards developing novel approaches to eliminate FLT3-ITD+ AML cells. For this purpose, we treated two AML cells, MOLM-13 and MV4-11, both possessing a common mutation on AML, namely FLT3, which harbors ITD mutation (22). Specifically, these two AML cells were chosen to help compare our combination therapy because they possess the same mutation. As a first step, we administered the MOLM-13 and MV4-11 cells with both cisplatin and SAHA alone in different dose ranges individually. Administration of 10 μM cisplatin resulted in a reduction in proliferation of less than 90% for both cells. On the other hand, 1 μM SAHA resulted in 90% of death cells for MOLM-13 and MV4-11 cell lines. Consistent with our data, the data revealed by Ma et al. indicated that administration of SAHA (0.01–10 μM) for 72 hours resulted in a dose-dependent reduction of cell proliferation of MOLM-13 cells (23). Furthermore, the study by Yao et al. revealed that when 10 μM of SAHA was applied, approximately 80% reduction in proliferation was observed for MOLM-13 and MV4-11 cells (24). Similar to our data, Zhang et al. showed that cisplatin has a dose- and time-dependent increasing anticancer efficacy in human AML cell lines MV4-11 and MOLM-13 (25). Altogether, our data revealed the inhibitory effect of both SAHA and cisplatin in terms of cell proliferation for two AML cells.

As a further study, we continued with the combination of SAHA and cisplatin and evaluated this combination on the proliferation of MOLM-13 and MV4-11 FLT3-ITD+ AML cells. The results clearly show that the combined use of these two drugs has a synergistic anti-proliferative effect on these two AML cells compared to control and single administrations of SAHA and cisplatin. Similar to our results, the combination of SAHA and cisplatin significantly reduced VAT-39 cell viability of hepatoid adenocarcinoma cells in a dose-dependent manner (26). In addition, Jin et al. reported the cytotoxic effect of SAHA and cisplatin combination therapy on HeLa cells (27). Moreover, the data revealed by Shen and his colleagues indicated that a combination of SAHA and cisplatin results in a marked increase in cytotoxicity of human oral squamous cell carcinoma and osteosarcoma cells (28, 29). Furthermore, the results have shown that SAHA significantly reduced cell viability and synergistically enhanced the anticancer effects of cisplatin in both the cisplatin-resistant head and neck cancer cells (30).

To further investigate, we evaluated the effect of the mentioned combinations in terms of programmed cell death; apoptosis. With this aim, the cells were treated with both alone and in combinations of IC50 value of SAHA, 1 μM of SAHA and IC20 value of cisplatin and analyzed by flow cytometer. Interestingly, following the IC50 values of SAHA and cisplatin, IC20 was not statistically significant; increasing the SAHA to 1 μM with IC20 of cisplatin resulted in induce total apoptotic cell death for both AML cells. Compatible with our data, while administration of SAHA (2.5 μM) or cisplatin (10 μM) alone induced moderate apoptosis on HeLa cells, co-administration of cisplatin (10 μM) and SAHA (2.5 μM) resulted in a significantly higher degree of induction of apoptosis when it compared to cisplatin alone (27). The co-treatment of SAHA and cisplatin led to an induced apoptosis in human oral squamous cell carcinoma cell lines after being evaluated by TUNEL assay (28). The study by Kyaw et al. showed that the number of apoptotic cells was 2.2 times higher in cells treated with cisplatin and 3.3 times higher in cells treated in combination with cisplatin and SAHA compared to control cells (26). However, single 1 μM SAHA resulted similarly to the combination with IC20 cisplatin in MV4-11 cells. This aforementioned situation led us to the idea that SAHA administration alone may also cause apoptotic cell death. To fully explain this, further analysis could be used to check for different apoptotic mechanisms, such as checking the expression level of anti-apoptotic or pro-apoptotic proteins to fully elucidate the apoptotic mechanism behind them. Although further studies about the mentioned combination effect on AML is needed, in conclusion, our study proposes simultaneous administration of SAHA and cisplatin to inhibit cell proliferation of MOLM-13 and MV4-11 cell lines in AML with induction of apoptotic cell death.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: E.B.G.A., Design: E.B.G.A., Data Collection or Processing: M.Ş., Ö.P., Analysis or Interpretation: M.Ş., Ö.P., Literature Search: M.Ş., Ö.P., Writing: E.B.G.A., M.Ş.

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Sexually transmitted infections (STIs)/HIV associated to human papillomavirus (HPV) in precancerous lesions among Cameroonian women

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Abstract

Cervical precancerous lesions signify cellular changes in the cervix linked to an escalated risk of cancer. These lesions often evolve towards malignancy influenced by various factors, including sexually transmitted infections (STIs), irrespective of human papillomavirus (HPV) presence. STIs represent a substantial global public health concern, affecting both sexes, with over 30 pathogens, comprising bacteria, viruses, and parasites, capable of causing STIs. The clinical presentation of these pathogens varies significantly. This study aimed to delineate the microbial profile within HPV-containing precancerous lesions. A prospective cross-sectional descriptive study was conducted in Cameroon's central region, particularly in the Endom district. Among 343 patients screened, 225 women were enrolled based on positive HPV cervical smear results. Cervical cell lesions were analyzed using the Papanicolaou technique, while parasitological and microbiological methods were utilized to identify other microbes. HPV genotypes were determined via multiplex PCR. The study revealed 65 HIV-positive and HPV-positive individuals (18.96%); 40 HIV-negative and HPV-positive individuals (11.65%); 25 HIV-positive and HPV-negative individuals (7.29%); and 213 HIV-negative and HPV-negative individuals (62.10%). Various cervical lesions were identified, including Low-grade Lesions (LSILs) (21%) and High-grade lesions (HSILs) (16%). *Trichomonas vaginalis* (TV) (51%), *Candida albicans* (CA) (28%), *Herpes simplex* (HS) (2%), *Gardnerella vaginalis* (GV) (17%), *Aspergillus* (A) (2%), and HIV were the primary infectious agents identified in these precancerous lesions, with significant distribution ($P<0.005$), suggesting their role as risk factors for precancerous lesions (OR=13.89, 95% CI 6.87-21.60). Multiple HPV genotypes were characterized in the same precancerous lesions, with approximately 25% (85) of women harboring either Low_Risk (LR) (6, 11) or High_Risk (HR) (16, 18, 45, 58) HPV genotypes ($P<0.001$). Coinfections such as HPV/TV/CA and HPV/HIV/TV were prevalent, indicating their potential as associated risk factors for precancerous lesions. Coinfections with other microbes, typically associated with cervicitis, appear to facilitate HPV infection. These findings contribute to understanding the microbial landscape of cervical samples and underscore the importance of integrated care approaches for HIV-positive individuals with HPV-related lesions.

Keywords: precancerous lesions, HPV, coinfections, cervical cytology, microbial composition

1. Introduction

Cervical cancer represents a substantial health burden for women worldwide(1). Among the cancers that strike people in their 20s and 30s, cervical cancer is highly prevalent(2). The World Health Organisation (WHO) estimates that human papillomavirus (HPV) affects 9% to 13% of the world's population, with over 6.2 million cases of the virus reported each year in the US alone (3). Among the more than 140 varieties of HPV, many serotypes (16, 18, 45, 58) are linked to 70% of cervical cancer cases, as well as precancerous lesions in the cervical region. The genesis of cervical tumors caused by HPV and HPV infection could be greatly impacted by the vaginal microbiota. Cervicitis is also caused by vaginal inflammation and oncogenic mutations in human cells, which are closely related to bacteria(4). An inflammation of the cervix is called cervicitis. It is typically sexually transmitted and caused by several infectious agents(5). The highest and lowest occurrences of pathogens were observed with *T. vaginalis* (71.0%) and *U. urealyticum* (6.45%), respectively. Coinfection exhibited a prevalence of 19.35% and 9.67% for three-organism and two-organism combinations, respectively. Significant correlations were identified between the presence of cervicitis and coinfection with other genital pathogens, abortion history, and inconsistent condom usage ($p < .05$).(6).

Another investigation unveiled numerous other sexually transmitted infections (STIs) linked to HPV infection: 36% tested positive for *Gardnerella vaginalis*, 35% for *Ureaplasma*

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parvum, 8% for *Candida albicans*, 6.7% for HPV, 4.6% for *Ureaplasma urealyticum*, 3.6% for *Mycoplasma hominis*, 2% for *Trichomonas vaginalis*, 0.8% for *Chlamydia trachomatis*, 0.4% for *Mycoplasma girerdii*, 0.2% for *Mycoplasma genitalium*, and 0.2% for Neisseria gonorrhoeae. Symptom absence was noted in 187 women (37%), among whom 61% were found to be infected. Of the 34 HPV-positive samples, 17 harbored high-risk HPV genotypes (HR-HPV); notably, types 16 (38%), 18 (21%), and 51 (18%) exhibited the highest prevalence. Among the 34 HPV-positive individuals, 29 carried HR-HPV. Associations with various risk factors were also documented(7). Sexually transmitted infections (STIs) often manifest without symptoms and can result in diverse complications, including pelvic inflammatory disease, infertility, ectopic pregnancy, cervical cancer, and congenital infections in infants born to infected mothers. Assessing the prevalence of STIs is crucial for preventing, managing, and effectively treating individuals afflicted with these infections(8). Persistent HPV infection is responsible for over 99% of cervical cancers. Annually, there are over 500,000 new cases of cervical cancer globally, resulting in around 250,000 deaths. Developing countries bear the brunt of this burden, with 80% of cases occurring in these regions(9). Several studies have reported associations between various microbial infections and the presence of HPV in cervical precancerous lesions. For example, Trichomonas vaginalis, *Candida albicans*, herpes simplex virus (HSV), *Gardnerella vaginalis*, and Aspergillus have been identified as common microbial agents found in conjunction with HPV infection in cervical lesions(10). These microbial coinfections have been suggested to influence the natural history of HPV infection, cervical lesion development, and progression to cervical cancer (11). It is therefore important to determine whether specific sexually transmitted infection (STI) co-factors are involved in the development and progression of precancerous cervical lesions associated with human papillomavirus (HPV) infection among Cameroonian women.

The aim of this study was to assess the prevalence and co-occurrence of specific sexually transmitted infections (STIs) alongside HPV infection in precancerous cervical lesions among Cameroonian women and to investigate the potential role of these STIs as co-factors in the development and progression of precancerous lesions."

2. Materials and Methods

2.1. Study sites

This prospective cross-sectional descriptive study was conducted in hospitals situated within the districts under study. The analyses were performed both on-site and at the biochemistry laboratory at the University of Douala

2.2. District of Endom

Endom District is located in the central region of Cameroon. It is characterized by its diverse landscape, which includes lush forests, fertile farmland, and picturesque hills. The district is home to several rural communities, each with its own unique culture and traditions. Economically, Endom District relies primarily on agriculture, with crops such as cocoa, coffee, and bananas being important sources of income for local residents. The district also has potential for tourism, with its natural beauty and opportunities for outdoor activities such as hiking and birdwatching. The infrastructure in Endom District is relatively basic, with limited access to amenities such as healthcare facilities and educational institutions. However, efforts are being made to improve infrastructure and services to better meet the needs of the local population.

Overall, Endom District is a rural area with a rich natural environment and cultural heritage but also faces challenges related to economic development and access to essential services. The health situation in Endom District, like many rural areas in Cameroon, faces various challenges and opportunities. Access to healthcare services, including primary care facilities, specialized medical care, and essential medications, may be limited in some parts of the district due to factors such as geographic isolation, inadequate infrastructure, and resource constraints. Common health issues in the Endom District may include infectious diseases such as malaria, respiratory infections, diarrheal diseases, and vaccine-preventable illnesses. Maternal and child health, including access to prenatal care, skilled birth attendants, and immunizations, are also important priorities for improving health outcomes in the district.

2.3. Study population and sample size

All women at various stages of development, with or without cancer, were the focus of these analyses.

Selection criteria

Inclusion

All Cameroonian women aged eighteen and above were eligible for inclusion. Participation in the study was contingent upon being sexually active, expressing willingness to take part, signing an informed consent form, not having undergone a hysterectomy, and presenting a positive cytology result.

Sampling method

A convenience sampling method was employed, involving the consecutive recruitment of potential participants at various sites.

Questionnaire

A semi-structured questionnaire administered by an investigator was used to gather data from each woman during a 15–20-minute individual interview.

Cervix sample collection and visual inspection

The present Bethesda system was utilized to classify cytological abnormalities into the following categories: negative, Atypical Squamous Cells of Undetermined significance (ASC-US), Atypical Glandular Cells (AGC), atypical squamous cells without High-Grade Intraepithelial Lesions (ASC-H), atypical squamous cells with low-grade squamous intraepithelial lesions (LSILs), and high-grade

squamous intraepithelial lesions (HSILs).

Microscopy

Trichomonas

Wet mount microscopy: A sample of vaginal discharge was collected, placed on a slide with a drop of saline solution, and observed under a microscope for the presence of the parasite.

***Candida albicans* identification**

Samples of affected tissue, discharge, or bodily fluids (e.g., vaginal swabs, oral swabs) were collected and examined under a microscope to determine the presence of *Candida albicans* yeast cells or hyphae.

Gardnerella vaginalis identification

A sample of vaginal discharge was collected and examined under a microscope to look for characteristic changes in the vaginal microbiota, such as the presence of "clue cells" (vaginal epithelial cells covered with bacteria), which are indicative of BV.

DNA extraction

Fresh cervical cells were utilized for DNA extraction employing the commercially available QIAGEN Multiplex PCR Kit. The extraction process followed the instructions provided by the manufacturer.

2.4. Statistical analysis

After the data were entered and checked for consistency in an Excel spreadsheet, Graph Pad Prism version 6 was used for analysis. One-way ANOVA and the independent chi-square test were used to compare the outcomes. In the tables and graphics, qualitative variables are displayed as percentages with 95% confidence intervals. A significance level of $P \leq 0.05$ was used.

3. Results

3.1. Sociodemographic information

Table 1 below displays the mean values of a certain parameter across different age groups. This study provides valuable insights into the relationship between age and the parameter being measured, highlighting the importance of considering age as a factor in research and clinical settings and the distribution of individuals across different marital statuses and education levels, along with the associated percentages and statistically significant values.

Table 1. Sociodemographic information of the study population

Age range	N (%)	Mean ± SD	P
[15-25[	14 (%)	19.30±2.6	0.001
[25-35[	11 (%)	35.17± 5.5	0.001
[35-45[	36 (%)	39.61±3.7	0.002
[45-55[	31 (%)	53.30± 4.9	0.001
[55-65[	8 (%)	61±0.9	0.05
CIVIL STATUS			
SINGLE	38(%)	-	0.05
DIVORCED	56(%)	-	
MARRIED	160(%)	-	
WIDOW	92(%)	-	
EDUCATION			
NONE	34(%)	-	**NS**
PRIMARY	178(%)	-	
SECONDARY	102(%)	-	
HIGHER	29(%)	-	

3.2. Distribution of Cervical Cytology Results: Normal, LSIL, and HSIL

Fig. 1 below presents the distribution of cervical cytology results among the categories "Normal", "LSIL" (low-grade squamous intraepithelial lesion), and "HSIL" (high-grade squamous intraepithelial lesion).

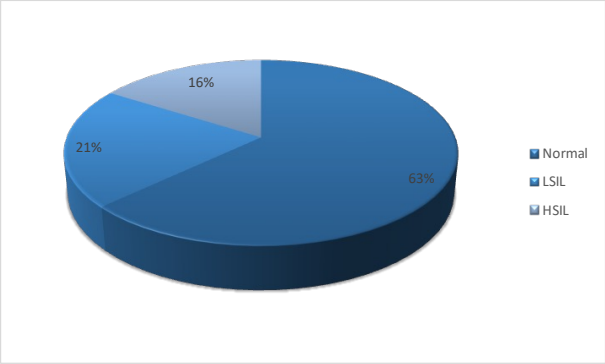

Fig. 1. Cervical lesions distribution
Normal: No atypical squamous cell
LSIL: Low-grade squamous intraepithelial lesion
HSIL: High-grade squamous intraepithelial lesion

3.3. Sexually Transmitted Infections (STIs) profile in lesions

Table 2 below illustrates the prevalence of various infections, including Trichomonas vaginalis, *Candida albicans*, Herpes simplex, *Gardnerella vaginalis*, Aspergillus, HPV, and HIV, among women with different cervical intraepithelial neoplasia (LSIL and HSIL) grades.

3.4. Human papillomavirus and STI coinfection

Table 3 provides data on the prevalence of different infections (Trichomonas vaginalis, *Candida albicans*, Herpes simplex, *Gardnerella vaginalis*, and Aspergillus) among individuals with LR (low-risk) genotypes of HPV 6 and HPV 11. There is a statistically significant association between Trichomonas vaginalis and candida albicans infection and LR cytology, Individuals infected with Candida albicans have twice the odds of having LR cytology compared to those without the infection. HIV infection shows a strong positive association with LR cytology. The odds of having LR cytology are nearly 14 times higher among individuals infected with HIV compared to those who are not.

Table 4 below presents the percentages (and corresponding numbers, n) of different microbial infections detected in association with various HPV genotypes (HPV 16, HPV 18, HPV 45, and HPV 58).

Table 2. Prevalence of various infections

	Trichomnas vaginalis n(%)	*Candida albicans* n(%)	Herpes simplex n(%)	*Gardnella vaginalis* n(%)	Aspergillus n(%)	HPV n(%)	HIV n(%)
Normal	**136(77.7%)**	**54(56.2%)**	**6(85.7%)**	**43(74.1%)**	**-**	**13(12.3%)**	**-**
LSIL	**27(15.4%)**	**42(43.8%)**	**1(14.3%)**	**15(25.9%)**	**7(100%)**	**75(71.4%)**	**63(70%)**
HSIL	**12(6.8%)**	**-**	**-**	**-**	**-**	**17(16.1%)**	**27(30%)**
Total	**175**	**96**	**7**	**58**	**7**	**105**	**90**
P value	**0.005**	**0.0001**	**NS**	**0.002**	**NS**	**0.0001**	**NS**

Table 3. Presentation of the different cervical lesions and the identified LR-HPV genotypes

LR Genotypes	HPV 6 % (n)	HPV 11 % (n)	OR
Trichomonas vaginalis	37.93 (11)	34.99 (7)	2.6
Candida albicans	31.01 (9)	14.99 (3)	2
Herpes simplex	27.59 (8)	25.01 (5)	1.02
Gardnella vaginalis	3.46 (1)	25.01 (5)	2.1
Aspergillus	100 (29)	100 (20)	-
HIV	3.46 (1)	1	13.89
TOTAL	12	46	

Table 4. Presentation of different cervical lesions and HR-HPV genotypes identified

LR Genotypes	HPV 16 % (n)	HPV 18 % (n)	HPV 45 % (n)	HPV 58 % (n)	OR
Trichomonas vaginalis	**40 (2)**	**20 (2)**	**54.5 (6)**	**0 (0)**	**4.2**
Candida albicans	**20 (1)**	**30 (3)**	**9.09 (1)**	**0 (0)**	**5.9**
Herpes simplex	**0 (0)**	**20 (2)**	**18.1 (2)**	**0 (0)**	**1.2**
Gardnella vaginalis	**20 (1)**	**30 (3)**	**18.1 (2)**	**0 (0)**	**3.3**
Aspergillus	**20(1)**	**-**	**-**	**0 (0)**	**-**
HIV	**(1)**	**(0)**	**(2)**	**0 (1)**	**13.89**
TOTAL	**5**	**10**	**11**	**1**	

4. Discussion

4.1. Sociodemographic information

The observed differences in age distribution among various brackets highlight the heterogeneity within the population under study. Similar age disparities have been documented in other populations, with younger individuals often presenting with distinct health needs and risk profiles compared to older age groups(12). For instance, a study by Smith *et al*. revealed that younger individuals were more likely to engage in risky health behaviors, such as substance use and unprotected sexual activity, which could impact their health outcomes over time (13).

The distribution of individuals across different civil status categories reflects the social dynamics within the population. Comparable patterns of civil status have been reported in previous studies, with variations in marital status linked to differential access to social support networks and healthcare resources(14). For example, divorced or widowed individuals may experience greater social isolation and have fewer resources available for managing health-related challenges (15).

4.2. Sexually Transmitted Infections (STIs) characterization in lesions

The distribution of cervical cytology results in this study, with a predominance of normal cytology and a smaller proportion of LSIL and HSIL cases, is consistent with findings from similar studies conducted in diverse populations (16). For example, a recent population-based study by Smith *et al*. reported comparable proportions of normal cytology and LSIL/HSIL cases among women undergoing cervical cancer screening (17). The predominance of normal cytology over low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL) in the Endom population may be influenced by several factors, such as the age distribution, reproductive history, sexual behaviour, and lack of certain behaviors, such as smoking and the presence of a younger population.

4.3. Sexually Transmitted Infections (STIs) profile in lesions

The greater prevalence of Trichomonas vaginalis among

individuals with LSILs than among those with HSILs is consistent with previous studies highlighting the association between Trichomonas vaginalis infection and cervical dysplasia(18). Recent research by Masha *et al*. revealed a similar trend, emphasizing the need for targeted interventions to address Trichomonas vaginalis infection in cervical health programs (19). The greater prevalence of Trichomonas vaginalis among individuals with LSILs may be multifactorial, involving a complex interplay of sexual behavior, immunological factors, the vaginal microenvironment, coinfections, and socioeconomic factors. This situation has also been observed for *Candida albicans*, and recent studies by Li *et al*. and Park *et al*. have explored the role of *Candida albicans* in cervical dysplasia, suggesting possible mechanisms for its involvement (20)**.** Herpes simplex (HSV) and *Gardnerella vaginalis*: Although Herpes simplex and Gardnella vaginalis exhibited varying prevalence between the LSIL and HSIL groups, their associations with cervical dysplasia warrant further investigation. Studies by Javanbakht *et al*. have explored the potential role of Herpes simplex and *Gardnerella vaginalis* in cervical health, highlighting the need for comprehensive diagnostic and management strategies(21). The high prevalence of HPV among individuals with both LSILs and HSILs is consistent with its established role as a primary driver of cervical dysplasia and cancer. Recent studies by de Sanjose *et al*. and Bruni *et al*. have provided comprehensive insights into the global burden of HPV infection and its association with cervical neoplasia(22). Additionally, the higher prevalence of HIV among individuals with HSILs highlights the intersectionality of HIV and cervical dysplasia, emphasizing the importance of integrated care approaches.

4.4. Human papillomavirus and STI coinfection

The prevalence of LR HPV genotypes alongside *Candida albicans* colonization reflects the complex interplay between fungal infections and HPV persistence. Studies have suggested a potential link between *Candida albicans* and HPV-related lesions, with some evidence indicating an association between *Candida albicans* biofilms and cervical dysplasia (23). The high prevalence of *Candida albicans* in cervical lesions may be attributed to several factors. The higher prevalence of *Candida albicans* in cervical lesions likely results from a combination of immunosuppression, hormonal fluctuations, antibiotic use, sexual activity, underlying medical conditions, and coinfections. The coexistence of LR HPV genotypes with HIV infection highlights the increased vulnerability of immunocompromised individuals to viral coinfections. Adler emphasized the importance of integrated care approaches for HIV-positive individuals with HPV-related lesions, underscoring the need for targeted interventions to improve health outcomes (24). Integrated care approaches for HIV-positive individuals with HPV-related lesions are essential for providing comprehensive, preventive, and patient-centered care. By addressing the unique healthcare needs of this population, integrated care models will contribute to improved health outcomes, reduced healthcare disparities, and efficient resource utilization. The data presented highlight the cooccurrence of specific HPV genotypes with various microbial infections in cervical tissue samples. These high-risk HPV genotypes are commonly associated with cervical cancer. The presence of Trichomonas vaginalis and *Candida albicans* alongside HPV 16 and HPV 18 raises questions about potential interactions between these pathogens. Gillet *et al*. suggested a possible association between Trichomonas vaginalis infection and increased risk of HPV acquisition and persistence(25). Similarly, the co-occurrence of *Gardnerella vaginalis* with HPV 45 and HPV 58 warrants an investigation into the relationship between BV and HPV infection. The absence of herpes simplex virus (HSV) and Aspergillus in HPV-positive samples suggests potential differences in their association with HPV infection compared to other microbial pathogens. Although limited research exists on the relationship between HSV or Aspergillus and HPV-related lesions, future studies could elucidate their role in cervical health and disease progression. The presence of HIV in a subset of HPV-positive samples has important clinical implications. HIV-positive individuals are at increased risk of HPV-related diseases, including cervical cancer. Integrated care approaches, as discussed previously, are essential for managing concurrent HIV and HPV infections and reducing the risk of cervical cancer development.

The findings from this study underscore the complex interplay between human papillomavirus (HPV) genotypes and microbial coinfections in cervical samples. Specific HPV genotypes exhibit varying degrees of association with microbial coinfections. While some genotypes, such as HPV 16 and HPV 18, demonstrate higher prevalence rates alongside certain pathogens, such as Trichomonas vaginalis and *Candida albicans*, others, such as HPV 45 and HPV 58, show different patterns of microbial co-occurrence. The presence of microbial coinfections, particularly those with established associations with HPV-related diseases, may have clinical implications for cervical health. Understanding the relationship between HPV genotypes and microbial pathogens can inform screening, diagnostic, and treatment strategies aimed at reducing the burden of cervical lesions and preventing cervical cancer development. Although this study provides valuable insights into the microbial landscape of cervical samples, further research is warranted to elucidate the underlying mechanisms driving HPV-microbial interactions. Prospective studies with larger sample sizes and longitudinal follow-up are needed to validate these findings and identify potential causative relationships between specific pathogens and HPV-related lesions. The integration of care for HIV-positive individuals with HPV-related lesions is essential for addressing the complex healthcare needs of this population. By providing comprehensive screening, treatment coordination, preventive measures, and psychosocial support, integrated care models

can improve health outcomes and quality of life for affected individuals.

Conflict of interest

The authors declare that they have no competing interests.

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Authors' contributions

Concept: E.L.E.E., Design: E.L.E.E., Data Collection or Processing: N.A.F.E., Analysis or Interpretation: E.R.M., Literature Search: M.C.B., Writing: E.L.E.E., A.H., K.M.M.L.

Ethical Statement

The study was conducted in accordance with the Helsinki Declaration and the Cameroonian Ministry of Public Health's guidelines for using human experimental models in clinical research. The National Ethics Committee of Cameroun, registration number 2022/12/312/CE/CNERSH/SP, granted ethical clearance to carry out this action. Regional delegations were also granted administrative clearance. Each woman received an explanation of the study's purpose and goals in the language they could best understand—French or English—as well as answers to any questions. Enrolment was restricted to women who had signed an informed consent form for their involvement. The study was entirely voluntary, and women had the right to refuse to answer any questions or to stop participating at any time.

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The value of EEG and SPECT in the assessment of juvenile migraine

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Abstract

Despite the cause of migraine headaches is not exactly understood, cerebral blood flow studies revealed new insights in the pathophysiology of migraine. The data from Single Photon Emission Computerized Tomography studies in juvenile group diagnosed according to the HIS criteria are very rare, also there are limited number of studies in literature in which SPECT and EEG findings are combined. This study aimed to evaluate changes in regional cerebral blood flow and EEGs in juvenile patients suffering from migraine, also to investigate the relationship between SPECT and EEG in respect to comparison of each other, the frequency of attacks and response to the treatment. We grouped 20 children with migraine, 9 with aura, 11 without aura, and obtained SPECT and EEG's in ictal and interictal periods. All patients received Propranolol after the studies were performed. Regional cerebral and cerebellar perfusion was evaluated both visually and semiquantitatively. In the study, visual observation of SPECT revealed that perfusion defect was found 88% for ictal, 55% for interictal period and 55% for ictal, 18% for interictal period in patients with and without aura, respectively. The corticocerebellar ratios obtained semiquantitativly revealed significant differences between ictal and interictal periods in patients without aura, ictal periods in patients with and without aura, interictal periods in patients with and without aura. EEG findings were not significant. All patients who received therapy recovered well. Overall, SPECT can be used as an additional test for diagnosis of migraine because it is useful for visualization of perfusion defect especially in ictal and interictal periods with aura, and in ictal period without aura in childhood. These finding suggest that the idea of the origins of migraines with or without aura might be similar. It is necessary to investigate in large series in order to clarify the pathogenesis of the disease further.

Keywords: juvenile migraine, eeg, spect, headache

1. Introduction

Despite many studies, pathogenesis of migraine is not completely understood and none of the theories proposed has gained certainity. Vascular and neuronal theories have been discussed in terms of apparent clinical and pathological resemblances. Inasmuch as vascular theory couldn't explain the whole migraine mechanism, it is supplemented by neuronal theory (1). The best studied phenomenon of migraine attack is the cortical spreading depression (CSD) pattern of Leao. This disturbance of the cerebral cortical function is associated with metabolic and haemodynamic changes which have been described well in some studies (2). It may be notified that migraine attacks occur with respect to exacerbations of preexisting changes in cerebral autoregulation due to innate or exogenous factors (3).

With the advances, regional cerebral blood flow (rCBF) studies revealed new insights into the pathophysiology which some showed hypoperfused areas especially in migraine patients with aura during interictal (3-6, 8-13) and ictal period (5-8), while others showed only nonspecific changes (4, 8, 14) or even hyperperfused areas (4, 6).

One of the relatively new techniques that describe cerebral blood perfusion alterations is Tc-99m HMPAO Single Photon Emission Computerized Tomography (SPECT), which the data in juvenile age group is very rare. Likewise EEG is one of the most commonly used neurophysiological method intended for migraine pathophysiology. That being said, to the best of our knowledge, there is no study combining both SPECT and EEG findings in juvenile migraine group diagnosed according to the International Headache Society (HIS) criteria in order to determine possible ictal and interictal vascular and electrophysiologic changes.

The aim of our study was to evaluate changes in rCBF and EEG's in juvenile patients suffering from migraine with aura ((MwA) and without aura (MwoA), also to investigate the relationship between SPECT and EEG in respect to comparisons of each other, the frequency of attacks and response to the treatment.

2. Materials and Methods

The study involved 20 juvenile patients, 9 with aura (mean age±sd: 13.5±2), 11 without aura (mean age±sd: 12.3±3) with the diagnosis of migraine who admitted to the Department of Pediatric Neurology in the Ondokuz Mayıs University School

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of Medicine between 1999 and 2000. Juvenile migraine was diagnosed on the basis of headache classification of the IHS. Patients with history of migraine lasting from at least a year who do not receive any prophylactic drugs other than propranolol (which stopped at least 3 months prior to the assessment) were included. Children who had abnormalities on neurologic examination and intracranial structural defects were excluded. Patients were also divided into three groups according to the attack frequency: ≤5 attact/month; 6-10 attact/month; >10/month. Propranolol treatment (1 mg/kg/day) has been given to all the patients after the study was performed.

Treatment response was evaluated at the third and sixth months of follow-up visits. SPECT and EEG studies were performed during headache free (interictal) and headache (ictal) periods to all patients during both ictal (in the first six hours after the headache had begun) and interictal periods (at least 24 hours for SPECT and 72 hours for EEG studies after the attack had stopped). Blood flow measurements were carried out by SPECT. A single head gamma camera (GE, starcam 4000i XCT, WI USA) fitted with a low energy high resolution collimator was used. Fifteen minutes after the bolus injection of Tc-99m HMPAO at a dose of 18,5 MBq/kg into the vein, brain acquisitions were collected. Cerebellar activity was chosen to normalize the brain slices. Cortico-cerebellar ratios (CCRs) were estimated by dividing the mean values detected from 10 different areas of the brain to ipsilateral cerebellum values. Transaxial slices parallel to orbitomeatal line were used to determine perfusion index (PI) of total 17x2 brain regions. The rectangular regions of interest (ROIs) (5x5 pixel) were set on the brain regions while irregular ROIs were drawn over the cerebellar hemisphere (Fig. 1).

EEGs were recorded at least for 20 minutes. The Wilcoxon test was used to compare the mean PI values. The Mann-Whitney U test was used to compare the mean PI values for ictal and interictal images in patients with and without aura. Evaluations within the groups were performed by paired-t test, between the groups by Student-t test. In our study, SPECT and EEG findings were interpreted with each other, frequency of attacks and response to the treatment by using chi-squared test, Fisher's exact test and Mc Nemar test. All the evaluations were performed by the same nuclear medicine specialist.

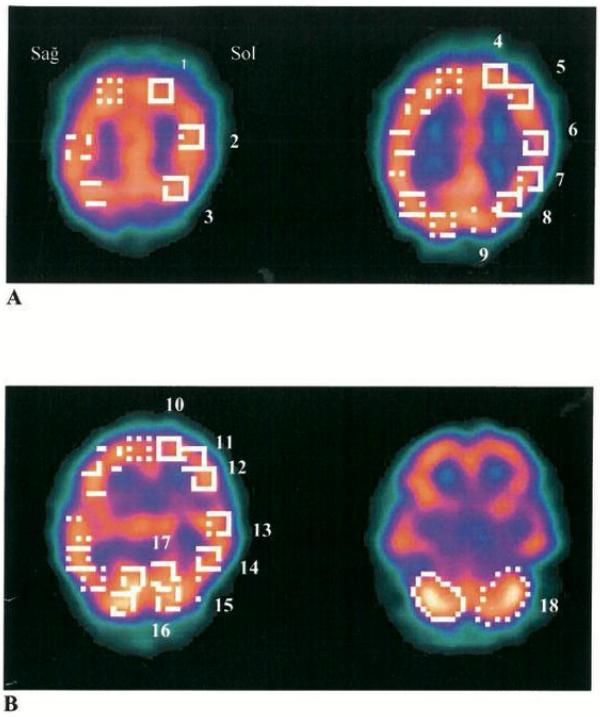

Fig 1 (A, B). Distribution of regions of interest (ROIs) used in semiquantitative evaluation according to the section. (1) Sup. Frontal 1, (2) Paracentral 1, (3) Sup. Parietal 1, (4) Sup. Frontal 2, (5) Middle Frontal 1, (6) Paracentral 2, (7) Sup. Parietal 2, (8) Inf. Parietal, (9) Cuneus, (10) Sup. Frontal 3, (11) Middle Frontal 2, (12) Inf Frontal, (13) Sup. Temporal, (14) Middle Temporal, (15) Occipital, (16) Occipital pole, (17) Visual cortex, (18) Cerebellum

3. Results

3.1. Patients and Study Design

General characteristics of study patients are given in Table 1. A total of 20 children, 11 girls (55%) and 9 boys (45%) aged 7 years to 16 years were included. Of the 20 patients, 9 had MwA (3 female, 6 male) and 11 (8 female, 3 male) had MwoA. The mean ages of cases with or without aura were 13,5±2 years (11-16) and 12,3±3 (7-16) years respectively. Attack frequency was less than 5 per month in half of the cases while it was 6-10 in 7 and greater than 10 in 3 patients. Half of the patients had unilateral headache. All of the patients with aura had visual symptoms. Neurologic symptoms were compatible with perfusion defect in 5 of 9 migraine patients with aura. Brain Computed Tomography (CT) was normal which was obtained in 7 patients.

Table 1. Clinical characteristics of patients

Patient number	Gender	Age (year)	Type of migraine	Period of illness (year)	Family history	Attack frequency	Attack duration	Headache localization	Associated neurological symptoms
1	G	16	W aura	2	+	3-4/m	1 d	Bil.O.F.	teichopsia, photopsy
2	B	15	W aura	1,5	Ø	4/m	1-2 d	Uni.O.	Teichopsia, blurred vision
3	B	13	W aura	1	Ø	1-2/m	3-4 h	Bil.F.	fortification
4	G	11	W aura	1	Ø	8-10/m	3-4 h	Uni.F.P.	photopsy scotoma
5	G	13	W aura	1	Ø	7-8/m	3-4 h	Uni.O.P.	Teichopsia, photopsy

6	B	11	W aura	1	+	7-8/m	2-3 h	Bil.O.	photopsy, scotoma
7	B	15	W aura	1	Ø	3/w	2-3 h	Bil. O.F.	blurred vision
8	B	12	W aura	2-3	Ø	3/w	3-4 h	Uni. F.	photopsy
9	B	16	W aura	4	Ø	1-2/m	1-2 d	Bil. F.	photopsy, scotoma
10	G	16	w/o aura	1,5	+	1-2/m	1 d	Uni. F.	
11	G	16	w/o aura	1,5	Ø	3/m	1 d	Uni. F. P.	
12	G	9	w/o aura	3-4	Ø	1/m	1-2 d	Uni.P. T.	
13	G	10	w/o aura	1	+	7-8/m	3-4 h	Bil. F.	
14	B	10	w/o aura	2	+	1/m	1-2 d	Uni. F.	
15	B	7	w/o aura	1	Ø	1/m	3-4 h	Bil. F.	
16	G	13	w/o aura	1	+	8-10/m	3-4 h	Uni. F. P.	
17	B	15	w/o aura	3	+	4-5/m	1 d	Bil. F.	
18	G	14	w/o aura	3	Ø	10/m	3-4 h	Bil. F.	
19	G	12	w/o aura	2	+	3/w	3-4 h	Bil. F.	
20	G	14	w/o aura	1,5	+	7-8/m	5-6 h	Bil. F. P.	

3.2. SPECT Findings

SPECT findings obtained by visual evaluations are given in Table 2. Out of 9 patients with aura, 8 (88%) showed perfussion defect during ictal period with 11,7% in pariatal lobe, 9,1% in frontal which was bilateral in 3 patients. Decreased rCBF was observed in all brain lobes. Images of hypoperfused areas during ictal period obtained from Patient 3 are shown in Fig. 2A, as for Patient 9 in Fig. 3A. In the group with aura, while perfusion defect during interictal period continued in 5 of the 9 cases (55%), it had normalized in other 4 patients. In Patient 3, hypoperfused areas were converted to normal in interictal period (Fig. 2B). Patient 9 showed continuation of ictal hypoperfused areas in interictal period as well, with relative increase in blood perfusion (Fig. 3B).

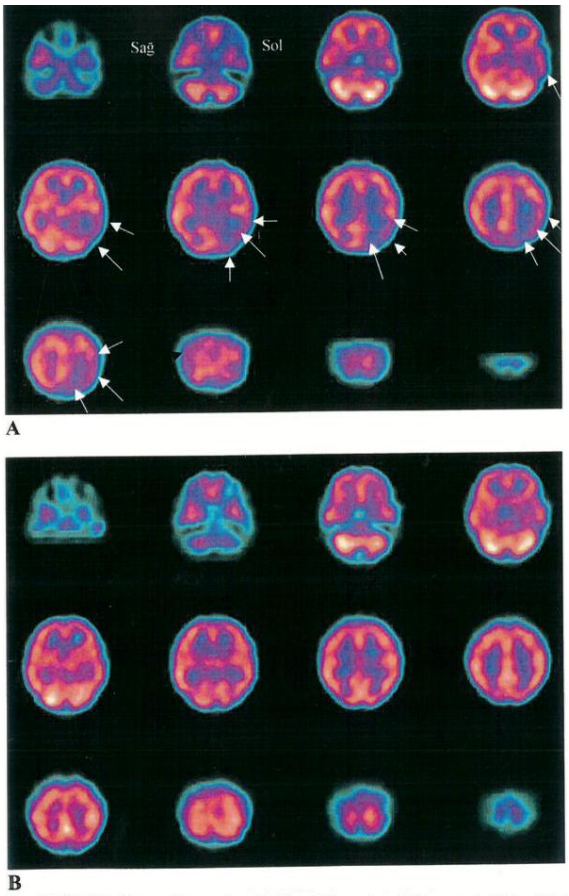

Fig. 2 (A,B). In Patient 3, hypoperfusion was observes in left parietal, temporal and occipital areas during ictal stage (A) with axial section by Tc-99m HMPAO SPECT. During interictal stage (B), braib perfusion was normal

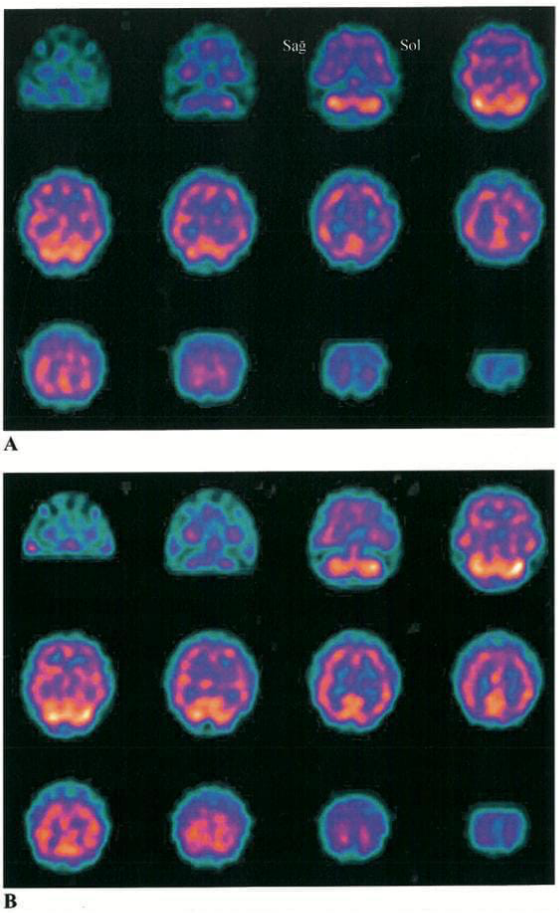

Fig. 3 (A, B). In Patient 9, widespread hypoperfusion was observed in the brain during ictal stage (A) with axial section by Tc-99m HMPAO SPECT. The same patient showed continuation of ictal hypoperfused areas in interictal period as well, with relative increase in blood perfusion (B)

Table 2. SPECT and EEG results obtained by visual evaluations

	Perfused areas		EEG		Relationship of perfusion to symptoms	CT
n	Ictal	Interictal	Ictal	Interictal	+	Not available
1	Bil. P.O.↓	Normal	Normal	Normal	+	Normal
2	Left F.P.T.O↓ RightO.↓	Left T.O.↓ Right O.↓	Voltage supression	Normal	+	Normal
3	Left P.T.O↓	Normal	Voltage supression	Normal	∅	Normal
4	Left F.P.T.↓	Normal	Normal	Normal	∅	Not available
5	Left F.P.↓ Right F.T↓	Bil.P.↓	Normal	Normal	∅	Not Available
6	Normal	Normal	Normal	Normal	∅	Normal
7	Left F.P.O.↓ Right F.↓	Left F. ↓	Normal	Normal	+	Not available
8	Bil. P.↓	Bil.P.↓	Voltage supression	Voltage supression	∅	Not available
9	Bil. F.P.T.O↓	Bil.F.P.T.O↓	Normal	Normal	+	Normal
10	Right P↓ Left P.T.O.↓	Right F.P↓ Left F.P.T.O↓	Normal	Normal		Not available
11	Bil. P.↓ Bil. F. ↑	Normal	Normal	Normal		Not available
12	Normal	Normal	Normal	Normal		Not availabl
13	Normal	Normal	Normal	Normal		Normal
14	Normal	Normal	Voltage supression	Voltage supression		Not available
15	Normal	Normal	Voltage supression	Normal		Not available
16	Normal	Normal	Normal	Normal		Normal
17	Bil.T.P.O↓	Normal	Normal	Normal		Not available
18	Bil.P.↓	Normal	Normal	Normal		Not available
19	Bil. P↓ Bil. O.↑	Bil.P.↓	Voltage supression	Normal		Not available
20	Left F.P.↓	Normal	Normal	Voltage supression		-

F:Frontal, P:Parietal, O:Occipital, T:Temporal, Bil: Bilateral, +:associated, ∅: nonassociated

In the group without aura, hipoperfusion were observed in 6 of 11 patients (55%) during ictal period. Regional CBF was reduced mainly in the parietal lobe. Slightly hyperperfused areas were observed in 2 patients as well (patient 11 and 19). Perfusion defect was bilateral in 5 patients. In interictal period, normal perfusion was observed in 4/6 while 2 patients still had hypoperfused areas. While one patient (Patient 10) showed new hypoperfused areas additional to the areas seen in ictal period, the other (Patient 19) had lesions to continue regressively.

Table 3. Mean perfusion indexes (PI) (SD) during the ictal and interictal phases in migraineurs with aura and without aura

	With Aura (n: 18)		Without Aura (n:22)					
	Ictal (Mean±SD)	Interictal (Mean±SD)	Ictal (Mean±SD)	Interictal (Mean±SD)	P1	P2	P3	P4
Sup. Frontal 1	0.96±0.07	0.98±0.08	1.02±0.09	1.03±0.07	≠	≠	*	≠
Paracentral 1	0.95±0.09	0.97±0.07	0.96±0.06	1.01±0.08	≠	**	≠	≠
Sup. Parietal 1	0.89±0.10	0.93±0.06	0.91±0.09	1.00±0.07	*	***	≠	≠
Sup. Frontal 2	0.93±0.07	0.91±0.07	1.02±0.09	0.99±0.07	≠	≠	**	**
Middle Frontal1	0.94±0.07	0.94±0.08	1.02±0.07	1.01±0.07	≠	≠	***	**
Paracentral 2	0.92±0.10	0.94±0.08	0.97±0.07	0.99±0.07	≠	≠	≠	≠
Sup. Parietal 2	0.93±0.11	0.94±0.06	0.96±0.07	0.98±0.05	≠	≠	≠	≠
Inf. Parietal	0.84±0.11	0.86±0.05	0.85±0.08	0.93±0.09	≠	**	≠	*
Cuneus	0.94±0.12	0.97±0.05	1.01±0.10	1.02±0.09	≠	≠	*	≠
Sup. Frontal 3	0.84±0.08	0.87±0.10	1.00±0.11	0.95±0.06	≠	≠	****	**
Middle Frontal 2	0.89±0.08	0.90±0.08	1.01±0.09	0.98±0.08	≠	≠	****	*
Inf. Frontal	0.91±0.06	0.92±0.07	0.98±0.07	0.96±0.08	≠	≠	***	*
Sup. Temporal	0.95±0.09	0.96±0.07	1.00±0.07	1.00±0.08	≠	≠	*	≠
Middle Temporal	0.91±0.09	0.93±0.07	0.94±0.08	0.97±0.07	≠	≠	≠	*
Occipital	0.91±0.10	0.93±0.07	0.95±0.07	0.97±0.09	≠	*	≠	≠
Occipital Pole	1.03±0.07	1.03±0.06	1.08±0.07	1.10±0.07	≠	≠	≠	≠
Visual Cortex	0.94±0.07	0.97±0.05	1.00±0.07	1.04±0.07	≠	**	*	≠

*p<0.05, ** p<0.01, *** p<0.001, , **** p=0.000, ≠p>0.05 **P1**: Comparison of PI values between ictal and interictal phases in patients with aura. **P2**: Comparison of PI values between ictal and interictal phases in patients without aura. **P3**: Comparison of ictal PI values between patients with and without aura. **P4**: Comparison of interictal PI values between patients with and without aura.

When SPECT results were evaluated by CCR scale (Table 3), statistically significant difference was observed only in superior parietal 1 area between ictal and interictal periods in MwA (p<0,05) while it was observed in paracentral, inferior parietal and visual cortex (p<0,01) with the highest significance in superior parietal area (p<0,001) in cases

without aura. When it was evaluated between migraine patients with and without aura ictally, statistically significant differences were obtained in frontal area, cuneus, superior temporal and visual cortex with the highest significance in superior and middle frontal areas (p=0.000) while it was observed significant in frontal area during interictal period (p<0.01).

When evaluating ictal and interictal SPECT findings among whole patients (Table 4), data comparing areas with normal perfusion and hypoperfusion qualitatively during ictal period showed that there was significance only in the areas of superior parietal 1 and superior frontal 3 (p<0,05).

Table 4. Mean perfusion indexes (PI) (±sd) during ictal and interictal phases comparing areas with normal perfusion and hypoperfusion

		Ictal			Interictal					
	n	Hypoperfused (Mean±SD)	n	Normal	n	Hypoperfused (Mean±SD)	N	Normal (Mean±SD)	P1	P2
Sup. Frontal 1	2	0.81±0.05	36	1.00±0.08	2	0.90±0.05	38	1.02±0.07	≠	Ø
Paracentral 1	5	0.86±0.06	35	0.96±0.07	2	0.94±0.03	38	1.00±0.08	≠	Ø
Sup. Parietal 1	11	0.79±0.06	29	0.94±0.07	4	0.85±0.02	36	0.98±0.07	*	****
Sup. Frontal 2	2	0.77±0.05	38	0.99±0.09	2	0.85±0.02	38	0.96±0.08	≠	Ø
Middle Frontal1	2	0.80±0.03	38	0.99±0.08	2	0.87±0.05	38	0.98±0.07	≠	Ø
Paracentral 2	3	0.78±0.00	37	0.96±0.08	2	0.86±0.07	38	0.97±0.07	≠	Ø
Sup. Parietal 2	6	0.83±0.07	34	0.97±0.08	2	0.93±0.02	38	0.96±0.06	≠	Ø
Inf. Parietal	7	0.77±0.07	23	0.90±0.06	9	0.83±0.03	31	0.91±0.08	≠	****
Cuneus	7	0.76±0.03	31	0.10±0.05	2	0.92±0.03	38	1.00±0.08	≠	Ø
Sup. Frontal 3	9	0.78±0.07	31	0.98±0.10	2	0.74±0.05	38	0.93±0.08	*	Ø
Middle Frontal2	7	0.81±0.05	33	0.99±0.09	3	0.81±0.04	37	0.96±0.08	≠	Ø
Inf. Frontal	4	0.81±0.04	36	0.96±0.06	4	0.85±0.05	36	0.95±0.07	≠	Ø
Sup. Temporal	4	0.84±0.07	36	1.00±0.07	3	0.90±0.02	37	0.99±0.08	≠	Ø
Middle Temporal	8	0.84±0.03	32	0.96±0.08	4	0.86±0.02	36	0.96±0.07	≠	Ø
Occipital	8	0.82±0.04	32	0.97±0.07	2	0.83±0.06	38	0.97±0.08	≠	Ø
Occipital Pole		--	40	1.06±0.09		--	40	1.07±0.07	≠	Ø
Visual Cortex	3	0.85±0.01	37	0.98±0.08	1	0.89±00	39	1.01±0.07	≠	Ø

*p<0.05, **** p=0.000, ≠p>0.05, Ø no statistical analysis has been made. **P1**: Comparison of PI values qualitatively obtained from normal perfused areas between ictal and interictal phases. **P2**: Comparison of PI values qualitatively obtained from hypoperfused and normal perfused areas during ictal phases.

3.3. EEG Findings

EEG findings are given in Table 2.

Voltage suppression was present in 3/9 patients (33%) in ictal, 1/9 (11%) in interictal period in patients with aura and 3/11 patients (27%) in ictal, 2/11 (18%) in interictal period in patients without aura, as within the same patient**.**

Comparison of SPECT and EEG data in patients with and without aura in ictal and interictal periods:

Out of 10 patients whose attack frequency 1-5/month in ictal period, 4 patients with aura showed perfusion defect in their SPECT (100%) and half of them had voltage suppression in their EEG (50%); out of the rest of 6 patients without aura, 3 showed perfusion defect in their SPECT (50%) and one third had voltage suppression in their EEG (33%). Analyzing 7 patients whose attack frequency 6-10/month in ictal period, 1 patient out of 3 with aura showed perfusion defect in their SPECT (33%), none showed voltage suppression in EEG (0%); half of 4 patients without aura (%50) showed perfusion defect in SPECT (50%) with none had voltage suppression in EEG (0%). When examined 3 patients with an attack frequency of 10 or more /month in ictal period, both two patients with aura had perfusion defect in SPECT (%100), one of them had voltage suppression in EEG. When compared whole patient's SPECT and EEG findings and attack frequency with each other, there was no statistical significance (p>0.05).

When evaluating response to propranolol treatment (1 mgr/kg/day), 18 patients out of 20 gave response to 3 or 6 months of medication. There was no statistical difference in patients with or without aura in relation to the treatment response with cerebral perfusion defect and their EEG (p>0.05).

4. Discussion

Migraine is a vascular disease which pathophysiology is not understood clearly in spite of several studies. Cerebral blood flow studies performed over the years have brought a new dimension to the debate. Studies combining SPECT and EEG findings in migraine patients with and without aura are limited and very few of them are in juvenile age group diagnosed according to the HIS criteria.

Cerebral perfusion studies performed in patients with and without aura in ictal and interictal periods are vary. Although early rCBF studies in the ictal phase in patients with MwA shows hyperperfusion, especially some studies performed with PET and SPECT have shown a pattern of reduced rCBF during the headache phase (4-7). Olesen J et al (6) studied in 63 migraineous patients with aura during the aura phase, headache phase and after the headache disappeared, and showed that the first observed event was a decrease in regional cerebral blood flow in the posterior of one cerebral hemisphere. The development of this process was accompanied by aura symptoms, followed by headache while the rCBF remained

low. In the headache phase, the CBF increased gradually from low to abnormally high while headache was disappeared in some patients. In the study of Soriani et al (4), perfusion abnormalities were found in 14 of 19 patients in the ictal period in patients with aura, of which 11 was hypoperfusion. In Lauritzen and Olesen's SPECT series of 33 patients with Xe-133 inhalation (7), unilateral hypoperfusion was found in 8 out of 11 in patients with aura during the attack. Studies performed in migraine patients during interictal period are substantially more. Even though normal perfusion studies of Xenon-133 and SPECT performed in MwA in interictal period were rarely reported (4, 15), studies with abnormal perfusion pattern were published (3-6, 9-13). In the interictal SPECT study of Battisella et al (8) on 19 juvenile migraineurs with and without aura, none of 10 patients without aura showed perfusion defect while hypoperfused areas were found in 4 out of 9 patients with aura and they suggested that insufficient regional cerebral vascular autoregulation may occur even in the interictal period in patients with classical and hemiplegic migraine. Schlake et al (3) detected hypoperfusion in 19 out of 23 adult patients with MwA during interictal period in their SPECT study and stated that the most significant change was in a patient with complicated migraine. In the SPECT study of Calamussi (12) in patients with aura, perfusion disorders were shown in 22 out of 28 patients during interictal period and concluded that SPECT is useful in the diagnosis of migraine. Maini et al (13) found decreased rCBF in their SPECT study in 13 of 14 patients with aura during interictal period. Contrary, in the brain SPECT study of Tc.09m HMPAO performed by Soriani et al (4) in a 30-person group of patients with juvenile MwA (19 in the ictal and interictal period, 11 in the interictal period only), hypoperfusion was found in 4 of 20 patients and normal perfusion in 16 of them in interictal period.

In our study, a significant decrease in cCBF was detected in the in ictal period in the majority of migraineurs with aura (%88). Decreased cCBF was observed in all brain lobes. In interictal period of 3/9 patients, rCBF had normalized while 5 others (%55) still showed hypoperfusion and continued with a decrease in number and width of the defects. Therefore, despite higher corticocerebellar ratios (CCRs) of 15 out of 17 brain regions, significant difference was not found between ictal and interictal brain perfusion by semicantitative evaluation (Table 3). The findings we obtained in interictal period in patients with aura are consistent with the literature (3-6, 9-13). In spite of limited number of studies conducted in the ictal period, our findings show consistency to some studies (4-7).

Although most of the studies conducted in the interictal period in MwoA found normal flow pattern (8, 11, 14), one large-series of SPECT study with Xe-133 inhalation showed significant asymmetry (15). Ferrari et al (14) did not observe significant regional cerebral perfusion asymmetry in the attack-free period, during the attack, and after the treatment with Sumatriptan in the Tc-99m HMPAO SPECT performed in a group of 20 patients with MwoA. In the interictal SPECT study conducted in 29 adult patients (24/29 migraineus without aura) by Mirza et al (9) revealed significantly reduced Tc 99m-HMPAO uptake in the right lower frontal, temporal, upper frontal and occipital regions. In Levin et al's interictal study (16), rCBF values measured by 133Xe inhalation were lower in migraineurs than in controls. Mean asymmetry index of the classic/complicated group was significantly higher than that of the controls but not different from that of the common migraine group. It was suggested that in the headache-free interval rCBF asymmetries exist in classic/complicated migraineurs variable in location and may be related to the cause or the effect of the focal neurologic dysfunction that occurs during an attack in these patients.

Despite early studies characterized hyperemia as occurring during the ictal phase of MwoA, large series studies conducted subsequently have revealed normal global or regional CBF (7, 14, 15). A normal cerebral blood flow pattern was observed in all 12 individuals with MwoA during the attack in Lauritzen and Olesen's SPECT series (7) of 33 patients with Xe-133 inhalation. Bednarczyk et al (17) used PET to quantify global CBF, oxygen metabolism, and oxygen excretion in a group of 9 participants and discovered that CBF was reduced throughout the headache phase. Cerebral oxygen metabolism and excretion remained severely diminished.

In our study, ictal hypoperfused areas were seen in 55% of patients without aura. Regional CBF was reduced mainly in the parietal lobe. Two patients showed slightly hyperperfused areas in addition to hypoperfused regions. Perfusion recovered to normal in 4 of 6 patients during the interictal period, while remained low in 2/6. This restoration to normalcy in brain perfusion observed during the interictal phase was confirmed semiquantitatively (ictal-interictal comparison) in 5 of 17 brain areas in CSO values (Table 3). It was shown that two individuals with MwoA (patients 11 and 19) exhibited both hyper and hypoperfused brain regions (bilateral frontal in patient 11 and bilateral occipital in patient 19).

Our findings in the interictal stage in a sample of migraine patients without aura are similar to several previous research (8, 11, 14). The rate of perfusion disorder that we found in a significant proportion of patients during the ictal period is consistent with the data from Bednarczyk's study (17) which is important in terms of supporting the idea that migraine with and without aura may be caused by the same disease process.

Our study did not look at the link between pain attacks and changes in brain perfusion over time.

Since the normal group was not formed in our study, a comparison between the patient groups and the normal ones could not be made. However, according to the qualitative evaluation results, it was observed that the mean CCR values of the brain regions considered hypoperfusion in the ictal period were considerably lower than the brain regions considered normal in the ictal period, and this difference was

quite significant in the areas that could be statistically analyzed (superior parietal I and inferior parietal) (p. =0.000, table 4). It was observed that the mean CCR values were close to each other in the brain regions considered qualitatively normal in the ictal and interictal periods, and the difference in 2 of the 17 regions (superior parietal 1 and superior frontal 3) was significant at the $p<0.05$ level. No comparison was made between other data in Table 4 due to the lack of sufficient number of pathological regions. However, it was observed that regions interpreted as hypoperfused in the interictal period had lower CCR values than regions evaluated as normal.

When our study was evaluated in general, it was determined that MwA showed more and significantly decreased cerebral blood flow in interictal and ictal periods than MwoA. It was observed that the hypoperfusion areas observed in MwA affected larger brain regions than in MwoA. Significant differences were detected in 9/17 brain regions between ictal images of patients with and without aura. There were also significant differences in 7/17 regions between interictal images of patients with and without aura. Since interictal SPECT showed reversal of the majority of ictal cCBF changes to normal in MwoA, we think that brain perfusion studies performed only during interictal period will be insufficient to show the changes in CBF in migraine patients especially without aura and this will cause false or negative interpretations.

Extensive data on EEG findings are available in patients with migraine, and the incidence of EEG abnormalities has been published, with rates as high as 70%. However, most EEG findings that are considered normal (posterior slowing, hyperventilation sensitivity, 14-6 Hz positive spike discharges) are found at similar levels in normal controls as well as in migraineurs. The results of D. De Carlo' EEG study (18) on 425 pediatric and adolescent chronic headache patients showed that EEG may show temporary abnormalities, especially in aural migraine patients in the ictal period (focal, hemispheral, or bilateral slowing and continuous beta activity) while it is often normal or may reveal some abnormality without clear clinical relevance during interictal period.

There are some publications showing abnormal EEG findings especially in patients with hemiplegic, basilar and complex neurological aura in the ictal period. Uri Kramer et al (19), in their literature review to investigate the importance of EEG in the evaluation of headache found that mild diffuse slowing of background activity occurs in 3-24% of the EEGs of patients with migraine. In their study, they stated that deceleration was significantly more pronounced in migraine patients than in tension-type headache patients. In the same literature review, it is stated that abnormalities occurring during hyperventilation were recorded in some studies, and it was reported that advanced EEG analyses using differential values (coherence values and alpha values) could distinguish migraine patients from controls. In the interictal and ictal period SPECT and EEG study conducted by S. Soriani et al (4), no abnormality was observed in the EEG recordings made in the interictal period, and only slow-wave activity was observed in 14 out of 19 migraine patients during ictal period.

Quantitative and topographic EEG studies give more accurate results which allow reduced subjectivity. However, we did not perform a quantitative EEG evaluation in our study.

In our study, we found diffuse voltage suppression in ictal period in 3 (33%) of 9 patients with MwA. While this finding was transient in 2 patients, it continued in interictal period in 1 patient. Widespread voltage suppression observed in 3 (27%) of 11 patients with MwoA in the ictal period persisted in 2 of them in the interictal period. Our findings are in agreement with some studies in the literature (18-20). However, the lack of quantitative EEG analysis and lack of diversity in the group of patients, also limited number of cases may have reduced the significance of the results.

There are limited number of studies in the literature in which SPECT and EEG findings are combined in patients with migraine and very few of them have been studied in the childhood age group (3, 4, 8, 9). In the juvenile age group study of Battistella (8) especially in patients with hemiplegic and classical migraine, slow-wave activity in the posterior region was shown consistent with contralateral neurologic symptoms in the ictal period, inconsistent with SPECT findings. In the study of Soriani et al (4) in the juvenile group, it was stated that the side agreement between EEG and brain SPECT asymmetry was high. Mirza et al (9) stated in their study that mild hypoperfusion was observed in patients with interictal EEG abnormalities while Schlake et al (3) revealed EEG findings at certain rates without a definite relationship with the topography of SPECT in adult series of migraine patients with aura. We could not find any study in the literature showing the relationship between SPECT and EEG findings, frequency of attacks, and response to treatment. In our study, we discovered that SPECT and EEG data were not connected to each other or the frequency of attack. Similarly, we found no significant relationship between the length of the patients' therapy and the SPECT and EEG data. However, larger studies with the variety of patients are needed for more significant results.

According to our findings, we think that SPECT can be utilized as an objective diagnostic approach in juvenile patients especially with aural migraine during ictal and interictal phase and in patients with MwoA during ictal phase. Diagnostic yield of EEG is low but could be useful as ictally in certain patients such as hemiplejic and basilar migraine, or as a differential from epilepsia. The presence of cerebral perfusion abnormality in migraine patients who do not have an aura appears to support the theory that both conditions might be due to the same disease mechanism.

Conflict of interest
The authors declared no conflict of interest.

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Authors' contributions
Concept: T.K., Design: T.K., Data Collection or Processing: T.K., M.S., Analysis or Interpretation: A.T.S., M.S., Literature Search: T.K., M.S., S.U., Writing: T.K.

Ethical Statement
Our study has been perfonmed between 1999 and 2000. Since there is no Ethical Committee before 2011, approval has not been taken for this study (no need for approval).

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Inoperability decision in GIS malignancies: How sufficient is CT alone?

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Abstract

The prognosis in gastrointestinal system malignancies is directly correlated with the stage of the disease at the time of presentation. There are debates and variations in the preferred methods for staging (3,4). Despite being part of the recommendations of international associations in preoperative staging, the adequacy of CT is controversial. In our study, we aimed to investigate the effectiveness of CT in the decision of inoperability based on our case series of patients who were admitted to our clinic with a preliminary diagnosis of gastrointestinal system malignancy and deemed inoperable. At the General Surgery Clinic of - University Training and Research Hospital, demographic data of patients with intraoperatively detected inoperable gastrointestinal malignancies between 2018 and 2023, preoperative CT and endoscopy findings, surgical reports, and neoadjuvant treatment applications were retrospectively analyzed. Preoperative endoscopic and cross-sectional imaging findings were compared with intraoperative findings. The study included 91 patients. The sensitivity of preoperative endoscopy was found to be 80.2%, while the sensitivity of preoperative CT was 69.2%. When comparing organ-based intraoperative findings with the distant invasion signs findings detected in preoperative investigations, no significant difference was found. When the correlation of intraoperative inoperable and widely accepted findings with preoperative treatment was examined, no statistically significant relationship was found in the statistical analysis. In our study, the sensitivity and specificity of CT were found within ranges consistent with the literature. However, the adequacy of CT in invasive and metastatic disease was again found to be low, in line with the literature. Therefore, to prevent misinterpretation, we believe that adding advanced imaging modalities (MRI, PET, EUS) during the preoperative period in addition to CT may help reduce unnecessary laparotomies.

Keywords: computed tomography, gastrointestinal malignancies, surgical, oncology

1. Introduction

The prognosis in gastrointestinal system malignancies is directly associated with the stage of the disease at the time of presentation. In localized disease, surgical resection is often sufficient for treatment (1,2). However, in metastatic disease, treatment modalities vary. Therefore, staging of the disease holds a crucial place in treatment protocols. There are debates and variations in the preferred methods for staging (3,4). Although computed tomography (CT), magnetic resonance imaging (MRI), gastroscopy, and colonoscopy are routinely used in preoperative staging, advanced investigations may be required for determining local recurrence, the extent of surgical resection, and the need for adjuvant and neoadjuvant therapy (5,6). Despite being part of the recommendations of international associations in preoperative staging, the adequacy of CT is controversial. It is known to be useful, especially in the presence of synchronous tumors, metastatic disease, and invasion of adjacent organs (7,8). In our study, we aimed to clarify the adequacy of preoperative CT in the decision-making process for inoperability by investigating the effectiveness of CT in determining inoperability in a series of patients who presented to our clinic with a preliminary diagnosis of gastrointestinal system malignancy and were deemed inoperable after laparotomy

2. Materials and Methods

In this retrospective study, 91 patients who underwent elective surgery with a preliminary diagnosis of gastrointestinal malignancy at the General Surgery Clinic of Samsun University Training and Research Hospital between January 1, 2018, and September 1, 2023, and were found to have inoperable gastrointestinal malignancy during surgery were included. Palliative operations such as tube gastrostomy, tube jejunostomy, gastroenterostomy, ileostomy, and colostomy were performed. Patients aged 18-90 years without a history of previous surgery for gastrointestinal malignancy, not referred to our center with laboratory and imaging findings, and undergoing elective surgery were included in the study. Data regarding patients' age, gender, preoperative endoscopy, preoperative imaging, neoadjuvant therapy, and surgical procedures were obtained from the archives. Preoperative endoscopic findings, CT findings including organ invasion and distant organ metastasis, whether preoperative neoadjuvant therapy was received, intraoperative procedures and duration, and intraoperative findings were recorded. Preoperative CT findings such as para-aortic lymph nodes, liver metastasis, lung metastasis, pancreatic invasion, peritoneal invasion, major vessel invasion, and bladder invasion were generally

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considered as distant invasion signs of malignancy. The effectiveness of neoadjuvant therapy, preoperative endoscopic and CT findings, and the distant invasion signs on CT were compared with intraoperative findings. The study was approved by the - University Clinical Research Ethics Committee on January 31, 2024, with protocol number -. Descriptive statistics were performed to provide information about the general characteristics of the study groups. Data for quantitative variables were described using mean and standard deviation, while data for qualitative variables were described using number (n) and percentage (%). Differences between groups for qualitative variables were evaluated using the Chi-square test. Agreement between two different times in evaluating categorical items was calculated using the Kappa statistic. The effectiveness of the diagnostic methods was evaluated using the True Positive Rate (Sensitivity) and True Negative Rate (Specificity). Statistical software (IBM SPSS Statistics 22, SPSS Inc., an IBM Co., Somers, NY) was used for calculations.

3. Results

Between January 1, 2018, and December 31, 2022, a total of 237 patients who underwent elective surgery with a preliminary diagnosis of gastrointestinal malignancy and were considered inoperable, subsequently undergoing palliative operations such as tube gastrostomy, tube jejunostomy, gastroenterostomy, ileostomy, and colostomy were identified. However, 18 patients were excluded due to pancreaticobiliary malignancy, 43 patients due to missing data, 23 patients due to previous malignancy operation history, and 62 patients due to emergency surgery. Consequently, 91 patients were included in the study (Fig. 1). Of these patients, 65 were female and 26 were male. The median age was found to be 66.44 years. The mean surgical duration was calculated as 100.87 minutes (Table 1). Upon examination of preoperative endoscopic findings, gastric malignancy was most commonly observed in 29 patients, followed by rectal malignancy in 14 patients. Analysis of the performed surgical procedures revealed that the most common procedure was feeding jejunostomy, performed in 30 patients, mostly in gastric malignancies (16, 55.2%), followed by esophageal malignancies (11, 78.6%); colostomy was performed in 22 patients, mostly in rectal malignancies (12, 75%); finally, enterotomy/enterostomy was performed in the right colon malignancies of 5 patients (83%) (Table 2). Preoperative CT findings revealed that in 13 patients (68.4%), organ-based diagnosis could not be established, and the distant invasion signs could not be predicted, while in 6 patients, although organ-based diagnosis could not be established, the distant invasion signs was predicted; in 18 patients (64.3%), gastric malignancy was suspected, but the distant invasion signs could not be predicted, and in 10 patients, distant invasion signs was detected. Liver metastasis was predicted in 5 of these patients (Table 3).

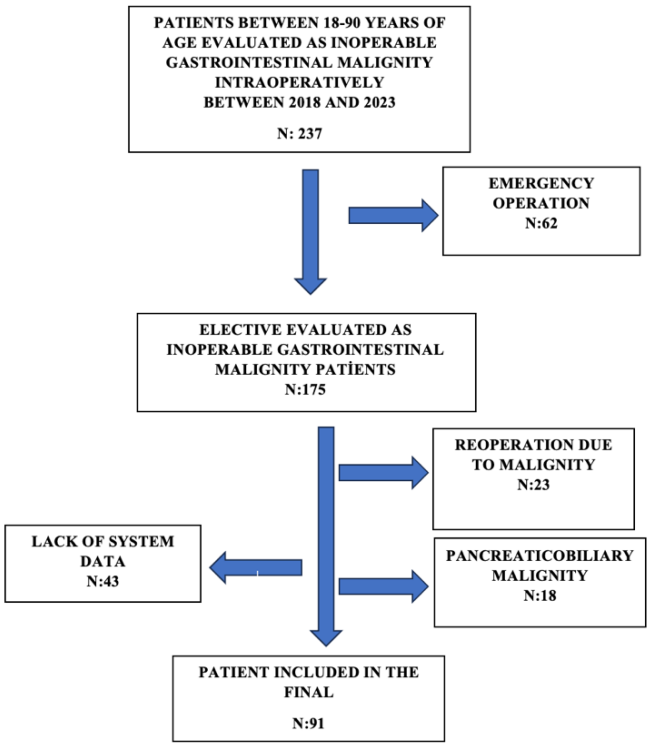

Fig. 1. Included and excluded patients

Table 1. Age, gender and operation duration

		Mean	Standard Deviation	Minimum	Maximum
Age		66.44	14.81	26.00	97.00
Operation Duration (min)		100.87	46.86	30.00	235.00
			n	**%**	
Gender	Female		65	71.4	
	Male		26	28.6	

Table 2. Preoperative gastroscopy/colonoscopy - surgery analysis

		Operation				
		Tube jejunostomy (30)	**Gastroenterostomy (15)**	**Tube gastrostomy (Surgery) (7)**	**İleostomy (17)**	**Colostomy (22)**
Preoperative Gastroscopy/Colonoscopy	**Normal**	-	1(100.0)	-	-	-
	Stomach	16(55.2)	10(34.5)	2(6.9)	-	1(3.4)
	Sigmoid colon	-	-	-	4(50.0)	4(50.0)
	Esophagus	11(78.6)	-	3(21.4)	-	-
	Rectum	-	-	-	4(25.0)	12(75.0)
	Left colon	-	-	-	-	1(100.0)
	Transverse colon	-	-	-	2(100.0)	-

	Right colon	-	-	-	5(83.3)	1(16.7)
	Duodenum	-	3(100.0)	-	-	-
	Stomach+Esophagus	2(40.0)	-	2(40.0)	1(20.0)	-
	Sigmoid colon+Rectum	-	-	-	-	2(100.0)
	Sigmoid colon+Left colon	-	-	-	1(100.0)	-
	Stomach+Transverse colon	1(100.0)	-	-	-	-
	Stomach+Duodenum	-	1(100.0)	-	-	-
	Right colon+Duodenum	-	-	-	-	1(100.0)
	Left colon+Sigmoid Colon+ Rektum	-	-	-	-	-

Table 3. Preoperative CT findings - distant invasion signs analysis

		Distant Invasion Signs							
		None	**Paraaortic Lymph Node**	**Liver**	**Lung**	**Pancreas**	**Peritoneum**	**Major Vessel Invasion**	**Bladder**
Preoperative CT / Preoperative Malignancy	**Normal**	13(68.4)	1(5.3)	2(10.5)	2(10.5)	1(5.3)	-	-	-
	Stomach	18(64.3)	1(3.6)	5(17.9)	-	-	4(14.3)	-	-
	Sigmoid colon	2(40)	-	3(60)	-	-	-	-	-
	Esophagus	2(28.6)	1(14.3)	-	4(57.1)	-	-	-	-
	Rectum	6(75)	2(25)	-	-	-	-	-	-
	Left colon	1(100)	-	-	-	-	-	-	-
	Transverse colon	2(100)	-	-	-	-	-	-	-
	Right colon	3(42.9)	-	3(42.9)	-	1(14.3)	-	-	-
	Duodenum	-	-	-	-	1(50)	-	1(50)	-
	Stomac+Esophagus	1(100)	-	-	-	-	-	-	-
	Sigmoid colon+Rectum	4(57.1)	-	1(14.3)	-	-	1(14.3)	-	1(14.3)
	Sigmoid colon+Left Colon	1(50)	-	-	1(50)	-	-	-	-
	Left Colon+Sigmoid colon +Rectum	-	-	-	-	-	1(100)	-	-

When comparing the organ-based agreement between preoperative endoscopy, preoperative CT, and intraoperative findings: the agreement between preoperative endoscopy and intraoperative findings (kappa: 0.761) ($p<0.001$) was found to be more significant compared to the agreement between preoperative CT and intraoperative findings (kappa: 0.625) ($p<0.001$). The sensitivity of preoperative endoscopy was found to be 80.2%, while the sensitivity of preoperative CT was 69.2% (Tables 4, 5). All patients were evaluated as inoperable intraoperatively. When comparing organ-based intraoperative findings with the signs of distant invasion on preoperative CT, no proportionally high signs of distant invasion were observed in gastric malignancies in 21 patients (63.6%), rectal malignancies in 7 patients (77.8%), and sigmoid colon and rectal malignancies in 6 patients (66.7%); however, proportionally high signs of distant invasion were observed in esophageal malignancies in 9 patients (69.2%), and no significant difference was found ($p=0.332$) (Table 6). When correlating the organ-based evaluation of intraoperative inoperable and distant invasion accepted findings with preoperative treatment, it was found that in gastric malignancies, the majority of patients (57.6%) received neoadjuvant treatment, in esophageal malignancies, 69.2% received neoadjuvant treatment; in sigmoid colon and rectal malignancies, 55.6% received neoadjuvant treatment, while in rectal malignancies, 6 (66.7%), transverse colon malignancies, 2 (100%), and right colon malignancies, 6 (85.6%) patients did not receive neoadjuvant treatment despite the presence of inoperable and distant invasion accepted findings. Statistical analysis revealed no significant relationship ($p=0.126$) (Table 6). Detailed analysis of preoperative CT signs of distant invasion and preoperative neoadjuvant treatment revealed that in the group not receiving neoadjuvant treatment, signs of involvement were not predominantly observed in preoperative CT in 27 patients (62.8%); in the group receiving neoadjuvant treatment, signs of distant invasion were not predominantly observed in preoperative CT in 26 patients (54.2%), and no significant difference was observed ($p=0.535$) (Table 7).

4. Discussion

In gastrointestinal malignancies, accurate diagnosis and staging are crucial for treatment selection. Endoscopy with diagnostic biopsy is the gold standard for diagnosis. CT is used for TNM staging, which evaluates tumor size, depth of invasion, extent of the tumor (T), metastasis (M), lymph nodes (N), and ascites. Controversial aspects of CT include local tumor invasion, normal-sized metastatic lymph nodes, and peritoneal invasion (9). In our study of patients operated on due to GI malignancy and intraoperatively found to be inoperable, preoperative CT findings showed normal CT findings in 14 (15.38%) out of 91 patients and signs of distant invasion in 37 (40.65%) patients, indicating that CT sensitivity for local invasion or distant organ metastasis was consistent with the literature (Table 3). For these reasons, although the role of CT in preoperative evaluation remains debatable, CT, positron emission tomography (PET), endoscopy, and endoscopic ultrasonography (EUS) are commonly used preoperative staging imaging procedures for gastrointestinal malignancies (10-11).

Table 4. Preoperative gastroscopy / colonoscopy - intraoperative malignancy compatibility

		Intraoperative Malignancy													
		Stomach	Sigmoid Colon	Esophagus	Rectum	Left colon	Transverse Colon	Right Colon	Duodenum	Stomach+ Esophagus	Sigmoid Colon+ Rektum	Sigmoid Colon+ Left colon	Left colon+ Rektum	Stomach+ Left colon+ Sigmoid Colon+ Rectum	Total
Preoperative Gastroscopy / Colonoscopy	**Normal**	1	0	0	0	0	0	0	0	0	0	0	0	0	**1**
	Stomach	28	0	0	0	0	0	0	0	1	0	0	0	0	**29**
	Sigmoid Colon kolon	0	5	0	0	0	0	0	0	0	1	1	0	1	**8**
	Esophagus	0	0	13	0	0	0	0	0	1	0	0	0	0	**14**
	Rectum	0	0	0	9	0	0	0	0	0	6	0	1	0	**16**
	Left colon	0	0	0	0	1	0	0	0	0	0	0	0	0	**1**
	Transverse Colon	0	0	0	0	0	2	0	0	0	0	0	0	0	**2**
	Right Colon	0	0	0	0	0	0	6	0	0	0	0	0	0	**6**
	Duodenum	0	0	0	0	0	0	0	3	0	0	0	0	0	**3**
	Stomach+ Esophagus	2	0	0	0	0	0	0	0	3	0	0	0	0	**5**
	Sigmoid Colon+ Rektum	0	0	0	0	0	0	0	0	0	2	0	0	0	**2**
	Sigmoid Colon+ Left Colon	0	0	0	0	0	0	0	0	0	0	1	0	0	**1**
	Stomach+ Transverse Colon	1	0	0	0	0	0	0	0	0	0	0	0	0	**1**
	Stomach+ Duodenum	1	0	0	0	0	0	0	0	0	0	0	0	0	**1**
	Right Colon+ Duodenum	0	0	0	0	0	0	1	0	0	0	0	0	0	**1**

Kappa: 0.761; p < 0.001*

(The Kappa test value ranges from -1 to +1. As it approaches -1, there is no agreement between the observations. A value of 0 indicates that the agreement is due to chance. As the value approaches +1, the agreement between observers increases.)

There was a significant (p <0.001) and positively substantial agreement between Preoperative Gastroscopy / Colonoscopy and Intraoperative Malignancy organ-based findings (Kappa: 0.761, according to Landis and Koch's Kappa Classification).

Sensitivity = 80.2%

Table 5. Preoperative CT findings - intraoperative malignancy compatibility

		Intraoperative Malignancy													o t
Preoperative CT Findings	**Normal**	6	1	6	1	0	0	1	1	1	2	0	0	0	**19**
	Stomach	25	0	0	0	0	0	0	0	3	0	0	0	0	**28**
	Sigmoid Colon kolon	0	4	0	1	0	0	0	0	0	0	0	0	0	**5**
	Esophagus	0	0	7	0	0	0	0	0	0	0	0	0	0	**7**
	Rectum	1	0	0	6	0	0	0	0	0	1	0	0	0	**8**
	Left colon	0	0	0	0	1	0	0	0	0	0	0	0	0	**1**
	Transverse Colon	0	0	0	0	0	2	0	0	0	0	0	0	0	**2**
	Right Colon	1	0	0	0	0	0	6	0	0	0	0	0	0	**7**
	Duodenum	0	0	0	0	0	0	0	2	0	0	0	0	0	**2**
	Stomach+ Esophagus	0	0	0	0	0	0	0	0	1	0	0	0	0	**1**
	Sigmoid Colon+ Rektum	0	0	0	1	0	0	0	0	0	6	0	0	0	**7**
	Sigmoid Colon+ Left colon	0	0	0	0	0	0	0	0	0	0	2	0	0	**2**
	Left Colon+ Sigmoid Colon+ Rectum	0	0	0	0	0	0	0	0	0	0	0	1	1	**2**
Total		**33**	**5**	**13**	**9**	**1**	**2**	**7**	**3**	**5**	**9**	**2**	**1**	**1**	**91**

Kappa: 0.625; p < 0.001* (The Kappa test value ranges from -1 to +1. As it approaches -1, there is no agreement between the observations. A value of 0 indicates that the agreement is due to chance. As the value approaches +1, the agreement between observers increases.) There was a significant (p < 0.001) and moderately positive agreement between Preoperative CT and Intraoperative Malignancy organ-based findings (Kappa: 0.625, according to Landis and Koch's Kappa Classification).

Sensitivity = 69.2%

According to McHugh's Kappa Classification (2012):	According to Landis and Koch's Kappa Classification (1977):
0.00-0.20: No Agreement	<0.00 : Poor Agreement
0.21-0.39: Minimal Agreement	0.00-0.20: Slight Agreement
0.40-0.59: Weak Agreement	0.21-0.40: Fair Agreement
0.60-0.79: Moderate Agreement	0.41-0.60: Moderate Agreement
0.80-0.90: Strong Agreement	0.61-0.80: Substantial Agreement
0.90< : Excellent Agreement	0.81< : Almost Perfect Agreement

Table 6. Intraoperative malignancy - distant invasion signs - treatment

		Distant Invasion Signs (P=0,332)		Treatment	
		Absent (n=53)	Available (n=38)	Absent(n=43)	Neoadjuvant Therapy(n=48)
Intraoperative Malignancy	**Stomach**	21(63,6)	12(36,4)	14(42,4)	19(57,6)
	Sigmoid Colon	2(40)	3(60)	3(60)	2(40)
	Esophagus	4(30,8)	9(69,2)	4(30,8)	9(69,2)
	Rectum	7(77,8)	2(22,2)	6(66,7)	3(33,3)
	Left Colon	1(100)	-	1(100)	0(0)
	Transverse Colon	2(100)	-	2(100)	0(0)
	Rİght Colon	4(57,1)	3(42,9)	6(85,7)	1(14,3)
	Duodenum	1(33,3)	2(66,7)	2(66,7)	1(33,3)
	Stomach+Esophagus	4(80)	1(20)	0(0)	5(100)
	Sigmoid Colon+Rectum	6(66,7)	3(33,3)	4(44,4)	5(55,6)
	Sigmoid Colon+Left Colon	1(50)	1(50)	1(50)	1(50)
	Left Colon+Rectum	-	1(100)	0(0)	1(100)
	Stomach+Left Colon+Sigmoid Colon+Rectum	-	1(100)	0(0)	1(100)

The data are presented as n(%).

p: Chi-square Test

There was no significant difference between intraoperative malignancy organ-based findings and preoperative CT signs of distant invasion (p=0.332)

Table 7. Distant invasion signs - treatment

		Preoperatif Neoadjuvan Treatment		p
		Absent	Available	
Distant Invasion Signs	**Absent**	27(62,8)	26(54,2)	0,535
	Available	16(37,2)	22(45,8)	

The data are presented as n(%).

p: Chi-square Test

The preoperative treatment status did not show a significant difference with the preoperative CT signs of distant invasion . (p=0.535)

When we compared the organ-based findings of preoperative endoscopies with intraoperative organ-based findings of patients identified as intraoperatively inoperable, a high level of concordance was observed, especially in gastric malignancies, followed by esophageal and rectal malignancies. According to kappa analysis, significant agreement was found (kappa: 0.761, p<0.001) (Table 4). The sensitivity rate of endoscopic examination in gastrointestinal malignancies in the literature ranges from 78% to 98% (14). In our study, the sensitivity rate of the endoscopic procedure was found to be 80.2%, consistent with the literature. When we examined the intraoperative organ-based findings of patients identified as intraoperatively inoperable and compared them with preoperative CT organ-based findings, it was observed that in 19 patients, preoperative CT findings were reported as normal, but malignancy was intraoperatively detected in all of these patients, most of whom had gastric malignancy. Upon further examination of the remaining patients, a high level of concordance was observed between the preoperative CT organ-based findings and intraoperative organ-based findings, especially in gastric malignancies, with 25 patients, followed by esophageal, rectal, and right colon malignancies. According to kappa analysis, significant agreement was found (kappa: 0.625, p<0.001) (Table 5). Minami et al. found the accuracy of abdominal CT in diagnosing early and advanced gastric cancer patients to be between 53% and 92% (15). Leufkens et al. determined the staging and accuracy rate of CT in colorectal cancers to be between 67% and 77% (16). In our study, the sensitivity of preoperative CT was found to be 69.2%, which is close to the literature.

In advanced stage and inoperable gastrointestinal malignancies, unnecessary laparotomies increase the risk of mortality and morbidity, and over the past 20 years, there have been numerous developments in diagnosis and staging to reduce unnecessary laparotomies. Many studies indicate that CT, MRI, Transrectal USG, Intraoperative USG, and EUS examinations are valuable methods for operability (12-13). Kwee et al. reported in a systematic review that the sensitivity of CT in detecting lymph node metastasis ranged from 62.5% to 91.9%, and its specificity ranged from 50.0% to 87.9% (17). The widespread use of CT during imaging for distant metastases in the pelvis, abdomen, and thorax simultaneously is a significant reason. In one study, the sensitivity and specificity of CT in detecting distant metastases in gastric malignancies were reported to be 14.3%-59.1% and 93.3%-99.8%, respectively (18). The detection of peritoneal metastasis with CT can be challenging, with a reported sensitivity of 28.3% and specificity of 93.3%-99.8% (19).

While distant organ metastasis plays a crucial role in determining treatment protocols, in cases where visceral metastatic disease is detected by CT, alternative methods such as colonic stenting can be used instead of laparotomy. There are also studies in the literature showing that CT has high sensitivity in detecting metastases (20). In our study, when organ-based findings were examined along with preoperative CT findings of distant invasion, preoperative CT did not find distant invasion signs in a high percentage of patients, especially in gastric malignancies (63.6%) and rectal malignancies (77.8%). A high rate of distant invasion sign findings (69.2%) was found in esophageal malignancies. There was no statistically significant relationship between organ-based findings and preoperative CT distant invasion sign findings (p: 0.332). The sensitivity of CT regarding distant organ metastases is consistent with controversial results in the literature. Neoadjuvant therapy before surgery for gastric, esophageal, and rectal malignancies is a commonly standardized method nowadays for tumor regression. In the EORTC 22921 study on rectal malignancies, tumor regression was found to be significant in patients receiving preoperative chemotherapy and radiotherapy, with rates of 14% and 5.3%, respectively ($p<0.001$) (21). In another Polish study, complete response rates with preoperative chemotherapy and short-term radiotherapy were shown to be 16% and 1%, respectively ($p<0.0001$) (22). When intraoperative malignancy findings were examined in patients considered inoperable intraoperatively, with preoperative neoadjuvant therapy, it was found that in 57.6% of patients with gastric malignancies, 69.2% of patients with esophageal malignancies, and 33.3% of patients with rectal malignancies, significant responses were not achieved despite preoperative neoadjuvant therapy, and they were evaluated as inoperable intraoperatively. In the statistical analysis of preoperative CT findings of distant invasion signs and preoperative neoadjuvant therapy, in the group not receiving neoadjuvant therapy, patients without distant invasion signs on preoperative CT account for a high percentage (62.8%), whereas in the group receiving neoadjuvant therapy, patients without distant invasion signs on CT account for 54.2%. Although the group without distant invasion sign findings was more frequently observed in the group not receiving treatment, there was no statistically significant difference between the groups (p: 0.535).

The data in our study were collected from the hospital database. The main limitations include limited access to certain patient data, selection of a limited number of patients with specifically gastrointestinal system malignancies, and the single-center, retrospective study design.

In advanced stage and inoperable gastrointestinal malignancies, unnecessary laparotomies increase the risk of mortality and morbidity. Although preoperative CT is the preferred imaging method due to its relatively high accuracy and widespread availability, we believe that adding advanced imaging modalities (MRI, PET, EUS) during the preoperative period may help reduce unnecessary laparotomies by preventing misinterpretation of distant organ metastasis and distant invasion sign findings.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: M.A.A, C.A. Design: M.A.A, M.G.B. Data Collection or Processing: Ö.F.B., M.G.B. Analysis or Interpretation: C.A, Ö.F.B., Literature Search: Ö.F.B., C.A. Writing: M.A.A., M.G.B.

Ethical Statement

Approval was obtained from Samsun University Non-invasive Clinical Research Ethics Committee, the study started. The ethics committee decision date is 31/01/2024 and the number of ethical committee decisions is 2024/3/1.

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Investigation of subtypes of hypertension and related factors in obese individuals

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Abstract

Both systolic and diastolic hypertension (HT) have been associated with insulin resistance. There are also studies showing a relationship between insulin resistance and systolic hypertension, but not with diastolic hypertension. This issue is controversial. In this study, we examined the relationship between hypertension subgroups and obesity and insulin resistance. Our study is retrospective and 391obese individuals were divided into 2 groups: normotension group and newly diagnosed hypertension group. The risk of HT in men was found to be 2.4 times higher than in women. The risk of HT was found to be 2.9 times higher in the group with insulin resistance. While there was a positive relationship between systolic blood pressure and CRP and HOMA-IR, no relationship was found with diastolic blood pressure. Metabolic inflammation and insulin resistance may be in the etiology of systolic HT rather than diastolic HT. Obesity-related hypertension is more common in men, with increasing age, and in those with insulin resistance. Systolic blood pressure may be associated with insulin resistance.

Keywords: obesity-related hypertension, systolic/diastolic hypertension, insulin resistance, metabolic inflammation

1. Introduction

Obesity is defined by excess body fat (adipose tissue) and is associated with a higher risk of developing serious diseases, including: type 2 diabetes, heart disease, and cancer.

The diagnosis of obesity is made by measuring people's weight and height and by calculating the body mass index (BMI): weight (kg)/height² (m²).

A BMI of 30 kg/m^2 to 34.9 kg/m^2 defines class 1 obesity, of between 35 kg/m^2 and 39.9 kg/m^2 constitutes class 2 obesity, and of at least 40 kg/m^2 marks class 3 obesity.

Waist circumferences (WC) of at least 102 cm in men and of at least 88 cm in women indicate abdominal obesity, which is associated with increased risk for adiposity-related disease (1, 2).

Define the degree of central adiposity based on waist-to-height ratio (WC/Height) as follows:

- Healthy central adiposity: waist-to-height ratio 0.4 to 0.49, indicating no increased health risks;
- Increased central adiposity: waist-to-height ratio 0.5 to 0.59, indicating increased health risks;
- High central adiposity: waist-to-height ratio 0.6 or more, indicating further increased health risks.

The health risks associated with higher levels of central adiposity include type 2 diabetes, hypertension and cardiovascular disease (3).

Insulin resistance refers to the reduced response of insulin-sensitive tissues to insulin signaling and is associated with the development of cardiovascular disease (4).

Insulin resistance occurring in obese individuals is explained by metabolic inflammation. This inflammation starts in the adipose tissue and causes insulin resistance by affecting the insulin receptor and intracellular pathways in the liver, muscle and fat tissue (5).

HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) is an attractive screening tool for insulin resistance in clinical practice. The formula includes: fasting insulin (mU/L)*fasting glucose (mg/dl)/405. Values of HOMA-IR ≥ 2.5 are strongly correlating with the presence of IR (6).

According to the ESC 2023 Hypertension Guideline, the diagnosis of hypertension (HT) is defined as systolic 140 mm Hg and diastolic 90 mm Hg above the mean blood pressure in at least 2 measurements in 2 different examinations in the office. 140-159/90-99 mm Hg is rated as grade 1, 160-179/100-109 mm Hg as grade 2, and ≥180/100 mm Hg as grade 3. Isolated systolic hypertension is defined as diastolic blood pressure being <90 mm Hg when systolic blood pressure is ≥140 mm Hg, and isolated diastolic hypertension is defined as diastolic blood pressure being ≥90 mm Hg when systolic blood pressure is <140 mm Hg (7).

The relationship between weight gain and hypertension is known (8). The risk of hypertension in obese individuals was found to be 3.5 times higher than in non-obese individuals (9).

Excessive visceral fat distribution is accepted as the cause

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of hypertension in obesity. The mechanisms of obesity and obesity-related hypertension are due to the sympathetic nervous system, to renal and adrenal function, to the endothelium, to the adipokines, and to the insulin resistance (10).

It has been shown that systolic and diastolic hypertension increases as body mass index increases. However, there is also a metabolically healthy obese group and there is no risk of hypertension in this group (11). There are also studies showing that body mass index is more associated with isolated diastolic hypertension (12).

The relationship between insulin resistance and hypertension is also known (13). Additionally, the risk of developing hypertension was found to be high in those with insulin resistance (14). Both systolic and diastolic hypertension have been associated with insulin resistance (15). There are also studies showing a relationship between insulin resistance and systolic hypertension, but not with diastolic hypertension (16, 17).

In this study, we wanted to examine the relationship between systolic HT and diastolic HT, which are subtypes of hypertension, and insulin resistance-metabolic inflammation in obese individuals. We aimed to evaluate this issue, which has not yet been fully clarified, in our own patient population. We also investigated other factors that may affect obesity-related hypertension.

2. Materials and methods

Our study is retrospective, and 391 obese (Body mass index =BMI>30) individuals who applied to Kayseri City Hospital Internal Medicine and Obesity outpatient clinic between August 1, 2022 and April 1, 2024 were included in the study. They were divided into two groups: normotensive group and newly diagnosed hypertensive group.

The diagnosis of hypertension was made in at least two examinations and the average of at least two measurements was over 140 mm Hg systolic and 90 mm Hg diastolic. Patients with blood pressure averages below these values were considered as the normotensive group. Echocardiography was not performed because the patients had no cardiac complaints and their cardiological examinations and electrocardiography findings were normal.

Comparisons were made between the groups in terms of body mass index, waist circumference, liver and kidney function tests, insulin resistance levels, and blood lipids. In addition, subgroup analysis was performed by dividing groups with subtypes of hypertension, namely systolic hypertension, diastolic hypertension, and both systolic and diastolic hypertension.

Those with systolic blood pressure >140 mm Hg were considered systolic HT, those with diastolic blood pressure >90 mm Hg were considered diastolic HT, and those with systolic blood pressure >140 mm Hg and diastolic blood pressure >90 mm Hg were considered both systolic and diastolic hypertension. Blood pressure was classified as 140-159/90-99 mm Hg is rated as grade 1, 160-179/100-109 mm Hg as grade 2, and ≥180/100 mm Hg as grade 3.

Those with a HOMA-IR score ≥2.5 were considered to have insulin resistance.

Correlation analysis was performed for factors that may affect systolic blood pressure and diastolic blood pressure.

Inclusion criteria

- Obese individuals with a body mass index >30 who apply to the obesity clinic between the ages of 18-70.

Exclusion criteria

- Under age 18 and over 70;
- Pregnant women;
- Those taking anti-hypertensive medications;
- Those with chronic diseases (Diabetes, hypertension, cardiovascular disease);
- Those with HgA1c > 6.5;
- Those with a history of drug use;
- Patients with cardiac complaints and pathological findings on cardiac examination and electrocardiography.

Data obtained from patient file archive records were entered into the SPSS program.

In comparison of groups: Pearson Chi-Square test was used for nominal parameters. Independent samples T-test was used for parametric data, and Mann-Whitney U test was used for statistics of non-parametric data. Spearman test was applied for correlation analysis.

3. Results

391 patients with obesity were included in the study. Of these, 344 were women (88%) and 47 were men (12%). Newly diagnosed hypertension was detected in 93 (23.8%) patients included in the study. Systolic HT was detected in 58 (62.4%) patients, diastolic HT was detected in 14 patients (15%), and both systolic and diastolic HT were detected together in 21 (22.6%) patients. Stage 1 HT was detected in 77 (82.8%) patients, stage 2 HT was detected in 15 (16.1%) patients, and stage 3 HT was detected in 1 (1.1%) patient.

Age was significantly higher in the HT group. While 17 of the men (36.2%) had HT, 76 of the women (22.1%) had HT. The risk of HT in men was found to be 2 times higher than in women (p=0.033, odd ratio=1.998). There were 73 (18.7%) people with obesity but not insulin resistance, and 318 (81.3%) people with insulin resistance. The risk of HT was found to be 2.6 times higher in the group with insulin resistance (p=0.011, odd ratio=2.553).

Age, Body mass index (BMI), Waist circumference (WC),

Waist circumference/Height (WC/Ht), Glucose, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), Total Cholesterol (T. Chol), low-density lipoprotein (LDL), Triglyceride (TG), and CRP were found to be significantly higher and Glomerular filtration rate (GFR) was significantly lower in the hypertensive group compared to the normotensive group (Table 1).

Table 1. Comparison of the hypertensive and normotensive groups

Parameters		Hypertensive group, n=93	Normotensive group, n=298	*p*
Blood pressure means, (mm Hg)	**Systolic, mean±SD**	144.41±11.80	120.68±10.09	**0.000**
	Diastolic, mean±SD	83.69±11.16	69.67±8.81	**0.000**
Age, (years), Median (min-max)		38 (18-68)	34 (18-68)	**0.014**
Gender	**Female, n (%)**	76 (81.7%)	268 (89.9%)	**0.033**
	Male, n (%)	17 (18.3%)	30 (10.1%)	
Insulin resistance	**Pozitive, n (%)**	84 (90.3%)	234 (78.5%)	**0.011**
	Negative, n (%)	9 (9.7%)	64 (21.5%)	
BMI, (kg/m^2), Median (min-max)		38.4 (30-61)	36 (30-53)	**0.001**
WC, (cm), Median (min-max)		111 (85-177)	105 (84-198)	**0.000**
WC/Ht, Median (min-max)		0.68 (0.52-1.11)	0.65 (0.53-1.17)	**0.002**
Glucose, (mg/dL), Median (min-max)		93 (74-124)	90 (60-148)	**0.012**
HOMA-IR, Median (min-max)		3.95 (1.11-50.86)	3.22 (0.75-49.98)	**0.002**
HgA1c, (%), Median (min-max)		5.6 (4.7-6.3)	5.5 (4.7-6.4)	0.082
Creatinine, (mg/dL), Median (min-max)		0.69 (0.39-1.18)	0.68 (0.35-1.14)	0.197
GFR, (ml/min), Median (min-max)		111 (65-152)	113 (66-146)	**0.045**
AST, (U/L), Median (min-max)		18 (10-41)	17 (9-195)	0.304
ALT, (U/L), Median (min-max)		20 (7-53)	18 (6-251)	0.143
T. Chol, (mg/dL), Median (min-max)		194 (130-302)	183 (107-476)	**0.011**
LDL, (mg/dL), Median (min-max)		125 (75-213)	115 (42-386)	**0.004**
HDL, (mg/dL), Median (min-max)		46 (23-87)	47 (23-102)	0.230
TG, (mg/dL), Median (min-max)		139 (37-457)	118 (34-535)	**0.003**
CRP, (mg/L), Median (min-max)		5.2 (0.4-34)	4.3 (0.3-49.9)	**0.033**
TSH, (mU/L), Median (min-max)		2.1 (0.3-24.2)	2.1 (0.1-9.6)	0.787

Pearson Chi-Square test was used for nominal parameters. Independent samples T-test was used for parametric data, and Mann-Whitney U test test was used for statistics of non-parametric data. The significance level is P=0.05

SD=Standart deviation, BMI=Body mass index, WC=Waist circumference, WC/Ht= Waist circumference/Height, HgA1C=hemoglobin A1C, HOMA-IR=Homeostatic Model Assessment for Insulin Resistance, GFR= Glomerular filtration rate, AST=Aspartate transaminase , ALT= Alanine transaminase, T.Col = Total Cholesterol , LDL=low-density lipoprotein , HDL=High-density lipoprotein , TG=Triglyceride , CRP= C-Reactive Protein, TSH=Thyroid stimulating hormone

In the correlation study, a positive correlation was found between systolic and diastolic blood pressure and age, glucose, BMI, HgA1c, WC, WC/Ht ratio, T. Chol and LDL; while a negative correlation was found between GFR. While there was a positive relationship between systolic blood pressure and CRP, HOMA-IR and creatine, no relationship was found with diastolic blood pressure. While there was a positive relationship between diastolic blood pressure and TG, no relationship was found with systolic blood pressure (Table 2).

Table 2. Correlation results of factors related to systolic and diastolic blood pressure

Paramaters	Systolic blood pressure		Diastolic blood pressure	
	Correlation Coefficient	*p*	Correlation Coefficient	*p*
Age	0.134	**0.008**	0.109	**0.030**
Glucose	0.183	**0.000**	0.141	**0.006**
BMI	0.224	**0.000**	0.173	**0.001**
HOMA-IR	0.172	**0.001**	0.077	0.136
HgA1c	0.175	**0.001**	0.112	**0.027**
WC	0.240	**0.000**	0.227	**0.000**
Creatine	0.103	**0.044**	0.078	0.128
GFR	-0.150	**0.003**	-0.140	**0.006**
Total Cholesterol	0.121	**0.018**	0.125	**0.014**
LDL	0.160	**0.002**	0.130	**0.011**
TG	0.092	0.073	0.108	**0.035**
CRP	0.121	**0.020**	0.009	0.867
WC/Ht	0.222	**0.000**	0.194	**0.000**

Spearman's test
Correlation is significant at the 0.05 level. BMI=Body mass index, WC=Waist circumference, WC/Ht= Waist circumference/Height, HOMA-IR=Homeostatic Model Assessment for Insulin Resistance , HgA1C=hemoglobin A1C, GFR= Glomerular filtration rate, T.Col = Total Cholesterol , LDL=low-density lipoprotein, TG=Triglyceride, CRP= C-Reactive Protein

4. Discussion

In our study, HT was detected in 23.8% of the patients. We found that the risk of HT in men is 2 times higher than in women. Age was significantly higher in the HT group. The risk of HT was 2.6 times higher in the group with insulin resistance. BMI, WC, WC/Ht, Glucose, HOMA-IR, T. Chol, LDL, TG, and CRP were found to be significantly higher; GFR was significantly lower in the hypertensive group compared to the normotensive group. While there was a positive relationship between systolic blood pressure and CRP, HOMA-IR, no relationship was found with diastolic blood pressure.

It is known that the risk of hypertension increases as age increases. In our study, the older age in the hypertensive group is compatible with the literature (18).

It is known that male gender is an independent risk factor for HT (11). Although there is a study (13) that found that HT in obese individuals is 2 times more common in women, in our study we found it to be 2.4 times more common in men.

Although there was a study (13) that found BMI to be associated with isolated diastolic hypertension, we found it to be associated with both systolic and diastolic hypertension.

In previous studies, isolated systolic hypertension was found to be higher up to the 6th decade (13). We found a positive correlation between age and both systolic and diastolic hypertension, consistent with the literature.

BMI, WC, WC/Ht, Glucose, HOMA-IR, T. Chol, LDL, TG, and CRP were significantly higher in the hypertensive group than in the normotensive group, indicating that hypertensive-obese individuals have more abdominal fat, insulin resistance, metabolic inflammation and shows that they are prone to atherosclerosis.

The finding of a significant relationship between systolic and diastolic blood pressure and male gender, age, BMI, T. Chol, LDL, TG levels was found to be similar to the literature (14). Differently, we found a significant relationship between HOMA-IR and systolic blood pressure, but not diastolic. It has also been found to be associated with diastolic blood pressure in the literature (14). The reason for this is that systolic HT is mostly due to vascular resistance secondary to atherosclerosis, while diastolic HT may be due to volume increase, sympathetic nervous system activation, and impaired renal salt excretion. The lower carotid intima media thickness in isolated diastolic hypertensive patients compared to patients with isolated systolic HT and systolic+diastolic HT supports our idea (20).

Obesity-associated inflammation is a systemic process that affects all metabolic organs (21). While there is a relationship between CRP and systolic blood pressure, there is no relationship with diastolic blood pressure, suggesting that metabolic inflammation may be the cause of the etiology of systolic hypertension.

Isolated diastolic HT also increases the risk of cardiovascular events, like isolated systolic HT. It has been shown that the development of cardiovascular events decreases with isolated diastolic HT treatment (22).

New studies are needed to elucidate the etiology of isolated diastolic HT and to develop treatment appropriate to the etiology.

The limitations of our study are that it is retrospective and the number of patients is small.

Obesity-related hypertension is more common in men, with increasing age, and in those with insulin resistance. We found a relationship between insulin resistance and systolic HT.

Ethical Statement

Ethics committee approval for the study was received from Kayseri City Hospital Non-Interventional Clinical Research Ethics Committee with date 07.05.2024 and decision number

77.

Conflict of interest

The authors declare no conflict of interest.

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None to declare.

Authors' contributions

Concept: S.B., Design: S.B., Data Collection or Processing: S.B., B.A., Analysis or Interpretation: S.B., Literature Search: S.B., B.A., Writing: B.A.

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Effects of different platelet-rich plasma administration methods on peripheral nerve regeneration: A histomorphometric study

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Abstract

Peripheral nerve damage is a common medical problem that frequently leads to significant functional limitations and long-term disabilities. Platelet-rich plasma (PRP), a growth factor-rich substance, promotes axonal regeneration in peripheral nerve injuries. This study aimed to investigate the effect of PRP on axonal regeneration in Wistar rats (*Rattus norvegicus*) after peripheral nerve injury via histomorphometric analysis. This study used a post-test-only control-group design. Twenty-five white rats were randomly assigned to the control or treatment groups (n=5). The control group consisted of the sham-operated and saline groups. The treatment groups consisted of local, intraperitoneal, or combined PRP. A single dose of PRP was administered after axonotmesis of the sciatic nerve. After 21 days, a surgical procedure was performed to extract sciatic nerve tissue, which was then stained with Luxol fast blue for histomorphometric analysis. The mean diameter of the fibers, diameter of the axons, number of axons, and myelin sheath thickness were analyzed using MANOVA ($p<0.05$). Compared with the saline group, the PRP treatment group presented a greater number of regenerating myelinated axons with larger fiber and axon diameters, and thicker myelin ($p<0.05$). The myelinated axons in the sham-operated group showed a typical morphology, whereas those in the saline group showed a decrease in myelinated axons, smaller fiber and axon diameters, and thinner, irregular myelin sheaths. Injection of a combination of PRP, both intraperitoneally and locally, promoted the regeneration of damaged nerves in rats.

Keywords: platelet-rich plasma, peripheral nerve injury, nerve regeneration, axonotmesis

1. Introduction

Peripheral nerve injuries occur frequently in clinical settings and can have various causes, such as violent acts with sharp objects or gunshots, recreational pursuits, motor vehicle accidents, and incidents that occur during surgical procedures (1–3). Traumatic peripheral nerve injuries are relatively common, occurring in approximately 2.8% of the population and affecting people under the age of 40 years. Almost 70% of patients fall into this age group (4). Among these patients, men were more frequently affected than women were, with an incidence rate of 1.52. The upper extremities were the most frequently affected regions (42.1%). In particular, the ulnar nerve (18%) and median nerve (12.8%) are frequent sites of injury in the upper extremities, whereas the sciatic nerve (10.8%) is a common site of injury in the lower extremities (2, 4, 5).

Peripheral nerve damage can lead to axonal demyelination, degeneration, or a combination of both. From a clinical perspective, demyelination and axonal degeneration can cause impaired motor and sensory functions or a combination of both. Damaged peripheral nervous systems have substantial structural and functional regenerative capacities (3, 6, 7). These injuries can significantly affect an individual's quality of life, functional ability, and work capacity (8).

Platelet-rich plasma (PRP) has been studied as a potential method to accelerate tissue regeneration. The advantages of PRP in the regeneration of injured nerves have been previously demonstrated (9–12). PRP was obtained from autologous blood by centrifugation. PRP is rich in growth factors, including PDGF, VEGF, and IGF-1, which are involved in degranulation of endothelial growth factor, and insulin-like growth factor (10, 13).

Several neurotrophic factors in PRP can accelerate axonal regeneration after peripheral nerve injury (14). Furthermore, PRP contains essential cytokines that regulate cell processes and cell differentiation and has neuroprotective effects. TGF-β, found in PRP, is a versatile cytokine that contributes to a

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variety of phenotypic changes in Schwann cells, enhances the intrinsic growth capacity of nerves, and modulates the permeability of the blood-nerve barrier, thereby promoting increased nerve regeneration (15).

The progression of peripheral nerve regeneration can be evaluated by histological analysis, with histomorphometric evaluation as a key component. Histomorphometry is used to measure cells or tissues and assess changes in cell shape. This involves assessing parameters such as the cell number, volume, thickness, length, and width (16). To evaluate axonal regeneration, histomorphometric analysis includes measuring variables, such as the number of axons, fiber diameter, and axon diameter within each nerve fascicle.

Local injection of collagen sponge-activated PRP and cytidine 5-phosphocholine improved mental nerve regeneration after injury, as demonstrated by histomorphometric analysis. These changes include increases in fiber diameter, axon diameter, and myelin thickness (17). Previous studies have demonstrated neuroprotective, neurogenic, and neuroinflammatory effects of PRP in the treatment and regeneration of peripheral nerves after local administration (18–20). However, the effects of PRP on post-axonomic axonal regeneration using various methods of administration have not been fully elucidated. This study examined the effects of PRP administered via different routes, specifically local, intraperitoneal, or a combination (local and intraperitoneal), on histomorphometric parameters of axonal regeneration after peripheral nerve injury.

2. Materials and Methods

2.1. Animals

Male Wistar rats (*Rattus norvegicus*) aged 3-4 months and weighing 250-300 grams were obtained from the Laboratory of Experimental Animals of the Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia. Throughout the study, the animals were kept under controlled conditions at room temperature with *ad libitum* access to standard food and water. The rats were acclimatized for two weeks before initiation of the experiments. In the post-test control-only group design, 25 male Wistar rats were randomly assigned to five groups (n=5). The sham-operated and saline groups served as controls, whereas the treatment groups consisted of local PRP, intraperitoneal PRP, or a combination of local and intraperitoneal PRP.

2.2. PRP collection and preparation

Ten donor rats were anesthetized with ether after 5 ml of intracardiac blood was collected in a tube containing 0.3 ml of anticoagulant. After whole blood was centrifuged twice, first at 1600 rpm for 10 min and then at 2000 rpm for another 10 min, the blood was effectively separated to obtain PRP. This method allows for the clear separation of blood into three distinct components: the lower layer consisting of erythrocytes, the upper layer comprising the supernatant, and the PRP layer placed in the middle. After the initial centrifugation, the erythrocyte layer was carefully removed. The subsequent centrifugation step resulted in the separation of the supernatant layer, producing 3 ml of pure PRP. PRP is activated by direct application of light and vibration activators shortly before each administration to animals (21).

2.3. Induction of peripheral injury by axonotmesis of the sciatic nerve

Axonotmesis injury to the sciatic nerve was induced by minor surgery, using a previously described protocol (22–24). White rats were anesthetized intraperitoneally with ketamine (50 mg/kg body weight) and xylazine (5 mg/kg body weight). The gluteal area of each rat was shaved, and the skin was sterilized with iodine solution (povidone) and prepared by draping at the site of the operation. An incision was made in the skin and superficial gluteus muscle to locate the sciatic nerve from the sciatic notch to the popliteal branch, identifying the right biceps femoris muscle. The injured nerve was marked with a 0.8 nylon suture at the epineurium. The nerve was then clamped using an arterial clamp for 60 seconds and placed approximately 1.5 cm from the sciatic foramen. The location of axonotmesis was marked with a 0.4 silk suture in the muscle, according to the site of axonotmesis of the sciatic nerve.

2.4. Experimental design

The saline and PRP control groups underwent sciatic nerve axonotmesis injury, whereas the sham-operated group did not. The sciatic nerve at the axonotmesis site in the saline control group was covered with a gelatin sponge that had been saturated with 0.2 ml of 0.9% saline solution. In contrast, the sciatic nerve in the treatment group was bandaged with a gelatin sponge that had been moistened with 0.2 ml of PRP. The group assigned to receive intraperitoneal treatment received only intraperitoneal PRP without the use of a gelatin sponge, which was administered five minutes after the axonotmesis procedure had been completed. In the sham control group, the sciatic nerve was exposed without axonotmesis, which is a form of acute compression injury. The wound was sutured using a thread. The entire procedure was performed by the same operator, using microsurgical instruments and a sterile environment. The animals that underwent treatment were subsequently relocated to their home cages, where they were observed for 21 days (25, 26).

2.5. Histomorphometric analysis of regenerated nerves

On day 22, rats were sacrificed by cervical dislocation. The sciatic nerves were subsequently surgically extracted. The nerve was divided into two separate sections, with the first segment beginning 5 mm proximal to the axonotmesis site and extending 10 mm distal. The second segment was obtained by cutting it 5 mm distal to the first section. To prepare histological specimens, 5-millimeter-thick nerve segments (segment 2) were immersed in 10% BNF solution. Sciatic nerve tissue sections measuring 5 µm in thickness were stained with Luxol fast blue (LFB).

Histomorphometric analysis was used to quantify axonal

regeneration by counting the axons and measuring the diameters of the fibers and axons within the nerve fascicles. Histological characteristics were assessed using a BX-51 microscope (Olympus, Japan) with a DP80 camera system (Olympus, Japan) and the Cell Sens software (Mitani Corp., Japan). Images were captured in three fields of view at 100x magnification, and the overall fiber diameter, axon diameter, and number of axons in each field of each group preparation were recorded.

2.6. Statistical analysis

All data are expressed as mean ± standard deviation (SD). Multivariate analysis of variance (MANOVA) was performed using SPSS software (version 25.0; IBM Corp.). Statistical significance was set at $p< 0.05$.

3. Results

The results of histomorphometric analysis of the sciatic nerve are presented in Table 1. The PRP-treated groups presented the highest mean values for all the evaluated parameters; however, these values were significantly lower than those observed in the sham-operated group. Although there were no significant differences in the mean diameter of the nerve fibers between the sham-operated and combined PRP treatment groups ($p>0.05$), there were significant differences in the diameter of the axon and myelin sheath thickness ($p<0.001$). No significant differences in the number of axons or myelin sheath thickness were observed between the local and intraperitoneal injection groups ($p>0.05$). Histomorphometric analysis of the sciatic nerve in the sham-operated group revealed myelinated axons with a normal morphology. In contrast, the saline control group showed a reduction in the number of myelinated axons, exhibiting signs of atrophy characterized by small fiber and axon diameters as well as thin and nonhomogeneous myelin (Fig. 1).

Table 1. Histomorphometric analyses of the sciatic nerve among the experimental groups

Groups	Fibre Diameter (µm)	Axon diameter (µm)	Axon counts (fb/mm^2)	Myelin sheath thickness (µm)
Sham	4.35±0.21^a	2.06±0.26^a	83.13±6.06^a	2.29±0.23^a
Saline	2.22±0.15^b	1.24±1.27^b	47.07±5.89^b	0.93±0.19^b
Local PRP	3.15±0.24^c	1.46±0.15^c	58.93±1.36^c	1.69±0.33^c
Intraperitoneal PRP	3.62±0.08^d	1.69±0.08^d	64.73±3.57^c	1.93±0.03^c
Combined PRP	4.20±0.22^a	1.98±0.03^a	71.53±4.16^e	2.16±0.36^d

Data presented as mean±SD. Note: $^{a-e}$ Within a column, means without a common superscript statistically differ ($p<0.05$)

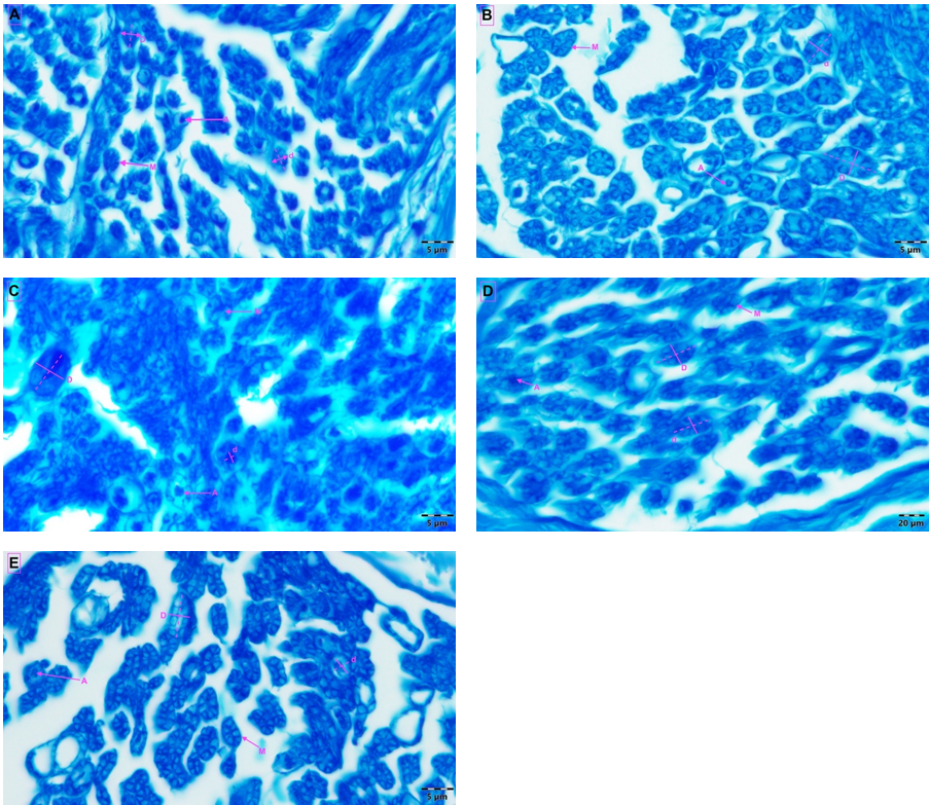

Fig. 1. Photomicrographs of histomorphometric analysis of the sciatic nerve after axonotmesis stained with LFB (100x objective lens). (A) saline group, (B) sham-operated group, (C) local PRP group, (D) intraperitoneal PRP group, and (E) combined PRP group. A: axon; M: myelin sheath; D: fiber diameter; d: axon diameter

4. Discussion

This study investigated the effects of PRP administered through various routes, namely, the intraperitoneal route and combined local and intraperitoneal routes, on axon regeneration after peripheral nerve injury. The number of axons in the saline group was the lowest among all groups. A

higher number of axons indicates a better regeneration process, accompanied by a larger axon diameter. However, when regeneration is poor, a considerable number of axons may be present, but their diameter may not be normal. The presence of a significant number of small-diameter axons was indicative of abnormal sprouting. Thus, it is crucial not only to assess the number of axons but also to assess the diameter and homogeneity of the myelin sheath. Excessive but suboptimal regeneration is defined by an abundance of axons with relatively small diameters, thin myelin sheaths, low density, and inconsistent distribution. The smaller number of axons in the saline group can also be caused by axons that sprout in an unorganized growth direction (not toward one target). This can be characterized by an increased distance between axons in nerve tissue sections (23).

Compared to the saline group, the PRP treatment group showed a higher number of regenerating myelinated axons with larger fiber and axon diameters and thicker myelin. A previous study showed that local administration of PRP leads to increases in both the diameter and number of regenerative axons in the distal portion (18, 19). Intracavernosal administration of PRP in rats after cavernous nerve injury leads to the concurrent acceleration of myelinated axon regeneration, reduced apoptosis, and increased proliferation of corpus cavernosum smooth muscle cells in the early stages (27). Histological analysis of nerve regeneration also revealed an increase in the number of inflammatory cells and endoneurial vacuoles in the PRP group (20). Furthermore, the administration of PRP to rats after injury significantly improves nerve conduction and increases the number of axons in the sciatic nerve (28).

Compared to local administration, intraperitoneal PRP has more effective systemic effects. This is due to the extensive surface area and the numerous blood vessels present in the abdominal cavity. This route of administration accelerates the direct absorption of PRP into the bloodstream, thereby facilitating its rapid absorption (29). According to Karakas et al., intraperitoneal administration of PRP demonstrated superior effectiveness in reducing postoperative peritoneal adhesions. This is achieved through tissue separation, reduction of inflammation, inhibition of matrix metalloproteinase and plasminogen activation, and obstruction of subsequent stages of adhesion formation (30).

Compared with the local or intraperitoneal administration of PRP alone, the use of PRP through intraperitoneal and local injections has shown superior results. Our findings showed that the combined PRP group exhibited a higher mean fiber diameter, axon diameter, axon count, and myelin sheath thickness than the local or intraperitoneal PRP groups. Furthermore, the mean fiber diameter, axon diameter, and myelin thickness in the combined PRP group were not significantly different from those in the sham-operated group. The group that received local and intraperitoneal PRP exhibited the thickest myelin sheath, which was similar to that observed in the sciatic nerve 21 d after axonotmesis. This significant result occurred because greater axon myelination results in faster transmission of neural impulses and better clinical outcomes (31).

In the combined group, the nerves showed an almost normal structure, although some nerve fibers still showed a thin myelin sheath. Our findings are consistent with those of a previous study that demonstrated that local and intraperitoneal administration of PRP after sciatic nerve injury effectively mitigated symptoms of neuropathic pain and improved motor function in mice, as indicated by the extensor postural thrust test (EPT) (32). Muttaqien reported that the combined application of local and intraperitoneal platelet-rich plasma (PRP) resulted in the greatest improvement in motor function in mice, as determined by the toe-out angle (TOA) test after sciatic nerve injury, exceeding the effects of administering PRP locally or intraperitoneally separately (26). The combination of local and intraperitoneal PRP not only exerts immediate effects on the injured sciatic nerve, but also triggers systemic effects by circulating through the blood vessels in the abdominal region. The use of PRP can increase the levels of several circulating growth factors such as IGF-1, VEGF, and FGF by activating specific biological pathways, thereby increasing the benefits of PRP (33, 34).

PRP shows substantial potential to improve wound healing and tissue regeneration owing to the rejuvenating abilities of platelets. When PRP is administered at the site of injury, it activates platelet-release factors that exhibit anti-inflammatory properties and prevent tissue degradation (35). The ability of PRP to promote axonal regeneration after injury is attributed to its ability to secrete growth factors, cytokines, and extracellular matrix components. This secretion facilitates the migration, proliferation, stabilization, and differentiation of various cell types, including Schwann cells, endothelial cells, fibroblasts, and mesenchymal cells (13). Furthermore, PRP has been found to induce nerve regeneration in injured nerves, leading to an increase in myelin thickness and a significant increase in the number of regenerating axons. PRP also induces the regeneration of injured nerves, leading to increased myelin thickness and a large number of regenerating axons (36). Furthermore, evidence suggests that PRP can promote nerve regeneration through neuroprotection, prevention of neuronal apoptosis, stimulation of vascular regeneration, promotion of axonal regeneration, and regulation of inflammatory responses in the microenvironment (37).

According to Moegni et al., PRP can be administered safely to patients with different pathological conditions without causing allergic reactions, infections, coagulation problems, or life-threatening situations. When administered systemically, calcium can be used as a PRP activator to prevent thromboembolism. This has been attributed to the ability of calcium to activate platelet granules, thereby suppressing

platelet activation (38, 39). PRP promotes peripheral nerve regeneration via various mechanisms. The following mechanisms are involved: increasing the capacity for axonal outgrowth, promoting the formation of new blood vessels, providing neuroprotection, preventing cell apoptosis, addressing the inflammatory environment, and reducing muscle atrophy in the denervated target areas. These results suggest that PRP plays a complex role in the restoration of peripheral nerves and that Schwann cell proliferation is a fundamental step in this regenerative process (40).

In this study, the type of nerve injury examined was characterized by axon transection or axonotmesis, which are more severe than neuropraxia. This type of injury results in damage and loss of axonal continuity as well as demyelination of the axon, damage to the endoneurium and perineurium, and Wallerian degeneration. After injury, nerve fibers undergo degeneration and regeneration. The diameter of the nerve fibers is an indicator of a successfully regenerated nerve, which is close to the normal size (23, 41). Axonotmesis-induced regeneration of the sciatic nerve highlights the neuroprotective and neuroregenerative properties of PRP, which contains an abundance of growth factors and cytokines that accelerate nerve regeneration.

In conclusion, PRP has been shown to be highly effective in improving the fiber diameter, axon diameter, myelin sheath thickness, and axon count in the sciatic nerve of Wistar rats (*Rattus norvegicus*) after sciatic nerve injury. Our study demonstrated that combined intraperitoneal and local PRP injections improved axonal regeneration in a rat model of sciatic nerve injury following axonotmesis.

Ethical Statement

All the experimental procedures were approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia (Ref. no: 168/KEPH/IX/2022, 14 September 2022).

Conflict of interest

The authors declare that they have no competing interests.

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Authors' contributions

Concept: M.M., Design: D.R.E., Data Collection or Processing: M.M., E.M., Analysis or Interpretation: M.M., E.E, Z.Z., S.R., Literature Search: D.R.E., E.M., Writing: M.M., S.H.

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Characteristics of ambulance crashes in Ankara: A retrospective analysis

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Abstract

One of the threats that Emergency Medical Services (EMS) personnel regularly face on the field is ambulance collisions and crashes. Ambulance crashes cause death and injury burden on both the EMS personnel and the civilians. In our study we aimed to evaluate the characteristics of ambulance crashes in Ankara, capital province of Türkiye. This retrospective study analyzed 812 ambulance crashes in Ankara, Türkiye, from 01/01/2022 to 31/12/2023 using data from the EMS Command Centre. Crashes were categorized by location, date, driver's gender and education, patient presence, and accident mechanism, revealing significant correlations between crash incidence and factors such as patient absence and urban settings with stationary objects. We found that there is no statistically significant correlation between the gender and the educational status of the driver and the risk of an ambulance crash. Our data analysis also points out that crashes occur involving a stationary object and EMS vehicle are more common than other mechanisms. Additionally, there is statistically significant correlation between the incidence of an ambulance crash and the absence of a patient inside the ambulance. Ambulance crashes in Ankara, Türkiye, frequently involve stationary vehicles on straight roads in urban areas and are more common when no patient is on board. These findings highlight the need for improved training and safety measures for ambulance drivers to prevent such accidents.

Keywords: ambulance crashes, emergency medical services, traffic accidents, driver training

1. Introduction

Emergency Medical Services (EMS) personnel face various safety hazards on the ground. Traffic accidents and crashes involving EMS vehicles and ambulances are among these hazards. According to a National Highway Traffic Safety Administration (NHTSA) report published in 2015, there are an estimated 1,500 ambulance crashes annually in the United States (1). A study conducted by Sanddal et al. reported that EMS personnel are at a higher risk for traffic accidents compared to other first responders, such as law enforcement officers and firefighters (2). The National EMS Memorial Service reports that between 1993 and 2010, 97 EMS personnel were killed in ambulance crashes in the United States (3).

Ambulance crashes pose threats not only to EMS personnel but also to the civilian population and patients. A study conducted by Kahn et al. reported that most injuries in ambulance crashes were inflicted upon those not in the vehicle itself, and those injured or killed in the ambulance are often the patients themselves or those in the rear compartment of the vehicle (4). Furthermore, ambulance crashes result in delays in EMS services and impose a financial burden on the system and the civilian population (5).

Alongside vehicle design, poor maintenance, and inadequate personnel training, factors such as fatigue, distraction, and stress are among the most common contributing factors to ambulance collisions (6,7). Various studies and sources also mention a correlation between the use of sirens and the incidence of ambulance crashes. One study by Isenberg et al. noted that EMS personnel using sirens are 15 times more likely to be involved in a crash (8,9). Excluding risk factors stemming from the design and maintenance of the vehicle itself, data from the aforementioned studies underline the importance of personnel-related contributing factors, such as training, fatigue, and stress. A study conducted by Boland et al. evaluated these factors by investigating the driving behavior and demographics of the EMS personnel in charge of the vehicle (10). It was found that regardless of gender and demographic factors, poor compliance with safety protocols while driving resulted in a higher risk of crashing.

The factors contributing to ambulance crashes differ by the location of the accident. Rural ambulance crashes tend to involve fewer other vehicles and occur due to environmental or vehicle factors, while urban ambulance crashes more often occur at intersections and involve other vehicles (4,11). A study conducted by Ray et al. (11) reported no difference in the severity of injuries between urban and rural crashes, while a study conducted by Weiss et al. showed that rural crashes tend to occur at higher speeds and result in higher injury severity compared to their urban counterparts (12).

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In our study, we aimed to evaluate the characteristics of ambulance crashes in Ankara, the capital province of Turkey. We recorded various factors, such as the weekday and hour of the accidents, the gender and license status of the driver, the location of the accident, the mechanism of the accident, and the presence of a patient in the crashing ambulance. By analyzing the actual hour of the shift at the time of the accident, we aimed to determine the effect of fatigue and stress on the incidence of ambulance crashes. By including other potentially contributing factors, we aimed to form a comprehensive study regarding the factors contributing to ambulance crashes. We aim to use this data to provide a better understanding of ambulance crashes and their mechanisms in Turkey.

2. Materials and Methods

2.1. Study Design and Setting

This study was designed as a retrospective study involving data collected from the EMS Command Centre of Ankara, the capital province of Turkey. As this was a retrospective study, historical data collected from previous incidents using the EMS Command Centre database were used, thus informed consent was waived. All researchers and authors involved in this study acted in accordance with the Helsinki Declaration while preparing the study protocol. This study was ethically approved by the Scientific and Ethical Review Board of Ankara Bilkent City Hospital with the approval number: TABED 1-24-283 and approval date: 22/05/2024.

We included all documented crashes that occurred within the provincial borders of Ankara between 01/01/2022 and 31/12/2023 in the study. This includes both rural and urban crashes. To provide a more detailed and meaningful analysis, we categorized the incidents according to the place and date of the accident, the ambulance driver's gender and educational status, and the presence of a patient inside the ambulance at the time of the accidents. We also divided the incidents according to the mechanism of the accident. Accidents involving a front-end or rear-end collision of different vehicles and collisions with stationary objects were categorized into different groups to determine if there is a prominent mechanism in accidents involving EMS vehicles.

2.2. Statistical Analysis

Data analysis was performed using the statistical package program IBM SPSS 27.0 (Armonk, NY: IBM Corp.). While evaluating the study data, in addition to descriptive statistical methods (frequency, percentage), Chi-Square test was used to compare qualitative data. In cases where differences were found in multiple comparisons, post-hoc Bonferroni correction was used. Statistical significance level was accepted as $p<0.05$.

3. Results

We analyzed a total of 812 ambulance crashes occurring in both rural and urban areas of Ankara between 01/01/2022 and 31/12/2023. Our data reveals that more crashes occurred in 2022 compared to 2023, but this difference did not have any statistical significance. Most crashes occurred while the EMS crew was en route to evaluate a case, with 249 crashes (57.4%) occurring this way (Table 1). We did not find any statistically significant difference or correlation between a certain month of the year and ambulance crash incidence. Our data showed that most crashes happened on weekdays rather than weekends, with a total of 605 (74.5%) accidents occurring on weekdays compared to 207 (25.5%) accidents occurring on weekends (Table 1). Our data analysis reports no statistically significant correlation between the educational status of the driver and the incidence of ambulance crashes. Additionally, we did not find any correlation between the gender of the driver and the incidence of ambulance crashes.

Table 1. Comparisons in accordance with the phase at the time of the accident

		The Phase at the Time of the Accident, n (%)						P
		Enroute to case	At he event scene	Transport Phase	At the Hospital Grounds	Returning from a case	Total	
		(249)	(108)	(165)	(98)	(192)	(812)	
Year	2022	143 (57.4)	57 (52.8)	79 (47.9)	59 (60.2)	97 (50.5)	435 (53.6)	0.190
	2023	106 (42.6)	51 (47.2)	86 (52.1)	39 (39.8)	95 (49.5)	377 (46.4)	
Month	January	22 (8.8)	10 (9.3)	18 (10.9)	16 (16.3)	21 (10.9)	87 (10.7)	0.640
	February	22 (8.8)	8 (7.4)	7 (4.2)	7 (7.1)	11 (5.7)	55 (6.8)	
	March	21 (8.4)	8 (7.4)	11 (6.7)	10 (10.2)	20 (10.4)	70 (8.6)	
	April	19 (7.6)	8 (7.4)	13 (7.9)	4 (4.1)	12 (6.3)	56 (6.9)	
	May	19 (7.6)	7 (6.5)	21 (12.7)	6 (6.1)	17 (8.9)	70 (8.6)	
	June	23 (9.2)	4 (3.7)	10 (6.1)	6 (6.1)	13 (6.8)	56 (6.9)	
	July	17 (6.8)	10 (9.3)	9 (5.5)	4 (4.1)	21 (10.9)	61 (7.5)	
	August	26 (10.4)	15 (13.9)	16 (9.7)	11 (11.2)	10 (5.2)	78 (9.6)	
	September	26 (10.4)	8 (7.4)	15 (9.1)	5 (5.1)	15 (7.8)	69 (8.5)	
	October	19 (7.6)	9 (8.3)	18 (10.9)	11 (11.2)	18 (9.4)	75 (9.2)	
	November	16 (6.4)	9 (8.3)	13 (7.9)	6 (6.1)	17 (8.9)	61 (7.5)	
	December	19 (7.6)	12 (11.1)	14 (8.5)	12 (12.2)	17 (8.9)	74 (9.1)	
Weekday /Weekend	Weekday	186 (74.7)	78 (72.2)	115 (69.7)	81 (82.7)	145 (75.5)	605 (74.5)	0.211
	Weekend	63 (25.3)	30 (27.8)	50 (30.3)	17 (17.3)	47 (24.5)	207 (25.5)	
Time Period	00:00 - 07:59	20 (8.0)	17 (15.7)	15 (9.1)	8 (8.2)	17 (8.9)	77 (9.5)	0.072
	08:00 - 15:59	136 (54.6)	56 (51.9)	83 (50.3)	41 (41.8)	87 (45.3)	403 (49.6)	

	16:00 - 23:59	93 (37.3)	35 (32.4)	67 (40.6)	49 (50.0)	88 (45.8)	332 (40.9)	
Driver Title	EMT	54 (21.7)	28 (25.9)	43 (26.1)	30 (30.6)	51 (26.6)	206 (25.4)	0.069
	Paramedic	110 (44.2)	47 (43.5)	65 (39.4)	34 (34.7)	78 (40.6)	334 (41.1)	
	Driver	68 (27.3)	28 (25.9)	39 (23.6)	18 (18.4)	53 (27.6)	206 (25.4)	
	Other	17 (6.8)	5 (4.6)	18 (10.9)	16 (16.3)	10 (5.2)	66 (8.1)	
Driver Gender	Female	34 (13.7)	17 (15.7)	16 (9.7)	10 (10.2)	23 (12.0)	100 (12.3)	0.548
	Male	215 (86.3)	91 (84.3)	149 (90.3)	88 (89.8)	169 (88.0)	712 (87.7)	

Chi-Square Test

We included an analysis of the location of the accidents and the ambulance crash incidence in urban and rural settings. Our findings suggest that in urban settings, accidents on straight roads are statistically significantly more common than those at intersections or traffic lights ($p<0.001$) (Table 2). Our data analysis reveals a statistically significant correlation between the mechanism of the accident and the incidence of ambulance crashes, with accidents involving collisions with stationary objects and EMS vehicles being more common ($p<0.001$) (Table 2). Our data analysis also reports a statistically significant correlation between the absence of a patient in the ambulance and the incidence of ambulance crashes ($p<0.001$) (Table 2).

Table 2. Comparisons in accordance with the phase at the time of the accident (continuation)

		The Phase at the Time of the Accident, n (%)						**P**
		Enroute to case	**At he event scene**	**Transport Phase**	**At the Hospital Grounds**	**Returning from a case**	**Total**	
		(n=249)	**(n=108)**	**(n=165)**	**(n=98)**	**(n=192)**	**(n=812)**	
Accident Place	Urban	243 (97.6)	103 (95.4)	156 (94.5)	97 (99.0)	183 (95.3)	782 (96.3)	0.252
	Traffic Light	7 (2.9)	0 (0.0)	11 (7.1)	0 (0.0)	12 (6.6)	30 (3.8)	**0.001**
	Intersection	33 (13.6)	4 (3.9)	31 (19.9)	14 (14.4)	29 (15.8)	111 (14.2)	
	Straight Road	203 (83.5)	99 (96.1)	114 (73.1)	83 (85.6)	142 (77.6)	641 (82.0)	
	Rural	203 (83.5)	99 (96.1)	114 (73.1)	83 (85.6)	142 (77.6)	641 (82.0)	0.252
	Traffic Light	0 (0.0)	0 (0.0)	1 (11.1)	0 (0.0)	1 (11.1)	2 (6.7)	0.711
	Intersection	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.3)	
	Straight Road	5 (83.3)	5 (100.0)	8 (88.9)	1 (100.0)	8 (88.9)	27 (90.0)	
Mechanism	Collision with a Vehicle	90 (37.0)	23 (22.3)	69 (44.2)	21 (21.6)	66 (36.1)	269 (34.4)	**<0.001**
	Collision from Behind	32 (13.2)	7 (6.8)	31 (19.9)	15 (15.5)	38 (20.8)	123 (15.7)	
	Collision with a Stationary Object	127 (52.3)	78 (75.7)	65 (41.7)	62 (63.9)	88 (48.1)	420 (53.7)	
Patient Presence at the Time of the Accident	Patient Absent	249 (100.0)	100 (92.6)	8 (4.8)	94 (95.9)	192 (100.0)	643 (79.2)	**<0.001**
	Patient Present	0 (0.0)	8 (7.4)	157 (95.2)	4 (4.1)	0 (0.0)	169 (20.8)	

Chi-Square Test

We separated the cases into two groups based on the presence of a patient within the ambulance and compared the location, mechanism, and timing of the accident alongside the demographics of the drivers. The subsequent data analysis revealed statistically significant correlations between the location of the accident in urban settings, the mechanism of the accident, and the ambulance schedule at the time of the accident. We did not find a statistically significant correlation between other variables and the incidence of ambulance crashes when comparing these separated groups (Table 3).

Table 3. Comparisons in accordance with the presence of the patients at the time of the accident

		When the crash occurred, there were, n (%)			P
		No Patients (n=643)	Patients (n=169)	Total (n=812)	
Year	2022	350 (54.4)	85 (50.3)	435 (53.6)	0.383
	2023	293 (45.6)	84 (49.7)	377 (46.4)	
Month	January	69 (10.7)	18 (10.7)	87 (10.7)	0.456
	February	48 (7.5)	7 (4.1)	55 (6.8)	
	March	56 (8.7)	14 (8.3)	70 (8.6)	
	April	42 (6.5)	14 (8.3)	56 (6.9)	
	May	47 (7.3)	23 (13.6)	70 (8.6)	
	June	46 (7.2)	10 (5.9)	56 (6.9)	
	July	52 (8.1)	9 (5.3)	61 (7.5)	
	August	62 (9.6)	16 (9.5)	78 (9.6)	
	September	54 (8.4)	15 (8.9)	69 (8.5)	
	October	59 (9.2)	16 (9.5)	75 (9.2)	
	November	48 (7.5)	13 (7.7)	61 (7.5)	
	December	60 (9.3)	14 (8.3)	74 (9.1)	
Weekday/Weekend	Weekday	487 (75.7)	118 (69.8)	605 (74.5)	0.141
	Weekend	156 (24.3)	51 (30.2)	207 (25.5)	
Time Period	00:00 - 07:59	62 (9.6)	15 (8.9)	77 (9.5)	0.865
	08:00 - 15:59	321 (49.9)	82 (48.5)	403 (49.6)	
	16:00 - 23:59	260 (40.4)	72 (42.6)	332 (40.9)	
Driver Title	EMT	163 (25.3)	43 (25.4)	206 (25.4)	0.387
	Paramedic	266 (41.4)	68 (40.2)	334 (41.1)	
	Driver	167 (26.0)	39 (23.1)	206 (25.4)	
	Other	47 (7.3)	19 (11.2)	66 (8.1)	
Driver Gender	Female	83 (12.9)	17 (10.1)	100 (12.3)	0.384
	Male	560 (87.1)	152 (89.9)	712 (87.7)	
Accident Place	Urban	621 (96.6)	161 (95.3)	782 (96.3)	0.565
	Traffic Light	19 (3.0)	11 (6.5)	30 (3.7)	**0.007**
	Intersection	80 (12.4)	31 (18.3)	111 (13.7)	
	Straight Road	522 (81.2)	119 (70.4)	641 (78.9)	
	Rural	22 (3.4)	8 (4.7)	30 (3.7)	0.565
	Traffic Light	2 (0.3)	0 (0.0)	2 (0.2)	0.545
	Intersection	1 (0.2)	0 (0.0)	1 (0.1)	
	Straight Road	19 (3.0)	8 (4.7)	27 (3.3)	
Mechanism	Collision with a Vehicle	202 (31.4)	67 (39.6)	269 (33.1)	**0.018**
	Collision from Behind	92 (14.3)	31 (18.3)	123 (15.1)	
	Collision with a Stationary Object	349 (54.3)	71 (42.0)	420 (51.7)	
The Phase at the Time of the Accident	Enroute to case	249 (38.7)	0 (0.0)	249 (30.7)	**<0.001**
	At the event scene	100 (15.6)	8 (4.7)	108 (13.3)	
	Transport Phase	8 (1.2)	157 (92.9)	165 (20.3)	
	At the Hospital Ground	94 (14.6)	4 (2.4)	98 (12.1)	
	Returning from a case	192 (29.9)	0 (0.0)	192 (23.6)	

Chi-Square Test

4. Discussion

In our study we aimed to analyze the incidence of ambulance crashes in Ankara, the capital province of Türkiye to understand if there is a correlation between driver demographics, environmental conditions and the presence of patients inside the ambulance at the time of the accident.

Ambulance crashes are important issues for the EMS personnel and the civilians involving in these accidents. When

compared to other traffic accidents, ambulance crashes are approximately four times more fatal for the personnel involving in them (13). Injuries and fatalities caused by the ambulance crashes affect both the patients and the EMS personnel, with the injury burden being the most severe on personnel being in the rear end of the vehicle at the time of the accident (14). Bystanders and civilians are also affected by ambulance crashes, with some studies reporting a higher burden of injury and death on those who are not in ambulance (4,15). Alongside the risk of death and injury, ambulance crashes cause delay and economic burden on the public services (7). Damage to civilian properties, lawsuits, loss of productivity and the delay in the EMS services result in high financial costs for the public (16).

Our data analysis reports no statistically significant correlation of the gender or educational status of the driver and the incidence of ambulance crashes. This result is similar to the findings of a study conducted by Boland et al. which also reports no statistically significant correlation between the gender and age of the driver and the incidence of ambulance crashes (10). In another study conducted by Studnek et al. it was stated that the driver's age and sleep problems affected the risk of being involved in an ambulance crash (17). The difference in these results could be explained earlier publishing date of Studnek et al.'s study when compared to Boland et al.'s study. We believe that later developments in driver training, workplace conditions and safety precautions may have a positive effect in terms of reducing the ambulance crashes in the demographic groups which were mentioned previously in Studnek et al.'s study.

Our data reveals that in urban settings, accidents involving crossroads and traffic lights are less common than those occurring at straight roads. This result bears similarity to the data mentioned in a study conducted by Biggers et al. which reports that %85,1 of ambulance crashes occurred outside intersections (18). In another study conducted by Ray et al. in 2007, it was found that intersections are much more prevalent in urban ambulance crashes when compared to rural ones (11). It is our belief that the difference between our data and Ray et al.'s 2007 study data stems from environmental issues. Most emergency vehicle crashes occur during daylight and dry road conditions. This is generally attributed to heavier traffic volumes and reduced driver attentiveness (5). This could also be a sign of the need for an improved ambulance driver certification and training system.

Our data analysis points out that accidents involving stationary objects and EMS vehicles are more common in urban setting. This result is contradictive of other studies which investigated ambulance accidents in a rural and urban setting. Studies conducted by Sanddal et al. and Ray et al. reported that crashes involving moving vehicles and EMS vehicles are more common in urban setting while more rural crashes involved stationary objects and EMS vehicles (2,11). This difference could be interpreted as a sign of the need for improved training system for the EMS personnel and better safety precautions.

Our data analysis highlight that crashes occur more commonly when ambulance does not have a patient inside. There are studies in the literature that report increased risks of accidents in the transport phase, which is the phase where ambulance has a patient assigned to it and is returning to the medical center with the said patient (8). However there are also different studies that mention no statistically significant correlation between the presence of a patient inside the ambulance and the risk of an ambulance crash (14,19).

Ambulance crashes cause significant death and injury burden alongside financial losses and emergency service delays. We aimed to understand the characteristics of the ambulance crashes in Ankara province of Türkiye. Our results showed that in Ankara province, ambulance accidents involving stationary vehicles on straight roads are more common in urban settings. Our data also reports that the incidence of ambulance crashes are higher if there isn't a patient inside the ambulance. The result from this study shows that more and better training for ambulance drivers as well as better safety precautions are needed to improve much more preventable accidents in the urban setting.

Conflict of interest
The authors declared no conflict of interest.

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Authors' contributions
Concept: RY, EDB, UMK, SF, Design: RY, SF, UMK, RBT, BY, Data Collection or Processing: BY, SD, HK, AFBK, SF, BY, Analysis or Interpretation: RBT, HK, SF, BY, MG, Literature Search: RY, EDB, MG, AFBK, RBT, HK, UMK, SD, Writing: RY, SF, EDB, MG, AFBK, SD.

Ethical Statement
Approval was obtained from Scientific and Ethical Review Committe of Ankara Bilkent City Hospital. The ethics committee decision date is 22/05/2024 and the number of ethical committee decisions is TABED 1-24-283.

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Evaluation of the efficacy of maternal hemogram parameters in predicting meconium presence at birth in healthy pregnancies

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Abstract

The presence of meconium-stained amniotic fluid (MSAF) is a critical indicator of fetal distress, associated with increased neonatal morbidity and mortality. The aim of this study was to evaluate the potential of maternal blood parameters to predict the presence of meconium-stained amniotic fluid (MSAF) in term pregnancies. Data were retrospectively analyzed from healthy pregnant women who presented to Samsun Education and Research Hospital between 2014 and 2023 and delivered either by normal spontaneous delivery or cesarean section. The study included healthy pregnant women aged 18 and over who gave birth between 37 and 42 weeks of gestation. The non-invasive laboratory parameters investigated were neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), systemic immune-inflammation index (SIRI), aggregate index of systemic inflammation (AISI), and procalcitonin levels. Results showed that NLR, MLR, SIRI, AISI, and procalcitonin levels were significantly higher in the MSAF group. The ROC curve analysis demonstrated that SIRI (cutoff: 3.55) had an AUC of 0.826 (sensitivity: 89.2%, specificity: 71.8%). AISI (cutoff: 1340.39) had an AUC of 0.749 (sensitivity: 75.8%, specificity: 70.9%). NLR (cutoff: 3.58) showed an AUC of 0.757 (sensitivity: 87.5%, specificity: 56.3%). MLR (cutoff: 0.55) presented an AUC of 0.822 (sensitivity: 87.5%, specificity: 74.8%). However, SII demonstrated negligible and statistically non-significant diagnostic value. PLR and procalcitonin exhibited lower diagnostic efficacy. This study demonstrates that NLR, MLR, SIRI, AISI, and procalcitonin are effective non-invasive biomarkers for predicting the presence of MSAF in term pregnancies. These parameters can assist clinicians in anticipating fetal distress and the risk of meconium aspiration syndrome (MAS), thereby improving perinatal outcomes through timely intervention.

Keywords: meconium, amniotic fluid, inflammation mediators, biomarkers

1. Introduction

Amniotic fluid surrounds the fetus within the uterus, providing a protective and low-resistance environment. Meconium is a dark green substance typically found in newborns, composed of bile, mucus, and epithelial cells. Under acute or chronic hypoxic conditions, the fetus may pass meconium into the amniotic fluid. The presence of meconium in the amniotic fluid is a serious indicator of fetal distress and is associated with increased neonatal morbidity and mortality (1). Even in women with a low risk of obstetric complications, meconium-stained amniotic fluid (MSAF) is common and is associated with a fivefold increase in perinatal mortality compared to low-risk patients with clear amniotic fluid (2).

MSAF is observed in approximately 13% to 16% of births (3). Several maternal and fetal factors contribute to the occurrence of MSAF, including hypertension, gestational diabetes mellitus (GDM), chronic respiratory or cardiovascular diseases, post-term pregnancy, preeclampsia, eclampsia, oligohydramnios, intrauterine growth restriction, and a poor biophysical profile (4, 5). MSAF is associated with higher rates of intervention during delivery, cesarean sections, low birth weight, fetal distress, the need for admission to the neonatal intensive care unit (NICU), and neonatal death (6). The presence of MSAF may indicate normal gastrointestinal maturation, but it can also be a sign of fetal distress due to acute or chronic hypoxic events (7, 8). Meconium aspiration syndrome (MAS) occurs when the baby aspirates meconium, affecting approximately 2% to 10% of all MSAF cases (7). Around 12% of infants with MAS result in neonatal death (9). The passage of meconium increases after the 37th week of pregnancy, and MSAF is associated with higher rates of cesarean delivery (1). Meconium aspiration can occur in the uterus, during delivery, or after birth. Causes of meconium passage in the uterus include increased motilin levels, a post-term fetus with normal gastrointestinal function, vagal stimulation caused by cord compression, and fetal stress in utero (10).

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Recent studies have highlighted the importance of various maternal hemogram and serum biochemistry parameters in predicting adverse pregnancy outcomes. The complete blood cell count, which is routinely performed and relatively inexpensive, can provide valuable insights into maternal and fetal health. Combined hematological indexes of inflammation, such as the neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte Ratio (MLR), platelet-to-lymphocyte ratio (PLR), systemic inflammation index (SII), systemic inflammation response index (SIRI), and aggregate inflammation systemic index (AISI), are already studied as biomarkers in several disorders (11-17).

For instance, studies have shown that NLR and PLR can predict fetal distress during labor and are associated with low APGAR scores (10, 18). Elevated levels of MLR and SIRI have also been observed in septic infants (19). NLR and MLR have been implicated in predicting preeclampsia and other pregnancy-related complications (5). Additionally, SIRI and AISI have been associated with gestational diabetes and intrahepatic cholestasis of pregnancy (18, 20). Research has demonstrated that MSAF is linked with increased maternal and fetal inflammation, highlighting the inflammatory nature of this condition (21).

Meconium births are generally associated with fetal distress or hypoxia, yet the relationship between this condition and maternal blood values is not clearly understood. Identifying conditions that can lead to fetal distress is crucial for reducing neonatal mortality and morbidity rates. Meconium is a cause of fetal distress that can complicate many births.

Early detection of meconium is crucial for preventing fetal distress. The ability of maternal hemogram parameters, including derived indices like PLR, NLR, MLR, SII, SIRI, and AISI, to predict meconium presence is critical for understanding the potential risks and management strategies for meconium-stained births. In our study, we aimed to identify a parameter from maternal blood values before birth that could predict meconium-stained births and enable early preventive measures to reduce meconium-associated complications such as neonatal asphyxia.

2. Materials and Methods

In our study, data from healthy pregnant women who attended the Samsun Training and Research Hospital Gynecology and Obstetrics Clinic between January 1, 2014, and December 31, 2023, and who had normal spontaneous deliveries or cesarean sections, were retrospectively analyzed. The data were obtained from the hospital's information system or by requesting files from the archive. The study included healthy pregnant women aged 18 and over who gave birth between 37 and 42 weeks of gestation. Pregnant women with conditions that could alter blood parameters, such as preeclampsia, intrauterine growth restriction, diabetes, chronic hypertension, and rheumatologic diseases, were excluded from the study.

The participants were divided into two groups: those who had meconium-stained deliveries and those who did not. The collected data were compared between these two groups. The evaluated parameters included age, body mass index, gravida, parity, mode of delivery, hemoglobin (Hb), hematocrit, white blood cell count (WBC), platelet count, lymphocyte-monocyte-neutrophil percentages, SII, SIRI, AISI, NLR, MLR, PLR, baby birth weight, APGAR scores at 1 and 5 minutes, presence or absence of meconium aspiration, need for neonatal unit admission, neonatal intubation status, gestational age, and procalcitonin levels. Maternal blood values were calculated based on the last blood samples taken for birth preparation before delivery and were compared accordingly.

The statistical analysis of the data obtained from the study was performed using the SPSS (v21.0, Illinois, US) program. The data were presented as mean ± standard deviation (SD) and median (min-max). The Kolmogorov-Smirnov test was used to analyze the assumption of normal distribution of quantitative results. Multiple group comparisons were performed using the Kruskal-Wallis H test. Bonferroni correction was applied for post-hoc pairwise comparisons following multiple group comparisons. Mann-Whitney U and Student's t-tests were used for pairwise comparisons. The relationship between variables was evaluated using Spearman's Rank correlation analysis. The ROC curve was used to determine the diagnostic value of the study data. The area under the ROC curve (AUC) was considered as a measure of the diagnostic test's discriminative power. Confidence intervals for AUC were calculated, and sensitivity and specificity values were determined. For all tests, a p-value of <0.05 was considered statistically significant.

3. Results

In this study, 120 pregnant women with MSAF and 103 healthy pregnant controls evaluated. Demographic, clinical, and biochemical data regarding the study groups has been presented in the table 1. No significant differences were observed in maternal age, gravidity, and parity. In the MSAF group median Body Mass Index (BMI) was significantly lower compared to healthy pregnant group ($p=0.013$). Vaginal deliveries were less common in the MSAF group (42.5%) compared to the control group (74.8%), with cesarean sections accounting for 57.5% in the MSAF group and only 25.2% in the control group ($p<0.001$).

In hemogram parameters, white blood cell counts and hematocrit ratio was comparable between the groups. Hemoglobin levels were lower in the MSAF group than in the control group ($p<0.001$). Lymphocyte and monocyte count also differed significantly, with the MSAF group presenting lower lymphocyte and higher monocyte and neutrophil counts ($p<0.001$). Analysis of the percentage distribution of white blood cell subtypes revealed statistically significant elevations in the rates of lymphocytes and monocytes in the MSAF group ($p<0.001$, $p=0.046$ respectively), despite higher absolute lymphocyte counts in the control group. Conversely, the

percentages of neutrophils did not show a significant difference between the groups. Besides, platelet counts were significantly lower in the MSAF group compared to control group (p<0.001). Detailed data has been presented in the table 1.

Table 1. Demographic, clinical and biochemical data of patients[a]

		MSAF (n=120)	Control (n=103)	*p*
Maternal characteristics				
Age (years)		26.5 (23.3-29.8)	27.1 (24.3-30.5)	0.164[b]
BMI (kg/m^2)		25 (23-28)	27 (24-30)	**0.013**[b, *]
Gravidity		2 (1-2)	2 (1-3)	0.787[b]
Parity		1 (0-2)	1 (0-2)	0.281[b]
Mode of delivery	**Vaginal**	51 (42.5%)	77 (74.8%)	**<0.001**[c, *]
	Cesarian section	69 (57.5%)	26 (25.2%)	
Hb (g/dl)		9.5 (9-10.2)	10.8 (9.3-11.9)	**<0.001**[b, *]
Hct (%)		29.9 (27.7-33.6)	30.3 (26.6-33.1)	0.465[b]
WBC (10^3/µl)		12.2 (10.7-14.1)	12.5 (9.7-15.3)	0.964[b]
Lymphocytes (10^3/µl)		1.3 (1.1-1.6)	1.9 (1.4-2.3)	**<0.001**[b, *]
Lymphocytes (%)		14.3 (11.8-18.2)	12.1 (9.3-15.2)	**<0.001**[b, *]
Monocytes (10^3/µl)		2.1 (1.3-3.0)	0.7 (0.5-1.1)	**<0.001**[b, *]
Monocytes (%)		6.2 (5.0-7.7)	5.8 (4.6-6.7)	**0.046**[b, *]
Neutrophils (10^3/µl)		7.1 (6.3-10.9)	5.9 (5.1-7.2)	**<0.001**[b, *]
Neutrophils (%)		80.7 (77.3-84.8)	78.8 (76.8-83.5)	0.052[b, *]
Platelets (10^3/µl)		238.5 (181.3-270.1)	381.5 (344.6-420.3)	**<0.001**[b, *]
SII		1415.3 (937.9-1904.3)	1303.6 (975.5-2012.6)	0.939[b]
SIRI		12.2 (5.6-21.3)	2.2 (1.6-2.1)	**<0.001**[b, *]
AISI		2930.7 (1359.3-4637.5)	822.8 (560.9-1805.5)	**<0.001**[b, *]
NLR		5.8 (4.2-8.4)	3.5 (2.6-5.4)	**<0.001**[b, *]
PLR		176.9 (136.5-235.9)	222.8 (169.2-266.7)	**0.001**[b, *]
MLR		1.7 (0.7-2.6)	0.4 (0.3-0.6)	**<0.001**[b, *]
PCT (ng/ml)		0.20 (0.17-0.26)	0.18 (0.16-0.22)	**0.018**[b, *]
Fetal characteristics				
Gestational age at birth (weeks)		39 (38-39)	38 (37-38)	**<0.001**[b, *]
Birth weight (g)		3370 (3152-3650)	3800 (3700-4060)	**<0.001**[b, *]
Apgar score (1st min.)		9 (7-9)	9 (9-9)	**0.037**[b, *]
Apgar score (5th min.)		10 (8-10)	10 (10-10)	**<0.001**[b, *]
NICU		43 (35.8%)	17 (16.5%)	**0.001**[c, *]
Intubation		20 (16.7%)	8 (7.8%)	**0.046**[c, *]

Abbreviations: MSAF, meconium-stained amniotic fluid; Hb, hemoglobin; Hct, hematocrit; WBC, white blood cell count; SII, systemic immune-inflammation index; SIRI, systemic inflammation response index; AISI, aggregate immune suppression index; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; MLR, monocyte to lymphocyte ratio; PCT, procalcitonin; NICU, neonatal intensive care unit

[a]Data are given as median (IQR) and as number (percentage)

[b]Mann-Whitney U Test

[c]Pearson $\chi 2$ test

[*]$p < 0.05$ indicates statistical significance

SII was comparable between the groups. However, SIRI and AISI were elevated in the MSAF group compared to controls [12.2 (IQR: 5.6-21.3) vs 2.2 (IQR: 1.6-2.1); 2930.7 (IQR: 359.3-4637.5) vs. 822.8 (IQR: 560.9-1805.5) respectively], both showing statistically significant differences (p<0.001). Similarly, NLR, and MLR were significantly higher in the MSAF group [5.8 (IQR: 4.2-8.4) vs 3.5 (IQR: 2.6-5.4); 1.7 (IQR: 0.7-2.6) vs. 0.4 (IQR: 0.3-0.6) respectively], indicating a heightened inflammatory state. In contrast, PLR was higher in the control group compared to MSAF group [222.8 (IQR: 169.2-266.7) vs 176.9 (IQR: 136.5-235.9)]. Additionally, procalcitonin (PCT) levels were found to be significantly higher in the MSAF group [0.20 ng/mL (0.17-0.26)] compared to the control group [0.18 ng/mL (0.16-0.22)] (p=0.018).

The median gestational age at birth was higher in the MSAF group compared to the control group [39 (IQR: 38-39) vs 38 (IQR: 37-38)] (p<0.001). Birth weights also differed, with MSAF infants having a lower median weight compared to control group [3370 (IQR: 3152-3650) vs 3800 (IQR: 3700-4060)] (p<0.001). NICU admissions and intubation rates were higher in the MSAF group, indicating more severe perinatal outcomes (NICU: 35.8% vs. 16.5%, p=0.001; Intubation: 16.7% vs. 7.8%, p=0.046).

In our study, significant correlations were observed between several inflammatory markers and key maternal and fetal outcomes (Table 2). Specifically, SIRI and AISI demonstrated positive, weak correlations with gestational age at birth (SIRI: r=0.288, p<0.001; AISI: r=0.245, p<0.001). Additionally, the MLR also showed a positive, weak correlation with gestational age (r=0.286, p<0.001). Concerning birth weight, there were notable negative, weak correlations with SIRI (r=-0.307, p<0.001), AISI (r=-0.220, p=0.001), NLR (r=-0.218, p=0.001), and MLR (r=-0.294, p<0.001). For the APGAR score at 5 minutes, a weak negative correlation was observed with PCT (r=-0.327, p<0.001).

Table 2. The correlation of inflammatory marker levels/indexes with other investigated maternal and fetal study parameters[a]

		SII	SIRI	AISI	NLR	PLR	MLR	PCT
Gestational age at birth	***r***	0.004	0.288‡	0.245‡	0.186†	0.104	0.286‡	0.072
	p	0.958	**<0.001***	**<0.001***	**0.005***	0.120	**<0.001***	0.282
Birth weight	***r***	0.070	-0.307‡	-0.220‡	-0.218‡	0.230‡	-0.294‡	-0.072
	p	0.297	**<0.001***	**0.001***	**0.001***	**0.001***	**<0.001***	0.282
Apgar score (1st min.)	***r***	0.035	0.078	0.093	0.012	0.051	0.106	-0.174
	p	0.599	0.247	0.166	0.854	0.452	0.113	**0.009***
Apgar score (5th min.)	***r***	0.169†	0.117	0.186†	0.054	0.256‡	0.162†	-0.327
	p	**0.012***	0.081	**0.005***	0.418	**<0.001***	**0.016***	**<0.001***

Abbreviations: SII,Systemic immune-inflammation index; SIRI, systemic inflammation response index; AISI, aggregate immune suppression index; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; MLR, monocyte to lymphocyte ratio; PCT, procalcitonin

[a]Spearman rank correlation

*p< 0.05 indicates statistical significance

†Very weak correlation

‡Weak correlation

On ROC curve analysis, diagnostic value of SII, SIRI, AISI, NLR, PLR, MLR, and PCT evaluated to determine optimal cutoff point for identifying increased risk of MSAF (Table 3 and Fig. 1). The ROC curve analysis showed that SIRI, with a cutoff value of 3.55, demonstrated high diagnostic performance with AUC of 0.826 (95% CI: 0.767-0.886, p<0.001), achieving a sensitivity of 89.2% and a specificity of 71.8%. Similarly, AISI displayed robust diagnostic potential with a cutoff of 1340.39, yielding an AUC of 0.749 (95% CI: 0.680-0.818, p<0.001), sensitivity of 75.8%, and specificity of 70.9%. The NLR also emerged as a significant marker with a cutoff of 3.58, showing an AUC of 0.757 (95% CI: 0.691-0.823, p<0.001), sensitivity of 87.5%, and specificity of 56.3%. MLR proved particularly effective with a cutoff of 0.55, where it presented an AUC of 0.822 (95% CI: 0.763-0.881, p<0.001), along with a sensitivity of 87.5% and a specificity of 74.8%. Conversely, PLR and PCT exhibited lower diagnostic efficacy. PLR, at a cutoff of 156.47, demonstrated an AUC of 0.629 (95% CI: 0.556-0.701, p=0.001) with sensitivity and specificity of 84.5% and 40.8%, respectively. PCT, with a cutoff of 0.23 ng/ml, reported an AUC of 0.592 (95% CI: 0.518-0.666, p=0.018), sensitivity of 42.5%, and specificity of 76.7%. SII demonstrated AUC of 0.503, indicating negligible diagnostic value with a statistically non-significant result (p=0.939).

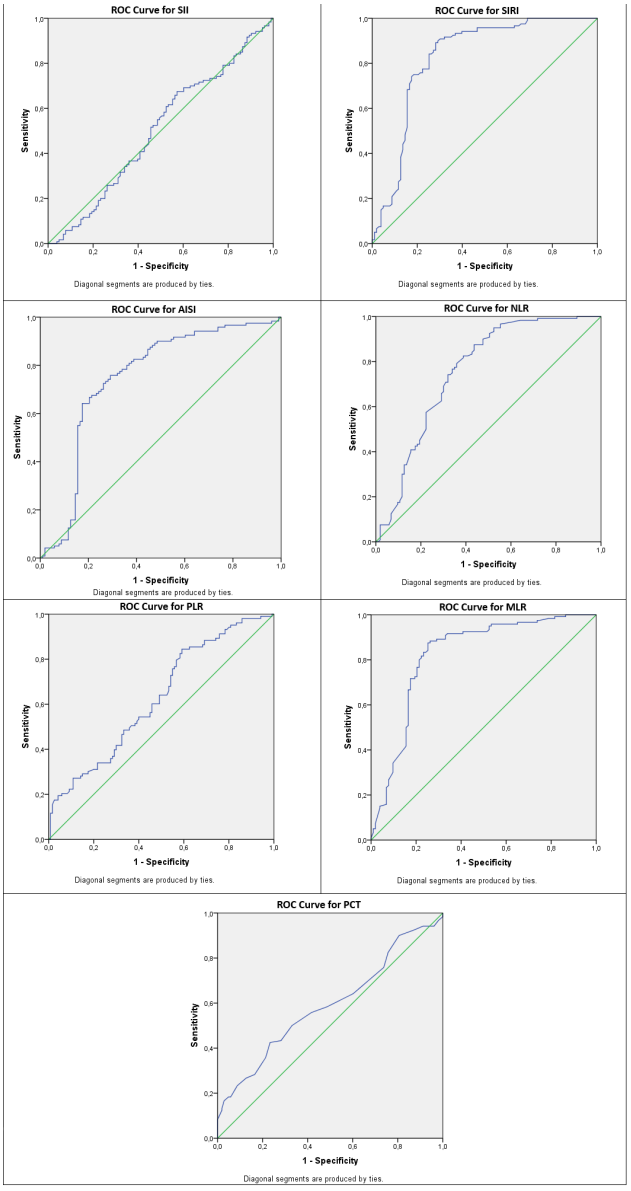

Fig. 1. Receiver operating characteristic curves for SII, SIRI, AISI, NLR, PLR, MLR, and PCT as screening tests for MSAF

Table 3. Details of the ROC curves

	Diagnostic scan					ROC curve		
Parameter	**Cutoff**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**AUC**	**CI (95%)**	***p***
SII	1156.64	65.8	43.7	53.89	56.10	0.503	0.426-0.580	0.939
SIRI	3.55	89.2	71.8	75.98	86.92	0.826	0.767-0.886	**<0.001**
AISI	1340.39	75.8	70.9	72.26	74.55	0.749	0.680-0.818	**<0.001**
NLR	3.58	87.5	56.3	66.69	81.83	0.757	0.691-0.823	**<0.001**
PLR	156.47	84.5	40.8	58.80	72.47	0.629	0.556-0.701	**0.001**
MLR	0.55	87.5	74.8	77.64	85.68	0.822	0.763-0.881	**<0.001**
PCT (ng/ml)	0.23	42.5	76.7	64.59	57.15	0.592	0.518-0.666	**0.018**

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; CI, confidence interval; SII, systemic immune-inflammation index; SIRI, systemic inflammation response index; AISI, aggregate immune suppression index; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; MLR, monocyte to lymphocyte ratio; PCT, procalcitonin

4. Discussion

The presence of meconium in the amniotic fluid can typically be detected during labor, but it remains a concerning condition due to its potential effects in the perinatal period (1, 2). Identifying markers that can provide early prediction would be highly beneficial for clinicians. In this study, we examined maternal hemogram parameters to predict MSAF in healthy term pregnancies for the first time in the literature and found that NLR, MLR, SIRI, AISI, and procalcitonin levels were significantly higher in the MSAF group. Additionally, in the MSAF group, gestational age at delivery was higher, the cesarean rate and birth weight were lower, and the 1st and 5th minute APGAR scores were lower. Moreover, the NICU admission rate and intubation rate were significantly higher. These results indicate that the severity of inflammation is directly proportional to the likelihood of detecting MSAF.

The passage of meconium can be a natural event indicating fetal maturity, but in some cases, it can also indicate increased vagal activity due to fetal hypoxia (oxygen deficiency) or umbilical cord compression. Amniotic fluid is sterile, but the passage of meconium into the amniotic fluid is critical due to the risk of MAS. It is also associated with adverse perinatal outcomes such as birth asphyxia, sepsis, neonatal depression, neurological sequelae, and neonatal death. Therefore, detecting the presence of meconium during delivery is crucial (10). Although electronic fetal monitoring can reflect fetal problems during the intrapartum period, there is interobserver variability in interpretation. Most studies in the literature focus on morbidity and mortality that develop in the neonatal period after meconium aspiration has occurred. Our study, unlike the literature, was designed to predict the likelihood of meconium in the fetus by utilizing maternal blood parameters in term pregnancies.

There are some changes in the hematologic system during pregnancy. Blood volume increases by 30-40%, and plasma and erythrocytes increase by 30%. While the half-life of platelets shortens, RDW (Red Cell Distribution Width) and MPV (Mean Platelet Volume) increase (10). The balance between the rising neutrophils as the first line of defense and the lymphocytes representing the regulatory and protective components of inflammation represents the inflammatory process. These ratios have been shown to be important in the prediction and monitoring of many diseases during pregnancy (5, 10, 20). In a study conducted by Gomez et al., amniotic fluid samples were collected from 15 women with suspected intra-amniotic infection and/or inflammation. They found that the neutrophils in the amniotic fluid were predominantly of fetal or maternal origin, or a mixture of both. These findings suggest that both the fetus and the mother participate in host defense mechanisms against intra-amniotic infection, indicating that both fetal and maternal neutrophils can invade the amniotic cavity (22).

In our study, NLR and MLR were found to be significantly higher in the MSAF group, while PLR was lower. We found that when we took an NLR cutoff of 3.58, it was 87.5% sensitive in predicting MSAF. It is known that the presence of MSAF can be secondary to fetal hypoxia in the intrauterine period (1, 2, 6). Similarly, Aksakal et al. demonstrated that NLR and PLR could be used to predict fetal distress during labor (10). In this study, the results of 124 pregnant women who developed fetal distress were compared with those of 126 healthy pregnant women. While APGAR scores were significantly lower in those with fetal distress, no difference was observed in terms of the need for NICU. However, the presence of intrauterine meconium in the infants was not questioned in this study (10). Karakoç et al. showed that NLR and PLR values were significantly higher in the group with APGAR <7 compared to the group with APGAR ≥7 in neonates. Studies on complete blood parameters during pregnancy in the literature have mostly been conducted on diseases such as preeclampsia and gestational diabetes (18). These studies have shown that elevated NLR and MLR provide a prediction for preeclampsia and gestational diabetes (5). Çakır et al. demonstrated the increase in MLR in septic newborns (19). However, different results exist in the literature for PLR. While an increase in PLR has been shown in preeclampsia and fetal distress, it has been observed to be unaffected in cases of premature rupture of membranes with normal amniotic volume (5, 10, 23). Taşkın et al. reported no difference in MPV, NLR, PLR, and MLR between groups with and without early sepsis in a study involving 272 babies with MSAF (24).

The use of SIRI and AISI parameters to determine inflammatory processes has been increasing in recent years. In our study, we found that SIRI and AISI parameters were significantly higher in the MSAF group. When we set the cutoff value for SIRI at 3.55, we found that it was 89.2% sensitive in identifying MSAF. Additionally, we found that birth weight was weakly negatively correlated with SIRI, AISI, NLR, and MLR. Previous studies have shown elevated SIRI in intrahepatic cholestasis of pregnancy and elevated SIRI and AISI in gestational diabetes (18, 20). Çakır et al. demonstrated that SIRI was significantly higher in the septic group in their study evaluating septic infants (19). Consonni et al. investigated MSAF and clear amniotic placentas in the African American population and evaluated the frequency, stage, and severity of the maternal inflammatory response (MIR) and fetal inflammatory response (FIR). They found that MIR and FIR were significantly higher in the MSAF group. MIR and FIR were present together in 35.8% of the MSAF group, whereas they were present together in 25.2% of the clear amniotic fluid group. In conclusion, they showed that MSAF is associated with an increase in both maternal and fetal inflammation. This histopathological study supports the increase in maternal inflammatory markers found in our study (21).

Procalcitonin, a precursor substance for calcitonin, acts as a prohormone involved in inflammatory processes. Serum levels of PCT rise faster than C-reactive protein (CRP) and can be detected in the blood within 2-4 hours, with a half-life of 24 hours. Normally, PCT levels are lower than 0.1 μg/L. During pregnancy, physiological levels of PCT increase from the first to the third trimester, reaching a cutoff value of 0.25 μg/L in the third trimester and at delivery. It has been reported that antibiotic treatment should be considered if values exceed this threshold (25, 26). New evidence in pregnancy suggests that PCT can complement clinical findings in the diagnosis of sepsis more effectively and promptly (27). In our study, the PCT serum level was found to be 0.20 (0.17-0.26) μg/L in the MSAF group and 0.18 (0.16-0.22) μg/L in the control group, with the difference being statistically significant. Although the values we detected were below the cutoff for a systemic inflammatory response, ROC analysis showed that with a PCT (ng/ml) threshold value of 0.23, sensitivity was 42.5% and specificity was 76.7% for predicting MSAF. Additionally, the 5-minute APGAR score was weakly negatively correlated with procalcitonin levels. Similarly, Taşkın et al. found high PCT levels in neonates with MSAF who were suspected of early sepsis (24). We believe that procalcitonin can be used in predicting maternal and fetal inflammation in this context.

In our study, a higher cesarean section rate was observed in patients with meconium-stained births among term pregnancies. High cesarean section rates in the presence of meconium have been reported in previous studies (1, 3, 4, 28, 29). Mundhra reported a cesarean section rate of 49.09% in a study including 165 MSAF and 190 term clear amniotic pregnancies. In these patients, the presence of a 1st minute APGAR score <7, fetal asphyxia, and the need for NICU were significantly higher (2, 30). High cesarean section rates in studies reflect not only abnormal fetal heart rates but also the concerns of the attending midwife or doctor about potential fetal morbidity and mortality. In contrast, Levin reported a cesarean section rate of 11.3%, and Wong SF reported 8.8%. Wong attributed these low rates to the inclusion of scalp pH sampling methods in their studies (29). In our study, consistent with the literature, MSAF was associated with increasing gestational age (1, 29). Mundhra and Rathoria observed that half of the meconium-stained patients were over 40 weeks and attributed this to the high frequency of unsupervised pregnancies and late hospital admissions (6, 30). However, in our region, the patients generally consisted of those who attended regular monthly check-ups, and there were no post-term patients.

In our study, 35.8% of term infants in the MSAF group required NICU, compared to 16.5% in the control group. Additionally, 16.7% of the MSAF group and 7.8% of the control group required intubation, and both rates were statistically significant. Similarly, Rathoria reported an NICU rate of 25.45%. This study showed that, when evaluating those with and without fetal distress in the MSAF group, the group with fetal distress had significantly higher rates of a low 1st minute APGAR score (≤5), emergency cesarean section, and NICU admission (4). In contrast, Levin reported a NICU need of 1.6% and a mechanical ventilation need of 0.5% in a study evaluating 11,329 births that included MSAF (31). The different results in the literature may be due to differences in sample sizes and whether the study groups included preterm and/or term patients.

MSAF is seen in one out of every seven babies, and MAS is observed in only 5% of these babies (6). Although studies have shown an increase in inflammatory markers in amniotic fluid and fetal serum following MAS exposure, to our knowledge, no studies have yet investigated maternal blood parameters in predicting MSAF. Asphyxia (antenatal and/or intranatal) has played a role in the pathogenesis of MFAS, as it has been shown that fetal breathing can lead to MSAF aspiration. In Lee's study, only 2 out of 12 neonates with MAS had an APGAR score <7 at 5 minutes after birth, and only 2 out of 12 cases had an umbilical artery pH <7.1. This suggested that the alternative mechanism of the disease might be inflammation (6). This study observed an increased risk of MAS in term infants with MSAF when funisitis was detected, whereas the MAS risk did not increase in MSAF infants without funisitis (6). Evaluating fetal systemic inflammation may target neonates born to MSAF mothers who show a suspicious clinical course for MAS. The rationale for this approach is that only 7.3% (6/82) of neonates without funisitis (one of the indicators of FIRS) developed MAS, whereas this risk increased fourfold (5/16) in neonates with funisitis. In light of these results, it can be inferred that biomarkers of fetal and maternal systemic inflammation can be used to predict MAS in

neonates exposed to MSAF.

The strength of our study is the ability to predict the presence of meconium in term pregnancies using non-invasive, simple inflammatory markers. The main limitation of our study is its retrospective design and the inclusion of a low-risk population. Different results may be obtained by evaluating the impact of the presence of meconium with additional risk factors on perinatal birth outcomes in studies including high-risk and preterm pregnancies. Furthermore, future studies that jointly evaluate samples taken from the amniotic fluid and fetal cord to examine fetal impact will provide guidance in elucidating the data we have obtained.

NLR, MLR, SIRI, AISI, and Procalcitonin have high sensitivity in predicting the presence of meconium-stained amniotic fluid in term pregnancies. The values of these parameters in maternal blood will guide clinicians in predicting the risk of fetal well-being deterioration and MAS.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: S.S.Ü., Y.C.Ü., Design: S.S.Ü., Y.C.Ü., Data Collection or Processing: Ö.D.Ü., Y.C.Ü., S.E., S.M.A., C.S.Ç., Z.Y., C.M.S., S.Ç., Analysis or Interpretation: S.S.Ü., S.Ç., Literature Search: Ö.D.Ü., C.S.Ç., Z.Y., C.M.S., S.Ç., Writing: Y.C.Ü., S.E., S.M.A.

Ethical Statement

Approval was obtained from Samsun University Non-invasive Clinical Research Ethics Committee, the study started. The ethics committee decision date is 22/05/2024 and the number of ethical committee decisions is 2024/10/2.

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The efficacy of antenatal maternal hemogram and coagulation test parameters in predicting atony-related postpartum hemorrhage

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Abstract

This study aims to evaluate the efficacy of maternal hemogram parameters in PPH in term pregnancies. A retrospective analysis was conducted on 84 patients with atony-related PPH and 96 control patients with healthy deliveries, who presented to the Gynecology and Obstetrics Clinic of Samsun Education and Research Hospital between January 1, 2008, and December 31, 2023. Patients' data were retrieved from the hospital's information system. Exclusion criteria included pregnant women under 18 years, those with preeclampsia, chronic hypertension, or bleeding disorders. Evaluated parameters included age, BMI, gravida, parity, gestational age, mode of delivery, prepartum and postpartum hemogram and coagulation parameters, and neonatal outcomes. The mean age was 32.1±6.9 years in the PPH group and 30.1±5.8 years in the control group. Hemoglobin and hematocrit levels were significantly lower in the PPH group (Hb: 9.4±1.0 ng/ml) compared to the control group (Hb: 11.5±1.4 ng/ml, $p<0.001$). Plasma fibrinogen levels were also significantly lower in the PPH group (392.6±69.2 mg/dl) compared to the control group (467.2±69.6 mg/dl, $p<0.001$). A significant positive correlation was observed between plasma fibrinogen levels and 1- and 5-minute Apgar scores ($p=0.002$ and $p<0.0001$, respectively). BMI ≥28.8 was associated with a higher risk of hemorrhage. Maternal hemogram parameters, particularly hemoglobin, hematocrit, and fibrinogen levels, are effective predictors of postpartum hemorrhage. The study emphasizes the importance of monitoring these parameters to identify and manage at-risk pregnancies, thereby reducing maternal morbidity and mortality associated with PPH.

Keywords: postpartum hemorrhage, fibrinogen, hemorrhage risk factors, blood coagulation tests

1. Introduction

Postpartum hemorrhage (PPH) is one of the leading causes of maternal morbidity and mortality related to childbirth (1, 2). PPH is an emergency obstetric condition, occurring in 3-5% of postpartum patients (3). The primary cause of postpartum hemorrhage is uterine atony. Due to the high blood flow in the uterine arteries during the late stages of pregnancy, uterine atony can lead to rapid and severe bleeding. Protocols for the step-by-step active management of postpartum hemorrhage improve outcomes (4,5). The first steps in managing PPH include manual examination of the uterus and injection of oxytocin. In cases of persistent atony, stronger uterotonic prostaglandin analogs are recommended. In severe postpartum hemorrhage cases, advanced interventions such as hemodynamic resuscitation, blood products, uterine artery embolization, and/or surgical arterial ligation or hysterectomy may be required (6).

Risk factors and management protocols for postpartum hemorrhage are continuously updated. In recent years, the importance of fibrinogen and fibrinolysin in major postpartum hemorrhages has been highlighted. Towards the end of pregnancy, the concentration of various clotting factors increases, and the activity of natural anticoagulants and fibrinolytic activity decreases (7, 8). In term pregnant women, fibrinogen levels rise to between 4 and 6 g/L, whereas in non-pregnant women, levels range from 2 to 4 g/L. During pregnancy, an increase in fibrinogen levels is observed, while standard indicators such as prothrombin time (PT) and activated partial thromboplastin time (aPTT) show very little difference. Coagulation plays a crucial role in postpartum hemostasis (9). Coagulation disorders are insufficiently evaluated risk factors for postpartum hemorrhage. In 2007, Charbit et al. demonstrated that fibrinogen levels were lower in women who developed severe postpartum hemorrhage compared to those with mild postpartum hemorrhage (6). Another study conducted in 2012 with 738 patients diagnosed with postpartum hemorrhage also provided informative data indicating that fibrinogen levels can serve as a warning to clinicians regarding postpartum hemorrhage (10). A meta-analysis published in 2023 evaluated hemogram parameters and fibrinogen levels. This study observed that patients with

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postpartum hemorrhage had lower prepartum platelet counts and suggested that parameters such as hemoglobin and fibrinogen could not predict postpartum hemorrhage (11).

Predicting postpartum hemorrhage can allow for early intervention and treatment for a potential bleeding situation, potentially improving patient survival by preventing unnecessary treatments. Despite treatment protocols, in cases where bleeding cannot be prevented, if no precautions are taken and the bleeding is not predicted, aggressive measures up to hysterectomy may be necessary. Being able to predict postpartum hemorrhage can save time in patient management, preventing the need for aggressive treatments and reducing mortality and morbidity rates. Numerous studies in the literature have focused on the risk factors for postpartum hemorrhage. In this study, we aimed to identify hemogram and coagulation parameters that could predict postpartum hemorrhage by comparing pregnant women with postpartum hemorrhage to those with healthy deliveries. The results obtained from routine blood samples taken before delivery from these women were retrospectively analyzed.

2. Materials and Methods

Our study included pregnant women between the 37th and 42nd weeks of gestation who applied for delivery to the Gynecology and Obstetrics Clinic of Samsun Training and Research Hospital between January 1, 2008, and December 31, 2023. The data of the patients were obtained from the hospital's information system and analyzed retrospectively. Eighty-four pregnant women with postpartum hemorrhage due to atony were included in the experimental group, and 96 healthy pregnant women were included in the control group. Pregnant women under 18 years old, and those with preeclampsia, chronic hypertension, or bleeding disorders were excluded from the study. The parameters evaluated included age, body mass index, gravida, parity, gestational age, mode of delivery, prepartum hemoglobin, hematocrit, white blood cell count, neutrophil-to-lymphocyte ratio, platelet count, fibrinogen levels, PT, aPTT , international normalized ratio (INR) levels, postpartum hemoglobin, hematocrit, platelet count, fibrinogen levels, whether hysterectomy was performed, baby's birth weight, 1-minute and 5-minute Apgar scores, presence or absence of meconium aspiration, whether the newborn was admitted to the neonatal unit, and whether the newborn was intubated.

The statistical analysis of the data obtained from the study was performed using the SPSS (v21.0, Illinois, US) program. The data were presented as mean ± standard deviation (SD) and median (min-max). The Kolmogorov-Smirnov test was used to analyze the assumption of normal distribution of quantitative results. Multiple group comparisons were performed using the Kruskal-Wallis H test. Bonferroni correction was applied for post-hoc pairwise comparisons following multiple group comparisons. Mann-Whitney U and Student's t-tests were used for pairwise comparisons. The relationship between variables was evaluated using Spearman's Rank correlation analysis. The ROC curve was used to determine the diagnostic value of the study data. The area under the ROC curve (AUC) was considered as a measure of the diagnostic test's discriminative power. Confidence intervals for AUC were calculated, and sensitivity and specificity values were determined. For all tests, a p-value of <0.05 was considered statistically significant.

3. Results

Demographic, clinical, and biochemical characteristics between the control group (n=97) and the PPH group (n=84) were detailed in table 1. Maternal age was significantly (p=0.040) higher in the PPH group (32.1±6.9 years) compared to the control group (30.1±5.8 years). Body mass index (BMI) also differed significantly, with the PPH group showing a median BMI of 28.8 (IQR 25.6-33.0) versus 24 (IQR 23-25) in the control group (p<0.001). No significant differences were observed in gravidity and parity between the two groups. The mode of delivery, categorized into vaginal and Cesarean sections, did not differ significantly between groups. Notably, hysterectomy was only performed in the PPH group (82.1% of cases). Plasma hemoglobin and hematocrit levels were lower in the PPH Group (9.4±1.0 ng/ml and 28.6±4.2%, respectively) compared to the control group (11.5±1.4 ng/ml and 35.4±3.7%, respectively) (p<0.001). PT and aPTT showed no significant difference between the control group and the PPH group. However, INR differed significantly, with the control group recording a median INR of 0.9 (IQR 0.9-1.0), while the PPH group had a median INR of 1.0 (IQR 0.9-1.0) (p<0.001). Serum calcium levels were significantly lower in the PPH group (8.6±0.7 mg/dl) compared to the control group (9.1±0.4 mg/dl) (p<0.001). Additionally, plasma fibrinogen levels were significantly lower in the PPH group (392.6±69.2 mg/dl) than in the control group (467.2±69.6 mg/dl), also showing statistical significance (p<0.001). The PPH group demonstrated significantly lower gestational age at birth, with a median of 38 weeks (IQR 37-39) compared to 39 weeks (IQR 38-40) in the Control Group (p=0.008). Similarly, birth weight was lower in the PPH Group, with an average weight of 3403.1±714.4 grams, compared to 3188.1±501.2 grams in the Control Group (p=0.022). Apgar scores at 1st and 5th minutes post-birth also differed significantly. The PPH group had lower scores at 1st minute with a median score of 7.5 (IQR 7-8) compared to 9 (IQR 9-9) in the control group (p<0.001). At 5th minutes, the scores remained lower in the PPH group with a median score of 8 (IQR 7.3-9) against 10 (IQR 10-10) in the control group (p<0.001). Additionally, the incidence of meconium-stained amniotic fluid was substantially higher in the PPH group, affecting 25 cases (29.8%) compared to only 3 cases (3.1%) in the control group (p<0.001). Admission to the neonatal intensive care unit (NICU) was more frequent in the PPH group, with 15 (17.9%) admissions versus 3 (3.1%) in the control group (p=0.001). Intubation rates were also higher in the PPH group, with 12 (14.3%) instances compared to 3 (3.1%) in the control group (p=0.006).

Table 1. Demographic, clinical and biochemical data of patients[a]

		Control Group (n=97)	PPH Group (n=84)	*p*
Maternal characteristics				
Age (years)		30.1±5.8	32.1±6.9	**0.040**[b,*]
BMI (kg/m²)		24 (23-25)	28.8 (25.6-33.0)	**<0.001**[c,*]
Gravidity		3 (2-4)	3 (2-4)	0.247[c]
Parity		2 (1-2)	2 (1-2)	0.435[c]
Mode of delivery	**Vaginal**	60 (61.9%)	57 (67.9%)	0.400[d]
	Cesarian section	37 (38.1%)	27 (32.1%)	
Hysterectomy	**Yes**	0 (0%)	15 (82.1%)	N.A
	No	97 (100%)	69 (17.9%)	
Plasma Hemoglobin (ng/ml)		11.5±1.4	9.4±1.0	**<0.001**[b,*]
Plasma Hematocrit (%)		35.4±3.7	28.6±4.2	**<0.001**[b,*]
Plasma WBC (10^3/µl)		10.6 (8.3-12.8)	12.4 (10.0-16.5)	**<0.001**[c,*]
Plasma PLT (10^3/µl)		228.0±61.6	186.8±46.8	**<0.001**[b,*]
Plasma PT (sec.)		10.9 (10.6-11.5)	11 (10.6-11.5)	0.449[c]
Plasma APTT (sec.)		23.9±3.0	24.5±3.1	0.166[c]
Plasma INR		0.9 (0.9-1.0)	1.0 (0.9-1.0)	**<0.001**[c,*]
Serum Calcium (mg/dl)		9.1±0.4	8.6±0.7	**<0.001**[b,*]
Plasma Fibrinogen (mg/dl)		467.2±69.6	392.6±69.2	**<0.001**[b,*]
Fetal characteristics				
Gestational age at birth (weeks)		39 (38-40)	38 (37-39)	**0.008**[c,*]
Birth weight (g)		3188.1±501.2	3403.1±714.4	**0.022**[b,*]
Apgar score (1st min.)		9 (9-9)	7.5 (7-8)	**<0.001**[c,*]
Apgar score (5th min.)		10 (10-10)	8 (7.3-9)	**<0.001**[c,*]
MSAF		3 (3.1%)	25 (29.8%)	**<0.001**[d,*]
NICU		3 (3.1%)	15 (17.9%)	**0.001**[d,*]
Intubation		3 (3.1%)	12 (14.3%)	**0.006**[d,*]

Abbreviations: PPH, post-partum hemorrhage; BMI, body-mass index; WBC, white blood cells; PLT, platelets; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio; MSAF, meconium stained amniotic fluid; NICU, neonatal intensive care unit
[a]Data are given as mean±SD, median (IQR) and as number (percentage)
[b]Student's T Test
[c]Mann-Whitney U Test
[d]Pearson $\chi 2$ test
[*]$p < 0.05$ indicates statistical significance

Table 2. The correlation of plasma fibrinogen, serum calcium levels and other coagulation parameters levels with other investigated maternal and fetal study parameters

		Age	BMI	Gravidity	Parity	Gestational age at birth	Birth weight	Apgar score (1st min.)	Apgar score (5th min.)
Plasma Fibrinogen	***r***	-0.123	**-0.164**[†]	-0.022	0.010	0.031	**-0.161**[†]	**0.233**[‡]	**0.305**[‡]
	p	0.099	**0.027**[*]	0.771	0.891	0.683	**0.030**[*]	**0.002**	**<0.000**[*]
Serum Calcium	***r***	**-0.223**[‡]	**-0.223**[‡]	-0.006	0.024	0.125	-0.054	**0.252**[‡]	**0.252**[‡]
	p	**0.003**[*]	**0.003**[*]	0.931	0.745	0.094	0.473	**0.001**[*]	**0.001**[*]
Plasma Hemoglobin	***r***	-0.043	**-0.364**[‡]	0.007	0.017	**0.197**[†]	-0.033	**0.298**[‡]	**0.420**[§]
	p	0.566	**<0.000**[*]	0.931	0.820	**0.008**[*]	0.659	**<0.000**[*]	**<0.000**[*]
Plasma Hematocrit	***r***	-0.076	**-0.342**[‡]	-0.012	-0.014	**0.210**[‡]	-0.022	**0.329**[‡]	**0.489**[§]
	p	0.308	**<0.000**[*]	0.867	0.852	**0.005**[*]	0.773	**<0.000**[*]	**<0.000**[*]
Plasma PLT	***r***	**-0.188**[†]	**-0.234**[‡]	**-0.178**[†]	-0.113	0.030	-0.067	0.070	**0.199**[†]
	p	**0.011**[*]	**0.002**[*]	**0.016**[*]	0.131	0.688	0.370	0.346	**0.007**[*]
Plasma PT	***r***	0.020	0.031	-0.125	**-0.148**[†]	-0.135	0.008	-0.124	-0.057
	p	0.794	0.675	0.093	**0.047**[*]	0.070	0.918	0.096	0.446
Plasma APTT	***r***	-0.031	-0.025	0.036	-0.023	0.124	0.022	-0.068	-0.059
	p	0.682	0.737	0.626	0.756	0.096	0.771	0.360	0.430
Plasma INR	***r***	0.129	**0.192**[†]	-0.109	-0.109	**-0.183**[†]	0.097	**-0.302**[‡]	**-0.277**[‡]
	p	0.084	**0.010**[*]	0.145	0.145	**0.014**[*]	0.193	**<0.000**[*]	**<0.000**[*]

Abbreviations: BMI, body-mass index; CNP, c-type natriuretic peptide
[*]$p < 0.05$ indicates statistical significance
[†]Very weak correlation
[‡]Weak correlation
[§]Medium correlation

The correlation analysis of plasma fibrinogen and serum calcium levels with various maternal and fetal parameters, detailed in Table 2, revealed several statistically significant relationships. Plasma fibrinogen exhibited significant but very weak negative correlations with body mass index (BMI) (r=-0.164, p=0.027) and birth weight (r=-0.161, p=0.030).

Additionally, it showed significant positive weak correlations with Apgar scores at the 1st minute (r=0.233, p=0.002) and 5th minutes (r=0.305, p<0.0001). Serum calcium also showed significant negative correlations with BMI (r=-0.223, p=0.003) and significant positive correlations with Apgar scores at both the 1st minute (r=0.252, p=0.001) and 5th minutes (r=0.252, p=0.001) assessments. For other coagulation parameters, several correlations were observed. Intriguingly, plasma hemoglobin levels and hematocrit showed medium positive correlations with the 5th minute Apgar score (r=0.420 and r=0.489 respectively; both p<0.0001). Detailed data have been.

Table 3. Details of the ROC curves for discrimination of thresholds of maternal plasma fibrinogen levels

Diagnostic scan					ROC curve		
Cutoff (mg/dl)	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**AUC**	**CI (95%)**	***p***
391.5	91.8	59.5	69.39	87.89	0.786	0.719-0.853	<0.001

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; CI, confidence interval

On ROC curve analysis, the optimal cutoff point for identifying increased risk of PPH were found to be 391.5 mg/dL (91.8% sensitivity and 59.5% specificity) for plasma fibrinogen. The positive predictive value (PPV) was 69.39%, the negative predictive value (NPV) was 87.89%. According to the ROC analysis, AUC was 0.786 (95% CI: 0.719-0.853) (Table 3 and Fig.1).

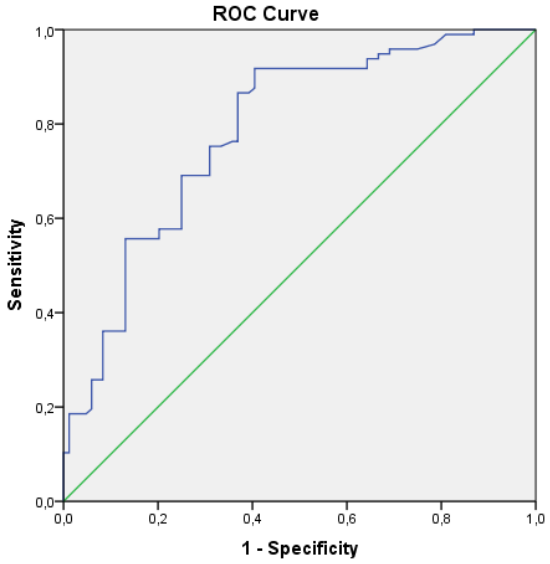

Fig. 1. Receiver operating characteristic curves for fibrinogen as screening tests for postpartum hemorrhage

4. Discussion

PPH is classified into early hemorrhage (within the first 24 hours after birth) and late hemorrhage (24 hours to 12 weeks after birth). Early hemorrhage is often severe and is associated with prolonged labor, retained placenta, and coagulation disorders (12). PPH is reported to occur in 3% to 5% of births (13). An analysis of population-based data from the United States National Inpatient Sample showed that the rate of PPH increased from 2.7% in 2009 to 4.3% in 2019 (13). The primary cause of postpartum hemorrhage is uterine atony.

In recent years, the incidence of PPH has been increasing worldwide (14). Due to its complex etiology and pathogenesis, preventing PPH is challenging. Early diagnosis and treatment are urgently required to prevent PPH. Some studies have reported that coagulation disorders are considered one of the four main causes of postpartum hemorrhage (15-17).

Our study included 84 individuals in the postpartum hemorrhage group and 96 individuals in the control group. The average age of women in our study was calculated as 32.1±6.9 years in the postpartum hemorrhage group and 30.1±5.8 years in the control group. Advanced maternal age is considered an independent risk factor for postpartum hemorrhage. In Japan, Ohkuchi et al., in their study of 10,053 women with singleton births, found through multivariate analysis that maternal age ≥35 years was an independent risk factor for postpartum hemorrhage (18). Ijaiya et al. from Nigeria found that the risk of postpartum hemorrhage in women aged ≥35 years was twice as high as in those aged ≤25 years (19). Okogbenin et al. from Nigeria also reported that obstetric hemorrhage increases with age (20). According to the authors, the risk is 0.1% at age 20 and increases to 0.7% at age ≥40. The results of our study also found that the average age in the postpartum hemorrhage group was significantly higher, consistent with the predictions of other studies.

Although grand multiparity has traditionally been considered a risk factor for postpartum hemorrhage, our study did not find a significant difference among cases analyzed based on parity. Ockuchi found that primiparity posed a risk for vaginal postpartum hemorrhage in his study (18). Additionally, no significant difference in postpartum hemorrhage was observed between patients who had vaginal deliveries and those who had cesarean deliveries. Similar results were found in a study by Zakaria et al. in 2019 (21).

In our study, there was no significant relationship between BMI and the amount of postpartum hemorrhage, similar to the findings of Kınay et al. (22). However, Budwick et al. found a moderate increase in the risk of postpartum hemorrhage in obese women (23). In our study, a BMI ≥28.8 was associated with a higher risk of hemorrhage.

In our study, hemoglobin and hematocrit levels were significantly lower in the postpartum hemorrhage group compared to the control group (PPH group; Hb: 9.4±1.0 ng/ml, control group; 11.5±1.4 ng/ml, p<0.001). Similar to our results, the literature indicates that anemic women have a higher risk of developing PPH due to uterine atony during childbirth. Studies have shown that severe anemia can impair myometrial contractility due to reduced oxygen transport to the uterus (24, 25). There are few studies addressing the causal relationship between severe anemia and PPH, and there is no consensus on the threshold hemoglobin value that may increase the risk of

uterine atony.Such research provides obstetricians with a new and improved tool to identify pregnant women at risk and thus provide them with standard care. The design of our study suggests that further research is needed in this area.

Fibrinogen (clotting factor 1) is normally found in the blood at levels of 200-450 mg/dl before pregnancy (26). During the activation of the coagulation cascade, thrombin converts fibrinogen into fibrin polymers that form the occlusive clots (27). During pregnancy, the average fibrinogen level rises to 500 mg/dl (28). Low fibrinogen levels or reduced function severely impair hemostasis. Observational studies suggest a relationship between low fibrinogen levels at the onset of postpartum hemorrhage and the subsequent severity of bleeding. During PPH, fibrinogen levels rapidly decrease due to two primary mechanisms: blood loss leading to the depletion of clotting factors and the consumption of factors associated with coagulation activation (29). In our study, plasma fibrinogen levels in the postpartum hemorrhage group (mean 392.6±69.2 mg/dl) were significantly lower than those in the control group (mean 467.2±69.6 mg/dl) ($p<0.001$). There was a significant correlation between plasma fibrinogen and serum calcium levels, similar to fibrinogen. A weak but significant negative correlation was found between plasma fibrinogen levels and BMI ($p=0.027$) and birth weight ($p=0.030$). Additionally, there was a significant positive correlation with the 1-minute and 5-minute Apgar scores ($p=0.002$ and $p<0.0001$). These values are close to those observed by Kaufner et al., with fibrinogen levels of 440 mg/dl and 330 mg/dl, respectively (30). In a study by Zakaria et al. in 2019, fibrinogen levels typically considered normal, ranging from 200 to 300 mg/dl, were still associated with a higher risk of severe postpartum hemorrhage. When fibrinogen levels were 200 mg/dl, the risk increased almost 12-fold (21). This result is consistent with Kaufner et al., showing that fibrinogen levels at the 200 mg/dl threshold have a 100% positive predictive value for severe postpartum hemorrhage (30). Karlson et al. in 2016 and Finlayson et al. in 2019 found that low fibrinogen levels were superior in indicating the risk of PPH, independent of other laboratory indicators (31, 32).

These studies and the literature indicate that fibrinogen levels are of vital importance during pregnancy. When evaluating fibrinogen levels during pregnancy, it should be kept in mind that they are higher compared to non-pregnant adult values. Furthermore, it should not be forgotten that fibrinogen is the best and earliest marker indicating the severity of postpartum hemorrhage. Although the primary cause of postpartum hemorrhage is not fibrinogen deficiency, decreases in fibrinogen levels contribute to the continuation of bleeding and the worsening of the clinical situation, as it is the fastest-decreasing factor in major hemorrhages. In our study, similar to the study by Kaufner et al., the only coagulation variable that remained independently associated with severe hemorrhage was fibrinogen level (30).

Clinical studies and experimental data from intensive care units show that the early use of fibrinogen can reduce the need for other blood derivatives. There is no consensus on the transfusion threshold value for fibrinogen during hemorrhage. The RCOG (Royal College of Obstetricians and Gynaecologis) recommends cryoprecipitate infusion when fibrinogen levels are ≤100 mg/dl. The Club d'Anesthésie et de Réanimateurs en Obstétrique recommends fibrinogen infusion when levels fall below 200 mg/dl. A recent in vitro study showed that a minimum fibrinogen concentration of 200 mg/dl is necessary for optimal clot formation. The study also suggests that even a threshold of 300 mg/dl could be beneficial (29). Larger studies are needed to determine the optimal fibrinogen threshold value.

Maternal hemogram parameters, particularly hemoglobin, hematocrit, and fibrinogen levels, are effective predictors of postpartum hemorrhage. Fibrinogen is a critical component of physiological coagulation and an important biomarker for assessing coagulation function in the body. For these reasons, this study aimed to determine the impact of antepartum blood fibrinogen and hemogram parameters on postpartum hemorrhages. The study emphasizes the importance of monitoring these parameters to identify and manage at-risk pregnancies, thereby reducing maternal morbidity and mortality associated with PPH.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: S.S.Ü., Y.C.Ü., Design: S.S.Ü., Y.C.Ü., Data Collection or Processing: Ö.D.Ü., Y.C.Ü., S.E., S.M.A., C.S.Ç., Z.Y., C.M.S., S.Ç., Analysis or Interpretation: S.S.Ü., S.Ç., Literature Search: Ö.D.Ü., C.S.Ç., Z.Y., C.M.S., S.Ç., Writing: Y.C.Ü., S.E., S.M.A.

Ethical Statement

Approval was obtained from Samsun University Non-invasive Clinical Research Ethics Committee, the study started. The ethics committee decision date is 22/05/2024 and the number of ethical committee decisions is 2024/10/2.

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The relationship between food addiction in obesity and anxiety, impulsivity, depression, and emotion regulation difficulty

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Abstract

We aimed to develop interventions that can prevent obesity and food addiction in the early period by investigating the relationship between food addiction and impulsivity, anxiety, depression, difficulties in emotion regulation, sociodemographic characteristics, exercise and diet behaviors. We included 88 consecutive patients with BMI ≥30 and above, aged between 18-65 years, who applied to the Obesity outpatient clinic and psychiatry outpatient clinic of "---" in 2022 in our study sample. Sociodemographic and Clinical Information Form, Yale Food Addiction Scale(YFAS), Beck Anxiety Inventory(BAI), Difficulties in Emotion Regulation Scale-Brief Form (DERS-16), Barratt Impulsiveness Scale and Beck's Depression Inventory(BDI) were applied to the patients. It was determined that 45 of 88 patients included in our study had food addiction according to the YFAS score, and 43 did not have a food addiction. There was a significant difference between the two groups in terms of age and education level. Mean scale scores for BAI, DERS-16, and Barratt Impulsiveness Scale were found to be significantly higher in the food addiction group than in the other group. The subscales of the Barratt Impulsiveness Scale were evaluated separately, it was observed that the sub-scale scores of "motor impulsivity" and "attention impulsivity" were significantly higher in the food addiction group compared to the other group. Food addiction is seen at a higher rate in people with a lower average age and higher education level. Anxiety, impulsivity, and Difficulties in Emotion Regulation correlate with food addiction.

Keywords: food addiction, impulsivity, anxiety, emotional dysregulation

1. Introduction

Food addiction, a recently defined type of addiction, refers to the intense and excessive consumption of foods with high calories, sugar, and fat content (1,2). Some studies observed that these foods with high calories, high sugar, and fat content cause craving symptoms and reported that individuals have difficulty controlling their eating behaviors (3). Eating foods high in fat and sugar may alter the brain's reward system, resulting in food addiction and obesity (4).

An experiment with rats with high-sugar foods revealed that consuming these foods in large amounts developed withdrawal, craving, and compulsive-seeking behaviors similar to other addictive substances. The changes in the opioidergic and dopaminergic systems of the brain caused by high-sugar foods are similar to addiction (5).

Many studies have shown that there is a relationship between addiction and impulsivity. Impulsivity is related to excessive food intake, high motivation, energy expenditure to find food, and compulsive eating. Studies have shown that people with food addiction symptoms show higher impulsivity than people who do not experience symptoms and that the addictive characteristics of delicious foods are a risk factor for increased impulsivity (6,7). It is widely accepted that eating behavior in humans varies according to various emotions, such as anxiety, joy, and anger. This eating behavior, which occurs in connection with the emotional state, has been defined as "emotional eating" and is associated with body weight in many studies (8). Food addiction is more associated with overeating, emotional eating, and problematic eating attitudes rather than high body mass index (4).

Other factors related to emotional, uncontrolled, and binge eating behavior were shown to be anxiety and depressive mood, and positive correlations were found between depression, anxiety and stress, and emotional eating (9). A study conducted with university students during the Sars-CoV-2 outbreak found that increased anxiety levels and depressive symptoms after the closure of their university were associated with food addiction (10).

The inability to regulate emotions following the current situation is defined as Difficulties in Emotion Regulation. Difficulty in emotion regulation can negatively affect the functionality and human relations of the individual, increase negative emotional experiences and decrease positive

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emotional experiences, resulting in maladaptive behavior patterns (11). The studies dealing with the relationship between eating disorders and emotional regulation difficulties argued that individuals with eating disorders had the insufficient ability to cope with negative emotions, and this inadequacy may cause deterioration in eating behaviors (12,13).

When all these data are evaluated, the relationship of food addiction with anxiety, impulsivity, difficulty in emotion regulation, and depression has been assessed separately and in limited amounts in the current literature. In our study, we aimed to evaluate all of these factors that are effective on food addiction together and to determine their relationship.

2. Materials and Methods

We included 88 of 108 consecutive patients between 18-65 years, with a BMI of 30 and above, who applied to the Obesity and psychiatry outpatient clinics in --- Hospital to this cross-sectional case study In 2022. A total of 8 patients left some parts of the forms blank, five patients had vision problems and therefore could not fill the forms, and seven took the forms to fill in but did not bring them back. We excluded these 20 patients from our study.

Firstly, we explained the purpose of the study to the patients and obtained their written informed consent for participation. Also, we clarified that they were free to refuse to participate or to terminate the interview at any point, and their records would be kept confidential.

Sociodemographic and Clinical Information Form for Patients, Sociodemographic and Clinical Information Form, Yale Food Addiction Scale (YFAS), Beck Anxiety Inventory(BAI), Difficulties in Emotion Regulation Scale-Brief Form (DERS-16), Barratt Impulsiveness Scale and Beck's Depression Inventory(BDI) was applied.

Patients between 18-65, literate, BMI of 30 and above, had the mental capacity to respond to the assessment tools, and agreed to participate were included in the study. People with a <30 BMI, did not have the mental capability to answer the assessment scales, had alcohol and substance addiction or any other diagnosed pscyhiatric disorders, and had a BMI below 30 were not included in the study.

2.1. Tools

Sociodemographic and Clinical Data Form: Following the purpose of the study, we developed this form to collect sociodemographic and clinical data, which includes age, gender, marital status, education level, economic income, habits, medications, history of concomitant mental and physical illness, family history of mental illness and obesity, childhood obesity, diet, and exercise habits.

Yale Food Addiction Scale (YFAS): It is a 27-item scale developed by Gearhardt et al. (13) that evaluates eating attitude with substance addiction symptoms (tolerance, withdrawal, loss of control, and others). There are two scoring methods. The first must meet at least one of the symptoms to qualify as an eating disorder. Three or more symptoms must be positive in the last year, and additional clinical deterioration must accompany the diagnosis of an eating disorder. A validity and reliability study was conducted by Bayraktar et al. (14) in our country.

Beck Anxiety Inventory (BAI): Beck et al. (15) developed this scale to determine the frequency of anxiety symptoms experienced by individuals. It is a Likert-type scale including 21 items and scored between 0-3. The high scores obtained from the scale indicate the severity of the anxiety experienced by the individual. A validity and reliability study was conducted in our country by Ulusoy et al. (16)

Difficulties in Emotion Regulation Scale-Brief Form (DERS-16): Bjureberg et al. (17) developed DERS-16 as the short form of the Dysregulation of Emotions Scale designed by Gratz and Roemer, which aimed to measure the difficulty level of emotion regulation of individuals. The scale consists of 16 items in total, and the items are scored on a Likert-type scale from 1 (rarely) to 5 (almost always). A high score implies a high emotion dysregulation symptom level. Turkish validity and reliability study of Difficulties in Emotion Regulation Scale-Brief Form (DERS-16) was conducted by Yiğit and Guzey Yiğit (18).

Barratt Impulsiveness Scale: This is a Likert-type scale consisting of 30 items. It has three subcales: attention-related impulsivity, inability to plan-related impulsivity, and motor impulsivity. A higher total grade implies a higher impulsiveness level of the practitioner. A high score for attention indicates that the person behaves more carelessly, a high motor score indicates an increase in motor activity, and a high unplanning score indicates instability in life planning and an inability to plan (19). The Turkish validity and reliability study of the Barratt Impulsivity Scale was conducted by Güleç et al (20).

Beck Depression Inventory (BDI): The Beck Depression Inventory, developed by Beck et al. (21) in 1978, measures the motivational, cognitive, emotional, and vegetative symptoms seen during the depression. Beck Depression Inventory is a 21-item self-assessment scale. Each item has a score between 0 and 3. The highest score is 63, and the lowest is 0. A high score on the scale indicates that the severity or level of depression is high. Hisli N. (22) translated the Beck Depression Inventory.

2.2. Statistical analysis

Whether there was a difference between groups with continuous variables in terms of independent variables was investigated using the Independent Groups T-Test for normally distributed variables and the Mann-Whitney U-Test for variables that did not fit the normal distribution. We evaluated normality with the Kolmogorov-Smirnov test. We used the Spearman test for correlation analysis. All data were evaluated with SPSS (Statistical Package for the Social Sciences) 22 computer programs. A p-value of <0.05 was considered statistically significant in all these analyses.

3. Results

We determined that 45 of 88 patients included in our study had food addiction according to the YFAS score, and 43 did not. The sociodemographic characteristics of the groups are shown in Table-1. According to the results, while there was a significant difference between the two groups by age and education level (p=0.038, p= 0.017, respectively), there was no significant difference between the groups for other sociodemographic characteristics. We observed that the mean age resulted in lower and the level of education higher in the group with food addiction (Table-1).

Table 1. Comparison of sociodemographic characteristics between patients with and without food addiction

	Food addiction	No food addiction	
Number, (n)	45	43	
Gender. n (%)			P= 0.375
• Female	38 (%84.4)	39 (%90.7)	
• Male	7 (%15.6)	4 (%9.3)	
Age. (year) (mean ± s.d.)	34.58 ± 12.77	40.02 ± 11.39	***P= 0.038***
Height (cm) (median; i.r.)	162; 11.5	162; 10	P= 0.841
Weight (kg) (mean ± s.d.)	111.62 ± 17.85	111.73 ± 20.44	P= 0.979
BMI (kg/m2) (mean ± s.d.)	41.80 ± 5.96	42.06 ± 7.27	P= 0.858
Marital status;			
• Married	28 (%62.2)	32 (%74.4)	
• Living apart	0 (%0)	1 ((%2.3)	
• Divorced	0 (%0)	3 (%7)	
• Widow	0 (%0)	2 (%4.7)	
• Single	17 (%37.8)	5 (%11.6)	
Education;			***P= 0.017***
• Literate	2 (%4.4)	4(%9.3)	
• Primary School	6 (%13.3)	14 (%32.6)	
• Middle School	7 (%15.6)	4 (%9.3)	
• High School	19 (%42.2)	17 (%39.5)	
• University	11 (%24.4)	4 (9.3)	
Work Status;			
• Unemployed	9 (%20)	23 (%53.5)	
• Student	5 (%11.1)	1 (%2.3)	
• Employee	19 (%42.2)	11 (%25.6)	
• Resigned	11 (%24.4)	4 (%9.3)	
• Retired	1 (%2.2)	4 (%9.3)	
Monthly income (Turkish Lira);			P= 0.055
• 4000 TL >	14 (%31.1)	6(%14)	
• 4000 TL <	31 (%68.9)	37 (%86)	
Cigarette smoking			P= 0.538
• Yes	10 (%22.2)	12 (27.9)	
• No	35(%77.8)	31 (%72.1)	
Alcohol consumption			p=1.0
• Yes	4 (%8.9)	4 (%9.3)	
• No	41 (%91.1)	39 (%90.7)	
Diet			P= 0.090
• Yes	16 (%35.6)	23 (%53.5)	
• No	29 (%64.4)	20 (%46.5)	
Exercise			P= 0.295
• Yes	17 (%37.8)	21 (%48.8)	
• No	28 (%62.2)	22 (%51.2)	

s.d.: standard deviation; i.r.: interquartile range

Independent samples t-test was applied for variables that follow a parametric distribution. Mean values and standard deviations are provided. Mann-Whitney U test was used for variables that do not follow a parametric distribution and ordinal variables. Median values and interquartile ranges are provided. Chi-square test or Fisher's exact test has been used for nominal variables. $p < 0.05$ is considered statistically significant.

Table-2 shows whether there is a difference between the groups in terms of scale scores. The mean scale scores were higher in the food addiction group than in the other group in BAI, DERS-16, and Barratt Impulsiveness Scale (p=0.007, p=0.006, p=0.011, respectively). In terms of BDI, there was no

significant difference between the two groups. When we evaluated the subscales of the Barratt Impulsiveness Scale separately, we found that the motor impulsivity and attention impulsivity subscales scores were significantly higher in the food addiction group than in the other group (p= 0.003 and p=0.030, respectively). But there was no statistically significant difference between the groups in the inability to plan.

When the subscales of the Difficulty in Emotion Regulation Scale were examined, it was found that the subscales of clarity, goals, impulse and strategies were significantly higher in the group with food addiction than in the group without food addiction. The difference between the groups in terms of the non-acceptance subscale is at the statistically significant level (Table 2).

Table 2. Comparison of scale scores between patients with and without food addiction

	Food addiction	No food addiction	
BAI (median; i.r.)	44 ; 30	33 ; 15	***P= 0.007***
BDI (median; i.r.)	19 ; 8	16 ; 12	P= 0.067
DERS-16 (median; i.r.)			
• Total score	33 ; 26	25 ; 14	***P= 0,006***
• Clarity	4 ; 4	3 ; 2	***P=0,005***
• Goals	7 ; 6	6 ; 4	***P=0,022***
• Impulse	5 ; 4.75	4 ; 2	***P=0,020***
• Strategies	10 ; 11	7 ; 5	***P=0,016***
• non-acceptance	5 ; 5,75	4 ; 3	P=0,051
BIS (median; i.r.)			
• Total score	29 ; 7	25 ; 7	***P= 0.011***
• Not making plans	11 ;4	9 ; 6	P=0.322
• Motor impulsivity	9 ; 3.5	7 ; 3	***P= 0.003***
• Attentional impulsivity	10 ; 3	9 ; 2	***P=0.030***

i.r.: interquartile range; BAI: Beck Anxiety Inventory; BDI: Beck Depression Inventory; DERS-16: Difficulties in Emotion Regulation Scale-Brief Form; BIS: Barratt Impulsiveness Scale

As all scale scores followed a non-parametric distribution, the Mann-Whitney U test was used for all of them. Median values and interquartile ranges are provided $p < 0.05$ is considered statistically significant.

Table 3. Examination of the correlation between Yale Food Addiction Scale scores and other scale scores

	YFAS	BAI	BDI	BIS	DERS-16
YFAS		r=0,503 p<0,001	r=0,332 p=0,002	r=0,308 p=0,004	r=0,441 p<0,001
BAI	r=0,503 p<0,001		r=0,613 p<0,001	r=0,285 p=0,007	r=0,566 p<0,001
BDI	r=0,332 p=0,002	r=0,613 p<0,001		r=0,347 p=0,001	r=0,656 p<0,001
BIS					
• Plannig	r=0,184 p=0,087	r=0,158 p=0,142	r=0,297 p=0,005	r=0,740 p<0,001	r=0,317 p=0,003
• Motor	r=0,402 p<0,001	r=0,406 p<0,001	r=0,327 p=0,002	r=0,598 p<0,001	r=0,420 p<0,001
• Impulsivity	r=0,203 p=0,058	r=0,141 p=0,189	r=0,149 p=0,167	r=0,780 p<0,001	r=0,225 p=0,036*
DERS-16					
• Clarity	r=0,367 p<0,001	r=0,562 p<0,001	r=0,684 p<0,001	r=0,467 p<0,001	r=0,722 p<0,001
• Goals	r=0,401 p<0,001	r=0,505 p<0,001	r=0,559 p<0,001	r=0,342 p=0,001	r=0,893 p<0,001
• Impulse	r=0,397 p<0,001	r=0,436 p<0,001	r=0,552 p<0,001	r=0,363 p=0,001	r=0,788 p<0,001
• Strategies	r=0,402 p<0,001	r=0,526 p<0,001	r=0,545 p<0,001	r=0,442 p<0,001	r=0,924 p<0,001
• Non-acceptance	r=0,300 p=0,005	r=0,386 p<0,001	r=0,541 p<0,001	r=0,276* p=0,010	r=0,805 p<0,001

BAI: Beck Anxiety Inventory; BDI: Beck Depression Inventory; DERS-16: Difficulties in Emotion Regulation Scale-Brief Form; BIS: Barratt Impulsiveness Scale; YFAS: Yale Food Addiction Scale

All scale scores followed a non-parametric distribution, so all correlations were performed using the Spearman Test.

*statistically significant with p<0.05

All other results were considered statistically significant with $p < 0.01$

Finally, we investigated whether the scale scores correlated with each other. As seen in Table-3, there is a statistically significant correlation between all scale scores. Especially, YFAS had moderate positive correlations with BAI and DERS-16 ($r=0.503$, $p<0.001$ and $r=0.441$, $p<0.001$, respectively) and low-moderate positive correlations with Barratt Impulsiveness Scale and BDI ($r=0.308$, $p=0.004$ and $r=0.332$, $p=0.002$, respectively). The subscales of Barrat Impulsivity Scale and Difficulty in Emotion Regulation Scales were also evaluated. Accordingly, it was found that there was a moderate positive correlation between the Yale Food Addiction Scale and the motor impulsivity subscale of the Barrat Impulsivity Scale ($r=0.402$; $p<0.001$), while no statistically significant correlation was observed with the inability to plan and attention impulsivity subscales ($r=0.184$; $p=0.087$ for inability to plan / $r=0.203$; $p= 0.058$ for attention impulsivity). When the subscales of Difficulties in Emotion Regulation were examined, it was found that the Yale Food Addiction Scale had a low-moderate positive correlation with the clarity, impulse and non-acceptance subscales of Difficulties in Emotion Regulation ($r=0.367$ for clarity; $p<0.001$ / $r=0.367$ for impulse ; $p<0.001$ / $r=0.397$; $p<0.001$ / $r=0.300$; $p=0.005$ for impulse / $r=0.300$; $p=0.005$ for non-acceptance), and a moderate positive correlation with the goals and strategies subscales ($r=0.401$; $p<0.001$ for goals / $r=0.402$; $p<0.001$ for strategies) (Table 3).

4. Discussion

In this study, we aimed to develop interventions that can prevent obesity and food addiction in the early period by determining the relationship between food addiction and impulsivity, anxiety, depression, emotional regulation difficulties, sociodemographic characteristics, exercise, and diet behaviors.

We found that food addiction was at a higher rate in the group with a lower average age and higher education level in our study. In a study with middle-aged and older women found that the prevalence of food addiction measured by YFAS ranged from 1 to 9%, and food addiction was inversely proportional to age (23). A cross-sectional study investigating the relationship between the prevalence of food addiction and stress and sleep quality among university students found that food addiction was negatively associated with age (24). Food addiction was inversely proportional to age in the literature, consistent with our study results, which we think may be due to the easier access of the young population to high-energy, flavor-enhanced, and processed foods.

The education level was higher in the food addiction group in our study. Although there are not many studies about the relationship between education level and food addiction in the literature, many studies show that the risk of obesity decreases as the education level increases (25-27). The study of Devaux et al. (25) links the positive effect of education level on obesity to the quick accessibility to health-related issues, awareness of risk perception in lifestyle, and the development of control. Another study examining the prevalence of food addiction among low-income women of reproductive age suggests that education level did not affect food addiction (26). There are different results between these studies and ours. We think that this may be related to the fact that these studies included people with a BMI<30 and only low-income women. However, another study reported that food addiction incidence was higher in individuals with high academic performance, in line with our results (27). As the level of education increases, the expectations of society, the environment, and even the expectations of the individuals increase. The higher the requirements, the higher the stress level, and the problematic eating behaviors as a way of coping with stress, may have caused this result.

We determined that the Barratt Impulsiveness Scale total scores and motor impulsivity and attention impulsivity subscales scores were significantly higher in those addicted to eating. In a study examining the relationship between food addiction and impulsivity in young adults, motor impulsivity and impulsivity total scores were higher in individuals with food addiction than those without food addiction (28). Meule et al. (29) reported that attention-related impulsivity and motor impulsivity predicted food addiction together in obese individuals. After evaluating the current findings, we think there is a clinical association between impulsivity and food addiction.

In our study, we evaluated the presence of depression with the BDI, and there was no difference between the groups with and without food addiction. In a meta-analysis, unlike our study, they reported a positive correlation between food addiction and depression (30). A study investigating the relationship between depression, food addiction, and body mass index in 793 university students reported that the level of depression was higher in the food-addicted group (31). The causes of the different results in our study may be that we included only obese individuals in our study group, and the sample sizes and depression scales used were different between studies.

Studies reported that anxiety was associated with emotional, uncontrolled, and excessive eating behaviors and health outcomes related to eating (9, 32). We evaluated the anxiety level of the subjects with BAI. The anxiety level was higher in the food addiction group, which was compatible with other studies in the literature. A study examining the longitudinal relationship between food addiction and anxiety in men and women found that food addiction affected anxiety longitudinally. The interventions that address food addiction reduce anxiety in men and women, while interventions that reduce anxiety help prevent food addiction in women (33).

The number of studies about how eating is affected by changes in people's emotions increased in recent years. Based on the idea that eating makes people happy, Smith et al.(34)

determined that eating reduces their negative affect and increases their positive affect in their 3-year study with adolescents. A study investigating the predictors of food addiction with 878 undergraduate students found that impulsivity and difficulty in emotion regulation were positively related to food addiction (35). A study examining the relationship between food addiction and difficulty in emotion regulation in 214 university students determined that the total score of difficulty in emotion regulation and sub-factors (refusal to accept, strategies, and impulse) were higher in food-addicted participants than non-food addicted participants (36). We found that difficulty in emotion regulation predicted food addiction positively in our study, which is consistent with the literature.

In our study, it was found that the scores of clarity, goals, impulse and strategies subscales of the Difficulty in Emotion Regulation Scale were significantly higher in the group with food addiction compared to the group without food addiction. In another study, it was found that the scores belonging to the subscales of Non-Acceptance, Impulse and Strategies were more related to food addiction. This shows that people with food addiction have greater difficulties in accepting their emotional reactions and controlling their impulses when they experience negative emotions. This is perhaps due to their limited access to effective emotion regulation strategies (37).

The limitations of the study: This study does not show a definite cause-effect relationship between the variables due to its cross-sectional nature and small sample size. Due to the lack of sufficient objective measurement tools for the diagnosis of food addiction, it was assessed only with the Yale Food Addiction scale and limited to the subjective interpretations of the participants.

While the food addiction rate is higher in the group with lower mean age and higher education level, other sociodemographic characteristics did not differ between groups with and without food addiction. While anxiety, impulsivity, and difficulty in emotion regulation were higher in the food-addicted group, there was no significant difference in terms of depression.

Our results suggest that psychiatric evaluation is crucial in people seeking obesity treatment. Providing psychiatric treatment and support to these individuals will contribute to the prevention and treatment of obesity and food addiction.

Psychological evaluation should not be neglected in patients who apply to non-psychiatry outpatient clinics for obesity treatment. Our study emphasises the importance of multidisciplinary evaluation in these patients.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: F.U., E.K., K.T.A., M.K.T., Design: F.U., E.K., K.T.A., M.K.T., Data Collection or Processing: F.U, E.K., M.A., Analysis or Interpretation: Ö.Ö., K.T.A., M.A., M.K.T., Literature Search: F.U., E.K., Ö.Ö., N.T., M.K.T., Writing: F.U., E.K., Ö.Ö., N.T., M.K.T.

Ethical Statement

We obtained the approval for this study from the "lstanbul Kartal Lütfi Kırdar City Hospital Ethics Committee" on 28.09.2022 with the number 2022/514/234/5. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

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Affordable and simple protocol for immunofluorescence staining of insulin secreting iGL cell line 2D and 3D

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Abstract

Immunofluorescence staining is a widely used technique to visualize the localization of specific proteins in cells. This protocol provides an affordable and simple method for immunofluorescence staining of the Insulin-GLase (iGL) cell line in both 2D and 3D culture conditions. We describe cost-effective alternatives for reagents and materials without compromising the quality of the results, such as the use of Bovine Serum Albumin (BSA) 3% as a blocking buffer instead of normal serum. Immunofluorescence staining on the iGL cell line is a valuable technique for visualizing the distribution and localization of target biomolecules. With careful consideration of cost-effective strategies and an understanding of the unique characteristics of the iGL cell line, it is possible to achieve reliable and informative immunofluorescence results even with budget constraints.

Keywords: cell culture, immunofluorescence, iGL, 2D and 3D culture

1. Introduction

Immunofluorescence staining is a powerful technique to visualize the distribution and localization of specific biomolecules within biological samples, such as cells and tissues. It involves using antibodies linked to fluorophores, which emit fluorescence when excited with specific wavelengths of light. Immunofluorescence can provide detailed information about the localization, expression levels, and distribution of target proteins within a sample (1-3). Insulin-GLase (iGL) is a cell line developed for studying insulin production and regulation. This cell line is derived from INS-1E rat insulinoma cells and MIN6 cells, which are known to display beta-cell characteristics and secrete insulin in response to glucose (4). It is important to understand the characteristics of the iGL cell line to optimize the immunofluorescence staining protocol effectively.

The iGL cell line is widely used for studying the expression and localization of specific biomolecules within the cells. Its unique features and growth patterns make it a valuable model for immunofluorescence studies. The immunofluorescence analysis of the iGL cell line allows researchers to visualize and quantify the expression of specific proteins, including insulin, within the cells (3).

Immunofluorescence was performed in the iGL cell line using various techniques, such as co-transfection with plasmids expressing FLAG-tagged ChREBP and Mlx and staining for insulin using specific antibodies (5-6). The immunofluorescence analysis of the iGL cell line revealed robust glucose-stimulated insulin secretion and the expression of ChREBP, a transcription factor involved in regulating insulin production. Furthermore, the iGL cell line displayed a uniform pattern of insulin immunofluorescence that closely resembled normal islets within the pancreas (7). Overall, immunofluorescence analysis of the iGL cell line provided valuable insights into the expression and regulation of insulin (5, 8-10).

This study can provide valuable insights into an affordable and simple protocol for immunofluorescence staining of the iGL cell line. Using immunofluorescence analysis, researchers can confirm the successful differentiation of iGL cells into functional beta cells that produce insulin. Additionally, immunofluorescence analysis can be used to evaluate the response of iGL cells to glucose stimulation. For example, if the iGL cell line exhibits robust glucose-stimulated insulin secretion, this suggests that the cells are responsive to changes in glucose levels and capable of producing insulin in response to physiological demand.

2. Materials and Methods

2.1. iGL cell 2D and 3D culture system

iGL cells derived from Cosmo Bio, IGL01C, Japan were thawed and cultured in complete media. The complete media

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was produced using the following recipe:

1. RPMI1640 (containing L-glutamine, phenol red, and HEPES) as the basal media (Gibco, 11875093,USA)

   Additives :

2. 5% of FBS (Fetal Bovine Serum) (PAN Biotech, P303306, South America)
3. 1 mM Sodium Pyruvate (Gibco, 11360070, USA)
4. 500 µM monothioglycerol (Sigma-Aldrich, 96275, USA)
5. 200 µg/mL G-418 (Sigma-Aldrich, 108321422, USA)

First, the base media was prepared: RPMI1640 containing L-glutamine, phenol red, and HEPES (Gibco, 11875093,USA). The media was then supplemented with 5% of FBS (Fetal Bovine Serum) (PAN Biotech, P303306, South America), 1 mM pyruvic acid (Gibco, 11360070,USA) 500 µM monothioglycerol (Sigma-Aldrich, 96275, USA), 200 µg/mL G-418 (Sigma-Aldrich, 108321422, USA) into the base media (Cosmo Bio, IGLM, Japan). After that, frozen cells were thawed in vials in a water bath at 37 °C for two minutes. (Cosmo Bio, IGLM, Japan). We added 1 mL of complete media into the vials, then transferred the cell suspension into 15 mL tubes (Corning, 430791, USA) with 9 mL of complete media. Next, the cells were centrifuged at a speed of 300 g for five minutes, and then the supernatant was discarded. Pellets of cells were resuspended with 1 mL of medium and counted with an automatic cell counter (LUNA Automated Cell Counter, Logos Biosystem, 0300119, South Korea). The cell suspension was seeded into a 100-mm dish (Corning, 430167, USA) and incubated at 37 °C in 5% CO_2. It was replaced with fresh medium every three to four days. After reaching 70-90% of confluency, the cells were passaged. (11)

After cell propagation, cells were collected and divided into two culture systems. The first is a 2D or monolayer culture which was conducted in 6-well culture plate (SPL, 13485, South Korea) with 50.000 cells per well confluency. The other is a 3D culture system using the hanging drop method. The cell suspension drops were formed by placing 25 µL cell suspension on the inner part of the bacterial petri dish lid of a 100×20 mm bacterial petri dish (SPL,10101, South Korea). The cell suspension drops were placed in a relatively identical distance at the inner part of the bacterial petri dish lid. Forty drops were prepared for each cell concentration performed in one petri dish. Once all the drops were completed, the lid was flipped quickly and carefully to maintain the drops. The petri dishes were placed in the incubator at 37 °C with 5% of CO_2 for two days. After the 3D culture system was harvested, we then continued with the 3D iGL cell line immunofluorescence method. (11)

2.2. 2D iGL cell line immunofluorescence method

After cell propagation, the cells were passaged up to the 9th passage. Subsequently, the cells were harvested, and cell staining was initiated. For iGL cell line immunofluorescence analysis in 2D, the following procedure was followed. After the cells were seeded and harvested in a 24-well cell culture plate (Corning, 3524, China), the iGL cells were fixed with glass coverslips (coverslips are optional). Next, they were rinsed once with warm PBS (Gibco, 18912014, USA) and allowed to sit briefly, and then the PBS solution was carefully discarded using a pipette. After that, the cells were covered to a 2–3 mm depth with a warm fixation solution of 10% Neutral Buffered Formalin (Leica Biosystem, 3800698, USA) and fixed for 15 minutes at room temperature.

After incubating for 15 minutes at room temperature, the fixation solution is carefully discarded using a pipette. The cells are rinsed three times in 1X PBS (Gibco, 18912014, USA) 300 µl per well for five minutes each time. Permeabilization solution 0.3% Triton X-100 is added in PBS (Invitrogen, HFHF10, USA) at 100 µl per well and incubated for five minutes at room temperature. Then, it is washed once with Milli-Q/aquabidest, 300 µl per well, and after that 200 µl per well of blocking buffer, Bovine Serum Albumin (BSA) 3% (Sigma-Aldrich, 1003269866, USA) is added to the chamber sections and incubated for one hour at room temperature. After incubating for one hour, the blocking buffer is discarded and the primary antibody solution is added in the amount of 200 µl per well to the chamber slides and incubated at room temperature or overnight at 4 °C. The recommended concentrations on the datasheet in the antibody dilution buffer are 1: 200 (PBS = 995 µl + connexin 36 = 5 µl), 1: 100 (PBS = 990 µl + connexin 36 = 10 µl), 1: 50 (PBS = 980 µl + connexin 36 = 20 µl), 1: 10 (PBS = 900 µl + connexin 36 = 100 µl). After being incubated overnight at 4 °C, it is washed three times in PBS (Gibco, 18912014, USA) 300 µl per well.

A fluorochrome-conjugated secondary antibody, goat anti-Rabbit IgG (H+L) Secondary Antibody, FITC (Invitrogen, 656111, USA), is prepared and diluted and added at 200 µl per well. The secondary antibody dilution consisting of (1: 1000; PBS = 999 µl + FITC 1 µl) is then incubated for two hours at room temperature in the dark. Next, it is washed three times with Milli-Q/aquabidest, 300 µl per well. Then, DAPI (Thermo Scientific™, 62248, USA) is added, diluted 200 µl per well, in the dark. The DAPI dilution consisting of (1: 1000; PBS = 999 µl + DAPI 1 µl) is then incubated for 10 minutes at room temperature in the dark, or the coverslips are mounted onto microscope slides using mounting media with a nuclear stain, such as DAPI, to visualize the cell nuclei.

After the staining steps are complete, we visualize and quantify the marker expression and localization within the iGL cell line using fluorescence microscopy (Nikon, Ts2 Ph Fl, Japan) integrated with a camera (Nikon, DS-Fi3, Japan).

2.3. 3D iGL cell line immunofluorescence method

After the cells were harvested (hanging drop method) in a 100×20 mm bacterial petri dish (SPL, 10101, South Korea) the first step in immunofluorescence staining for the 3D iGL cell line is to prepare a pap pen circle onto object glass. Then, the

spheroid area is carefully transferred using a micropipette (Appendorf, USA) inside the pap pen and incubated for 30 minutes at room temperature. After that, cells are covered to 2–3 mm depth with a warm fixation solution of 10% Neutral Buffered Formalin (Leica Biosystem, 3800698, USA) and fixed for one hour at room temperature. After incubating one hour at room temperature, the fixation solution is discarded. The cells are then rinsed three times in 1X PBS (Gibco, 18912014, USA) for five minutes each time. Permeabilization solution 0.3% Triton X-100 in PBS (Invitrogen, HFHF10, USA) is added, 300 µl per object glass (pap pen area) and incubated for five minutes at room temperature. Then, the cells are washed once with Milli-Q/aquabidest, and then 200 µl blocking buffer, Bovine Serum Albumin (BSA) 3% (Sigma-Aldrich, 1003269866, USA), is added to the chamber sections and incubated for one hour at room temperature.

After incubating for one hour, the blocking buffer is discarded and the primary antibody solution is added in the amount of 200 µl to the chamber slides and incubated at room temperature or overnight at 4 °C. The recommended concentrations on the datasheet in the antibody dilution buffer are 1: 200 (PBS = 995 µl + connexin 36 = 5 µl), 1: 100 (PBS = 990 µl + connexin 36 = 10 µl), 1: 50 (PBS = 980 µl + connexin 36 = 20 µl), 1: 10 (PBS = 900 µl + connexin 36 = 100 µl). After incubating overnight at 4 °C, wash three times in PBS (Gibco, 18912014, USA). A fluorochrome-conjugated secondary antibody, goat anti-Rabbit IgG (H+L) Secondary Antibody, FITC (Invitrogen, 656111, USA), is prepared and diluted and 200 µl is added per object glass (pap pen area).

The secondary antibody dilution consisting of (1: 1000; PBS = 999 µl + FITC 1 µl) is then incubated for two hours at room temperature in the dark. Next the cells are washed three times with Milli-Q/aquabidest. Then, DAPI (Thermo Scientific™, 62248, USA) is added, diluted, 200 µl per object glass (pap pen area). This is done it in the dark. The DAPI dilution consisting of (1: 1000; PBS = 999 µl + DAPI 1 µl) is then incubated for 10 minutes at room temperature in the dark, or the coverslips are mounted onto microscope slides using mounting media with a nuclear stain, such as DAPI, to visualize the cell nuclei. After the staining steps are complete, the marker expression and localization within the iGL cell line are visualized and quantified using fluorescence microscopy (Nikon, Ts2 Ph Fl, Japan) integrated with a camera (Nikon, DS-Fi3, Japan).

3. Results

3.1. Microscopic observation results of 2D and 3D cultures of iGL cells

The monolayer culture of iGL cells reached more than 70% confluency after two days of incubation (Fig. 1). This type of adherent cells aggregated to each other as per typical characteristics in natural conditions. In the 3D culture system, the aggregated cells formed a compact round-shaped structure called a spheroid (Fig. 2). The spheroid began to form from the first day after cell seeding with average size.

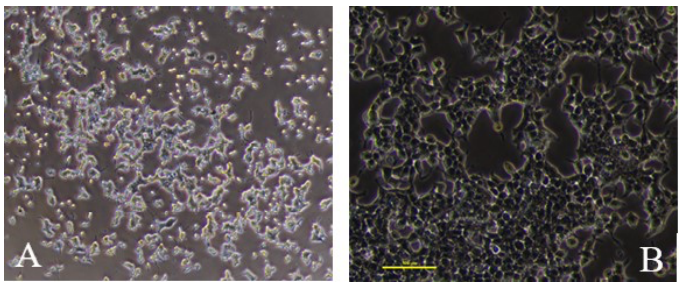

Fig. 1. Monolayer cell culture of iGL cell. Phase contrast microscope, mag. 40x (A) and 100x (B)

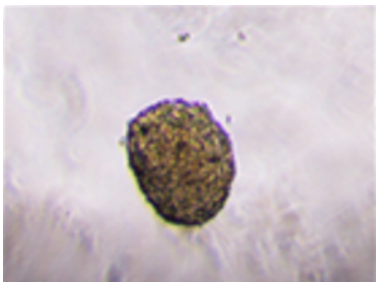

Fig. 2. 3D cell culture of iGL cell. Phase contrast microscope, mag. 40x

3.2. Microscopic observation results of immunofluorescence staining of iGL cell line 2D

Fluorescence microscopy revealed the distribution and localization of the target proteins within the iGL cells. The cells showed positive staining for the target biomolecule, indicating its presence within the cells. This suggests that the immunofluorescence staining technique successfully identified the target biomolecule in the iGL cell line. Representative images show the expression patterns of the markers in the 2D culture condition (Fig. 3). The fluorescence signal was observed in the cytoplasm of the iGL cells, confirming the presence and localization of the target biomolecule.

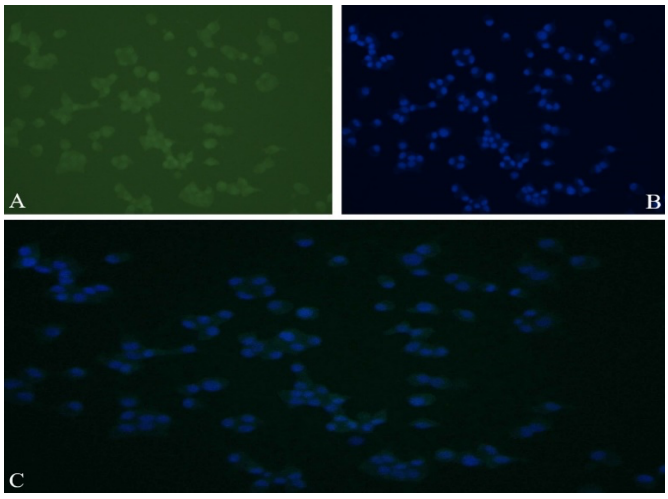

Fig. 3. Immunofluorescence staining of iGL cell line 2D. (A) Cx-36-FITC (green panel), (B) DAPI (blue panel), (C) merged

3.3. Microscopic observation results of immunofluorescence staining of iGL cell line 3D

In the 3D culture condition, immunofluorescence staining revealed the spatial organization and distribution of the target biomolecule within the iGL cells. The fluorescence signal was

observed throughout the 3D structure, indicating widespread expression of the target biomolecule. This suggests that the immunofluorescence staining technique successfully identified and localized the target biomolecule in both 2D and 3D culture conditions, providing valuable insights into its spatial distribution within the iGL cell line. Fluorescence microscopy revealed the distribution and localization of the target proteins within the iGL cells. Images depict the spatial organization and morphology of iGL cells within the 3D matrix. Representative images show the expression patterns of the markers in the 3D culture condition (Fig. 4).

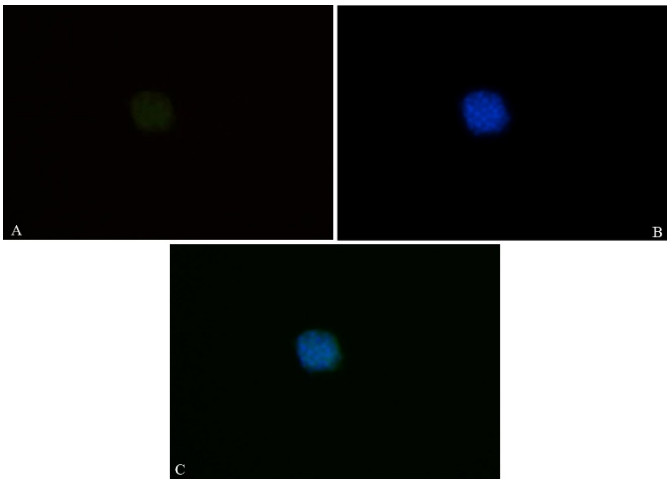

Fig. 4. Immunofluorescence staining of iGL cell line 3D. (A) Cx-36-FITC (green panel), (B) DAPI (blue panel), (C) merged

4. Discussion

The iGL cells, in either a monolayer or 3D culture system, were growing well until two days of incubation. This is in accordance with previous research by (11) that showed iGL cells with a stable morphology and viability could be derived from 50-100 cells / µL with two days of incubation. The stable morphology and viability are both critical aspects when considering an immunofluorescence assay. Cell morphology may provide valuable information about the shape, size, and organization of the cell. On the other hand, cell viability proposes the proportion of live and healthy cells within in a population (12).

The distinctive aspect of our study is that the cells are different. The iGL cells used in this study are still new and there are not yet many references using them as a research subject, so it is hoped that this protocol study can serve as an additional reference for knowledge regarding iGL cells, especially regarding methods/protocols for developing these iGL cells.

In the blocking section, the materials used are different. We use BSA, while in the literature normal serum/skim milk is used. The reason BSA is used is that it is less expensive than normal serum/skim milk and can still produce a fairly good fluorescent image.

In the antigen retrieval section, sodium citrate is normally used, but now we use a permeable solution (0.3% Triton X-100 in PBS. To prepare 100 mL: add 300 µl Triton X-100 to 100 mL PBS and mix.)

The similarity of our study to others is that we use viable 3D cell culture samples, that are fixated right away before immunostaining. We also use the same buffer, formaldehyde 10% and antibody dilution. (2)

The advantage of the method in this study is that we use inexpensive, easier materials and goods that are easy to obtain, while the disadvantage of this study is that this method cannot be directly compared with existing methods due to limited studies to date about iGL cells.

For microscopic observation of immunofluorescence staining of iGL cell line in 2D and 3D, images were captured using appropriate filter sets for each fluorophore (FITC for green, DAPI for blue).

To perform immunofluorescence staining on the IGL cell line in an affordable and simple setting, certain tips can be helpful:

1. Utilize cost-effective alternatives for reagents and materials without compromising the quality of the results.

2. Consider optimizing the staining protocol by adjusting the concentrations of antibodies and reagents to minimize wastage.

3. Explore using alternative mounting media and blocking buffers that are affordable and readily available.

4. Take advantage of economical options for fluorescent secondary antibodies without compromising the specificity of the staining.

In addition to the cost-effective alternatives mentioned above, there are further options for conducting immunofluorescence staining on a limited budget. For example, consider exploring local suppliers for discounted reagents and materials, and collaborating with other research groups to share resources and reduce costs. Additionally, optimizing the use of equipment and recycling certain consumables can contribute to cost savings without compromising the quality of the staining results. By leveraging these low-budget options and effectively managing resources, it is possible to achieve successful immunofluorescence staining outcomes while working within budget constraints. By continuing to prioritize meticulous technique and attention to detail, we will be able to generate valuable immunofluorescence data for research on the iGL cell line.

Immunofluorescence staining on the iGL cell line is a valuable technique for visualizing the distribution and localization of target biomolecules. With careful consideration of cost-effective strategies and an understanding of the unique characteristics of the iGL cell line, it is possible to achieve reliable and informative immunofluorescence results even with budget constraints.

Ethical Statement

The ethical approval for this study was obtained from the Ethics Committee of the Faculty of Medicine - Universitas Prof. Dr. HAMKA on June 18, 2021, with the approval number KEPKK/FK/003/07/2021.

Conflict of interest

The authors declare that there is no conflict of interest was declared by the authors.

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Authors' contributions

Concept: E.N.S., S.S.N., Z.N., N.D.P., Design: E.N.S., S.S.N., Z.N., N.D.P., Data Collection or Processing: E.N.S., S.S.N., N.D.P., Analysis or Interpretation: E.N.S., S.S.N., N.D.P., Literature Search: T.Y., W.S., Writing: E.N.S., T.Y., W.S.

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Management of acute respiratory deterioration in the intensive care unit during the COVID-19 pandemic: Prospective analysis of retrospective data collected from a tertiary intensive care unit

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Abstract

Coronavirus disease-2019 (COVID) is a highly contagious viral disease, spread predominantly by airborne particles. Due to the high dissemination risk of COVID-19, aerosol generating procedures such as orotracheal intubation and bronchoscopy may result in viral spread. Therefore, performing bronchoscopies in infected patients, with insufficient evidence, is controversial. The aim of this study was to assess the requirements for emergency bronchoscopy in COVID-19 patients treated with invasive mechanical ventilation in the ICU, and to evaluate the applicability of the procedure and its effects on clinical progress. Patients with confirmed COVID-19 diagnoses who received invasive mechanical ventilation in the intensive care unit between March 26, 2020 and February 1, 2021, and who underwent emergency bronchoscopy were included into the study. Respiratory parameters before and after the procedure were analyzed with the paired t test. Among 395 patients diagnosed with COVID-19 pneumonia who received mechanical ventilation, 45 (mean age:48, 17F/28M) underwent an emergency bronchoscopy. The major indication for bronchoscopy was forced mechanical ventilation that did not respond to different measures. In 91.6% of the bronchoscopies there were positive findings, the most common were mucus secretions (82.4%), hematoma secretions (17.7%), mucous plugs (17.6%), and extensive mucosal hyperemia (11.4%). Patients who underwent bronchoscopies were noted to have significant improvements in respiratory mechanics and PO_2/FiO_2 ratios ($p<0.05$). Bronchoscopy can be safely applied in COVID-19 patients to solve the complications of mechanical ventilation, provided that the rules of personal protective equipment are complied with. Immediate improvements in respiratory parameters can be attained with bronchoscopy. In the absence of X ray findings, causes of the acute respiratory deterioration in COVID-19 patients can be investigated with bronchoscopy.

Keywords: COVID-19 pandemic, adult respiratory distress syndrome, critical care, bronchoscopy, occupation safety

1. Introduction

COVID-19 was first described as a pneumonia caused by SARS-COV-2 virus. The disease spread rapidly, becoming a pandemic affecting the whole world. Due to the surge of cases both nationally and internationally, the infrastructure of the health system was challenged against such a pandemic. Therefore, optimal use of resources for an effective professional action against the pandemic, in addition to the limited amount of healthcare workers and the need for uninterrupted care challenged the health systems (1, 2). Due to the high risk of viral dissemination, the use of some procedures (respiratory function test, bronchoscopy, etc.) was limited during the pandemic. Bronchoscopy is widely applied for diagnostic and therapeutic purposes. It is considered a relatively safe procedure due to the low risk of severe complications, and is very useful in the diagnosis and management of respiratory problems (3). In critically ill COVID-19 patients admitted to the intensive care unit (ICU), bronchoscopy may be required to manage complications including atelectasis or hemoptysis, solve problems related to mechanical ventilation, or exclude a superinfection. Working during the coronavirus disease 2019 (COVID-19) pandemic changed the habits of healthcare workers performing bronchoscopies. Guidelines prepared immediately after the spread of COVID-19 stated the common opinion that in patients with known or suspected COVID-19 patients, the use of fiberoptic bronchoscopy (FOB) should be limited and regarded as a relative contraindication due to the risk of spread to healthcare workers and the generation of aerosols during the procedure (4, 5).

COVID-19 may be asymptomatic or manifest itself with a wide array of symptoms including cough, fever, fatigue, or pneumonia causing severe acute respiratory distress syndrome (ARDS). Criteria for admission to the intensive care unit differ between countries and institutions, and the rates of admission are between 5-32% (1). Intensive care may become necessary in severe pneumonia, ARDS, myocarditis, arrythmias,

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cardiogenic shock, cerebrovascular disease, or acute renal failure. In those patients having predominantly hypoxemic respiratory failure, hypercapnic respiratory failure due to mucous plugs may be also observed. Similar to the experiences reported by other centers during the first wave of the pandemic, our observations revealed that especially in ARDS patients requiring mechanical ventilation the needs for bronchoscopy may be increased for various causes, particularly bronco aspiration (6).

The aim of this study was to evaluate the results of endoscopic procedures in COVID-19 patients who were mechanically ventilated in the ICU and developed acute deterioration in the oxygenation status without an identifiable cause after exclusion of pneumothorax and other causes.

2. Materials and Methods

A retrospective analysis of the demographic and respiratory parameters of COVID-19 patients, diagnosed using polymerase chain reaction (RT-PCR) of nasopharyngeal swab samples, hospitalized in the ICU between 26 March 2020 and 1 February 2021, received invasive mechanical ventilation, and underwent bronchoscopy, was performed.

The exclusion criteria were age under 18, diagnosis of COVID-19 not verified with a PCR test, no history of mechanical ventilation, presence of hemodynamic instability, elevated intracranial pressure, bleeding diathesis, thrombocytopenia, thrombocyte function disorder, and severe anemia. The main indication was the presence of severe refractory hypoxemia despite maximum mechanical ventilator support, with respiratory or systemic deterioration under minimal invasive mechanical ventilation (plateau pressure (Pplat)<28 cmH_20, Driving pressure <15 cmH_20). The maximum ventilatory settings were peak pressure (Ppeak>40 cm H_2O, the fraction of inspired oxygen (FiO2) 1, oxygen saturation <80%, respiratory rate ≥36/min, and pressure regulating volume control mode.

2.1. Bronchoscopy Procedure

The standard fiberoptic bronchoscope used for diagnosis and treatment in the ICU is Pentax FB-18V (Pentax Co.: Medical Inst. Division, Japan). The procedures are carried out in supine position, under normal intravenous sedation (Propofol %2 by Fresenius, 1mg/kg/dose) with neuromuscular blocking agents (Rocuronium by Polifarma, 0,5 mg/kg/dose), in pressure regulated volume control (PRVC) mode, and using the minimum number of staff required. Before the procedure, saline, mucolytic drugs, the materials for microbiologic sampling, and all the necessary equipment were prepared outside the patient's room. A negative pressure room was not always available due to the increased demands for intensive care. The staff were protected with the best personal protective equipment recommended for use during bronchoscopy, including N95 or FFP3 masks, goggles, and disposable medical protective uniforms with two layers in the head and neck (7). In COVID-19 patients the inner aspect of the bronchoscope was cleaned with enzymatic detergent and 70% alcohol, the outside was cleaned with antiseptic wipes and attached to the screen.

2.2. Statistical Analysis

The data were analyzed with the SPSS 25.0 software (SPSS Inc., Chicago, IL). Demographic characteristics of the patients were analyzed with the descriptive statistics methods. Normality analysis was made with different methods including histogram, kurtosis and skewness test. Parametric paired t test was used in the analysis of pre procedure and post procedure respiratory parameters. $P<0.05$ was accepted statistically significant.

3. Results

During the study period, 45 of the 495 patients admitted to the ICU underwent emergency bronchoscopy for the indications stated in the methods section. Mean age of the patients (23 males and 22 females) who required emergency bronchoscopy was 53. The patients had comorbidities with chronic diseases; 14 (40%) had hypertension, 8 (27%) had diabetes, 5 (33%) had obesity, 4 had chronic renal failure (CRF), 2 had cancer, and 3 had COPD (chronic obstructive pulmonary disease). All procedures were carried out for the removal of mucous plugs in patients receiving mechanical ventilation. Atelectasis was detected in 4% of the patients who developed sudden worsening in hypoxemia. The results are summarized in the Table 1. During the bronchoscopy examination, the position of the orotracheal tube was checked, the tracheal and bronchial mucosas were assessed, the secretions were removed with airway irrigation. There were no complications related to the bronchoscopy. None of the staff who joined the procedure developed a COVID-19 positivity within the first 3 months following the procedure.

Table 1. Changes in respiratory parameters before and after bronchoscopy were statistically meaningful. p-value ≤ 0.05 "statistically significant"

	Before Bronchoscopy	After Bronchoscopy	P value
pH	7.17	7.39	<0.05
PCO_2	80.39	43.15	<0.05
PO_2/FiO_2	53.15	124.71	<0.05
P_{PEAK}	63.97	28.02	<0.05

Among 45 patients who developed sudden deterioration in the respiratory mechanics and worsening of hypoxemia, 43 were noted to have mucous plugs in the carina. The plugs were removed successfully.

Due to the difficulty in attaining peak pressure ventilation, pressure alarm thresholds were exceeded. The values prior to bronchoscopy were: PO2 112-125 mmHg, Ppeak 30 +/- 5 cmH_2O, and pCO2 36 +/- 6. When an indication for bronchoscopy emerged, the mean values were as follows: $P0_2$ 40-55 mmHg, Ppeak 65+/-15 cmH_2O, PCO_2 90+/-12 mmHg.

In our study, the reasons for performing an emergency bronchoscopy were near total occlusion of the airway by a

mucous plug, difficulties in ventilating the patient, and failure to extract the plug. Assessment of arterial blood gases before and after FOB showed significant improvements in PO_2, PCO_2, SpO_2 levels. Mean values are given in Table 1.

The bronchial mucosa was hyperemic in bronchoscopies. Most of the patients had very thick, dry plugs that were hard to aspirate, resembling limestones. In 45 cases, muco-hematic plugs that occluded the main or lobar bronchi were seen and they were extracted after instilling saline and mucolytic substance. The mucous plugs appeared to be trapped in the tube, the endotracheal tubes were clogging rapidly and required renewal more frequently than normal.

4. Discussion

In this study we report on our experience with emergency bronchoscopy on 45 among 395 patients who had laboratory confirmed COVID-19 infections and acute respiratory failure, and who were admitted to the ICU and received invasive mechanical ventilation. Patients who experienced acute respiratory deterioration during ICU stay for Covid 19 pneumonia significantly recovered from acute respiratory distress, and none of the health care workers were infected with virus.

Can age, presence of comorbid diseases, or a delay in hospital referral be factors in patients who are admitted to the hospital with acute respiratory failure which is present at admission or which develops later, and who have deterioration in the overall status? The answer to this question remains unclear because the natural course of COVID-19 is still not fully understood (8).

It has been shown that COVID-19 related ARDS is different from the typical ARDS (9, 10). The most important aspect of the former is preservation of respiratory parameters despite severe hypoxemia (11). While the disease itself causes severe hypoxemia, we showed that in patients who have improvements in inflammatory parameters and oxygenation a response to FOB could be attained after extraction of mucous plugs by emergency bronchoscopy, performed due to acute deterioration in oxygen status. Because it is regarded necessary and urgent due to the severity of the clinical condition, the difficulty of performing bronchoscopy during COVID-19 period is emphasized (Fig. 1).

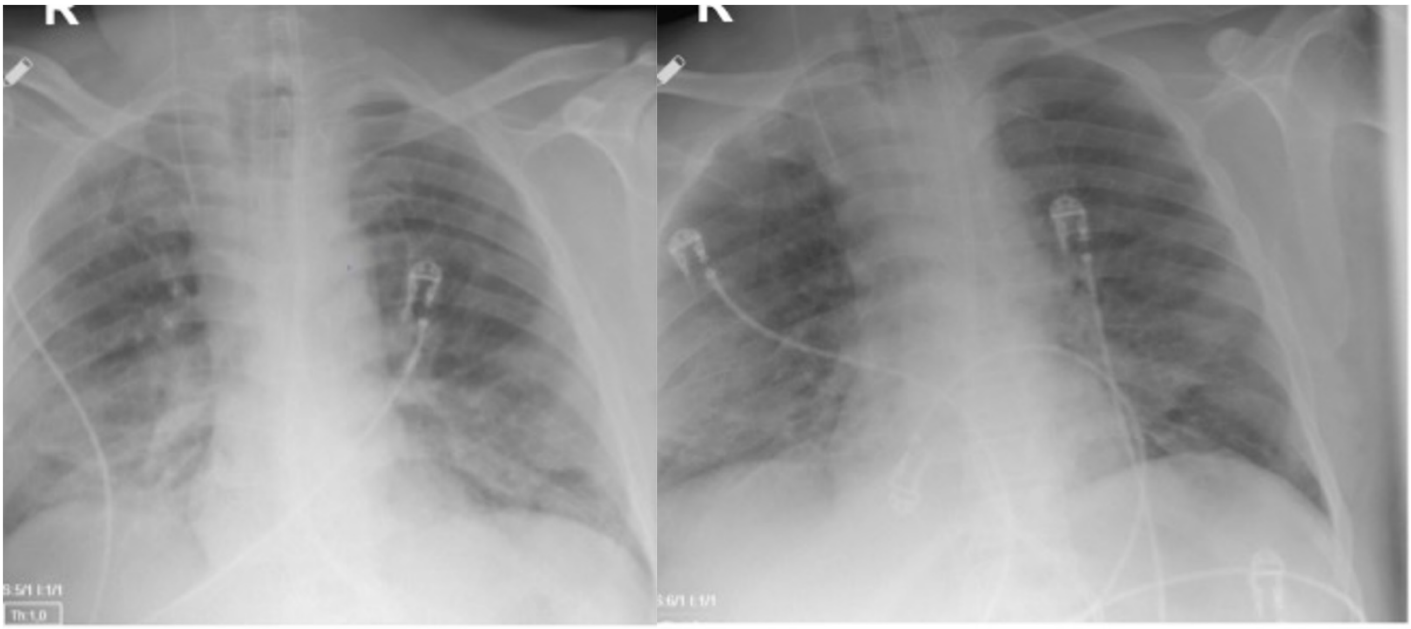

Fig. 1. On the left side is the patient's X-Ray before bronchoscopy, and on the right side is the X-Ray after bronchoscopy. Although fibrinous plug made clinical changes on respiration, the X-ray did not show these changes

The high contagiousity of the SARS-CoV-2 virus, the long incubation period, and possibility of asymptomatic infection has led to numerous deaths. There has been a great concern for the safety of healthcare workers who are especially under risk of contracting the SARS-CoV-2.

The first transmission to healthcare workers was reported in January 2020, and the first COVID-19 related death was of an ear nose throat specialist in Wuhan city, China (12). The airways of people infected with COVID-19 were seen to contain a high viral load (13). Any intervention on the airways will aerosolize the virus, which significantly increases the risk of infection in healthcare workers. Regarding the safety of healthcare workers, those who attended the bronchoscopies did not show any COVID-19 symptoms during a 3-month observation period, which suggested that protective equipments were adequately safe. Performing these procedures with protective equipment is cumbersome, with sweating, mucosal dryness, and extra fatigue. Putting the protective equipment on and off is time consuming and adds to workload (14). Despite all these difficulties, the infection risk is reduced by safety precautions.

Although the Association of Bronchology recommends

single use of bronchoscopes, due to the difficulties in the supply of materials and high costs we used the available bronchoscope on only COVID-19 patients, complying with the disinfection recommendations (4).

The most striking finding in the bronchoscopies of COVID-19 patients was the presence of thick secretions in many cases (23%), which were hard to aspirate and which formed real endobronchial molds (4, 5). As the authors stated, the high rates of mucous plugs in critically ill COVID-19 patients may be explained by the limitation of aerosolization and humidification treatments in compliance with current recommendations (6). The management of these thick mucous plugs required maneuvers that significantly prolonged the procedure times, which were around 45-60 minutes even in the hands of experienced bronchopists.

Although scientific societies recommend limiting the aerosolization and humidification treatments due to air spread risk of the coronavirus, a decision was made to initiate active humidification in all patients receiving mechanical ventilation in order to minimize the presence of these mucous plugs. Although the management of bronchial secretions in these patients continue to be the main indication for bronchoscopy, the secretions became thinner and easier to aspirate. Thus, the bronchoscopy time decreased significantly to approximately 10-20 minutes.

Most of international pulmonology societies do not recommend therapeutic bronchoscopy except in selected patients such as those with hemorrhage or lung atelectasis. On the other hand, our study showed that radiologic changes may lack the sensitivity for the detection of prominent mucous plugs and atelectasis may be missed. It is likely that at least a fraction of the ground glass appearances observed in COVID-19 patients represent, whether a segmental atelectasis is present or not, congestion of the alveolar spaces with mucus and a possibly worse outcome.

A study by Torrego et al. confirmed the presence of mucus during bronchoscopy in the airways of 95% of 101 COVID-19 patients with mean ventilation period of 6.6 days (6). Importantly, Earhart et al. showed that mucolytic dornase alfa improved the results and shortened the ventilation times in COVID-19 patients (15). A randomized clinical study in COVID-19 patients receiving oral mucolytic bromhexine showed that the benefits of bromhexine were maximized when the drug was initiated early. It also decreased the respiratory symptoms, requirements for intubation and intensive care unit hospitalizations, times for mechanical ventilation, and mortality (16).

Although our paper has the advantage of reporting on the experience from a large number of cases, it has also limitations. The first is the nature of our study. Due to long work hours our data were collected retrospectively and evaluated prospectively. The indication for bronchoscopy procedure was based primarily on the first author's experience which depended on the previous ICU and bronchoscopy practices. Bronchoscopy procedures were performed for additional support to the patients, with the author's decision contrary to the recommendations in the guidelines. The number of patients who underwent bronchoscopy in ICU seems to be low but when compared with the previous studies, however it is important to underline the ICU set up and single center single physician experience.

In conclusion, Removal of mucous plugs may be lifesaving, and may decrease ventilator days or even mortality. In the absence of X ray findings, causes of acute respiratory deterioration in COVID-19 patients can be investigated and managed with bronchoscopy.

In this study we showed that during the treatment of COVID-19 patients who have persistent hypoxemia, when an acute worsening in respiratory parameters occurs, mucous plugs should come into mind before a clinical deterioration is considered. The treatment of airway obstruction by removal of mucous plugs using the FFB may be lifesaving and improve acute respiratory deterioration. It has been shown that when personal protective equipment is used, FFB is a reliable and safe method that assists the treatment of COVID-19 patients.

Ethical Statement

All procedures performed in studies including human participants were according to the ethical standards of the institutional and national research committee and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Clinical Research Ethics Committee of Istanbul Kanuni Training and Research Hospital (Date: 03.2022, No: KAEK 2022/03/65).

Conflict of interest

The authors declare that they have no competing interests.

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None to declare.

Authors' contributions

Concept: E.K., Ö.K., A.S.Ş., Design: E.K., Ö.K., A.S.Ş., Data Collection or Processing: E.K., S.D., Analysis or Interpretation E.K., Ö.K., Literature Search: A.S.Ş., Writing: A.S.Ş.

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Research Article

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Reconstructive options and analysis in breast reconstruction: Our clinical experience

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Abstract

Breast cancer ranks first worldwide in cancer-related deaths in women. The percentage of breast cancer in Turkey has almost doubled in the last decade. Breast cancer treatment encompasses a broad spectrum of oncological treatments, including radiotherapy, chemotherapy and oncoplastic surgical treatments. Oncoplastic reconstruction is an integral part of breast cancer treatment, providing favorable aesthetic and psychological outcomes. In our study, the breast reconstructions of 166 patients who underwent mastectomy due to tumoral pathologies, performed by our institute between 2005 and 2023, were analyzed from various aspects. Based on our 19 years of experience, the number of breast reconstruction surgeries has increased dramatically in the last ten years; however, the repair trend has shifted to immediate and also implant-based reconstruction procedures.

Keywords: breast reconstruction, breast cancer, immediate, delayed, alloimplant, autologous

1. Introduction

Breast cancer ranks first worldwide in cancer-related deaths in women (1). In 2018, around 2.1 million new breast cancers were diagnosed worldwide, and 1 in 4 cancer diagnoses was reported as breast cancer (4). Breast cancer is the most commonly diagnosed cancer in the extensive majority of countries (154/185 countries) and is the leading cause of cancer-related deaths in more than 100 countries (5). The incidence of breast cancer in Turkey has nearly doubled in the last decade (24/100,000 in 1994 and 43.8/100,000 in 2015) (2, 3). In addition, in developed countries, mammography screening and increased attention have increased the diagnosis of early-stage and nonpalpable breast cancer (6). Breast cancer treatment is a broad spectrum covering oncological treatments such as radiotherapy, chemotherapy, and oncoplastic surgical treatments. Significant changes have occurred in the surgical modality in our country over time. In 2008, only 35% of patients underwent breast-conserving surgery, while this rate increased to 39% in 2014 and 57% in 2018 (7). Today, breast-conserving surgery is a secure treatment for most females with early-stage breast cancer and can now be safely performed in 70-80% of cases where surgery is required (8). With the evolution of breast ablation therapy to a less aggressive form, the number of breast reconstructions has also increased enormously (1, 9). Breast reconstruction is a major part of breast cancer treatment with favorable aesthetic and psychological outcomes. Reconstruction covers a broad spectrum, from the creation of a breast-like organ after a mastectomy performed years ago to the immediate reconstruction of the patient's breast following a mastectomy completed a few minutes ago. Breast reconstruction can be implant-based, autologous (locoregional, free flap), or a combination. In addition, breast reconstruction is nowadays an individual treatment modality shaped by the timing of surgery (immediate or delayed) and the patient's condition and desire (10).

2. Materials and Methods

In our study, breast reconstructions of mastectomy patients due to tumoral pathologies performed by our institute between 2005 and 2023 were analyzed from various aspects. Patients who underwent mastectomy caused by breast tumor or genetic predisposition to the tumor and underwent reconstruction between 01.01.2005 and 31.12.2023 were screened using the hospital database program. Our inclusion criteria were that the patients had completed at least six months of follow-up and that the etiology of mastectomy was tumoral pathologies. Patients who did not complete six months follow-up and patients who were operated on for breast agenesis, trauma, or primary aesthetic reasons other than tumoral pathologies were excluded from the study. Thus, 166 patients who fulfilled the criteria were included in our study. One hundred sixty-six patients were operated on from the beginning of 2005 until the end of 2023. All of our patient groups consisted of Caucasian women. The mean age of the screened patients was 44 years, with a minimum of 27 years and a maximum of 70 years. Date,

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age, mean follow-up period, neoplasm histology, immediate and delayed repair, repair method (autologous, implant, or combined repair), and complications were recorded.

2.1. Statistical Analysis

The IBM SPSS 26.0 package program evaluated the collected data. Mean, typical deviation, median, minimum, and maximum values for quantitative (numerical) variables and frequency (%) values for qualitative (categorical) variables were used as descriptive statistics. Yates Chi-Square analyses were performed to compare the proportions. Significance was evaluated at $p<0.05$ level in all comparisons.

3. Results

One hundred sixty-six patients were operated on from the beginning of 2005 until the end of 2023 (Fig. 1). All of our patient groups consisted of women. The mean age of the screened patients was 44 years, with a minimum of 27 years and a maximum of 70 years. The mean follow-up period was 22 months (min. six months – max. 96 months). Seventy-one patients were operated on the right breast, 51 on the left breast, and 44 on both breasts. One hundred twelve patients were operated on immediately, and 54 were operated on as delayed.

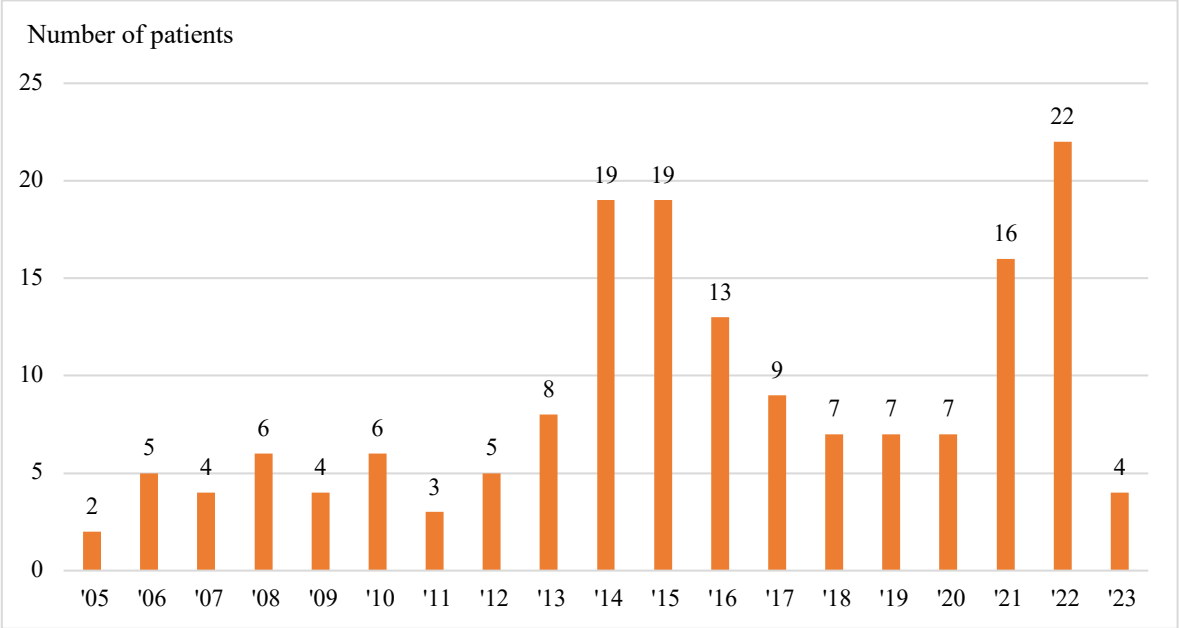

Fig. 1. Distribution of the number of patients by years

Between 2005 and 2013, 43 patients underwent breast reconstruction surgery; this number increased to 123 in 2014-2023 (Fig. 1). A total of 54 patients underwent delayed repair; 26 were performed until 2014, and 28 underwent delayed reconstruction surgery from 2014 to 2023. In contrast, while there were 17 immediate reconstructions until 2013, this number increased to 95 between 2014-2023 (Fig. 2). The number of delayed reconstruction operations performed in the period before 2014 is significantly higher than the period after 2014 ($p<0.001$). The number of immediate reconstruction operations performed after 2014 is significantly higher than before 2014 ($p<0.001$). Twenty-four patients (14%) were operated with autologous flaps (ten free deep inferior epigastric artery-based perforator (DIEP) flap, nine pedicled transverse rectus abdominis myocutaneous (TRAM) flap, three pedicled latissimus dorsi myocutaneous flap, and one free latissimus dorsi muscle flap, 107 patients (64%) were operated with implant-based repair technique (with direct prosthesis (70) or expander (37)), and 35 patients (22%) were operated with the combined repair technique of latissimus dorsi myocutaneous flap and prosthesis, which we mostly use as a salvage method in our clinic (Fig. 3). The flap of one patient who was reconstructed with DIEP was deemed non-viable due to early pedicle thrombosis, the flap was debrided, replaced with an expander and then reconstructed with a final prosthesis.

Of the 24 patients reconstructed with autologous flaps, 16 were performed in the period until 2013 and eight between 2014 - 2023. There will be a dramatic increase in implant-based reconstructions after 2014. Until 2014, 17 patients were reconstructed with implants; this number increased to 90 by 2023. It is observed that all implant-based reconstructions have increased significantly in the last decade.

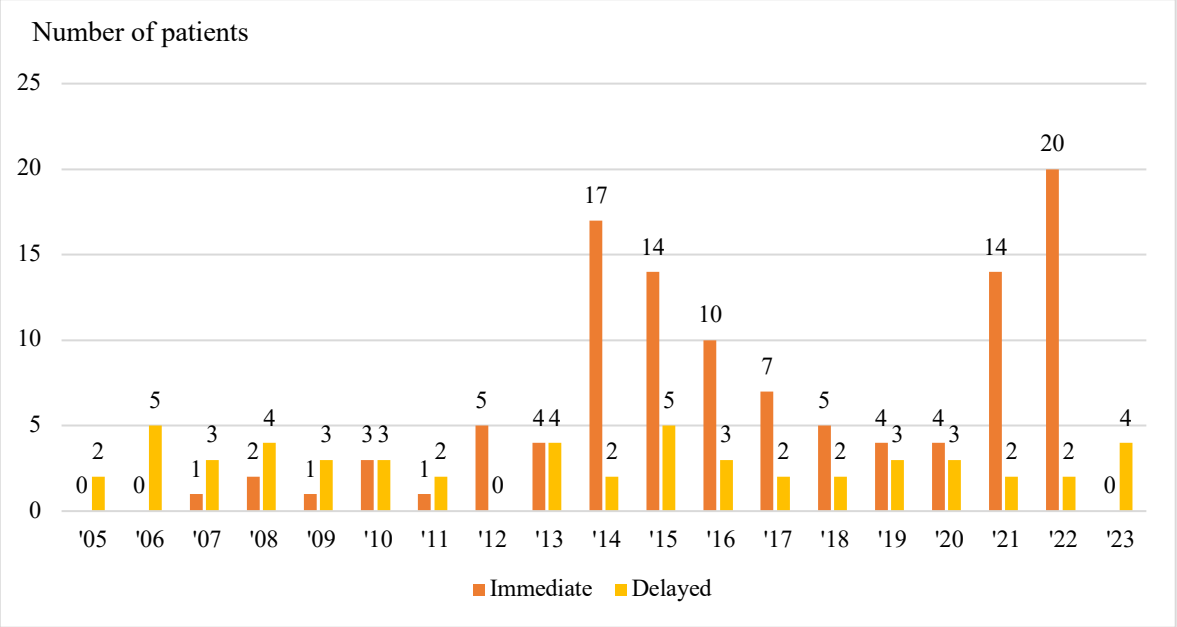

Fig. 2. Comparison of immediate and delayed reconstruction by years

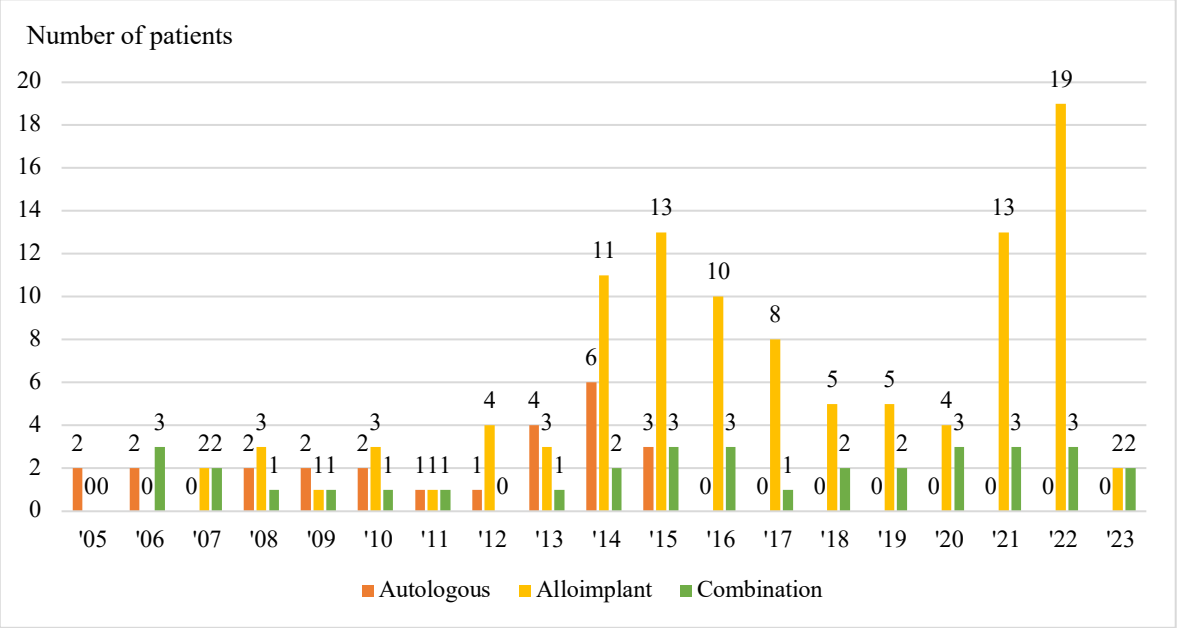

Fig. 3. Type of reconstructive methods by years

One hundred sixteen patients with invasive ductal carcinoma (70%), 17 patients with ductal carcinoma in situ (DCIS) (10%), ten patients with invasive lobular carcinoma (6%), six patients with fibrocystic breast disease (3%), four patients with mucinous carcinoma (2%), four patients with mixed breast carcinoma (2%), three patients underwent mastectomy for phylloides tumor (1.8%), and one patient each for fibroadenoma, bilateral intraductal hyperplasia, sclerosing adenosis, medullary carcinoma, adenoid cystic carcinoma, and reconstruction surgery was performed by us. One patient with BRCA-1 positivity underwent bilateral mastectomy for prophylaxis and reconstruction (Fig. 4).

A whole of 112 patients experienced immediate reconstruction. Seventeen were until 2014; 95 patients underwent immediate reconstruction from 2014 to 2023. After 2014, the number of breast reconstructions performed has shown a statistically significant increase compared to the previous period (p<0.001).

In our study, the use of implants in immediate reconstructions is by far more prominent (92 all implants vs. seven autologous flaps) (p<0.001). The modality of breast reconstruction in our study has significantly shifted towards implants and simultaneous repair over the years.

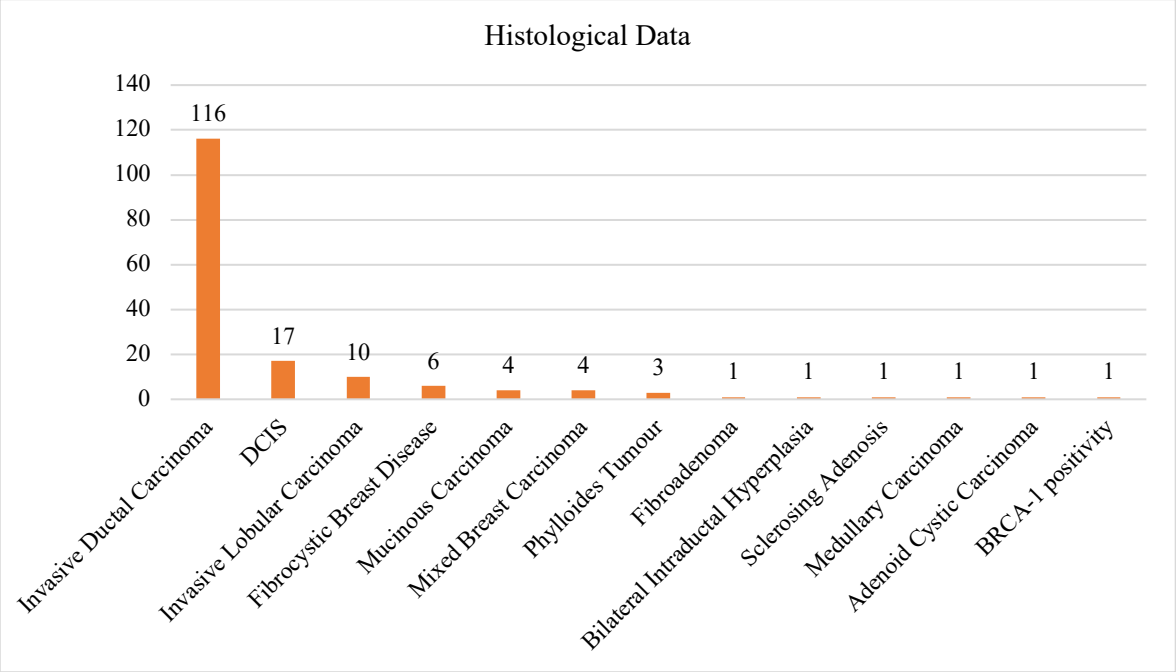

Fig. 4. Etiology of mastectomy

Complications were observed in 45 of 166 patients (27%). Complications were found in 36 immediate and nine delayed reconstruction patients. No statistical significance was observed between the complication rates of our immediate and delayed operations (p>0.05). Various complications were observed in 36 patients who underwent repair with all implants and six patients who underwent repair with autologous flaps. Our complications in cases who underwent repair with autologous flaps are as follows: two total flap necrosis, one seroma, one fat necrosis, and one infection, totaling five DIEP flap complications. The necrotic DIEP flap was replaced with an expander in the early period, and then salvage reconstruction was performed with a final prosthesis. One incisional hernia was observed at the donor site of the patient who underwent reconstruction with a pedicled TRAM flap.

Of the complications seen after repair with alloimplants, 23 occurred in the patient who underwent repair with direct prosthesis and 13 occurred in the patient who underwent expander placement. The prosthesis was exposed after necrosis of the skin flaps in seven of the breasts reconstructed with direct prosthesis in the acute period, and all of them were reconstructed with latissimus dorsi muscle-skin flap as a salvage method. Apart from these, partial necrosis of five nipple-areola complexes, four capsular contractures, two infections, two-incision dehiscence, one fat necrosis, one hematoma, and partial skin necrosis in one breast was observed.

In the patients who were primarily expander implanted, a total of four partial necrosis of the nipple-areola complex, one expander perforation, three expander exposures (implants were removed), two seroma, two capsular contracture, and one wound infection were observed.

It was observed that there was no noteworthy difference between the complication rates of our cases who underwent reconstruction with direct prosthesis and tissue expander placement first (p>0.05).

4. Discussion

One hundred sixty-six patients were operated on from the beginning of 2005 until the end of 2023. The mean age of the screened patients was 44 years. According to a study conducted on 20,000 women in Turkey, the mean age was 45-49 years, and this result is consistent with our study (11).

Between 2005 and 2013, 43 patients underwent breast reconstruction surgery; this number increased to 123 in 2014-2023. Recent studies also show that an increasing percentage of mastectomy patients are undergoing breast reconstruction, that patients are more informed about breast reconstruction and that they are requesting breast reconstruction (6).

Of the 24 patients reconstructed with autologous flaps, 16 were performed in the period up to 2014 and eight up to 2023. There will be a dramatic increase in implant-based reconstructions after 2014. Until 2014, 17 patients were reconstructed with implants; this number increased to 90 by 2023. In the USA, allogenous reconstructions currently account for about 65% of all breast reconstructions (12). Implant reconstruction is becoming an attractive option Considering advantages such as acceptable, good cosmetic results, and patient satisfaction (13, 14).

One hundred sixteen patients with invasive ductal carcinoma (70%) were identified in our patient group. In a comprehensive study of 20,000 women in Turkey, 77% of the neoplasm histology was reported as invasive ductal carcinoma, which is consistent with our study (11).

After 2014, the number of breast reconstructions performed

has shown a statistically significant increase compared to the previous period (p<0,001). Immediate breast reconstruction has become a more trendy repair method over time (15, 16) , and more than 70% of breast reconstructions are achieved presently with mastectomy (17).

In our study, the use of implants in immediate reconstructions is by far more prominent (92 all implants vs. seven autologous flaps) (p<0.001). The modality of breast reconstruction in our study has significantly shifted towards implants and simultaneous repair over the years. When considered globally, it is undeniable that breast reconstructions have evolved in this direction (6).

Complications were observed in 45 of 166 patients (27%). Complications were found in 36 immediate and nine delayed reconstruction patients. No statistical significance was observed between the complication rates of our immediate and delayed operations (p>0.05). Various complications were observed in 36 patients who underwent repair with all implants and six patients who underwent repair with autologous flaps. Our complications in cases who underwent repair with autologous flaps are as follows: two total flap necrosis, one seroma, one fat necrosis, and one infection, totaling five DIEP flap complications. The necrotic DIEP flap was replaced with an expander in the early period, and then salvage reconstruction was performed with a final prosthesis. One incisional hernia was observed at the donor site of the patient who underwent reconstruction with a pedicled TRAM flap. According to literature, it was found that total flap loss in autologous reconstructions was less than 1-4% (this rate was 8% in our study); major complications such as total-partial flap loss, fat necrosis, donor site morbidity were between 7.7% and 28.3% (17% in our study); total complications were between 22.6% and 44.3% (25% in our study) (Table 1) (18–21). Although some studies have shown that autologous reconstruction is significantly more complicated than implants, no noteworthy difference was found in our study (p>0.05) (6, 22).

Table 1. Comparison of complications of autologous reconstructions

	Related articles	Our study
Total flap loss	<1-4%	8%
Major complications -total-partial flap loss, -fat necrosis, -donor side morbidity	7.7% - 28.3%	17%
Total complications	22.6% - 44.3%	25%

Of the complications seen after repair with alloimplants, 23 occurred in the patient who underwent repair with direct prosthesis and 13 occurred in the patient who underwent expander placement. The prosthesis was exposed after necrosis of the skin flaps in seven of the breasts reconstructed with direct prosthesis in the acute period, and all of them were reconstructed with latissimus dorsi muscle-skin flap as a salvage method. Apart from these, partial necrosis of five nipple-areola complexes, four capsular contractures, two infections, two-incision dehiscence, one fat necrosis, one hematoma, and partial skin necrosis in one breast was observed.

In the patients who were primarily expander implanted, a total of four partial necrosis of the nipple-areola complex, one expander perforation, three expander exposures (implants were removed), two seroma, two capsular contracture, and one wound infection were observed. In studies, the rate of implant explantation in patients undergoing allogeneic reconstruction ranged between 2.7% and 3.8% (3.7% in our study). Major complication rates such as implant extrusion, premature explantation, capsular contracture, migration, and device malfunction ranged from 4% to 30.4% (16% in our study). Total complication rates are between 5.8% and 49% (33.6% in our study) (Table 2) (18, 19, 21). It was observed that there was no noteworthy difference between the complication rates of our cases who underwent reconstruction with direct prosthesis and tissue expander placement first (p>0.05). Ilonzo et al. also reported no distinction between reconstructions performed with direct prosthesis or expander (6). In addition, a systematic review meta-analysis comparing direct implant reconstruction and conventional two-step implant breast reconstruction indicated no significant difference between the two repair methods (23).

Table 2. Comparison of complications of alloimplant based reconstructions

	Related Articles	Our Study
Implant explantation	2.7% - 3.8%	3.7%
Major complications -implant extrusion, -premature explantation, -capsular contracture, -device malfunction	4% - 30.4%	16%
Total complications	5.8% - 49%	33.6%

This study is retrospective and represents patients from a single center. Also, 2005 was the year of transition of our institution from the classical file system to the digital database programme. Therefore, demographic information such as residence, occupation, marital status, job, socioeconomic status and education level of some patients could not be obtained because they were not entered into the digital database programme. These demographic parameters could not be included in our study. For the same reason, the use of dermal substitute or mesh in patients undergoing allomimplant-based reconstruction was not evaluated as it could not be confirmed.

Treatment planning and management of an individual diagnosed with breast cancer is a complex process involving oncological, socioeconomic, psychological, and reconstructive variables. Breast reconstruction is an integral part of holistic breast cancer treatment. While reconstructive surgery is an oncologically safe treatment, it reduces the impact of the

diagnosis and treatment of breast cancer on the patient's mental health, thereby improving the patient's quality of life, social participation, and self-esteem. According to our 19 years of experience, the number of breast reconstruction surgeries has increased greatly in the last ten years; however, the repair trend has shifted to immediate and also implant-based reconstruction procedures.

Ethical Statement

The study protocol was approved by the Clinical Research Ethics Committee of Ondokuz Mayıs University (Date: 27.03.2024/No:2024/153).

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: A.D., İ.A., Design: A.D., İ.A., Data Collection or Processing: İ.A., M.S.O., Analysis or Interpretation: İ.A., E.S., M.S.O., M.B.G., Literature Search: İ.A., C.G., Writing: İ.A.

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Research Article

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The effect of selenium therapy on semen parameters in infertile men with varicocele

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Abstract

Oxidative stress and varicocele have been linked to male infertility. The aim of this study was to assess the effect of selenium therapy on semen parameters in infertile men with varicocele. Forty infertile men with varicocele (Group 1) and forty infertile men without varicocele (Controls, Group 2) with oligoasthenospermia who fulfilled the selection criteria were included in the study. All participants received selenium (200 µg/day) orally for three months. Seminal fluid analysis (WHO 5th criteria) was performed at baseline and after three months of selenium treatment. The results were compared between groups before and after therapy. Following three months of selenium therapy, sperm progressive motility significantly increased in patients with and without varicocele ($P<0.01$, $P<0.05$ respectively; 32.7% change vs. 24.1% respectively) but the improvement was higher in patients with varicocele. Similarly, sperm total motility significantly increased in patients with and without varicocele ($P<0.01$, $P<0.05$ respectively; 23.6% change vs. 12.6% respectively) and the increment was higher in infertile men with varicocele. Sperm concentration and normal sperm morphology; however, did not significantly change before and after selenium therapy. In conclusion, treatment of infertile men with varicocele and oligoasthenospermia with selenium (200 µg/day) for 3 months improves sperm progressive and total motility and the increment is higher than in infertile men without varicocele. Thus, selenium could be an adjuvant treatment for varicocele-associated male infertility.

Keywords: selenium, male infertility, varicocele, semen

1. Introduction

Infertility is a global health issue affecting 8-15% of couples and approximately 48 million individuals worldwide with medical, psychological, and financial consequences (1). Infertility is diagnosed when the couple fails to conceive after twelve months of regular unprotected intercourse. Male factor accounts for approximately half of infertility cases. The underlying causes of male infertility encompass a wide range of conditions including undescended testis, varicocele, infections, endocrinopathies, systemic diseases, testicular tumors, radiotherapy, chemotherapy, immunological, genetic, and environmental factors (2–4). Reduced semen parameters are defined as sperm concentration below 15 million/ml, sperm progressive motility below 32%, total motility below 40%, and normal sperm morphology below 4% according to WHO 2010 5th criteria (5). A combination of low sperm concentration and motility is known as oligoasthenospermia (OA).

Varicocele is a condition characterized by the enlargement of veins within the scrotum. Varicocele has been found in 15% of fertile men but the prevalence among infertile men is approximately 40%(6). Several studies have demonstrated an association between varicocele and altered semen parameters(7,8). Varicocele is associated with decreased sperm concentration, motility, and morphology. These alterations could be attributed to increased scrotal temperature, hypoxia, venous stasis, accumulation of toxic metabolites, and elevated levels of reactive oxygen species (ROS) in semen, leading to oxidative stress (OS), sperm DNA damage (SDF), and impaired fertility (8,9). Varicocele may also have detrimental effects on ART outcomes(10).

OS is the result of prooxidants/antioxidants imbalance. A variety of antioxidant medications have been tried to treat OS and SDF and resulted in a reduction of ROS, lower SDF levels, and improvement in semen parameters and seminal antioxidant markers (11). However, antioxidant therapy is limited by the lack of consensus on a standardized treatment regimen including the type, dose, and duration of antioxidant therapy.

Selenium is an essential micronutrient that plays a key role in male reproduction including testosterone metabolism and sperm selenoproteins (12). Selenium also plays structural and enzymatic roles and is also a cofactor for many enzymes involved in antioxidant, anti-inflammatory, endocrine, immune modulation, DNA, and RNA synthesis (13). Sperm selenoproteins also maintain sperm structural integrity and glutathione peroxidase (GPx) antioxidant activity against ROS. Specifically, GPX4 has been found in the sperm nucleus where it performs stabilization of the condensed chromatin during spermatogenesis (14). Studies have reported decreased selenium levels in infertile men and that selenium therapy was associated with increased semen parameters (14,15). Therefore, this study aimed to explore the impact of selenium on semen parameters in infertile men with varicocele after three months of treatment as compared to the infertile men

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without varicocele.

2. Materials and Methods

2.1. Patients

In this prospective controlled clinical study, 40 infertile men with varicocele and OA (Group 1) were recruited from Infertility Clinic Babylon, Iraq from April to July 2023. Varicocele was diagnosed by scrotal examination in upright positions before and during Valsalva's maneuver and was confirmed by Doppler sonography. The control group (Group 2) consisted of 40 infertile men without varicocele and have OA. Participants with other known predisposing factors for male infertility such as undescended testicles, endocrinopathies, systemic diseases, genital infection, smoking, alcoholism, and recent antioxidants intake were excluded.

Seminal fluid analysis (WHO 2010 5th criteria) was performed at baseline and after 3 months (5) . All patients received selenium (America Medic & Science, Washington USA) 200 µg/day orally for 3 months. The selenium dose adopted in this study was used previously (14). Semen parameters were compared between the two groups at baseline and after three months of selenium therapy. Ethical approval was issued by the University of Babylon and informed consent was received before the start of the study.

2.2. Semen analysis

All the patients were asked to refrain from sexual activity for two to three days before the collection of the semen sample. The collection of semen samples was achieved by masturbation and the patients were instructed to provide a complete sample. Following collection, the semen sample was held at 37°C for liquefaction and then analyzed according to WHO 2010 5th criteria for all semen parameters (5). All the analyses were undertaken by the same investigator and for all participants, two semen samples were assessed at baseline and after three months, and their average value was used for this study.

2.3. Statistical analysis

Data analysis was performed with MedCalc software (v.22). Data normality was assessed with Kolmogorov–Smirnov test. The results were expressed as mean ± SD. Paired t-test was used to compare the results before and after selenium therapy in each group. The magnitude of change in semen parameters was expressed as percentage. P<0.05 was considered statistically significant.

3. Results

The mean age for patients in groups 1 and 2 was 34.3± 9.2 and 32.4±11.5 years respectively (Table 1). The mean for duration of infertility in groups 1 and 2 was 7.3±6.2 and 6.4±4.9 years respectively. After three months of selenium therapy, patients with varicocele and without varicocele demonstrated significant increment in sperm progressive motility but the improvement was significantly higher in patients with varicocele (P<0.01, P<0.05 respectively; 32.7% change vs. 24.1%) (Table 1 and fig. 1). Similarly, total sperm motility significantly increased in both groups but the increment was significantly higher in patients with varicocele as compared to those without varicocele (P<0.01, P<0.05 respectively; 23.6% change vs. 12.6%).

Table 1. Patients characteristics and semen parameters before and after selenium therapy

	Patients with varicocele (n=40)			Patient without varicocele (n=40)		
Age	34.3± 9.2		% Change	32.4±11.5		% Change
Duration of infertility (years)	7.3±6.2			6.4±4.9		
Concentration (million/ml)	7.8±2.6	9.1±3.5	16.6%	7.4±2.9	8.8± 4.1	18.9%
Progressive motility (%)	16.8±7.6	22.3±8.5**	32.7%	18.6±8.2	23.1±7.8*	24.1%
Total Motility (%)	21.6±6.9	26.7±6.5**	23.6%	23±6.2	25.9±6.8*	12.6%
Normal morphology (%)	8.5±4.2	10.7± 6.4	14.1%	7.8±3.3	8.5± 4.1	8.9%

* P<0.05 as compared to baseline
** P<0.01 as compared to baseline

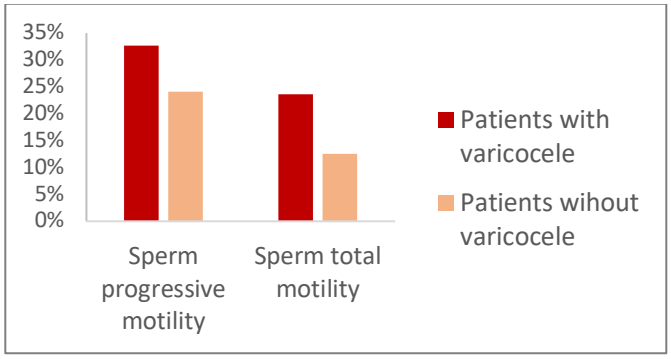

Fig. 1. Percentage change in sperm motility patient with and without varicocele after selenium treatment

4. Discussion

Recent years have witnessed advances in the management of infertile men including sperm function tests, sperm retrieval, and sperm selection techniques for ART treatment. Further, several studies have tried different oral antioxidants to treat men with idiopathic male infertility but with inconsistent results. Recently, systematic reviews and a meta-analysis by our group and other colleagues have also illustrated the beneficial effects of oral antioxidants on semen measures. However, there is no standardized treatment regimen. Surgery is the main therapy for varicocele but other modalities of treatment including antioxidants have been also tried. The

WHO 2010 5th criteria for semen analysis were used to define seminal fluid abnormalities. While conventional semen analysis is the standard initial assessment method for male infertility worldwide, semen analysis has many limitations. The main drawbacks stem from the fact that the reference values of semen analysis in the WHO manual were obtained from fertile rather than infertile. Another limitation is the adoption of the 5th centile as a cut-off value to distinguish fertile from infertile men while there is an overlapping of the values of semen parameters between fertile and infertile subjects (16). In addition, semen analysis cannot assess sperm function and also has geographical, genetic, and ethnic variations (17).

The current study results are in agreement with previous studies which illustrated higher semen measures following oral antioxidant therapy (18–20). Moslemi and Tavanbakhsh explored the impact of selenium (200 µg/day) in combination with vitamin E (400 units) for 100 days in infertile patients with idiopathic asthenoteratospermia and showed increased sperm motility and morphology post-therapy (21). This increment was attributed to the reduction in intracellular anion superoxide and sperm apoptosis. Further, in our previous study, a comparison was made for the effect of selenium (200 µg/day) with coenzyme Q10 (200 mg/day) in patients with idiopathic oligoasthenoteratospermia for three months in patients with idiopathic male infertility and demonstrated increased sperm concentration, motility, and seminal antioxidant markers in both groups although coenzyme Q10 produced higher increment (15). In one study; however, the administration of selenium (300 µg/day) for 48 weeks produced no significant changes in semen parameters (22). In addition, a high dose of selenium may produce detrimental effects on semen parameters (23).

Other studies have explored selenium therapy in combination with other antioxidants and illustrated variable beneficial effects on one or more semen parameters (24–26). It is essential to mention that combined antioxidant formulations in these studies have a wide range of selenium doses ranging from 10 to 225 µg (19). This fact in association with the heterogeneity of study design, type, dose, duration of treatment, and patient group makes the comparison more challenging. Higher semen measures detected post-selenium therapy in the present study could be due to the antioxidant properties of selenium, upregulation of GPx activity, reduction of OS, and the role of selenoproteins in sperm function (27). These improvements could be also explained by the recent observations of anti-inflammatory, endocrine, sperm mitochondrial membrane potential, genetic, and immune modulation effects for selenium that affect male fertility potential (13,23).

In the current study, selenium therapy in infertile men with varicocele and OA resulted in increased sperm motility which was higher than in those without varicocele. Our findings are congruent with other studies that demonstrated improved semen parameters in infertile men with varicocele following different regimens of antioxidant therapy (6,28). In a controlled study that assessed the effect of micronized purified flavonoid fraction in patients with painful varicocele, improvement in all semen parameters following 6 months of therapy was reported (29). However, treatment with pentoxifylline with zinc and folic acid (for 12 weeks) in men with varicocele-related infertility resulted in increased normal sperm morphology only (30). Studies on the effect of selenium on semen parameters in infertile men with varicocele are scarce. Oral selenium therapy in normal and varicocelized rats was associated with increased antioxidant capacity and reduced damage to testicular architecture (31). Another study illustrated improved testicular biochemical and morphological parameters in rats with experimental varicocele after lycopene and selenium therapy (32)

It is essential to mention that studies which have explored the impact of selenium on seminal SDF are scarce and many previous studies have investigated selenium in combination with other micronutrients. In addition, previous studies were heterogeneous, and various doses and durations of treatment were used in patients with one or more seminal abnormalities. This heterogeneity makes a direct head-to-head comparison of the findings and the impact of antioxidant therapy on male fertility challenging.

Previous studies that explored the impact of antioxidant therapy on semen measures in infertile men also illustrated different study designs. Some of these studies were before and after studies while the others were controlled trials. It is worth mentioning that a variety of antioxidant therapy were used in these studies either as monotherapy or a combination of various antioxidants. A lack of consensus on the dose of antioxidants was also demonstrated in these clinical studies. A variation in dietary intake for each patient could also affect the results of these studies. Some of these studies considered dietary intake using various questionnaires while others did not include dietary assessment in their design. Dietary assessment; however, could be influenced by recall bias as well as the complex interaction between different dietary constituents. The parameters measured were also different in these studies. Some studies explored the effect of antioxidants on semen measures only while others have additionally assessed OS markers or sperm DNA fragmentation. Using different primary endpoints in different clinical studies makes a direct comparison of these studies more difficult.

Limitations of the current study include a small number of participants (which is due to consent issues) and a lack of dietary assessment of selenium intake. Also, the results of the present study may not be generalizable to all areas as it was performed in a single location.

Treatment of infertile patients with varicocele and OA with selenium (200 µg/day) for 3 months improves sperm

progressive and total motility and the increment was higher than in infertile men without varicocele. Thus, selenium could be an adjuvant treatment for varicocele-associated male infertility.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: A.T.A., Design: A.T.A., Data Collection or Processing: A.T.A., Analysis or Interpretation: A.T.A., Literature Search: A.T.A., Writing: A.T.A.,

Ethical Statement

Approval was obtained from University by Babylon Ethics Committee, the study started. The ethics committee decisions is EC/12-2023.

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Research Article

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Attitudes of medical school students toward artificial intelligence in medical education: A survey study

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Abstract

This study aimed to assess medical students' knowledge and attitudes towards artificial intelligence (AI) models and their potential role in medical education and healthcare. A cross-sectional study was conducted among 398 students of Ondokuz Mayıs University Medical Faculty using an online questionnaire administered through Google Forms. Participants' awareness of AI applications, their usage patterns, and their opinions on AI's role in medical education were evaluated. The study found extensive awareness of AI applications among participants, with ChatGPT being the most recognized (%95). The majority of students believed that AI models could benefit medical education and assist in diagnosis and treatment when collaborating with doctors. However, concerns were raised regarding the potential for incorrect results, patient safety, and ethical issues. While students recognized the potential of AI-supported education to enhance learning motivation, they expressed concerns about its impact on creativity. Significant differences were found between classes regarding the acceptance of the proposition that artificial intelligence models will take over our profession in the future (p=0.001). Participants aware of AI applications exhibited a higher agreement rate with the proposition that AI-assisted education is more advantageous than traditional methods (p=0.022). Medical students recognize the potential benefits of AI models in clinical practice and medical education but also highlight concerns about ethical considerations and potential limitations. These findings emphasize the importance of a balanced approach to integrating AI into medical education and practice.

Keywords: artificial intelligence, medical education, medical students, surveys and questionnaires

1. Introduction

Artificial intelligence (AI) models are a very new technology that enables computers to have human-like intelligence by utilizing data analysis and learning capabilities (1). AI models are creating new divisions of labor between humans and machines in the working world. The World Economic Forum (WEF) predicts that the increased use of AI since the 2020 pandemic will disrupt 85 million jobs globally by 2025 and create 97 million new job roles, marking this period as the "augmented workforce" era of AI (2).

AI models (such as ChatGPT developed by OpenAI and Bard by Google), which can generate text and visuals based on user commands, provide translation capabilities, analyze and summarize information, and stay up-to-date, are being widely used and adapted by various individuals and organizations in the healthcare system. Healthcare professionals, other professions, and even patients can benefit from AI models (3). AI models, gaining popularity due to their creativity, ease of use, and accessibility, are increasingly playing a significant role in medical education. Integrating AI models into the education process of medical students offers potential benefits such as enriching students' experiences, quickly filtering related results from hard-to-access sources, and presenting relevant information. These developments offer the option of using AI-based tools alongside traditional learning methods in medical education (4).

AI models can play an important role in developing skills for medical students during their education, including case studies, clinical comparisons between similar cases, literature reviews from sources inputted by physicians, diagnosis, guidance in practical applications, and creating treatment plans (5). Additionally, these models can assist students in integrating theoretical knowledge with practical applications through simulation projects and support clinical decision-making processes (6). However, the role of AI models in medical education must align with the fundamental principles of medical education, including the importance of ethical values, maintaining the doctor-patient relationship, and the significance of human judgment (7). The possibility of developing AI models that support physicians' roles and perform these tasks, alongside considering the future of medicine as a human profession, is also an aspect that needs evaluation (8). Despite the increasing use of AI models in medical education in recent years, sufficient data regarding their use in our country is lacking. The aim of our study is to assess medical students' knowledge levels regarding AI models and evaluate their behaviors regarding its potential role in

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medical education.

2. Materials and Methods

This cross-sectional study was conducted through an online questionnaire using Google Forms from March 7th to March 24th, 2024. The study population was students of Medical Faculty of Ondokuz Mayıs University. The study protocol was approved by the Ondokuz Mayıs University Clinical Research Ethics Committee (protocol code: OMU-KAEK 2024/39,).

Since the number of individuals in the population was unknown and there was no known prevalence in the literature regarding the research topic, a sample size calculation was made for 50% prevalence, determining that at least 385 participants needed to be reached. Considering potential data loss, a total of 398 medical faculty students were contacted. The students of the Ondokuz Mayıs University Medical Faculty were contacted via social media applications (WhatsApp, Instagram, LinkedIn) and face-to-face interactions; those who agreed to participate in the study were asked to answer the survey questions online. Participants were not asked to state their names. The first part of the survey consisted of six questions querying sociodemographic characteristics and participants' knowledge levels about AI applications. In the second part, participants were asked to mark one of the options "agree," "undecided," or "disagree" according to their agreement status regarding 10 propositions related to AI and its relationship with medical education/practice.

Statistical analysis was performed using the IBM SPSS v21 software. In data analysis, measurement data were expressed as mean-standard deviation, and frequency data were expressed as numbers and percentages. To compare the answers given by the participants to the statements about AI according to the class, gender and artificial intelligence usage; the χ^2 test and linear by linear χ^2 analysis were used. The statistical significance level was set at $p < 0.05$.

3. Results

The mean age of the 398 medical students who participated in the study was 22.1 ± 2.5 years. 57.3% of the participants were female. The most common participants were in Class 2 (21.1%) and Class 4 (21.9%). The sociodemographic characteristics of the participants are presented in Table 1.

Table 1. Distribution of the sociodemographic characteristics of the participants (n=398)

Sex, n (%)	
Male	170 (42.7)
Female	228 (57.3)
Age, year, (mean ± SD) (min-max)	22.1 ± 2.5 (17-41)
Class, n (%)	
Class 1	41 (10.3)
Class 2	84 (21.1)
Class 3	63 (15.8)
Class 4	87 (21.9)
Class 5	77 (19.3)
Class 6	46 (11.6)

Only 5 (1.3%) students were unaware of artificial intelligence applications. 95% of the participants were aware of ChatGPT, while 38% were aware of other AI applications (Bard, YouChat, Jasper, Bing, Leonardo AI, CoPilot). There was no statistically significant difference in awareness of ChatGPT based on gender and class. The percentage of males aware of AI applications other than ChatGPT was statistically significantly higher than females (44.7% and 33.3%; respectively) ($p<0.05$).

85.0% of the participants stated that they used ChatGPT, 23.6% used other applications, and 10.6% did not use any artificial intelligence applications. The use of artificial intelligence was statistically significantly higher in male students compared to females (87.6% and 78.1%; respectively) ($p<0.05$). While Class 6 students were the most frequent users of AI applications (93.5%), Class 5 students were the least frequent users (66.2%). There was a statistically significant difference in the frequency of artificial intelligence use among classes ($p<0.05$), which was attributed to the difference between Class 6 and Class 5 students.

74.8% of the participants stated that they used artificial intelligence applications for studying, doing homework; 40.9% for literature review; 24.1% for learning languages; and 4.02% for other purposes (entertainment, translation, text summarization, software, visual creation, etc.).

Among the propositions given in the study, the statement "I think artificial intelligence models are beneficial in medical education" had the highest agreement rate at 80.2%, while the lowest agreement rate was 24.4% with the statement "I think artificial intelligence models will take over our profession in the future." The frequencies of participants' responses to the propositions are shown in Table 2.

The frequency of those who disagreed with the proposition "The use of artificial intelligence models may reduce our creativity" was statistically significantly higher in females compared to males ($p=0.006$).

The highest agreement with the proposition "I think artificial intelligence models will take over our profession in the future" came from Class 3 and Class 4 students (41.3% and 39.1% respectively). The disagreeing parties were Class 4 and Class 2 students (48.3% and 45.2% respectively). There was a statistically significant difference between the agreement rate to this proposition and the class of the participants ($p=0.001$).

82.9% of Class 1 students and 47.8% of Class 6 students agreed with the proposition "Artificial intelligence models can be used in collaboration with doctors for diagnosis and treatment." There was a statistically significant difference between the agreement rate to this proposition and the class of the participants ($p=0.012$).

Table 2. Distribution of participants' answers to propositions about artificial intelligence(%)

	Agree	Disagree	Undecided
I think AI models are beneficial in medical education.	80.2	3.8	16.1
I think AI models can produce incorrect results.	69.1	4.8	26.1
I think AI models will take over our profession in the future.	24.4	37.4	38.2
AI models can be used in collaboration with doctors for diagnosis and treatment.	72.9	5.5	21.6
The use of AI models can raise ethical issues.	57.0	10.3	32.7
The use of AI models may reduce our creativity.	52.8	14.6	32.7
In a hospital setting, the utilization of artificial intelligence models may pose a problem in terms of patient safety.	51.3	13.8	34.9
AI-supported learning is more advantageous than traditional education methods.	48.5	15.6	35.9
AI-assisted education can increase medical students' learning motivation.	58.8	12.1	29.1
The use of AI models can decrease doctors' professional responsibility.	54.3	13.1	32.7

The agreement rate with the proposition "Artificial intelligence-assisted education is more advantageous than traditional education methods." was statistically significantly higher among those who were aware of artificial intelligence applications compared to those who were not (p=0.022)

The agreement rates with the propositions "I think artificial intelligence models are beneficial in medical education." "Artificial intelligence models can be used in collaboration with doctors for diagnosis and treatment." "Artificial intelligence-supported learning is more advantageous than traditional education methods." and "Artificial intelligence-assisted education can increase medical students' learning motivation." were statistically significantly higher among users of artificial intelligence applications compared to non-users (p<0.05).

There was no statistically significant difference in the agreement rates with other propositions based on participants' gender, class, knowledge about AI, and usage. The responses of the participants to the propositions based on these characteristics are shown in Table 3.

Table 3. The comparison of participants' responses to propositions about artificial intelligence according to some groups *(%)*

	Agree	Disagree	Undecided	p value
I think AI models are beneficial in medical education.				
Sex				0.203
Female	82.9	2.6	14.5	
Male	76.5	5.3	18.2	
Class				0.168
Class 1	80.5	2.4	17.1	
Class 2	75.0	3.6	21.4	
Class 3	87.3	3.2	9.5	
Class 4	82.8	3.4	13.8	
Class 5	71.4	3.9	25.7	
Class 6	89.1	6.5	4.3	
Awareness of artificial intelligence				<0.001
Yes	80.9	3.1	16.0	
No	20.0	60.0	20.0	
Use of artificial intelligence				<0.001
Yes	83.1	2.0	14.9	
No	54.8	19.0	26.2	
I think AI models can produce incorrect results.				
Sex				0.443
Female	68.0	3.9	28.1	

Male	70.6	5.9	23.5	
Class				0.592
Class 1	68.3	2.4	29.3	
Class 2	61.9	6.0	32.1	
Class 3	73.0	6.3	20.6	
Class 4	67.8	3.4	28.7	
Class 5	72.7	2.6	24.7	
Class 6	73.9	8.7	17.4	
Awareness of artificial intelligence				0.818
Yes	69.0	4.8	26.2	
No	80.0	0.0	20.0	
Use of artificial intelligence				0.197
Yes	68.0	5.3	26.7	
No	78.6	0.0	21.4	
I think AI models will take over our profession in the future.				
Sex				0.4442
Female	23.7	36.4	39.9	
Male	25.3	38.8	35.9	
Class				0.001
Class 1	19.5	31.7	48.8	
Class 2	13.1	45.2	41.7	
Class 3	41.3	22.2	36.5	
Class 4	20.7	48.3	31.0	
Class 5	20.8	40.3	39.0	
Class 6	39.1	23.9	37.0	
Awareness of artificial intelligence				0.714
Yes	24.5	37.0	38.6	
No	22.7	45.5	31.8	
Use of artificial intelligence				0.899
Yes	24.8	37.0	38.2	
No	22.5	39.4	38.0	
AI models can be used in collaboration with doctors for diagnosis and treatment.				
Sex				0.228
Female	71.5	4.4	24.1	
Male	74.6	7.1	18.2	
Class				0.012
Class 1	82.9	4.9	12.2	
Class 2	73.8	6.0	20.2	
Class 3	76.2	0.0	23.8	
Class 4	72.4	6.9	20.7	
Class 5	79.2	5.2	15.6	
Class 6	47.8	10.9	41.3	
Awareness of artificial intelligence				0.177
Yes	73.3	5.3	21.4	
No	40.0	20.0	40.0	
Use of artificial intelligence				0.046
Yes	74.7	5.3	19.9	
No	57.1	7.1	35.7	
The use of AI models can raise ethical issues.				
Sex				0.096
Female	58.8	7.5	33.8	
Male	54.7	14.1	31.2	
Class				0.383
Class 1	58.5	7.3	34.1	
Class 2	53.6	7.1	39.3	
Class 3	55.6	12.7	31.7	
Class 4	56.3	11.5	32.2	
Class 5	68.8	10.4	20.8	
Class 6	45.7	13.0	41.3	
Awareness of artificial intelligence				0.538
Yes	56.7	10.4	32.8	
No	80.0	0.0	20.0	
Use of artificial intelligence				0.260
Yes	55.9	10.1	34.0	
No	66.7	11.9	21.4	
The use of AI models may reduce our creativity.				
Sex				0.006

Female	53.1	10.1	36.8	
Male	52.4	20.6	27.1	
Class				0.910
Class 1	46.3	17.1	36.6	
Class 2	56.0	15.5	28.6	
Class 3	57.1	11.1	31.7	
Class 4	50.6	13.8	35.6	
Class 5	57.1	14.3	28.6	
Class 6	43.5	17.4	39.1	
Awareness of artificial intelligence				0.139
Yes	52.7	14.2	33.1	
No	60.0	40.0	0.0	
Use of artificial intelligence				0.527
Yes	53.7	14.0	32.3	
No	45.2	19.0	35.7	
In a hospital setting, the utilization of artificial intelligence models may pose a problem in terms of patient safety				
Sex				0.136
Female	47.8	13.2	39.0	
Male	55.9	14.7	29.4	
Class				0.416
Class 1	43.9	17.1	39.0	
Class 2	44.0	13.1	42.9	
Class 3	52.4	11.1	36.5	
Class 4	51.7	11.5	36.8	
Class 5	55.8	19.5	24.7	
Class 6	60.9	10.9	28.3	
Awareness of artificial intelligence				0.399
Yes	50.9	14.0	35.1	
No	80.0	0.0	20.0	
Use of artificial intelligence				0.270
Yes	52.2	12.9	34.8	
No	42.9	21.4	35.7	
AI-supported learning is more advantageous than traditional education methods.				
Sex				0.053
Female	43.9	15.4	40.8	
Male	54.7	15.9	29.4	
Class				0.054
Class 1	31.7	17.1	51.2	
Class 2	34.5	17.9	47.6	
Class 3	55.6	12.7	31.7	
Class 4	57.5	14.9	27.6	
Class 5	54.5	15.6	29.9	
Class 6	52.2	15.2	32.6	
Awareness of artificial intelligence				0.022
Yes	48.9	15.0	36.1	
No	20.0	60.0	20.0	
Use of artificial intelligence				0.015
Yes	50.8	14.3	34.8	
No	28.6	26.2	45.2	
AI-assisted education can increase medical students' learning motivation.				
Sex				0.175
Female	61.8	9.6	28.5	
Male	54.7	15.3	30.0	
Class				0.364
Class 1	53.7	12.2	34.1	
Class 2	57.1	9.5	33.3	
Class 3	65.1	7.9	27.0	
Class 4	62.1	9.2	28.7	
Class 5	61.0	14.3	24.7	
Class 6	47.8	23.9	28.3	
Awareness of artificial intelligence				0.092
Yes	59.3	11.7	29.0	
No	20.0	40.0	40.0	
Use of artificial intelligence				<0.001
Yes	62.4	11.0	26.7	
No	28.6	21.4	50.0	
The use of AI models can decrease doctors' professional responsibility.				

Sex				0.477
Female	56.1	11.4	32.5	
Male	51.8	15.3	32.9	
Class				0.288
Class 1	48.8	12.2	39.0	
Class 2	47.6	16.7	35.7	
Class 3	66.7	11.1	22.2	
Class 4	58.6	9.2	32.2	
Class 5	46.8	13.0	40.2	
Class 6	58.7	17.4	23.9	
Awareness of artificial intelligence				0.795
Yes	54.5	13.0	32.5	
No	40.0	20.0	40.0	
Use of artificial intelligence				0.242
Yes	55.6	12.4	32.0	
No	42.9	19.0	38.1	

p value according to χ^2 analysis

4. Discussion

In this study, our aim was to investigate the attitudes of medical students toward artificial intelligence, which is increasingly gaining importance in our lives, and to examine how they perceive its positive and negative effects on their professional lives.

The majority of participants in our study (80.2%) believed that artificial intelligence applications were beneficial in medical education. Similarly, in a study conducted among medical students and doctors in Korea, about half of the participants reported that they would use artificial intelligence more intensively in the future, and they indicated that the most useful areas would be diagnosis and treatment planning (9). Another study conducted in our country revealed that the majority of students believed that hospitals using artificial intelligence were more advantageous both in diagnosis and treatment, and they expressed their desire to use artificial intelligence in their future medical careers, suggesting a trend towards more intensive use of artificial intelligence applications in medicine(6). In the literature, it is considered necessary to incorporate artificial intelligence into medical education (10,12).

The increasing accessibility of data in healthcare and the rapid development of analytical methods have made successful applications of artificial intelligence possible in healthcare. Powerful artificial intelligence models guided by relevant clinical questions can direct physicians towards potential diagnoses. In our study, 72.9% of the participants believed that artificial intelligence models could be used collaboratively with physicians in diagnosis and treatment. Similarly, Esteva and colleagues have shown that training artificial intelligence applications to diagnose skin cancer is comparable to dermatologists' skin cancer classification (12). In another study, the majority of participants believed that artificial intelligence could potentially detect pathologies in radiological examinations (10).

In our study, only 48.5% of the participants believed that artificial intelligence-supported education would be more advantageous than traditional educational methods. However, considering that the amount of information in medical literature doubles every three years, it is estimated that a physician would need to spend 29 hours a day reading books if they wanted to stay completely up-to-date (13). The belief that artificial intelligence-supported education would be advantageous compared to the traditional method was statistically significant among those who had knowledge of and used artificial intelligence, compared to those who did not. One of the reasons for this could be the concern among those who think that it could create ethical issues (57%). There are some ethical issues in the application of artificial intelligence in healthcare, patient care, and medical research. Issues such as the absence of informed consent forms, reliability of information, and lack of emphasis on patient privacy, as well as uncertainty about who would be blamed in case of an error, can lead to ethical problems (3,14). Furthermore, it should be transparent and free from discrimination (18). In addition to ethical issues, 69.1% of participants believed that artificial intelligence models could produce incorrect results. Among these concerns are cases of providing incorrect references, indicating the necessity for human verification of the accuracy of the information provided (15).

Approximately 59% of the participants believed that artificial intelligence-supported education could increase medical students' learning motivation. There are several advantages to using artificial intelligence in medical education, including providing a wide range of information sources, supporting the learning process, enhancing language skills, offering personalized learning experiences, and providing 24/7 access with problem-solving and analytical abilities (3). These advantages can not only support and enhance students' learning processes but also boost their motivation. Personalized content delivered through artificial intelligence technology, engaging experiences, and instant feedback can capture students' attention and make the learning process more effective. Offering content tailored to students' interests and ensuring easy access can also increase their participation in the learning process. Therefore, using artificial intelligence can enhance students' motivation and make their learning experiences more enjoyable. Participants in Yılmaz et al.'s study also believe that

they can provide better healthcare to patients by using artificial intelligence (16). In our study, it is also believed that artificial intelligence-supported education can increase learning motivation.

The majority of students participating in our study believe that the use of artificial intelligence models can reduce the professional workload of physicians. A study suggests that with the advancement of artificial intelligence technology, it can be used to compensate for shortages, especially in areas where there is a shortage of doctors and healthcare workers, and it can be beneficial in diagnosis and treatment. However, it has been noted that along with the decrease in responsibility, doctors fear becoming unemployed (13). In fact, in another study, one-sixth of participants who initially considered radiology as their first choice do not consider radiology due to concerns about artificial intelligence (17). In our study, only 37.4% of participants believe that it could take away their profession.

Artificial intelligence technology has the potential to reduce the number of errors in clinical settings and minimize differences in opinions among doctors. Moreover, through the analysis of large datasets obtained from clinical practices by artificial intelligence applications, new models discovered may lead to the development of beneficial markers for diagnosis and treatment (10). However, just as drugs and other medical devices need to undergo thorough verification of safety and efficacy before being used on patients, artificial intelligence technology also needs to undergo comprehensive clinical validation to determine its accuracy and ensure that it can provide care to patients without causing harm. In addition, the extent to which artificial intelligence ensures patient safety is also subject to debate. Processing, storing, and even altering information such as patients' identities, medical histories, lifestyles, and habits can lead to confusion about how much and under what circumstances artificial intelligence will access this information (3). Participants in our study, at 51.3%, also believe that the use of artificial intelligence models may pose problems in terms of patient safety.

In today's fast-paced world where everything is consumed rapidly and our attention spans are decreasing, it is possible to access information quickly through artificial intelligence. The ability of artificial intelligence to provide examples within specific patterns and generate new content leads to more than half of students believing that the use of artificial intelligence models will reduce our creative thinking abilities.

Our study has several limitations. Firstly, it was conducted solely among students of Ondokuz Mayıs University Faculty of Medicine, thus the findings cannot be generalized at a national level. Another limitation is that participation in the study was voluntary, potentially resulting in a bias towards individuals more interested in AI.

In conclusion, the students participating in our study expressed awareness of AI applications, with all those knowledgeable about such applications indicating familiarity with ChatGPT. They believe that the use of AI models in medical education could be beneficial, potentially reducing the workload of physicians and aiding in diagnosis and treatment when collaborating with doctors. However, they also acknowledge the potential for these models to produce incorrect results and raise concerns about patient safety and ethical issues. Regarding education, they agree that AI-supported education could enhance medical students' learning motivation but express concerns that the use of AI models may diminish their creative thinking abilities. Overall, while the students acknowledge the advantages of using AI models in clinical settings and medical education, they also highlight shortcomings, particularly in ethical considerations.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: B.D., Ö.Z.T., Design: B.D., H.B.Ç., Data Collection or Processing: G.N.A., M.Ü., C.T.Ü., E.Ş., B.Y., G.Z.A., L.K., Analysis or Interpretation: Ö.Z.T., B.D., Literature Search: All authors, Writing: All Authors.

Ethical Statement

Approval was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 31/01/2024 and the number of ethical committee decisions is 2024/39.

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Applications of total hip prosthesis to patients with coxarthrosis that has developed due to developmental hip dysplasia

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Abstract

The aim of this study was to evaluate the clinical and radiographic results of total hip arthroplasty (THA) for coxarthrosis secondary to developmental dysplasia of the hip (DDH). Examinations were made of 58 hips in 47 patients with DDH who were operated on between 1990-2000 (patients with anatomic centred hips with or without roof graft and/or femoral shortening). The patients were evaluated using the Modified Harris Hip Score pre- and postoperatively. The average Harris score increased from 47.7 preoperatively to 89.2 postoperatively. The average age of 43 female and 4 male patients was 48 years (24-65). All the patients presented with a limp, which was resolved after surgery in 34% and partially improved in others. The average medialization was 24 mm, and the average distal placement was 32 mm. A total of 28 (48%) complications were observed in 26 patients. Intraoperatively, fissures were detected in the proximal femur in 5, and were fixed with cerclage wiring. One patient with femoral shortening had hip dislocation one week later, so plate screw fixation was revised with open reduction. One patient had anterior thigh pain, heterotopic ossification was observed in three cases, and polyethylene wear in one case. All 20 patients who had superolateral acetabular grafting showed incorporation but there was observed to be resorption in a 20% section that was non-weight-bearing in 3 cases. THA is an effective treatment option for coxarthrosis in neglected DDH. Patients should be informed about the problems they may encounter after surgery and care should be taken in terms of complications such as dislocation, prosthesis infection, and vascular and nerve damage.

Keywords: developmental hip dysplasia, coxarthrosis, total hip arthroplasty, Harris hip score

1. Introduction

Early diagnosis and treatment are crucial for managing developmental dysplasia of the hip (DDH). In young patients who do not receive early diagnosis and treatment, interventions such as femoral and pelvic osteotomies, roof interventions, and hip arthrodesis are used as an alternative to total hip arthroplasty (THA). Compared to the normal adult population, the development of coxarthrosis is seen more in adult patients with DDH due to the prolonged exposure to body weight (1). Currently, THA is the option usually applied to these deformities.

As DDH patients are younger and have a wide range of anatomic abnormalities of the acetabulum and femur, this can present great challenges in THA. Complications such as limb length discrepancy, non-union in the osteotomy region, nerve damage, postoperative dislocation of the hip joint, valgus deformities in the knee, and aseptic loosening continue to be the main problems related to DDH (2).

The aim of this study was to review the clinical and radiographic results of THA applied to patients with Hartofilakadis type B and type C coxarthrosis, which had developed on the basis of DDH, and to evaluate the complications and their resolution.

2. Materials and Methods

A retrospective examination was made of patients with Hartofilakidis type B and type C dislocation who underwent THA in the 2nd Orthopaedics and Traumatology Clinic of Dışkapı Yıldırım Beyazıt Training and Research Hospital between 1990 and 2000. As the procedures related to THA in patients with Hartofilakidis type A were similar to those for primary coxarthrosis, these patients were not included in the study.

The study included 58 hips of 47 patients in which THA was reduced to the real acetabulum without grafting or shortening, those who underwent acetabular roof graft and those who underwent subtrochanteric femoral shortening. Of the cases, 43 (93%) were female and 4 (7%) were male. The average age at the time of surgery was 48 years, ranging from 24 to 65 years. The mean follow-up period was 37.6 months, with a range of 17 to 124 months (Table 1).

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Table 1. Demographic and clinical data of patients

	Early/mid-term
N (hips) n,	58
Age (year)	
Mean±SD	48.01±21.12
Range	24-65
Sex F/M n (%)	43 (%91) / 4 (%9)
Follow-up time (month)	
Mean±SD	37.6±29.16
Range	17-124
Side n (%)	
Right	7 (%15)
Left	29 (%62)
Bilateral	11 (%23)
Hartofilakidis type B/C, n (%)	35 (%60) / 23 (%40)
FSO (n)	7
AG (n)	20
Medialization distance of the hip (mm)	
Mean±SD	24,06±1.05
Range	14-36
True acetabular reduction distance of the hip (mm)	
Mean±SD	32,14±1.9
Range	25-55
Femoral osteotomy healing time (weeks)	
Mean±SD	12.06±3.9
Range	10-14
Modified Harris Hip Score	
Preoperative	**Early/mid-term**
44.73 ±11.3	89.28±8.3

SD: Standard deviation; M: Male; F: Female; FSO: Femoral shortening osteotomy; AG: Acetabular grafting

Of the 58 hips applied with THA, 35 (60%) were type B, and 23 (40%) were type C. Bilateral THA was performed on 11 (18.9%) patients. In 51 cases, THA was performed without shortening, reduction to the true acetabulum by making subtrochanteric osteotomy (STO) in 7 cases, and acetabular roof graft to 20 cases. In one 19-year-old case applied with subtrochanteric femoral shortening, Schanz osteotomy was performed in another centre (Fig. 1-3).

2.1. Preoperative Planning

Each case underwent clinical and radiographic preoperative evaluation focusing on abductor mechanism function, limb length discrepancy, hip centre migration, the potential need for shortening osteotomy, the choice of suitable implants, and the achievement of primary stability. Additionally, antibiotic, and deep vein thrombosis prophylaxis were administered.

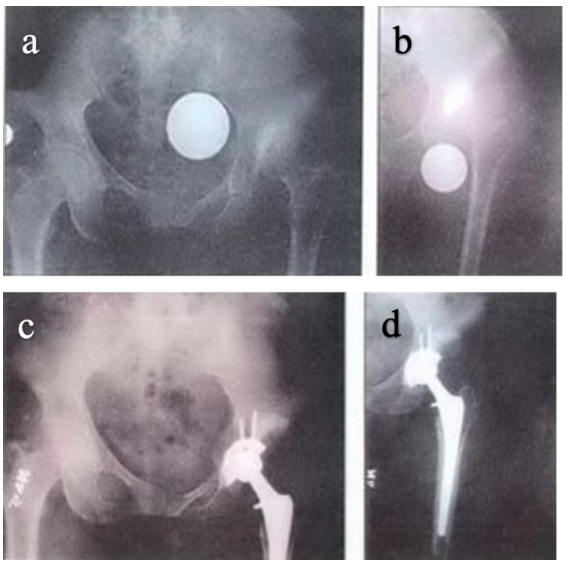

Fig. 1. A 49-year-old female patient with left coxarthrosis secondary to DDH, preoperative anteroposterior pelvic x-ray (a), anteroposterior left hip x-ray (b), and 26-month postoperative control pelvic x-ray (c) and anteroposterior left hip x-ray (d)

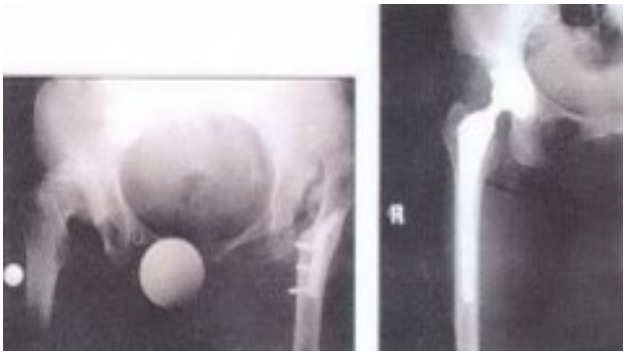

Fig. 2. 38-year-old female patient. Total hip arthroplasty was performed for bilateral coxarthrosis secondary to DDH. Preoperative anteroposterior pelvic radiograph (a), 6-month postoperative right hip radiograph (b) and 19-month postoperative left hip radiograph

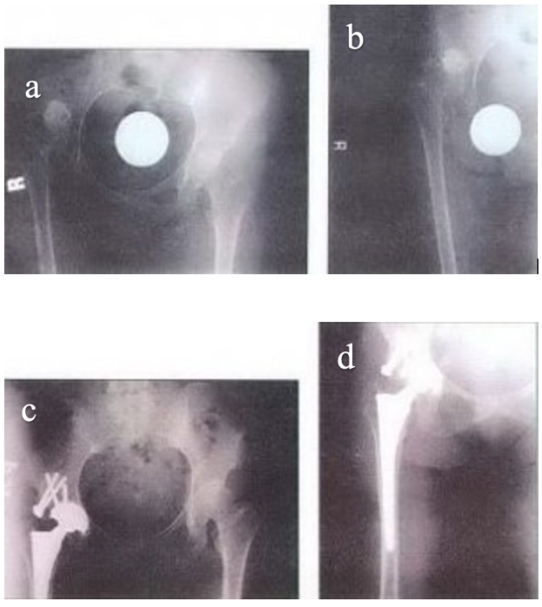

Fig. 3. A 49-year-old female patient with coxarthrosis secondary to DDH: preoperative anteroposterior pelvic x-ray (a), preoperative right hip x-ray (b), postoperative 24-month anteroposterior pelvic x-ray (c) and postoperative 24-month right hip x-ray (d)

2.2. Surgical Technique

All patients were positioned on their sides and posterolateral entry was made and the true acetabulum was prepared for the placement of the acetabular component. In the event that several tenotomies were necessary for reduction of the femur, adductor, iliopsoas tenotomy and loosening of the gluteus maximus from the linea aspera, as well as femur head-neck resection at the level of the trochanter minor were performed. Subsequently, the joint capsule was dissected to reach the true acetabulum. The hypertrophic capsule was completely excised in order to provide a wide view of the acetabulum. The proximal sections of the true acetabulum, including the pubic and ischial bones, were then exposed. The true acetabulum was widened and enlarged using small reamers (40-44 mm) to address the underdevelopment of the joint. The acetabulum was reamed gradually with hemispheric reamers until the medial wall of the true acetabulum was reached, with bleeding spongious bone. In uncemented cases, a porous-coated acetabular component was placed in the anatomic acetabular position using the press-fit technique, with the option of fixation with domed screws when necessary. In the event that acetabular bone was absent, autograft derived from the resected femoral head was employed to ensure adequate coverage of the acetabular component.

In the present study, subtrochanteric femoral shortening osteotomy was conducted on seven hips (15.9%) with a high dislocation incidence. When STO was performed, aggressive soft tissue loosening was not required. The degree of shortening was determined using preoperative radiographic measurements and intraoperative traction assessments. In one patient with a previous Schanz osteotomy, the shortening was combined with the correction of angulation from the prior procedure and adjustment of femoral anteversion. Following hip reduction, a 4-6-hole dynamic compression plate was positioned on the lateral femoral cortex, and unicortical screws were utilized for fixing the femoral osteotomy. Due to insufficient acetabular coverage in 20 (34.4%) hips, autografts from the femoral head were secured to the superolateral acetabulum using two spongious screws.

2.3. Implants and Fixation

Cemented prostheses were used in 3 (5%) hips, hybrid in 8 (14%), reverse hybrid in 3 (5%), and uncemented in 44 (76%). The external diameter of the acetabular cups varied between 40 mm and 54 mm. No supplementary rings, reconstruction cages, or special structure implants were used in any case.

The acetabular components used were acetabular component with screws (J&J, Aesculap, Zwei-Müller, Osteonisc) using domed screws (screw number varied between 0–3) in 52 cases. Cemented acetabular component (Exeter, Elite Plus) was used in 6 cases. Uncemented femoral component (AML, J&J, Aesculap, Zwei-Müller, Osteonisc, ABG, Medor) was used in 47 cases, and cemented femoral component (J&J ve Exeter) in 11 cases. Femoral head size was 28mm in 35 cases, 22mm in 21, and 32mm in 2.

2.4. Postoperative Care

Postoperatively, the patients were positioned supine, and a triangular pillow was placed between the legs to prevent hip adduction and internal rotation. Drain was removed after 24-48 hours, and the sutures were removed on the 15th day.

Prophylactic antibiotics were initiated one hour before surgery and were given twice daily for a duration of three days. Thromboembolism prophylaxis was administered as subcutaneous low-molecular weight heparin injection for 5 days, then 300 mg oral acetylsalicylic acid for 6 weeks. Active leg exercises were performed without the use of anti-embolic stockings.

Partial weight-bearing with underarm crutches was permitted for the first 6 weeks postoperatively, and in the subsequent weeks full weight-bearing was allowed. Patients who underwent acetabular grafting and shortening were permitted partial weight-bearing in the 6th week and full weight-bearing was reached gradually after 12 weeks.

Patients were discharged from hospital at mean 5 days (range, 4-8 days) and were then called for follow-up at 1.5 months, 3, 6, and 12 months postoperatively, and annually thereafter.

2.5. Clinical Evaluation

At the preoperative and postoperative examinations, all the patients were evaluated according to the modified Harris Hip Score (3) (Table 1). The strength of the abduction was evaluated using the Trendelenburg test.

2.6. Radiographic Assessment

Anterior-posterior (AP) and lateral radiographs of all the hips were taken preoperatively and postoperatively. On the AP radiographs, a line was drawn from the teardrops and the vertical distance of the centre of the femoral head from this reference line was compared (4). Horizontal migration was determined as the distance of the centre of the femoral head from the teardrop along this line. Acetabular inclination was measured on AP radiographs and recorded.

Evidence of loosening was evaluated according to the radiological follow-up parameters of Callaghan on the AP pelvis radiographs taken postoperatively and at the follow-up examinations. Radiolucent lines and sclerotic lines were evaluated in the areas explained by Gruen (5), and by Delee (6) and Charnley (7) for the acetabulum. The radiological Brooker classification was used in the classification of heterotopic ossification (8).

2.7. Statistical Analysis

Data obtained in the study were analysed statistically using SPSS software. Preoperative and postoperative continuous data were analysed using the two-sided, matched Student's t-test. Data were presented as mean and range values. A value of $p<0.05$ was accepted as statistically significant.

3. Results

The mean modified HHS increased from 44.73 preoperatively to 89.28 postoperatively (Table 1). The mean amount of medialization of the centre of hip rotation was 24.06 mm, and the mean distal placement was measured as 32.14 mm. Union was observed in all the structural grafts taken from the femoral head to support the acetabulum, and the STOs healed at mean 12 weeks (range, 10-14 weeks). The complaint of limping was present in all the patients preoperatively, and this complaint was completely resolved after surgery in 34% and partially improved in the others. The acetabular cup size used varied between 40 and 52 mm (mean 44mm). The mean inclination of the acetabular cup was measured as 48.23° (range, 37°-58°).

3.1. Complications

A total of 28 (48%) complications were observed in 22 patients (Table 2). These complications developed during the operation in 7 (12%) cases and postoperatively in 21 (36%). Femoral fissures occurred in 5 cases intraoperatively, which were addressed with cerclage wiring and patients were not permitted to bear weight for 6 weeks. Union was observed in all the cases during follow up. During reaming of the acetabulum in one patient, a medial wall fracture developed, so autogenous grafting was applied. Union of the fracture was observed during follow up and there was not seen to be any problem with the acetabular component.

Table 2. Complications

	Early/mid-term n (%)
Infection	1 (%1.7)
Deep Vein Thrombosis	1 (%1.7)
Intraoperative Fracture	6 (%10.3)
Polyethylene wear	1 (%1.7)
Hip Dislocation	3 (%5.17)
Lower Limb Length Discrepancy	4 (%6.9)
Sciatic nerve injury	1 (%1.7)
Anterior thigh pain	1 (%1.7)
Heterotopic ossification	4 (%6.9)

Lengthening of the leg on the side to which THA was applied was seen to be 2cm in two patients, 3cm in one, and 4cm in one. Postoperatively, a lesion developed in the peroneal branch of the sciatic nerve in the patient with 4cm lengthening. This partially recovered in the 20-month follow-up period. The patients with limb length discrepancy of ≥2cm were given a boot to eliminate the shortness.

Dislocation developed in 3 patients. Closed reduction was performed in the case that developed dislocation in the 6th postoperative week. In one patient who underwent femoral shortening, left-side dislocation occurred after 7 days, and revision was performed with open reduction and plate-screw fixation. This patient developed acute renal failure after the operation, then recovered after 3 cycles of hemodialysis.

There was anterior thigh pain in one patient and this complaint decreased by the end of the first year. Heterotopic ossification was determined as Brooker grade 1 in one patient, and grade 2 in three patients. As movement was not restricted to the extent that would prevent daily activities no surgical treatment was performed.

In one patient followed up without pain, polyethylene wear was determined by the 10th year. In all 20 cases that underwent superolateral grafting of the acetabulum, union was obtained and there was observed to be resorption in a 20% section that was non-weight-bearing in 3 cases.

Two-stage revision was recommended for one patient with infection determined in the 6th postoperative month, but the patient refused this treatment. Deep vein thrombosis was determined in one patient in the 3rd week after surgery, and this was treated appropriately.

4. Discussion

In the preoperative and postoperative clinical evaluations of the patients in this study, the most significant improvement was in pain. The results obtained confirmed that hip-origin pain is the primary indication for the application of THA to patients with DDH.

Trochanteric overgrowth may result from avascular necrosis, which occurs when the femoral head fails to grow properly. This can be caused by impaired growth or previous treatments in patients with developmental dysplasia of the hip (DDH) (9). In such cases, the trochanter major moves superior to the centre of the femoral head. In this situation, the neck length of the femoral component can be adjusted to raise the femoral head to a higher level. An increase in offset of the femoral component can help to improve abductor arm function (10,11).

In instances of high dislocations, notable anatomical changes may be observed on either the acetabular or femoral side. The acetabulum is typically shallow and underdeveloped, accompanied by a heightened acetabular anteversion angle and diminished anterosuperior bone stock. Additionally, the femoral head is smaller, and the neck is shorter. The femoral canal is narrow and straight, and the trochanter major is situated posteriorly. These structural abnormalities, along with a high hip centre, cause complications such as abductor muscle failure in the adjacent soft tissues, capsular thickening, and the shortening of neurovascular structures, resulting in improper placement (12). Femoral shortening addresses these issues by protecting neurovascular structures, correcting excessive femoral anteversion, and properly positioning the abductor arm laterally (8,13–17).

For patients with DDH, the acetabular component should be positioned in the true acetabulum where the bone stock is optimal (7,16–18). Hartofilakidis et al. (19) found that placing the acetabular component in the false acetabulum causes the moment arm of the body's center of weight to be longer than that of the abductor mechanism. This results in a reduction of strength in the abductor arm and increased loading on the hip. In the current study, due to the positive effects on the abductor mechanism of taking the centre of hip rotation medial and

inferior, the acetabular component was placed in the true acetabulum, at mean 32 mm distal and 24mm medialised.

In the long-term follow up of 129 dysplastic hips applied with THA, Linde et al. (20) reported loosening at the rate of 13% in components placed in the true acetabulum, and at the rate of 42% in components placed in the false acetabulum. In the current study, the acetabular component was placed in the true acetabulum in all the cases.

Garvin et al. concluded that to reduce exposure of the graft to stress and provide sufficient bone support to the prosthesis, it is necessary to use a small acetabular component and to achieve medial placement of the acetabular component (21). To be able to have sufficient acetabular coverage, 80% of the acetabular component must be covered with intact bone (22,23). The remaining section is grafted with femoral head autograft (the spongious part of the graft to come to the subchondral bone). In cases where femoral head autograft is used, grafting is made from bone preferably taken from the femoral neck if the head is cystic. Iano and Matsuno stated that corticocancellous graft taken from the femoral neck is superior to femoral head graft (24). In the current study, as acetabular coverage was insufficient in 20 (34.5%) cases, autograft taken from the femoral head was fixed with two spongious screws to the superolateral of the acetabulum. The autografts used supported a 30-40% section of the acetabular component. Although radiological signs of graft union emerge in 2-4 months, a period of 4 years is required for complete fixation (24,25). During the radiological follow up, there may be resorption in the weight-bearing region of the graft (25). In the current study, resorption was observed at the rate of 20% in the non-weight-bearing section of the graft in 3 (5.17%) cases.

The complication rate following THA applied to patients with DDH is higher than that of cases with primary osteoarthritis (26–28). In a study by Ferguson, the revision and complication rates were found to be higher in patients who had previously undergone femoral osteotomy (29). There is a high risk of fracture forming in the femur during placement of the prosthesis or in the reaming procedures associated with femoral deformities in dysplastic hips. In fractures including the proximal third of the femur, the fracture region is opened, anatomic reduction is performed, and cerclage is applied (30,31). A fracture that did not pass the trochanter minor developed in 3 (6.81%) of the current study cases, all of whom were treated with cerclage following reduction.

Nerve lesions following THA have been reported at the rate of 0.5-2%, and this rate has been reported as 3-15% in patients with dysplasia (32–38). Lesions in the peroneal branch of the sciatic nerve constitute 80% of all nerve lesions. When lowering the acetabulum to the true level, femoral shortening may be necessary to reduce the risk of sciatic nerve lesion. To avoid increasing the risk of sciatic nerve lesion, the femur should generally not be lengthened by more than 4 cm (14,21,35). In one case in the current study, a lesion developed in the peroneal branch of the sciatic nerve, and this showed improvement in the 20-month follow-up period.

Femoral shortening is not limited to the protection of neurovascular structures, but also provides correction of excessive femoral anteversion and lateral placement of the abductor arm (14,15). By removing the thin femur segment seen in some cases and good fixation, subtrochanteric osteotomy provides easier placement of the femoral component. In 7 patients with Hartofilakidis Type C in the current study, transverse subtrochanteric femoral shortening osteotomy was performed and fixed with plate-screws.

The dislocation rate after THA in dysplastic hips has been reported to be 5-11% (38–40). The accepted mean incidence for dislocation in all THAs is 2.7%. Yetkin et al. reported that the main determinants of hip dislocation after THA in patients with DDH are previous hip surgery, a high hip centre and a more vertical acetabular inclination, and stated that the surgical approach does not affect the dislocation rate (41). In the current study, dislocation was seen in 3 (5.17%) cases.

Deep vein thrombosis (DVT) is the most frequently seen complication in patients not administered prophylaxis, at a frequency of 50-70%. The rate of pulmonary emboli as a result of DVT is 6-19%, and the rate of deaths due to pulmonary emboli is 1-3%. Mechanical and pharmacological methods are used in thromboembolism prophylaxis. The most common pharmacological prophylaxes are acetyl salicylic acid and low-molecular weight heparin (LMWH). The period of greatest risk for thromboembolism has been reported to be the postoperative second and third weeks (41). In our clinic, LMWH was administered as prophylaxis throughout the hospital stay (mean 5 days), followed by 300 mg acetyl salicylic acid for six weeks after discharge.

Limitations of this study can be said to be the retrospective design and that the cases, the surgical techniques applied, and the implants used were not homogenous.

Despite the technical difficulties and high complication rates, the aim of THA is to reduce pain and for patients to regain the activities and social life that have been restricted.

Patients must be informed in detail preoperatively about problems that may be encountered such as shortening osteotomy and limb length discrepancy, and care must be taken in respect of the main complications such as dislocation, prosthesis infection, and vascular and nerve damage.

In patients with coxarthrosis that has developed on the basis of DDH, THA seems to be an effective treatment option.

Conflict of interest
The authors declared no conflict of interest.

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Authors' contributions

Concept: H.G., F.İ.P., Design: H.G., A.A., F.İ.P., Data Collection or Processing: A.A., Analysis or Interpretation: H.G., A.A., Literature Search: H.G., Writing: H.G., A.C.B., F.İ.P.

Ethical Statement

Approval was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 31/01/2024 and the number of ethical committee decisions is 2024/39.

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The evaluation of the affect of seventh staging system of esophagus cancers to the prognosis and survival

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Abstract

The exact staging of esophagus cancers are very important for the planning of treatment and analyse of prognosis. The aim of our study is the evaluation of last staging criteria of esophagus cancers and the comparison of sixth and seventh TNM staging systems. The eighty five patients who underwent resection due to esophagus cancer in the Thoracic Surgery Department of 19 Mayıs University, Faculty of Medicine between January 2003 and December 2014 were evaluated retrospectively. The clinical staging and the factors that effects the survival are evaluated both by the 6th and 7th TNM by using radiological imaging and upper gastrointestinal system endoscopy. The postoperative mean survival time is evaluated as 44.46 ± 6.71 months is measured for 85 patients who were resected by the diagnosis of esophagus cancer. The most common cause of admission to the hospital are dysphagia and weight loss. The 76.5 % of the patients are squamous cell carcinoma and 23.5 % of the patients are adenocarcinoma as a histopathological diagnosis of cancer. The most highest postoperative survival (57. 50 ± 27.86 months) is reported after the McKeown surgery. According to the seventh staging system the tumor depth, the number of metastatic nodes and metastasis affect the postoperative prognosis and ratio of survival negatively.It is showed that the effected lymph nodes and the number of effected lymph nodes are the most valuable criterias in the seventh TNM staging system and it is evaluated that more powerful results are gained when it is compared with the sixth TNM staging system.

Keywords: esophagus cancer, stage, resection, survival

1. Introduction

Accurate staging, treatment planning, and prognosis determination are crucial in the management of esophageal cancer. Due to its anatomical features, esophageal cancer often remains asymptomatic in the early stages, leading to delayed diagnosis and presentation at advanced stages. Patients with esophageal cancer are frequently diagnosed at advanced stages, making early detection challenging. The patients with the longest survival in esophageal cancer are those with early-stage disease who undergo surgical treatment. Therefore, accurate staging, treatment planning, and prognosis determination are essential.

Currently, the 7th edition of the TNM classification system developed by the American Joint Committee on Cancer (AJCC) is used for staging esophageal cancers. This system incorporates major changes in TNM categories and takes into account histological grade, tumor localization and histopathological type.

Our study aims to evaluate the 6th and 7th editions of the TNM staging systems for esophageal cancer in terms of treatment, prognosis, and survival. Additionally, we seek to investigate and compare factors influencing survival.

2. Materials and Methods

This study was approved by The Medical Research Ethics Committee with the reference number: (Ref. No. OMU MREC 2015/289). 135 patients who were treated for esophageal cancer in our clinic between January 2003 and December 2014 were evaluated retrospectively. Fifty patients who underwent palliative treatment and were considered “unresectable as a result of preoperative examinations" were excluded from the study. Eighty-five patients who underwent anatomical resection and lymph node dissection were included in the study. Exclusion criteries are shown in table 1.

Table 1. Exclusion criteries

Patients with distant metastases
Presence of distant and/or multiple and/or unresectable extracapsular spread of lymph nodes,
Medically inoperable patients
Patients who refuse surgical treatment

Demographic data of the patients were obtained from the OMU Medical Faculty digital database. Preoperative PET-CT and Brain MRI, routine laboratory tests, pulmonary function tests (PFT), electrocardiograms (ECG), upper gastrointestinal tract endoscopy and esophagus stomach duodenum radiograph were performed in all patients. Patients were evaluated separately according to 6th and 7th TNM staging system according to Thorax CT, PET CT, Brain MRI results. In addition, the effects of gender, age, habits, additional systemic diseases, presence of second malignancy and stage on all outcomes were evaluated. Transhiatal esophagectomy, laparotomy, thoracotomy, mediastinal lymph node dissection, three-site esophageal resection, esophagogastrostomy lymph node dissection were applied to the patients who were evaluated as resectable tumors according to the results. The

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definition of complete resection was evaluated according to the absence of microscopic or macroscopic tumor at the resection margins and complete mediastinal lymph node resection. The patients were called for outpatient clinic control every 3 months in the first year and every 6 months in the following years. In the controls, the patients were evaluated with complaints, physical examination findings, routine laboratory values, 2-way chest X-rays, thorax and abdominal computed tomography and/or PET CT.

The statistic analysis was done with SPSS for Windows 15.0 program. While evaluating the data, continuous variables were expressed as mean ± standard deviation, median (smallest-largest), number (%). Survival time after resection was calculated by Kaplan-Meier test. Log rank (Mantel – cox) analysis test was used to calculate survival times for different variables. Statistical significance level was accepted as $p<0.05$ for all tests.

3. Results

85 patients who underwent esophagectomy-esophagogastrostomy and lymph node dissection with the diagnosis of esophageal cancer between January 2003 and December 2014, were evaluated. All patients underwent open surgical procedures by the surgery types as Ivor lewis (49 patient), Orringer (17 patient), McKeown (4 patient), esophagolaryngectomy (7 patient), esophagojejunostomy (8 patients) were performed, respectively.

57.6% (n=49) of the patients were male and 42.4% (n=36) were female. Postoperative mean life expectancy was calculated as 44.46 ± 6.71 months for both genders ($p>0.05$). The mean age was calculated as 53.93 ± 11.72 (21-76) years. Survival for age was not statistically significant ($p>0.05$).

The most common complaint was dysphagia (95.3%, n=81). Other complaints were weight loss, pain, hoarseness and dyspeptic complaints. Survival according to symptoms was not statistically significant ($p>0.05$).

Although survival decreased in patients with comorbid disease and another primary malignancy, these results were not statistically significant in terms of esophageal cancer ($p>0,05$).

Squamous cell carcinoma was diagnosed in 76.5% (n=65) and adenocarcinoma in 23.5% (n=20) of the patients. The relationship between histopathological type, tumor location and survival (Table 2) was not statistically significant ($p>0.05$).

Table 2. The relationship between tumor location, histopathological type and survival

	Tumor Location	n=85	%	Postoperative mean survival time (month)	1 year survival (%)	5 year survival (%)
Squamous cell carcinoma	Cervical	9	13.8	29.55 ± 13.43	44	11
	Upper Thoracic	13	20.0	39.13 ± 12.09	53	28
	Middle Thoracic	22	33.8	40.49 ± 11.26	54	22
	Lower Thoracic	21	32.3	38.71 ± 11.32	43	28
Adenocarsinoma	Middle Thoracic	2	10.0	37.50 ± 10.96	-	-
	Lower Thoracic	18	90.0	44.72 ± 13.43		

Postoperative mean life expectancy was the highest in patients who underwent McKeown's method. Survival was statistically significant according to the surgical treatment method ($p<0,05$). The most common postoperative complication in the patients in the study group was anastomotic stenosis. All of stenosis were treated by balloon and/or bugia dilatation. Other complications are fistula, infection and hernia. In our study, survival times according to complications were not statistically significant ($p>0.05$).

The patients in the study group were evaluated according to the 6th TNM staging to evaluate the effect of the tumor depth (T) factor on survival. When evaluated according to the 6th TNM staging based on the T factor and postoperative average survival time based on the T factor (Table 3, Fig. 1a), survival was found to be statistically significant ($p<0.01$). As the tumor depth increases, the postoperative average life expectancy decreases.

Table 3. T and survival by 6th TNM

6.TNM T Factor	n=85	%	Postoperative mean survival time (month)	1 year survival (%)	5 year survival (%)
T1	5	5.9	46.20 ± 21.40	60	20
T2	20	23.5	54.16 ± 14.16	64	33
T3	49	57.6	45.56 ± 8.51	75	29
T4	11	12.9	6.00 ± 1.17	9	0

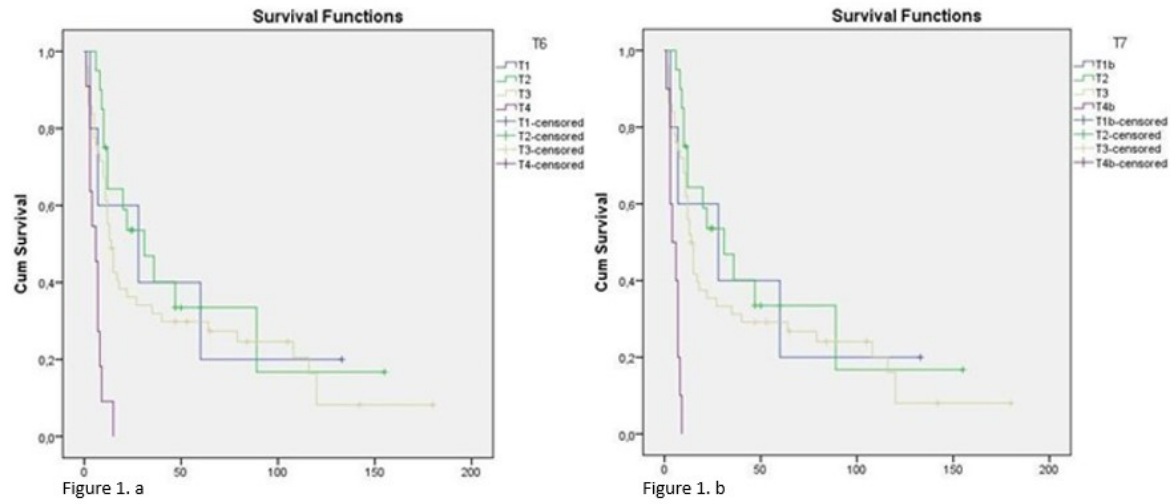


Fig. 1. a. Survival by T in 6th TNM

b. Survival by T in 7th TNM

The patients in the study group were evaluated according to the 7th TNM staging to evaluate the effect of the tumor depth (T) factor on survival. When evaluated according to the 7th TNM staging based on the T factor and postoperative average survival time based on the T factor (Table 4, Fig. 1b), survival was found to be statistically significant ($p<0.01$). As the tumor depth increases, the postoperative average life expectancy decreases.

Table 4. T and survival by 7th TNM

7.TNM T Factor	n=85	%	Postoperative mean survival time (month)	1 year survival (%)	5 year survival (%)
T1b	5	5.9	46.20 ± 21.40	60	20
T2	20	23.5	54.16 ± 14.16	64	33
T3	50	58.8	44.89 ± 8.35	56	29
T4b	10	11.8	5.1 ± 0.83	0	0

Patients in the study group were evaluated according to the 6th TNM staging to evaluate the effect of lymph node involvement (N) factor on survival. When evaluated according to the 6th TNM staging based on the N factor and postoperative average survival time based on the N factor (Table 5, Fig. 2a), survival was found to be statistically significant ($p<0.05$). It can be seen that the postoperative mean life expectancy is significantly longer in those without lymph node involvement.

Table 5. N and survival by 6th TNM

6. TNM N Factor	n=85	%	Postoperative mean survival time (month)	1 year survival (%)	5 year survival (%)
N0	38	44.7	61.43 ± 12.23	73	32
N1	47	55.3	31.28 ± 6.40	40	20

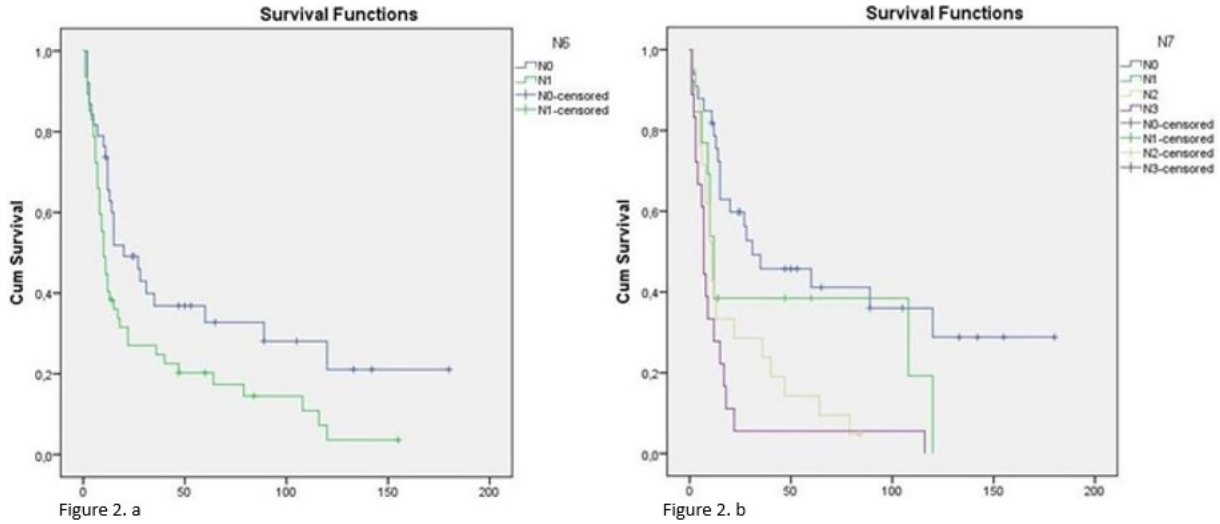


Fig. 2. a. Survival by N in 6th TNM

b.Survival by N in 7th TNM

Patients in the study group were evaluated according to the 7th TNM staging to evaluate the effect of lymph node involvement (N) factor on survival. When evaluated according to the 7th TNM staging based on the N factor and postoperative

average survival time based on the N factor (Table 6, Fig. 2b), survival was found to be statistically significant (p<0.01).The postoperative mean survival time was calculated to be significantly longer in those without lymph node involvement.

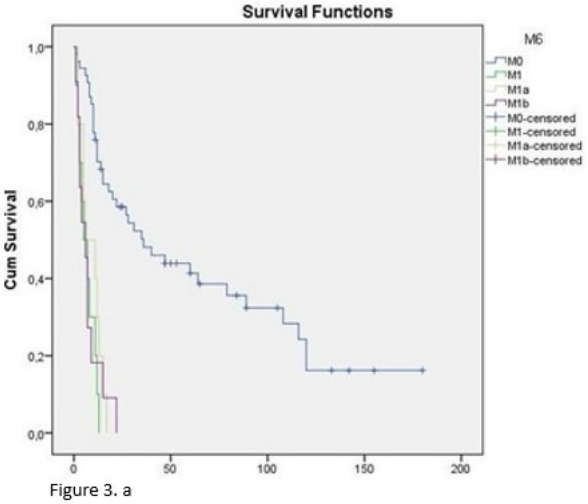
Table 6. N and survival by 7th TNM

7. TNM N Factor	n=85	%	Postoperative mean survival time (month)	1 year survival (%)	5 year survival (%)
N0	33	38.8	76.21 ± 13.83	78	41
N1	13	15.3	48.61 ± 15.23	38	19
N2	21	24.7	22.90 ± 5.42	38	9
N3	18	21.2	14.33 ± 6.15	27	0

Patients in the study group were evaluated according to the 6th TNM staging to evaluate the effect of the metastasis (M) factor on survival. When evaluated according to the 6th TNM staging based on the M factor and postoperative average survival time based on the M factor (Table 7, Fig. 3a), survival was found to be statistically significant (p<0,01). The postoperative mean survival time was found to be significantly longer in those without metastasis.

Table 7. M and survival by 6th TNM

6. TNM M Factor	n=85	%	Postoperative mean survival time (month)	1 year survival (%)	5 year survival (%)
M0	54	63.5	66.03 ± 9.41	70	41
M1a	15	17.6	9.26 ± 1.19	26	0
M1b	16	18.9	5.87 ± 1.39	12	0



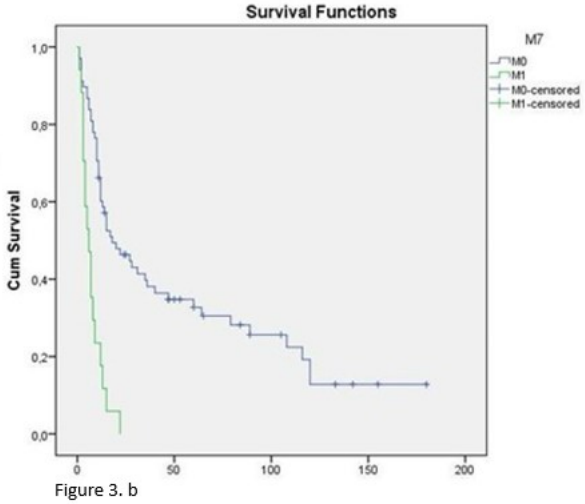


Fig. 3. a. Survival by M in 6th TNM

b. Survival by M in 7th TNM

The patients in the study group were evaluated according to the 7th TNM staging to evaluate the effect of the metastasis (M) factor on survival. When evaluated according to the 7th TNM staging based on the M factor and postoperative average survival time based on the M factor (Table 8, Fig. 3b), survival was found to be statistically significant (p<0,01). The postoperative mean survival time was found to be significantly longer in those without metastasis.

Table 8. M and survival by 7. TNM

7. TNM M Factor	n=85	%	Postoperative mean survival time (month)	1 year survival (%)	
M0	68	80	53.88 ± 8.0	60	32
M1	17	20	7.29 ± 1.33	17	0

The patients in the study group were evaluated according to the 7th TNM staging to evaluate the effect of the Grade (G) factor on survival. When evaluated according to the 7th TNM staging based on the G factor and postoperative average survival time based on the G factor (Table 9, Fig. 4), according to histological grade, although there are significantly longer differences in postoperative average survival times, statistical calculation couldn't be performed due to the low number of Grade 4 patients.

Table 9. Grade according to 7th TNM

Histological Grade	n=85	%
Well differentiated	53	62.4
Moderately differentiated	27	31.8
Poorly differentiated	4	4.7
Undifferentiated	1	1.1

The pathological stages of the patients were staged according to the 6th and 7th TNM by distinguishing the squamous cell carcinoma and adenocarcinoma. Because the tumor location is not taken into account in the staging of

adenocarcinoma according to the 7th TNM, while the stage changes according to the tumor location in squamous cell carcinoma.

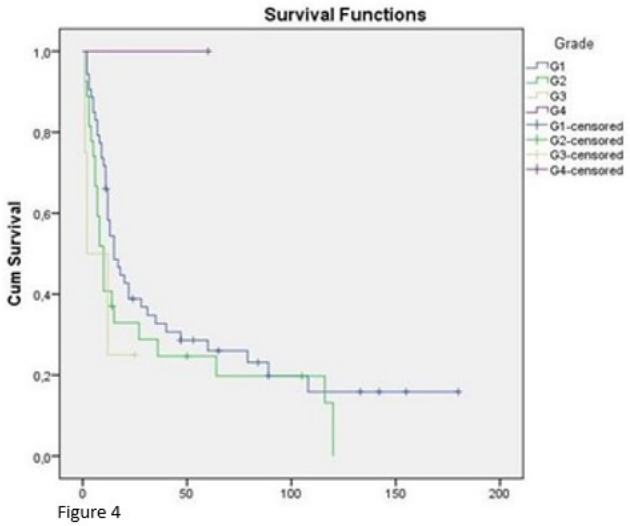


Fig. 4. Survival by grade in 7th TNM

The patients in the study group were evaluated according to the 6th TNM staging to evaluate the effect of pathological stages on survival of patients with squamous cell carcinoma. When evaluated according to the 6th TNM staging based on pathological stages and postoperative average survival time based on pathological stages (Table 10, Fig. 5a), it was found that the postoperative mean survival time decreased significantly as the stage increased, and a statistically significant difference was detected (p<0,01).

Table 10. Stage and survival according to 6th TNM in patients with squamous cell carcinoma

6. TNM Stage	n=65	%	Postoperative mean survival time (month)	1 year survival (%)	5 year survival (%)
I	4	6.1	56.00 ± 24.41	75	25
IIA	21	32.3	67.57 ± 16.08	80	42
IIB	5	7.6	51.40 ± 24.08	60	20
III	11	17	52.97 ± 16.18	45	36
IVA	14	21.6	8.85 ± 1.20	21	0
IVB	10	15.4	4.70 ± 1.25	10	0

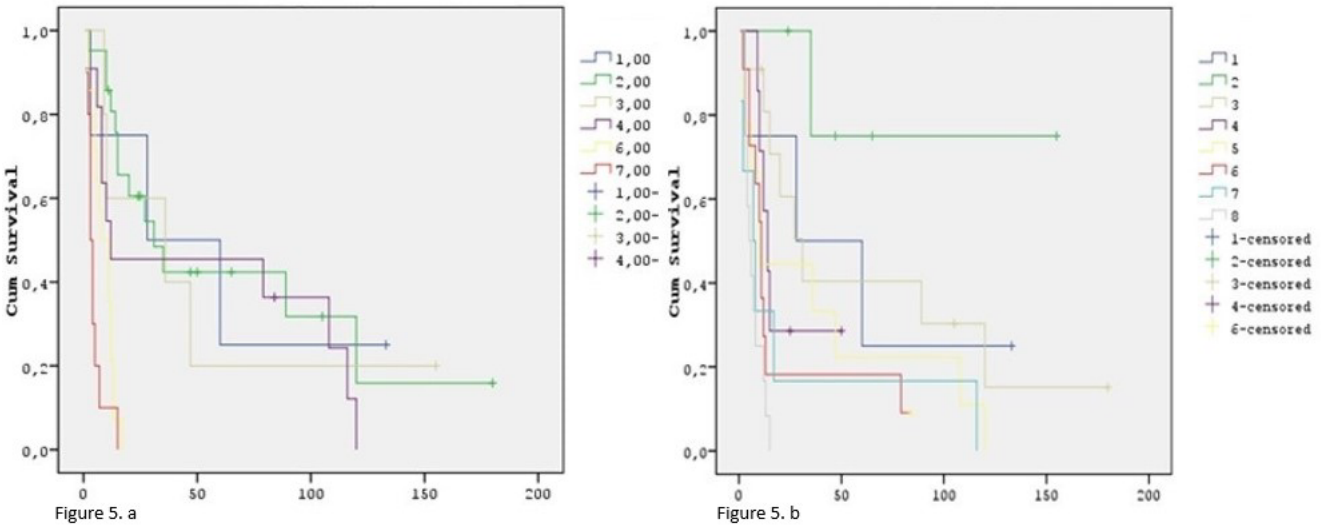


Fig. 5. a. Survival by stage in 6th TNM in patients with squamous cell carcinoma **b.** Survival by stage in 7th TNM in patients with squamous cell carcinoma

The patients in the study group were evaluated according to the 7th TNM staging to evaluate the effect of pathological stages on survival of patients with squamous cell carcinoma. When evaluated according to the 7th TNM staging based on pathological stages and postoperative average survival time based on pathological stages (Table 11, Fig. 5b), a significant difference was found (p<0.01).

Table 11. Stage and survival according to 7th TNM in patients with squamous cell carcinoma

7. TNM Stage	n=65	%	Postoperative mean survival time (month)	1 year survival (%)	1 year survival (%)
IA	4	6.2	56.00 ± 24.41	75	25
IB	5	7.7	125 ± 25.98	-	75
IIA	11	16.9	65 ± 20.53	80	40
IIB	7	10.8	22.85 ± 6.52	57	28
IIIA	9	13.8	37.77 ± 15.35	44	22
IIIB	11	16.9	21.81 ± 8.54	27	18
IIIC	6	9.2	25.16 ± 18.31	33	16
IV	12	18.5	6.91 ± 1.22	16	0

The patients in the study group were evaluated according to the 6th TNM staging to evaluate the effect of pathological stages on survival of patients with adenocarcinoma. When evaluated according to the 6th TNM staging based on pathological stages and postoperative average survival time based on pathological stages (Table 12, Fig. 6), it was found that the postoperative mean survival time decreased significantly as the stage increased and there was a statistically significant difference ($p<0,05$).

Table 12. Stage and survival according to 6th TNM in patients with adenocarcinoma

6. TNM Stage	n=20	%	Postoperative mean survival time (month)	1 year survival (%)	5 year survival (%)
IIA	4	20	109.25 ± 28.36	75	50
IIB	5	25	32.20 ± 10.37	60	40
III	5	25	34.25 ± 11.37	75	25
IVB	6	30	7.83 ± 3.09	16	0

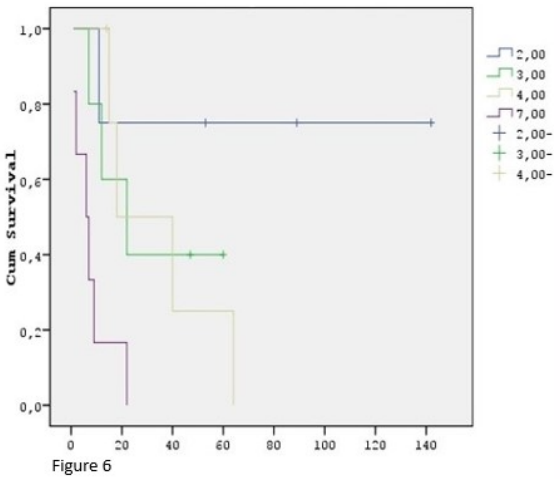


Fig. 6. Survival by stage in 6th TNM in patients with adenocarcinoma

The patients in the study group were evaluated according to the 7th TNM staging to evaluate the effect of pathological stages on survival of patients with adenocarcinoma. When evaluated according to the 7th TNM staging based on pathological stages and postoperative average survival time based on pathological stages (Table 13), it is seen that the postoperative mean survival time decreases significantly as the stage increases, but it could not be calculated statistically in the groups with a low number of patients.

Table 13. Stage and survival according to 7th TNM in patients with adenocarcinoma

7. TNM Stage	n=20	%	Postoperative mean survival time (month)	1 year survival (%)	5 year survival (%)
IIB	9	45	92.39 ± 21.81	88	50
IIIA	1	5	22 ± 0	-	-
IIIB	1	5	40 ± 0	-	-
IIIC	5	25	11.6 ± 2.29	40	20
IV	4	20	4.75 ± 1.93	0	0

4. Discussion

Correct staging in cancer treatment ensures the correct treatment protocol, and the correct treatment ensures successful results. Today, TNM staging system is used in staging of esophageal cancers, based on tumor invasion depth (T), lymph node involvement (N) and systemic metastasis (M).

The study conducted by Manzoni et al. on 116 esophageal adenocarcinoma patients, was demonstrated that advanced age, male gender, and an increased number of involved lymph nodes were negative prognostic factors (1). In our study, the postoperative mean survival time was found to be 57.31 ± 11.86 months in females, 33.42 ± 6.60 months in males, 53.11

± 9.30 months in patients under 60 years old, 28.13 ± 7.50 months in patients over 60 years old and 41.67 ± 7.22 months in patients diagnosed with squamous cell carcinoma, while it was 47.09 ± 13.15 months in patients diagnosed with adenocarcinoma. Although the differences were not statistically significant, similar survival times to the literature were determined.

In their study, Rice et al. Emphasized that previous staging systems based on TNM criteria for anatomical evaluation of T, N, and M were inadequate in terms of cancer biology. They highlighted that besides anatomical criteria, the evaluation of non-anatomical data is also important for survival and prognosis in esophageal cancer staging. The study emphasized that assessing tissue biological activity (histological grade), localization especially in esophagogastric junction tumors and histopathological cell type would lead to more accurate results in determining survival time (2).

In the staging of esophageal cancer concerning the T factor; T insitu was converted to high-grade dysplasia, and all non-invasive epithelial lesions were included in staging. In the 6th edition of TNM, patients classified as T1 were further divided into T1a and T1b. T4 tumors were classified as T4a and T4b. In the 6th TNM, tumors categorized as T4, thus Stage III and IV, were reassigned in the 7th TNM where T4a corresponds to Stage IIIA-Stage IIIC and Stage IV, and T4b corresponds to Stage IIIC and Stage IV. In our study, according to the T factor (independent from histological type), all patients classified as T1 in the 6th edition were classified as T1b in the 7th edition, hence the 5 patients classified as T1 according to the 6th TNM (46.20 ± 21.40 months) were also classified as T1b according to the 7th TNM (46.20 ± 21.40 months), and among the 11 patients classified as T4 (6.00 ± 1.17 months) according to the 6th TNM, 10 were classified as T4b (5.1 ± 0.83 months), and one was classified as T3. It was observed that changes in the T criteria in the 7th TNM from T1a to T1b and T4a to T4b did not make a difference in the number of patients, thus their survival remained the same. In the 6th staging, all patients classified as Stage I remained in Stage IA in the 7th staging, resulting in the same survival (56.00 ± 24.41 months). Therefore, while survival in the 6th staging was Stage IVA: 8.11 ± 1.76 months and Stage IVB: 6.40 ± 2.27 months, survival in the 7th staging was calculated as Stage IIIC: 25.16 ± 18.31 months and Stage IV: 6.91 ± 1.22 months. It was observed that as the T criterion increased, survival decreased, and statistically significant differences were found. In light of our results, when comparing the 6th and 7th stagings, it was observed that changes in the T criterion indicating the depth of tumor invasion did not have a sufficient effect on survival time in the staging system.

In T2-3 N0 M0 patients, survival remained the same according to the T criterion; however, in overall staging, it was observed that while patients were Stage IIA in the 6th staging, in the 7th staging, the stage changed based on grade for adenocarcinoma patients and based on grade and tumor location for squamous cell carcinoma patients. Increased histological grade is associated with decreased survival in early-stage cancers. For adenocarcinoma, differentiation into G1 (well) and G2 (moderate) in Stages I and IIA cancers is more important compared to G3 (poor) differentiation. Differentiation into G1, G2, G3 is also important for Stage I and II cancers for squamous cell carcinoma. Regarding tumor localization (upper-middle-lower thoracic), while T2 - T3 tumors in the lower thorax in the 6th staging shift one stage back in overall staging, the stage remains unchanged for tumors in the middle and upper regions. Although there is no difference in survival according to the T criterion between the 6th and 7th TNM, patients' stages change based on grade and location.

In their study, Kim et al. investigated 202 resected adenocarcinoma patients in terms of monotonicity (decreasing survival with increasing stage), different survival values between different stages, and homogeneity (presence of homogeneous survival values among patients in the same stage). They noted that in evaluations belonging to the 7th TNM system, survival rates decreased as the stage progressed. They also mentioned that although only Stage IIA was considered a more advanced stage compared to IA and IB, it could exhibit better survival values, which might be dependent on the tumor's histological grade. Despite evaluating that G3 had lower survival values compared to G1 and G2, they found that histological grade differences did not create a statistically significant difference according to the 7th staging system (3). In our study, according to tumor localization; when the survival values of 21 patients classified as Stage IIA in the 6th TNM were calculated in the 7th TNM, it was observed that 11 patients remained in Stage IIA and thus had similar survival values (65 ± 20.53 months), 5 patients shifted to Stage IB, resulting in increased survival (125 ± 25.98 months), and 5 patients classified as Stage IIA shifted to Stage IIB, leading to decreased survival (22.85 ± 6.52 months). In our study, survival according to histological grade and cancer localization, based on the obtained stage, exhibited contrasts to the literature as in similar studies (4,5,6).

However, the effects of histological grade on survival were not found to be statistically significant. It was observed that the survival of Stage IIA patients (65 ± 20.53 months) was higher than the survival of Stage IA patients (56 ± 24.41 months). The overall higher survival of patients in Stage IB may be attributed to the insufficient number of data for statistical evaluation. Similar to the results of Kim et al., monotonicity, homogeneity, and different survival rates according to stages were found in our study as well (107). Similar studies in the literature have also not considered histological grade and tumor localization as significant survival factors (4,7,8). Further investigations are required to evaluate the impact of histological grade and tumor localization on prognosis.

In our study, survival time in squamous cell carcinomas varied depending on tumor location, with median survival times observed in the midthorax, upperthorax, lowerthorax and cervical locations respectively. The shortest postoperative survival time was observed in cervical location (29.55 ± 13.43 months). While one-year survival rates showed similar durations, the 5-year survival was lowest in the cervical location. The shorter survival times in cervical and lower thoracic squamous cell carcinomas compared to other locations can be explained by the presence of closely located lymph nodes and a greater number of lymph nodes in these regions. Doki et al. demonstrated similar 5-10 year survival rates for upper, middle, and lower thoracic esophageal squamous cell carcinomas (9). While many studies indicate an increase in survival as tumors progress distally, these studies also include adenocarcinomas at the lower end and gastroesophageal junction, thus not reflecting the effect of localization on survival in patients with thoracic squamous carcinoma (6,10). Results based on cancer localization were not found to be significant (5,10,11).

In a study where Rice et al. evaluated 4627 patients who underwent esophagectomy for esophageal cancer, they found the 5-year and 10-year survival rates for adenocarcinoma and squamous cell carcinoma cases to be very similar according to the 7th staging. In this study, while squamous cell carcinomas and adenocarcinomas showed similar survival rates for Stage III (C, B, A) - II (A, B) and IB, in accordance with staging criteria for esophageal adenocarcinomas, the 5-year and 10-year survival rates for Stage IA and Stage 0 were found to be higher compared to squamous cell type. Unlike squamous cell carcinomas, Stage IA in squamous cell carcinomas has higher values both in terms of 5-year and 10-year survival rates compared to Stage 0 (2). In our study, the survival rates for adenocarcinomas were high (23.5%). According to our study, survival based on tumor cell type was found to be 47.09 ± 13.15 months for adenocarcinoma and 41.67 ± 7.22 months for squamous cell carcinoma.

In a study conducted by Hsu et al., they compared the 6th and 7th staging systems in 392 patients with esophageal cancer. When the 42 patients in Stage I were reclassified according to the new staging, 11 individuals were classified as Stage IA and 31 individuals as Stage IB. While survival was calculated as 70 months in the 6th staging, they reported 122 months for Stage IA and 70 months for Stage IB in the new staging. In Stage IIA, when 122 patients were re-staged, 7 patients were classified as Stage IB, 29 patients as Stage IIA, and 86 patients as Stage IIB. The survival of the 86 patients whose stage increased was calculated as 33 months, while the survival of the 7 patients whose stage decreased was reported as 70 months. The widest distribution of patients was observed in Stage IV; when 74 patients were classified according to the new staging, 9 patients were classified as Stage IIB, 19 patients as Stage IIIA, 20 patients as Stage IIIB, 26 patients as Stage IIIC and 9 patients as Stage IV (4).

In the 7th staging, the definition of regional lymph nodes has been restructured, including cervical and celiac lymph nodes in the paraesophageal lymph node definition. While in the 6th staging, celiac and similar non-regional lymph nodes considered metastases were evaluated as M1a; in the 7th staging, they were determined as N1, N2, N3 based on the number of metastatic lymph nodes. Thus, tumors classified as Stage IVA based on lymph node metastasis in the 6th staging regressed to Stage IIB, IIIA, IIIB, IIIC for N1 and N2 cases in the 7th staging and to Stage IIIC for N3 cases. Evaluation together with lymph node involvement and number allowed for a more precise assessment of patient survival. Retrospective studies have shown that evaluating the number of involved lymph nodes is a better criterion than classifying lymph node involvement as present or absent (12,13). When compared with the 6th TNM system, advanced stages in the 6th staging are corresponded to lower stages in the 7th staging, resulting in longer survival times than expected. Survival times obtained in the 7th TNM were found to be closer to reality. In our study, the survival of 12 patients classified as Stage IVA due to non-regional lymph node involvement in the 6th TNM (8.11 ± 1.76 months) was calculated as follows in the 7th TNM: 1 patient as Stage IIB (22.85 ± 6.52 months), 1 patient as Stage IIIA (37.77 ± 15.35 months), 6 patients as Stage IIIB (21.81 ± 8.54 months), and 4 patients as Stage IIIC (25.16 ± 18.31 months). The values obtained in our study are consistent with the literature and it is believed that the N criterion of the 7th staging is more effective in survival values. In a study conducted by Talsma et al., out of 64 patients classified as Stage 4 in the 6th TNM system due to celiac lymph node involvement being considered metastasis, it was observed that in the 7th staging, 6 patients were Stage IIB, 15 patients were Stage IIIA, 19 patients were Stage IIIB, and 16 patients were Stage IIIC. When survival values were calculated, it was observed that the 7th TNM staging more accurately reflected the survival values of the patients(14).

Xu et al. Reported that the presence and number of pathological lymph nodes are the most important criteria in both the 6th and 7th staging systems (15). Manzoni et al. Emphasized that among these criteria, the number of involved lymph nodes is the most important prognostic factor, and they highlighted the importance of the localization of involved lymph nodes in determining prognosis even when the number is equal. They also emphasized that in the presence of nodal involvement, the significance of tumor depth in assessing survival diminishes (1).

The similarity in survival between N2 and N3 patients explains why Stage IIIB (T3N2M0) and Stage IIIC patients show similar survival rates, which is not surprising. In the 7th staging, lymph node evaluation plays the most important role. Proper lymph node dissection and pathological examination help prevent discrepancies in lymph node staging (4). It has been observed that there is a wide difference in survival rates between N0 and N1 patients. This suggests that N1 patients

might actually include N2 or N3 patient groups due to inadequate lymph node sampling, resulting in significant differences in survival values between the two groups (14). A study by Peyre et al. reported that at least 23 regional lymph nodes should be sampled for the sampled lymph node count to be significant for survival (16). The Western Esophageal Cancer Consortium (WECC) stated that sampling at least 10 lymph nodes for T1, 20 for T2, and 30 or more for T3 and T4 would yield accurate results (17). This effect is not observed in N0 patients, so the number of removed lymph nodes does not create a significant difference in survival values (14).

In the 7th staging, the M factor was redefined, considering cervical and celiac lymph nodes as regional lymph nodes, which resulted in Stage IV patients transitioning to Stage III. In the 6th TNM staging, it was observed that the staging of M1a changed with the N factor, leading to a regression in stage and an increase in survival. On the other hand, in the 7th staging, M1b, indicating other distant metastases, was also considered as M1, resulting in no change in stage, which was consistent with the survival of Stage 4 patients. The survival value of Stage IVB patients in the 6th staging was found to be 6.40 ± 2.27 months, while in the 7th staging, the survival value of Stage IV patients was also 6.40 ± 2.27 months. The current results indicate that evaluating non-regional lymph nodes as M1a and M1b is unnecessary, and only patients with distant metastases should be considered Stage IV patients.

Patients who transitioned from Stage IV to early Stage III showed similar survival to Stage III patients. These patients were considered inoperable in the 6th TNM staging but became candidates for resection or neoadjuvant therapy in the 7th TNM staging (18). Patients with distant metastases were still considered inoperable (8).

Mehta et al. evaluated the prognostic and survival reliability of the 6th and 7th staging systems in 243 patients with esophageal cancer. They highlighted the importance of the TNM classification for both the 6th and 7th staging systems. They found that the changes in the 7th staging system provided more accurate values in determining treatment selection, prognosis and survival rates. They also observed that as the TNM classification worsened and the number of involved nodes increased, survival rates decreased (19). Both Hsu et al. and Gaur et al. confirmed that the 7th staging system was a better model in terms of survival values (4,20).

In our study, when esophageal squamous cell carcinomas were evaluated according to the 6th and 7th staging systems based on T status, grade, tumor localization, lymph node metastasis, and distant metastasis, it was observed that changes in T and M resulted in similar survival values, whereas changes in N factor leading to a decrease in stage prolonged survival time. It was also found that grade and tumor localization were not effective criteria for survival.

In conclusion, the 7th staging system is considered non-anatomic cancer criteria. By incorporating tumor histopathology, localization, and grade into staging, the aim was to improve the quality of life of patients. The 7th staging system is believed to show similarities to the 6th staging system in many aspects. Nevertheless, it is considered superior to the previous staging system in terms of homogeneity, monotonicity and discriminability (4). The 7th staging system has been found to be more successful in determining treatment protocols and predicting prognosis and survival with proportional and accurate estimations. Additionally, we found that evaluating both the presence and the number of involved lymph nodes in the 7th staging system contributes to more accurate results. Cancer staging is a dynamic process and as our understanding of cancer biology improves, staging systems will need to be updated accordingly.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: M.G.P., A.T.Ş., Y.B., A.B., Design: M.G.P., A.T.Ş., Y.B., A.B, Data Collection or Processing: M.G.P., A.T.Ş., Analysis or Interpretation: M.G.P., A.T.Ş., Literature Search: M.G.P., A.T.Ş., Writing: M.G.P., A.T.Ş., Y.B.,

Ethical Statement

This study was approved by The Medical Research Ethics Committee with the reference number:(Ref. No. OMU MREC 2015/289)

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Prognostic spotlight on N1 disease in NSCLC patients and its relation with tumor characteristics

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Abstract

Lung cancer is still one of the most common and potentially lethal cancers. N1 is considering a bad prognostic factor. We aimed to re-identify its prognostic aspects and its relation with the T descriptor. We have operated on 865 patients who were operated on for non-small cell lung cancer (NSCLC) between 2005-2019. Patients with any mediastinal lymph node and distal metastasis were excluded. A total of 667 T1-4N0-1M0 patients were investigated. Survival analysis was made via Cox regression. Five-year survival rate was 72% months in N1 patients. We have shown that increased tumor size and T score were related to higher incidence of N1 positivity T1 has the lowest rate while T3 and T4 have the highest ($p<0.001$). Lymphovascular invasion decreases overall survival (OR:0.540, $p=0.008$). The number of parenchymal and hilar lymph nodes was associated with survival regardless of the lymph node positivity ($p=0.017$). The mean survival of solitary hilar lymph node metastasis 129 months (95% Confidence Interval: 114-143 months) like N0 patients who had overall survival of 133 months (95% Confidence Interval: 125-142 months). Higher T stage is related with higher chance of N1 disease. Surgical and pathological LND from N1 stations are more important in these cases. N1 diseases with STAS and LVI positivity should be assessed with care, and one shall beware with potentially lower survival. Solitary hilar lymph node metastasis with no parenchymal lymph node metastasis had similar survival outcome to N0 patients which may indicate a need for reevaluation in staging.

Keywords: NSCLC, survival, lymph node dissection, parenchymal lymph nodes, N1 disease

1. Introduction

According to the estimations of the American Cancer Society, lung and bronchial cancers are defined as the second most common cancer type in both genders and the cancer type that causes the most deaths (1). Non-small cell lung cancers account for 80% of all lung cancers (2).

Historically, the TNM staging system was first developed by Denoix in 1952 and CF Mountain adapted this classification for lung cancer staging in 1974 and 1997(3-5). The 7th staging system for lung cancer, which was proposed for the first time on an international patient database, was published by the International Association for Lung Cancer Research (IASLC) in 2010 (6). Subsequently, the 8th staging system, based on an expanded dataset of 94,708 cases from 19 countries and 46 centers, was unveiled by the IASLC in 2017 (7).

According to the results of the studies, no change was proposed to T descriptive factors from the previous edition. Sub-classifications of N2 diseases (mediastinal lymph node metastasis) will be proposed including N2a as single mediastinal lymph node metastasis and N2b as multiple N2 disease. No change was offered in terms of N1 (hilar and parenchymal lymph node) metastases (8).

Our study aimed to aimed to analyze the prognostic value of N1 disease, the prevalence and its relation with tumor characteristics.

2. Material and Methods

Non-small cell lung cancer patients who were operated on between January 2005 and December 2019 were evaluated.

2.1. Data Collection

Demographic details, lung cancer types, resection methods, surgical approaches, additional pathological findings such as tumor size, metastatic lymph node stations, presence of lymphovascular invasion (LVI), perineural invasion (PNI), visceral pleural invasion (VPI), spread through air spaces (STAS) etc, TNM stages according to the latest 8th edition, smoking history, follow-up information were recorded. No additional tests or intervention was performed to the patients.

2.2. Patient Population

Patients with small cell components, with insufficient follow-up data and cases who were considered inoperable for either oncological or medical reasons were excluded from the study. There were 804 patients remaining. In addition, patients who received neoadjuvant therapy and patients with any N2 and M1 disease were excluded (Fig. 1). A total of 667 patients (546 males and 121 females) with T1-4N0-1M0 were considered eligible for our study.

The majority of our patients (n=558; 83.7%) underwent

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lobectomy. The most common histopathological subtype was adenocarcinoma with 287 patients and squamous cell carcinoma with 257 patients (43% and 38.5% respectively) (Table 1).

Even though minimally invasive approaches are more common nowadays, since the study includes a wide range of years, open thoracotomy was the leading surgical approach with 471 patients while VATS was performed on 196 patients (70.6% and 29.4% respectively) (Table 1).

Our patient population was mostly in the early stages. Among them 307 patients were stage 1 and 243 patients were stage 2 (45.9% and 36.6% respectively). The percentage of stage 3 patients was 17.5% (n=117) (Table 1).

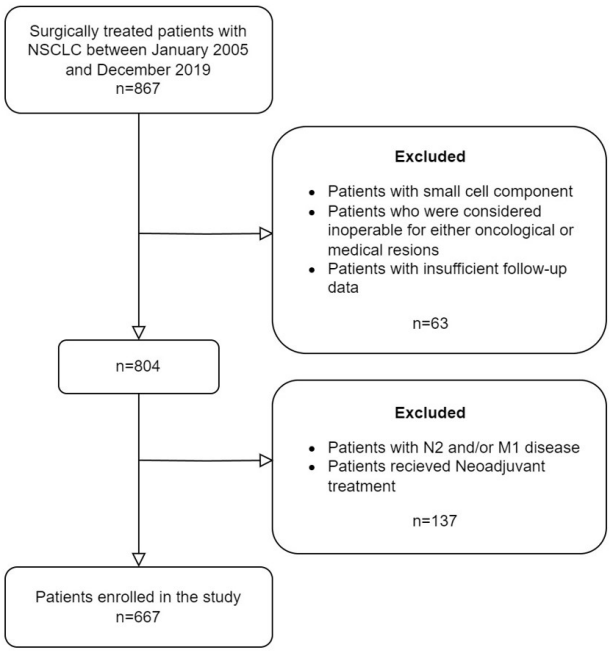

Fig. 1. Flow-chart of patient selection

2.1. Statistical Anaylsis

The Kaplan-Meier method was used for survival analysis. Univariate (log-rank) and multivariate analysis (Cox) were done for the disclosure of prognostic factors. Independent t test or Mann-Whitney U test was performed where appropriate. The relationship between categorical variables was evaluated with the chi-square test. Pearson correlation test was used to analyze the relationship of independent variables.

Continuous variables were shown as mean ±standard deviation and interquartile range. Categorical variables were shown as numbers and percentages. Odds ratios are presented with 95% confidence intervals. SPSS® for Windows version 25.0 (IBM, Chicago, IL, United States) proram was used for statistical analysis. Statistical significance level was accepted as $p<0.05$.

Table 1. Includes patient demographic data. number, mean and Percentage values are given

Patient Characteristics	n (%)
Age	
≤ 65 years	402 (60.3%)
> 65 years	265 (39.7%)
Gender	
Male	546 (81.9%)
Female	121 (18.1%)
Histopathology	
Adenocarcinoma	287 (43%)
Squamous Cell Carcinoma	257 (38.5%)
Carcinoid Tumor	25 (3.7%)
Adenosquamous	22 (3.3%)
Pleomorphic	19 (2.9%)
Large Cell	18 (2.7%)
Mucoepidermoid	10 (1.5%)
Other NSCLC	29 (4.4%)
Resection Type	
Sublobar	12 (1.8%)
Lobectomy	558 (83.7%)
Pneumonectomy	97 (14.5%)
Surgical Method	
Video-Assisted Thoracic Surgery	196 (29.4%)
Thoracotomy	471 (70.6%)
Pleural Invasion	
0	328 (49.2%)
1	187 (28%)
2	75 (11.3%)
3	77 (11.5%)
STAS	
Negative	555 (83.2%)
Positive	112 (16.8%)
Lymphovascular Invasion	
Negative	159 (22.3%)
Positive	518 (77.7%)
Perineural Invasion	
Negative	423 (63.4%)
Positive	244 (36.6%)
T status	
1a	40 (6%)
1b	101 (15.1%)
1c	98 (14.7%)
2a	148 (22.2%)
2b	68 (10.2%)
3	136 (20.4%)
4	76 (11.4%)
N status	
0	496 (74.4%)
1	171 (25.6%)
Stage	
1A1	36 (5.4%)
1A2	93 (13.9%)
1A3	81 (12.1%)
1B	97 (14.5%)
2A	48 (7.3%)
2B	195 (29.3%)
3A	117 (17.5%)

3. Results

The mean overall survival time in all patients was 136 (95% CI: 129-143 months). Five and 10-year survival rates were 72% and 57.3% respectively.

Overall survival was found to be shorter in males (mean 132 months; 95% CI: 124-140 months) than females (mean

146 months; 95% CI: 131-162 months) in all cases (p=0.032).

Patients older than 65 years old (n=246) had a significantly lower survival rate with a mean OS 115 months (95% CI: 103-127 months) compared to that of younger patients (n=375) with a mean OS of 149 months (95% CI: 140-159 months) (p<0.001).

In all patients N1 positivity had lower survival although not statistically significant (OS=110 months, 95% CI: 98-121 months) compared to that of N0 patients (OS=138 months, 95% CI: 129-147 months) (p=0.17). The survival of stage 2 patients (IIA and IIB) was statistically significantly lower overall survival (129 [95% 119-139 months] versus 145 months [95% CI: 134-157 months] (p=0.024)) compared to patients with stage 1 NSCLC.

STAS positivity was found to be a statistically significant prognostic factor (110 ±10 vs 136 10 months; OR: 0.673; 95% CI:0.474 - 0.955) (p=0.025). Similarly, lymphovascular invasion indicated worse survival (128 ±4 vs 138 ± 15 months [RR:2.11 95%CI: 1.42 - 3.20] (p<0.001). Perineural invasion was also found to be a prognosticator cases (n=244, 36.6%) related to lower survival with (121 ±7 vs 140 ±10 months; RR; 0.611; 95% CI: 0.464 - 0.806) (p<0.001).

Pleural invasion is also found to be associated with poor prognosis with significantly lower OS of 117 ±5 months (p<0.001) (Table 2).

Multivariate analysis showed that, older age, male gender, LVI and pleural invasion were independently associated with lower survival (Table 2).

Table 2. Cox regression analysis for overall survival among groups by age, sex, STAS, LVI, perineural invasion and pleural invasion status

	Univariate Analysis			Multivariate Analysis		
	Risk Ratio	95% CI	p Value	Risk Ratio	95% CI	p Value
Age (years)						
≤ 65 / > 65	0.555	0.422 - 0.729	<0.001*	0.548	0.411 - 0.731	<0.001*
Gender						
Female/Male	0.646	0.431 - 0.968	0.032*	0.596	0.382 - 0.932	0.023*
STAS						
Negative/Positive	0.673	0.474 - 0.955	0.025*	0.736	0.505 - 1.071	0.109
LVI						
Negative/Positive	0.473	0.317 - 0.705	<0.001*	0.540	0.342 - 0.853	0.008*
Perineural Invasion						
Negative/Positive	0.611	0.464 - 0.806	<0.001*	0.843	0.623 - 1.141	0.268
Pleural Invasion						
NegativE/Positive	0.572	0.423 - 0.774	<0.001*	0.664	0.486 - 0.908	<0.001*

STAS: Spread Through Air Spaces , LVI: Lymphovascular Invasion , CI: Confidence Interval
*: *p values smaller than 0.05 were considered as statistically significant.*

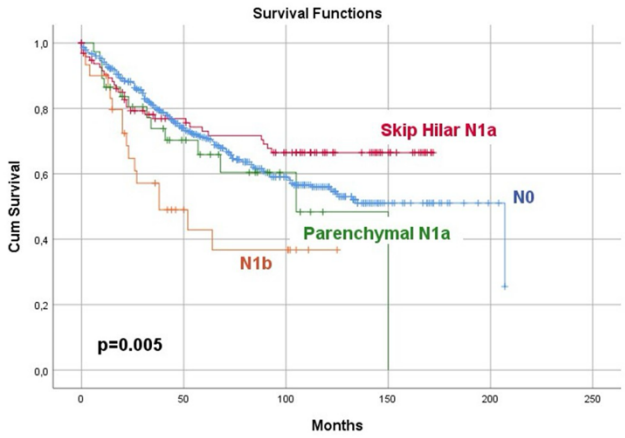

Fig. 2. Survival of NSCLC patients with N0 and skip hilar and other N1 metastasis

Higher T factor was found to be associated with N1 lymph node positivity(p<0.001).

Of patients with N1 disease, 79 patients (46.2%) had solitary hilar lymph node (station #10) metastases, 50(29.2%) and 42 patients had solitary pulmonary lymph node (station 11 to 14) metastases and 42 patients (24.6%) had single(N1a) and multiple N1(N1b) involvement respectively. Overall survival of the patients with single and skip hilar lymph node metastasis was 129 months (95% Confidence Interval: 114-143 months) which is similar to that of N0 patients (overall survival of 133 months [95% Confidence Interval: 125-142 months]) (Fig. 2). Those survivals were statistically significantly better than the overall survival of patients with N1a and N1b disease (p=0.017) (Fig. 2).

4. Discussion

In our study, we investigated the prognostic importance of N1 involvement and histopathological factors in patients who underwent resectional surgery for non-small cell lung cancer. In our study, a strong correlation was detected between the increase in T stage and the presence of N1. There was a higher rate of N1 presence in patients at T3 and T4 stages. Considering this data, it is plausible to perform systematic lymph node dissection with hilar and interlobar lymph node dissection in addition to mediastinal lymph node dissection to avoid undermine the N1 involvement in patients with T3 and T4 tumors. It would improve the accuracy of N staging.

Pleural invasion was one of the negative prognosticators (9,10). It was first included in the T descriptive factors in the 7th staging system (11). Pleural invasion can be designated as PL1, PL2, and PL3 according to the degree of invasion of the primary tumor to the pleural surface (12). A tumor with

invasion at the PL1-2 level is considered an upstage for a tumor of 3 cm or less in diameter from T1 to T2 (12). Invasion at the PL3 level is defined as T3 even for a tumor with 5 cm diameter or smaller (12). In our study, the presence of pleural invasion has been identified as a poor prognostic factor for survival in both univariate and multivariate analyses, independent of T and N stages. Although pleural invasion appears to be associated solely with the T stage, its impact on poor prognosis should not be overlooked.

Lymphovascular invasion and perineural invasion have been included in the pathological evaluation and it provides information about the invasive nature of the tumor (13). In a systematic review and meta-analysis published in 2014, Mollberg et al showed that LVI can be deemed as a prognostic marker in patients with Stage I non-small cell lung cancer (14). Wang et al. recommend that Stage IA NSCLC tumors with LVI should be upgraded to Stage IB (15). In our study, the identification of lymphovascular invasion as a poor prognostic factor in multivariate analyses underscores the importance of tumor characteristics. In addition, prognostic factors such as lymphovascular invasion can play a role in patient follow-up.

Intra-alveolar tumor dissemination, or STAS, was introduced in the WHO classification in 2015 and began to take place in pathological evaluations (16). This new histopathological finding, which has caused controversy since that year, was presented by two large independent cohort studies (17). In these studies, STAS was found to be a poor prognostic element. However, it also was stated that STAS is an artifact that can be encountered during sectioning (18). Blaauwgeers et al. designed a prospective study and argued that mechanical artifacts may arise while obtaining tissue material and observations of tumor cells in the airways should not be considered as an invasion criterion (18). The prevalence of STAS positivity in lung cancer reported differently in various studies ranging between 14.8% and up to 46.3% (19). The low percentage (16.8%) of STAS positivity in our patient group may be due to technical factors or population-based disparity. We confirmed in our case series that positivity for STAS was a poor prognostic finding in the survival analysis. However, in the multivariate analysis STAS presented only a borderline significance.

In addition to the currently accepted modalities in the surgical treatment of Lung Cancer, lobectomy, and systemic lymph node dissection (LND), there is an increasing interest in sublobar resections (20). Segmentectomy is considered a viable option in cases with low respiratory function, without preoperative histopathological diagnosis, as well as in patients with smaller tumors (<2 cm in diameter) and patients with small subsolid lesions (21).

Lymph node staging is considered as a very important prognostic factor in NSCLC cases, and the role of lymph node sampling or dissection for a correct staging has been well defined (22). Some studies suggest examining intersegmental lymph nodes with frozen sections during the operation and proceeding to lobectomy in cases with N1 lymph node positivity (23). If LND is neglected it may lead to under-detection of possible N1 disease and it may lead to failure to apply the correct treatment modality (24). We also have found that patients with T3 or T4 tumors had a higher chance of having N1 disease. Our findings suggest that surgical and pathological lymph node dissection is even more important in these patients since the rate of N1 involvement is higher.

The lung patients with NSCLC with skip hilar lymph node metastasis had a similar survival to those of N0 disease and this survival was significantly better than those with non-skip N1 disease. The mechanism behind this prognostic advantage and verification of our findings needs further studies with larger patient series. There are limitations in our study. It was single-centered trial of surgical NSCLC patients. We also were not able to analyze recurrence-free survival of the patients.

N1 involvement patterns and histopathological factors can be accounted for new (9th) staging system for lung cancer. The first recommendation articles for this new staging system were published in early 2024 and this staging is scheduled to take effect by the year 2025 (8,25).

The results of our study were obtained from a single center, and more accurate results can be revealed with IASLC lung cancer staging project study. Their validity and efficacy can be confirmed by adapting them to larger datasets across the globe. We hope that the results we obtained may be guiding in future staging efforts and other prognosis studies.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: İ.S., A.T., Design: İ.S., A.T., Data Collection or Processing: İ.S., Ş.B., B.K., V.K., E.E., Analysis or Interpretation: İ.S., G.Ö.I., A.T., Literature Search: K.K., A.T., Writing: İ.S., K.K., A.T.

Ethical Statement

This retrospective cohort study was approved by the Medical Research Ethics Committee of Istanbul University-Cerrahpaşa, approval number: 17109671-600-85480) and patients' personal

data were processed in accordance with confidentiality principles according to the Declaration of Helsinki and Patient Rights Regulation.

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Research Article

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The relationship between polypharmacy and quality of life in kidney transplant patients

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Abstract

Chronic kidney disease (CKD) is becoming more widespread globally. Thanks to renal replacement therapy (RRT) methods, the life expectancy of CKD patients is gradually increasing. Today, the notion of quality of life has come to the forefront in evaluating treatment efficacy with increasing life expectancy. Among the RRT methods, kidney transplantation is the one that improves the quality of life the most. Polypharmacy is a concept that has been on the agenda recently and is known to have adverse effects on quality of life. Kidney transplant patients are frequently exposed to polypharmacy due to immunosuppressive drugs and comorbidities, particularly during the initial stages of transplantation. In our study, the KDQOL-SF questionnaire was administered to 128 kidney transplant recipients who visited the Nephrology outpatient clinic between October 2023 and November 2023. Those taking ≥6 medications were defined as the group exposed to polypharmacy. Polypharmacy was considered present in 74 of the patients involved in the study. The mean age of the patients was 51.8 ±11.5 years in the polypharmacy group and 42.4 ±12.5 years in the non-polypharmacy group. In renal transplant patients, the quality of life was notably reduced in the polypharmacy group. The presence of polypharmacy showed a statistically significant correlation with age, comorbidity, multimorbidity, marital status, and primary disease. The group with polypharmacy had notably lower scores in all sub-parameters of the KDQOL-SF. In conclusion, our study determined that the presence of polypharmacy had a negative impact on the quality of life of renal transplant patients. Polypharmacy was found to be associated with age, comorbidity and multimorbidity.

Keywords: kidney transplantation, polypharmacy, quality of life, renal replacement

1. Introduction

The prevalence of chronic kidney disease (CKD) is on the rise due to the increasing elderly population world population. However, the number of patients developing end-stage renal disease (ESRD) is also increasing(1). Treatment methods used to replace lost kidney function relatively are called renal replacement therapy (RRT). The RRT method that improves quality of life the most is renal transplantation(2).

The drug burden in renal transplant patients is relatively high, especially in the early stages of transplantation(3). In renal transplant recipients, immunosuppressive agents often cause polypharmacy, along with other medications needed to manage comorbidities(4). Polypharmacy increases the risk of drug non-compliance and causes acute rejection and poor prognosis(5). Polypharmacy has also been linked to diminished quality of life in patients who have undergone successful renal transplantation (6).

Today, the fact that it is now possible to treat many diseases has led to an increase in life expectancy and living longer with chronic diseases. As a result, the notion of quality of life has come to the forefront in evaluating treatment effectiveness (7). The assessment of quality of life is also carried out through questionnaires in which patient participation is at the forefront and has taken its place alongside physiological measures in evaluating treatment efficacy(8). Although there are many questionnaires used for this purpose, the Kidney Disease Quality of Life Short Form (KDQOL-SF), a scale specific to renal patients, was used in our study.

Studies on polypharmacy in renal transplant patients are very limited in the literature(6). Studies in other disease groups have confirmed that polypharmacy is associated with medication adherence. Such studies are essential to draw attention to the effect of polypharmacy, which has become a significant public health problem, on the quality of life of renal transplant patients. This study aimed to assess the impact of polypharmacy on the quality of life in renal transplant patients.

2. Material and Method

This study included 128 patients who visited Ondokuz Mayıs University Faculty of Medicine Nephrology outpatient clinic between September 2023 and November 2023 and who agreed to participate in the study. Information form and KDQOL-SF v1.3 translated into Turkish and proven effective form were used by face-to-face interviews with the patients. The Excel spreadsheet for scoring the KDQOL-SF available on the website was used to score this questionnaire. Each section is scored between 0-100 in different sub-scores.

Blood pressure, weight and height information were obtained from the nurse interviews with the patients before the

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examination. Complete blood count, basic biochemical parameters, urine protein creatinine ratio, and medication levels on the day the questionnaire was completed were obtained from the hospital information system. Patient statements, anamnesis notes of the patients and biopsy results were taken into consideration while evaluating the etiology of CKD. Patients were evaluated for comorbidities such as diabetes mellitus, hypertension, coronary artery disease and other chronic diseases requiring regular medication. Patients with more than one disease in these defined conditions were defined as having concomitant multimorbidity.

Patients were categorized in terms of the number of drugs they used daily. Although there are various definitions of polypharmacy, patients who used ≥6 drugs previously utilized frequently in studies for polypharmacy were defined as the polypharmacy group. Patients using five or fewer drugs were considered as the non-polypharmacy group.

2.1. Statistical analysis

Statistical significance was determined as $p<0.05$. IBM SPSS 24.0 package programme was used for data entry and analyses. In analyses, the correlation of quantitative variables with polypharmacy status was determined by the Pearson chi-square test. The conformity of continuous variables to normal distribution was evaluated by histogram graphs and Kolmogorov-Smirnov and Shapiro-Wilk tests. If the continuous variables were suitable for normal distribution, the Student-t test was applied for the comparisons of the groups with and without polypharmacy; if the continuous variables were not suitable for normal distribution, the Mann-Whitney u test was applied for the comparisons of the groups with and without polypharmacy.

3. Results

Among the 128 patients in our study, 77 (60.2%) were male and 51 (39.8%) were female. Moreover, among the patients who participated in the study, 95 (74.2%) were married and 33 (25.8%) were single. The mean was 47.8±12.8 years. When the patients were classified according to the number of medications, 74 patients (57.8%) were grouped as polypharmacy group, and 54 patients (42.3%) were grouped as non-polypharmacy group. Age was statistically significantly higher in the polypharmacy group. When the participants were compared regarding polypharmacy status, no statistically significant result was found between gender and polypharmacy status. When the patients were compared in terms of marital status, the presence of polypharmacy was found to be statistically significantly higher in married individuals than in single individuals (Table 1).

When the study participants were evaluated in terms of the number of drugs, it was observed that the patients used an average of 6.7±2.4 drugs. When the participants were evaluated in terms of daily drug load, it was observed that the mean drug load was 11±3.9 tablets.

Table 1. Relationship between polypharmacy status and epidemiological data

		Polypharmacy				p
		None		Yes		
		Number	Percentage	Number	Percentage	
Gender	Male	28	36.4	49	63.6	0.101
	Female	26	51.0	25	49.0	
Marital status	Married	35	36.8	60	63.2	**0.038**
	Single	19	57.6	14	42.4	

When the participants were evaluated in terms of donor type, it was observed that 80 (62.5%) of 128 patients received renal transplantation from living donors and 48 (37.5%) from cadaveric donors. There was no statistically significant difference between polypharmacy and donor type.

The KDQOL-SF questionnaire results of the patients participating in the study were evaluated regarding polypharmacy status. Patients were evaluated under 20 subheadings, including nine under the quality of life related to renal disease and nine under the quality of life-related to general health and SF-12 components. Higher scores in the questionnaire results were evaluated to be associated with better quality of life. When the results of the patients' questionnaires were evaluated, it was found that the non-polypharmacy group had statistically significantly better quality of life in all sub-headings. In other words, quality of life was lower in the group with polypharmacy in every subheading (Table 2).

4. Discussion

Polypharmacy has become a significant health problem today due to increasing life expectancy and accompanying chronic diseases. Polypharmacy is known to be an essential public health problem with an impact on quality of life(9). Renal transplant patients are also exposed to polypharmacy, particularly during the initial post-transplant period, and polypharmacy is considered to affect the quality of life and medication compliance of renal transplant patients. However, there have not been sufficient studies evaluating the impact of polypharmacy on quality of life, especially in the renal transplant group.

Table 2. Relationship between polypharmacy status and KDQOL-SF questionnaire parameters

	PF	Ave.	SD	Med.	Min.	Max.	p
Relationship between Renal Disease and Quality of Life							
Symptom problem list	None	95.2	8.0	98.9	61.4	100.0	**<0,001**
	Yes	87.9	11.7	89.8	38.6	100.0	
Renal disease effect	None	92.8	10.8	96.9	59.4	100.0	**<0,001**
	Yes	80.3	15.8	81.3	25.0	100.0	
Renal disease burden	None	75.6	22.4	81.3	6.3	100.0	**<0,001**
	Yes	49.7	30.7	43.8	0.0	100.0	
Labour status	None	61.1	37.2	50.0	0.0	100.0	**0.001**
	Yes	37.8	40.4	50.0	0.0	100.0	
Cognitive function	None	82.5	16.5	83.3	40.0	100.0	**0.004**
	Yes	73.7	16.9	73.3	33.3	100.0	
Quality of social interaction	None	69.3	11.7	66.7	26.7	93.3	**0.029**
	Yes	64.4	14.4	66.7	20.0	100.0	
Sexual function	None	84.2	16.1	87.5	50.0	100.0	**0.001**
	Yes	65.2	24.8	62.5	,0	100.0	
Sleep	None	82.9	14.2	85.0	42.5	100.0	**0.001**
	Yes	74.4	16.2	72.5	2.5	100.0	
Social support	None	90.7	19.6	100.0	0.0	100.0	**<0,001**
	Yes	71.6	24.5	66.7	0.0	100.0	
Relationship between General Health and Quality of Life							
General health perception	None	27.6	4.3	30.0	20.0	30.0	**<0,001**
	Yes	23.2	5.0	20.0	10.0	30.0	
Physical functionality	None	87.4	18.3	94.4	11.1	100.0	**<0,001**
	Yes	74.5	21.5	77.8	16.7	100.0	
Physical problems	None	75.5	30.5	75.0	0.0	100.0	**<0,001**
	Yes	41.2	38.0	50.0	0.0	100.0	
Pain	None	93.2	14.1	100.0	22.5	100.0	**<0,001**
	Yes	81.9	19.7	90.0	20.0	100.0	
General health	None	73.0	21.0	80.0	15.0	100.0	**<0,001**
	Yes	55.0	25.7	50.0	15.0	95.0	
Emotional well-being	None	74.6	11.9	76.0	44.0	100.0	**0.002**
	Yes	66.2	17.5	64.0	12.0	100.0	
Emotional problems	None	84.6	32.8	100.0	0.0	100.0	**<0,001**
	Yes	57.7	47.2	100.0	0.0	100.0	
Social functionality	None	86.3	19.3	87.5	25.0	100.0	**<0,001**
	Yes	67.4	26.0	62.5	0.0	100.0	
Energy fatigue	None	70.3	16.8	75.0	30.0	100.0	**0.001**
	Yes	60.9	16.8	60.0	25.0	100.0	
SF-12 Components							
SF12 Physical	None	51.5	6.0	54.2	32.1	57.2	**<0,001**
	Yes	46.3	6.7	46.0	32.4	60.7	
SF12 Mental	None	50.6	7.0	52.9	31.0	62.4	**<0,001**
	Yes	44.5	9.6	45.5	26.0	62.5	

In our study, no significant difference was found between male and female gender in terms of the presence of polypharmacy. In a study by Schmidt et al. evaluating the burden of polypharmacy in patients with chronic kidney disease, polypharmacy was more common in females(10). In another study by Wozniak et al., in which polypharmacy was analyzed in renal transplant patients, there was no difference in the frequency of polypharmacy between male and female gender(6). There were different results in the literature regarding the relationship between gender and polypharmacy. In our study, the frequency of polypharmacy was higher in married individuals. In a study conducted in 2021 by Balkhi et al. covering all disease groups, polypharmacy was found to be higher in married participants when all participants were evaluated. When the study was evaluated in the elderly group, no relationship was found between marital status and polypharmacy(11). In our study, since the mean age of the married group was 52±10.4 years and the mean age of the

single group was 35.9±11.6 years, it was interpreted that this relationship was related to age. In our study, a notable correlation was observed between age and polypharmacy. In a study conducted by Adhikari et al. in renal transplant patients, a direct correlation between age and drug burden was reported, and it was thought that this was due to comorbidities increasing with age(12). In a study conducted by Ameri et al. in a group of elderly patients, it was shown that the frequency of polypharmacy increased with age, and in the same study, it was also shown that the frequency of comorbidity increased with age(13). In our study, it was interpreted that the increasing frequency of polypharmacy with increasing age was related to the presence of multimorbidity.

In our study, it was observed that there was no significant correlation between drug levels and polypharmacy. When the literature was reviewed, no other study was found examining the relationship between drug levels and polypharmacy in renal transplant patients. Our study is the first study in the literature in this respect and further large-scale and multicentre studies on this subject will help to clarify the relationship between drug level and polypharmacy.

When the results of the KDQOL-SF questionnaire used in our study were evaluated, both general health-related quality of life parameters, renal disease-related quality of life parameters and SF-12 components were lower in patients exposed to polypharmacy. Wozniak et al. evaluated the effect of polypharmacy in renal transplant patients using the KDQOL-SF, and patients were analyzed in 3 groups according to the number of drugs: those using less than five drugs, those using between 5-9 drugs and those using more than ten drugs and each group was compared with each other. In the study conducted by Wozniak et al., physical functioning, pain, social function, energy/fatigue, burden and impact of renal disease were directly lower in patients taking at least ten medications compared to the other two groups. In other statistical studies, an inverse correlation was found between symptom/problem list, physical functioning, pain, physical problems, general health, social functioning, energy/fatigue and number of medications.

Multivariate regression analyses showed that the number of medications acted as an independent variable for physical, pain, and social functioning. In addition, it was found that drug burden and drug cost harmed quality of life. As a result, Wozniak et al. concluded that polypharmacy negatively affects quality of life in renal transplant patients through the use of the KDQOL-SF quality of life questionnaire (6). In a study published by Adjeroh et al. with CKD patients who were not receiving dialysis treatment, the participants' quality of life was evaluated, and the patients were evaluated in terms of physical and mental components. The study found that polypharmacy was associated with lower quality of life in CKD patients not receiving dialysis(14). Although this study was not conducted in renal transplant patients, the relationship between polypharmacy and quality of life was shown in a CKD patient group, similar to our study.

In conclusion, polypharmacy is one of the factors that negatively affect the quality of life of renal transplant patients. Considering that renal transplantation is the most important RRT treatment that improves the quality of life of patients with CKD, improving the quality of life of patients and its follow-up is vital for patients. We believe that a multicentre study investigating the effect of polypharmacy on quality of life in renal transplant patients with more participants would give us better results.

Our study has demonstrated that polypharmacy reduces the quality of life in kidney transplant recipients. To prevent this, we recommend reviewing the medications used in every visit of kidney transplant patients to assess polypharmacy, discontinuing inappropriate medications, and evaluating the patients' quality of life at regular intervals using appropriate scales.

Our study's limitations include being conducted at a single center and not having a sufficient number of patients, despite being consistent with patient numbers reported in the literature. Additionally, patients' lack of awareness about the importance of survey studies can be considered a reason for their reluctance to participate in the study and respond to the questions.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: F.Ç., A.K., N.A., E.T., Design: F.Ç., A.K., N.A., Data Collection or Processing: F.Ç., A.K., Analysis or Interpretation: F.Ç., A.K, Literature Search: F.Ç., A.K, E.T., Writing: F.Ç., A.K., N.A., E.T.

Ethical Statement

Approval was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 24/08/2023 and the number of ethical committee decisions is 2023/172.

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Serum magnesium and C-peptide levels in Iraqi women with polycystic ovary syndrome

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Abstract

Magnesium (Mg) is the second most frequent intracellular cation in humans with critical role in insulin metabolism and glucoregulation. It is an antioxidant and acts as a cofactor for several enzymes. Evaluate serum Mg levels Iraqi women with PCOS and assess its relationship with C-peptide and BMI. This is a case control study that included 80 women with PCOS and 80 healthy volunteers (as control group) in the age range between 18-45 years old. C-peptide was measured using ELISA technique, while Mg was measured using a spectrophotometric technique. Serum C-peptide levels were significantly higher in PCOS patients compared to the control group. In PCOS patients, serum magnesium concentration was significantly lower compared to the control group. This experiment found no correlation between Mg, C-peptide, or BMI. The ROC analysis area under the curves for Mg and C-peptide were 0.803 and 0.875, respectively. These figures demonstrate analysis method reliability. PCOS patients had lower blood magnesium and higher C-peptide levels. No statistically significant relationship was found between these factors. Due to the low magnesium levels in PCOS women's serum. The correlation between lower serum magnesium and higher C-peptide levels in PCOS patients was not significant. Lower serum magnesium levels in women with PCOS need further study and treatment to determine its role and association with PCOS.

Keywords: magnesium, C-peptide, polycystic ovary syndrome, ELISA

1. Introduction

Polycystic ovarian syndrome (PCOS) is a common and diverse illness characterized by symptoms that vary depending on age and require personalized treatment approaches. The syndrome is defined by excessive levels of male hormones (hyperandrogenism) together with irregular or infrequent menstrual cycles (chronic oligo-anovulation), small cysts on the ovaries (micropolycystic morphology), and impaired ovarian function (1). Approximately 75% of women with polycystic ovary syndrome are affected by anovulatory infertility. Additionally, individuals who undergo in vitro fertilization (IVF) have a higher likelihood of developing ovarian hyperstimulation syndrome. Moreover, it is important to highlight that these women are also at a significantly higher risk of developing gestational diabetes and experiencing miscarriage (2). Polycystic ovary syndrome (PCOS), despite being rather common, is frequently undetected and often requires numerous consultations with different doctors or visits. The process of identifying PCOS usually takes more than a year. The process is quite vexing for the patient. Delaying the identification of comorbidities can hinder the adoption of lifestyle treatments, which are essential for improving PCOS symptoms and improved quality of life (3, 4). Personality disorder 2 (PCOS) is associated with various negative health issues, including cardiovascular risk, depression, obstructive sleep apnea (OSA), endometrial cancer, nonalcoholic fatty liver disease/nonalcoholic steatohepatitis, infertility, and metabolic syndrome. There are different screening recommendations for each of these diseases. However, doctors should be vigilant and conduct further inquiry in patients with polycystic ovary syndrome (5, 6) if any symptoms are present. Functional ovarian hyperandrogenism (FOH) is the primary cause of the majority of PCOS patients. Functional ovarian hyperandrogenism, observed in about two-thirds of instances of polycystic ovary syndrome (PCOS), is characterized by an excessive response of 17-hydroxyprogesterone (17-OHP) to gonadotropin stimulation and abnormal control of androgen production. In cases with residual polycystic ovary syndrome (PCOS) with atypical functional ovarian hyperandrogenism (FOH), there is no excessive response of 17-hydroxyprogesterone (17-OHP). However, an increase in testosterone levels can indicate the presence of atypical FOH if the production of adrenal androgens is suppressed. Approximately 3% of individuals diagnosed with PCOS also exhibit isolated functional adrenal

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hyperandrogenism (7). These individuals do not show any signs of abnormal steroid production; most of them are overweight, which doctors believe may be the reason for their unusual PCOS symptoms. Currently, conducting targeted tests for the FOH subgroup has minimal clinical usefulness (8, 9). Insulin acts as the primary hormone that controls the balance of glucose in the body and the production of lipids. Insulin acts as a hormone that stimulates cellular growth, in addition to its effects on the metabolism of carbohydrates, lipids, and proteins (10). Insulin's effects are carried out by insulin receptors, which are present in multiple tissues along the hypothalamic-pituitary-ovarian axis. Insulin increases the action of trophic hormones that are associated with steroidogenesis in steroidogenic tissues such as the ovary and adrenal cortex (11, 12). Hyperinsulinemia is the main cause of increased androgen synthesis because insulin indirectly enhances GnRH and mimics the effects of LH. Insulin decreases the amounts of sex hormone binding globulin (SHBG), a crucial protein in the bloodstream that regulates testosterone levels. Therefore, lower levels of SHBG would lead to higher amounts of unattached androgens, which are responsible for the appearance of clinical symptoms related to PCOS, such as excessive hair growth, hair loss, and acne (13, 14). There is a lot of documentation available that shows a strong connection between magnesium (Mg) and insulin resistance (IR) in people who have been diagnosed with metabolic syndrome and diabetic mellitus (DM) (15, 16). To be more precise, there is a correlation between a lack of magnesium and insulin resistance (IR). The aim of this study was to assess the serum levels of magnesium and C-peptide in patients with polycystic ovarian syndrome (PCOS), compared to control subjects who did not take magnesium supplements during the study period. Furthermore, explore the correlation between magnesium levels, C-peptide, and body mass index in individuals diagnosed with PCOS.

2. Materials and methods

This is a case-control study that was conducted in Department of Chemistry and Biochemistry at the College of Medicine/Al-Nahrain University and the Gynecological out-patient clinic at AlImmamain Alkadhmain Medical city during the period from 1/12/2021 to 1/9/2022. The participants included 80 PCOS women and 80 controls aged 18–45 years. Women with inflammatory conditions, congenital adrenal hyperplasia, hyperprolactinemia, Cushing's syndrome, and women taking Mg supplementation or other medication, were excluded from the study. Each participant was subjected to a physical examination. All individuals had their height and weight measured being the most widely accepted, the Rotterdam criterion is currently used to diagnose policy. The body mass index (BMI) is measured in kilograms per square meter. The diagnosis of polycystic ovarian syndrome (PCOS) was made based on the Rotterdam criteria. It requires the selection of two of the three following criteria: 1. oligo or anovulation, 2. clinical and/or biochemical signs of hyperandrogenism, and 3. polycystic ovaries (either 12 or more follicles measuring 2–9 mm in diameter, or an ovarian volume of >10 cm3). Approximately 5mL of venous blood was withdrawn from all participants and converted into a serum separated tube (Gel Tube) for magnesium and C-peptide measurement. Serum sample preparation was done by letting the whole blood sit for 20-30 minutes at room temperature (25°C) then the sample was centrifuged at 2000-3000 rpm for 20 minutes. The separated serum was stored in deep freeze (-20ºC) for subsequent measurement of serum magnesium (by using Serum Magnesium kit/ Linear /Germany) and C-peptide (by using Serum Human C-Peptide kit/ BioSource/USA).

2.1. Statistical analysis

SPSS for Windows, version 26, was employed to evaluate the findings (SPSS Inc. Chicago, Illinois, United States). The normality distribution of all variables was analyzed. The findings were tabulated as mean and standard deviation (SD). Following the ANOVA test, the Post Hoc test was employed. Pearson correlation was implemented to investigate the relationship degree between various parameters. $P<0.05$ was designated as the threshold for significance.

3. Results

3.1. Comparison of the study parameters between PCOS patients and controls

This study showed no significant difference in BMI between PCOS (26.10±4.12) and control (26.08±3.43) groups. The mean ages of the control and PCOS groups were (29.61±7.09) and (29.00±6.49) years, respectively. The two-tailed independent samples t-test result was not significant, $t(158) = -0.51$, $p=0.609$, indicating that the mean age was not significantly different between the PCOS and control groups. The level of C-Peptide for PCOS patients (3.53±1.04 ng/ml) was significantly higher than for control group (2.20±0.86 ng/ml), $t(152.69) = 8.79$, $p<0.001$. A two-tailed independent samples t-test was conducted to examine whether the mean of magnesium was significantly different between the PCOS and control groups. The serum level of magnesium for PCOS (1.06±0.23 mmol/L) was significantly lower than for control group (1.48±0.75 mmol/L), $p<0.001$. Instead of using Student's t-test, which is less reliable when dealing with different sample sizes and variances between two samples (Ruxton, 2006) (17), Welch's t-test was used. With an α value of 0.05 and a t-test result for two-tailed independent samples [$t(93.06) = -4.81$, $p<0.001$], the statistical analysis indicates that the null hypothesis can be rejected due to the high degree of significance. The finding demonstrates a substantial difference in the average magnesium levels between the PCOS and control groups. The results are presented in Table 1, while the bar charts illustrating the average values for each parameter may be seen in Fig. 1-4.

Table 1. Comparison of the study parameters between PCOS patients

and controls

Variable	*PCOS*	*Control*	two-tailed independent samples *t*-test		
	M±*SD*	*M*±*SD*	*t*	*p*	*d*
Age (Years)	29.06±6.47	29.61±7.09	-0.51	.609	0.08
BMI	26.15±4.11	26.08±3.43	0.11	.914	0.02
C-Peptide (ng/ml)	3.53±1.04	2.20±0.86	8.79	**<.001**	1.39
Magnesium (mmol/L)	1.06±0.23	1.48±0.75	-4.81	**<.001**	0.76

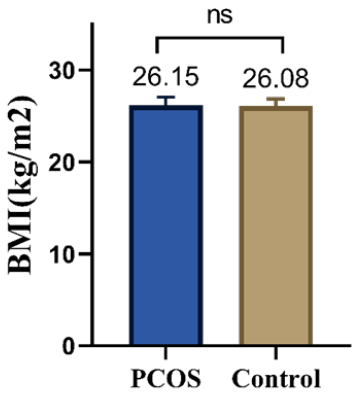

Fig. 1. The mean of BMI (ns: non-significant difference)

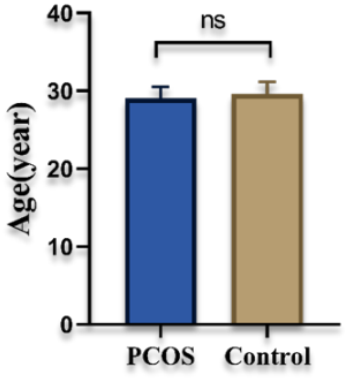

Fig. 2. The mean of Age (ns: non-significant difference)

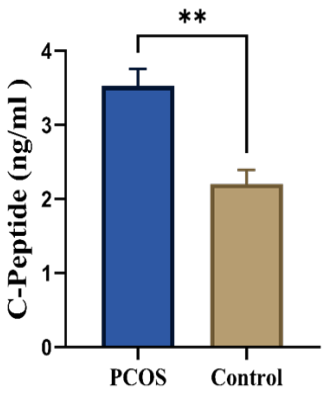

Fig. 3. The mean of C-peptide (**: significant difference)

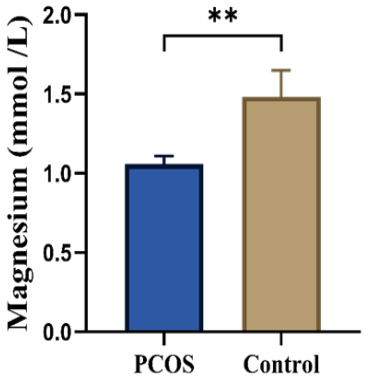

Fig. 4. The mean of Mg (**: significant difference)

3.2. Spearman Correlation Results among Magnesium, C-Peptide, BMI, and Age in PCOS Group

The results pertaining to the relationship between magnesium and C-peptide levels in women with polycystic ovarian syndrome indicate that the connection is feeble and does not possess statistical importance. The Spearman correlation coefficient (r) for this group was -0.03, with a 95% confidence range ranging from -0.25 to 0.19. The value of (p) is 1.000. Likewise, the relationship between magnesium and body mass index (BMI), as well as magnesium and age, showed no statistical significance with correlation coefficients of .13 with similar confidence intervals and p values equal to 1.000. It was also noted that there was no statistical correlation between C-peptide and body mass index, with a correlation coefficient of -.06, a confidence interval of [-.27, .17], and a p value of 1.000. While a strong and statistically significant negative correlation was recorded between C-peptide and age, with a correlation coefficient (r) equal to -.79, a confidence interval ranging between [-.86, -.70] and a value (p) of less than .001. Finally, a weak correlation was found between body mass index and age, with a correlation coefficient equal to .16, a confidence interval between [-.06, .37] and a (p) value equal to 1.000, which indicates that there is no statistical significance for this correlation (Table 2, Fig. 5).

Table 2. Spearman Correlation Results among Magnesium, C-Peptide, BMI, and Age in PCOS Group

Combination	*r*	95.00% CI	*p*
Magnesium-C-Peptide	-.03	[-.25, .19]	1.000
Magnesium-BMI	.13	[-.09, .34]	1.000
Magnesium-Age	.13	[-.10, .34]	1.000
C-Peptide-BMI	-.06	[-.27, .17]	1.000
C-Peptide-Age	-.79	[-.86, -.70]	< .001
BMI-Age	.16	[-.06, .37]	1.000

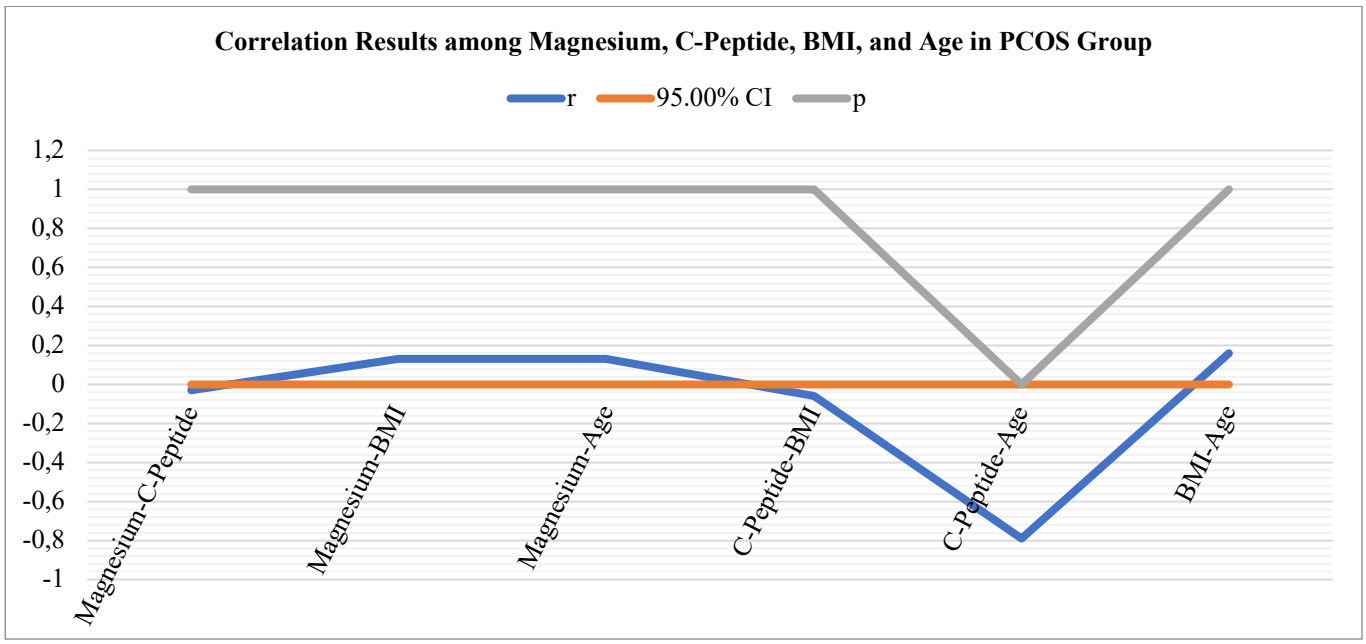

Fig. 5. Spearman Correlation Results in PSOC

3.3. Spearman Correlation Results among Magnesium, C-Peptide, BMI, and Age in control Group

In the statistical correlation study of the control group, the results show a very weak correlation between magnesium and C-peptide levels (r=.03) with a 95% confidence interval of [-.19, .25] and a p-value of 1.000, indicating no evidence A statistic that indicates an association. The relationship between magnesium and body mass index (BMI) was also weakly negative (r=-.06) according to a 95% confidence interval of [-.28, .16] with p=1.000. While there was a weak correlation between magnesium and age (r=.15) with a confidence interval ranging between [-.08, .36] with p=1.000. The results indicated a negative correlation between C-peptide and BMI (r=-.14) with a confidence interval [-.35, .09] and p=1.000. However, the negative correlation between C-peptide and age was more pronounced (r=-.52) with a confidence interval ranging between [-.66, -.34] and a p-value less than .001, indicating a significant and strong statistical correlation between them. Finally, the table showed no statistically significant correlation between BMI and age (r=.10) with a confidence interval [-.12, .32] and p=1.000 (Table 3, Fig. 6).

Table 3. Spearman Correlation Results among Magnesium, C-Peptide, BMI, and Age in control Group

Combination	r	95.00% CI	p
Magnesium-C-Peptide	.03	[-.19, .25]	1.000
Magnesium-BMI	-.06	[-.28, .16]	1.000
Magnesium-Age	.15	[-.08, .36]	1.000
C-Peptide-BMI	-.14	[-.35, .09]	1.000
C-Peptide-Age	-.52	[-.66, -.34]	< .001
BMI-Age	.10	[-.12, .32]	1.000

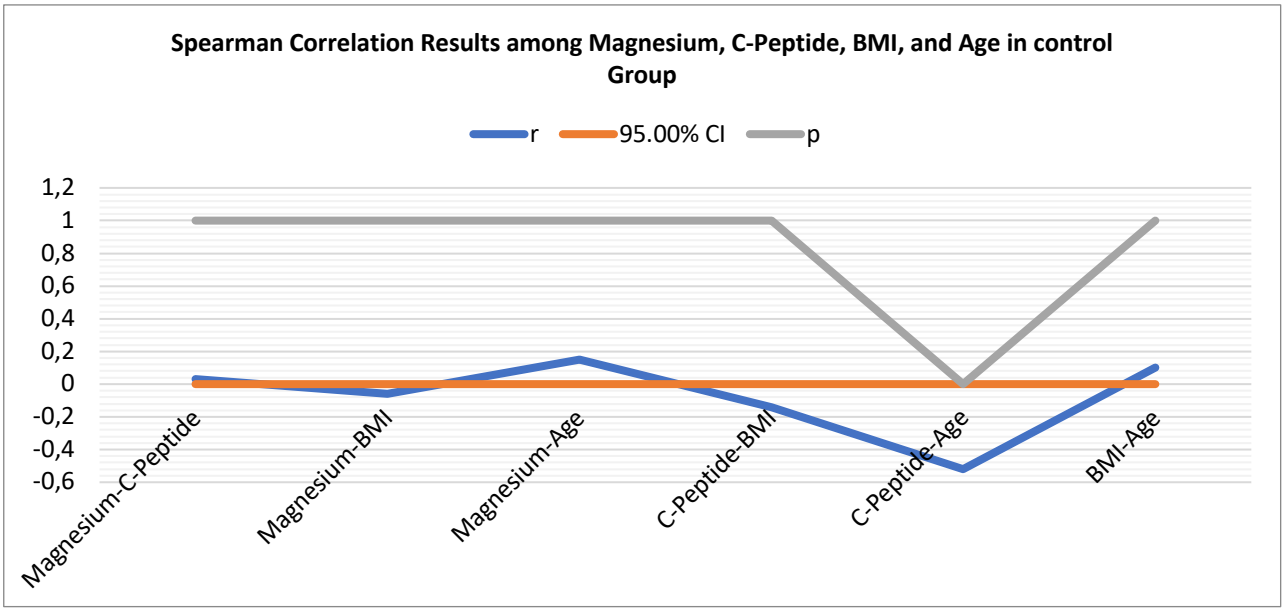

Fig. 6. Spearman Correlation Results in control Group

3.4. ROC curve characteristics of the study parameters as they classify PCOS from control subjects

The ROC curve characteristics of the study demonstrate its ability to classify polycystic ovary syndrome (PCOS) cases from controls, with magnesium levels having an area under the curve (AUC) of 0.803 with a standard error (SE) of 0.0352 and a 95% confidence interval extending from 0.733 to 0.862. The p value was less than 0.001, Cutoff criterion ≤1.184, and showed a sensitivity of 81.25% and a specificity of 73.75%. In contrast, C-PEPTIDE levels showed a higher AUC value of 0.875 with a SE of 0.0286, a 95% confidence interval between 0.814 and 0.922, and a p value of less than 0.001, and the criterion was set at (Cutoff) >2.592, with a sensitivity of 87.50% and a specificity reported at 80.00. %. These results indicate a high efficiency of these indicators in distinguishing between the study group and the control group regarding polycystic ovary syndrome (Table 4, Fig. 7 and 8).

Table 4. ROC curve characteristics of the study parameters as they classify PCOS from control subjects

Variable	AUC	SE	95% CI	p	cutoff	Sensitivity	Specificity
Magnesium	0.803	0.0352	0.733 to 0.862	<0.001	≤1.184	81.25	73.75
C-peptide	0.875	0.0286	0.814 to 0.922	<0.001	>2.592	87.50	80.00

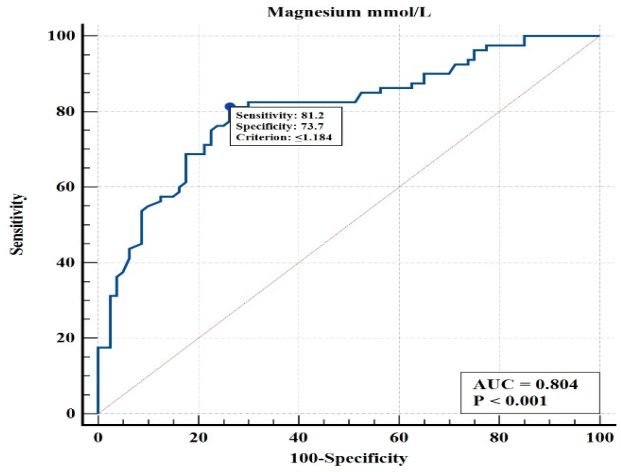

Fig. 7. ROC curve analysis of Mg as it differentiates between PCOS from control groups

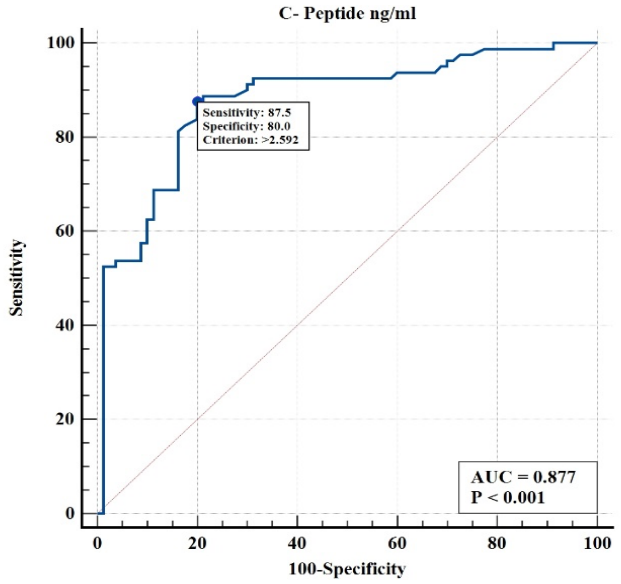

Fig. 8. ROC curve analysis of C-peptide as it differentiates between PCOS from control groups

4. Discussion

In this study, the serum level of C-peptide was significantly higher in PCOS patients compared to control group. This study agrees with a study done by Momo et al. (18), in which women with PCOS had a high C-peptide level and HOMA-IR index compared to matched controls suggesting greater insulin resistance. As anticipated, the PCOS group exhibited significantly higher C-peptide levels, which are indicative of insulin production, compared to the control group. This suggests the presence of hyperinsulinemia in this particular population. Women with PCOS exhibit elevated rates of basal insulin secretion. On the other hand, when faced with a high amount of glucose, the body's ability to produce insulin is usually not enough, resulting in a lower glucose disposition index compared to individuals of the same age and BMI who do not have this issue (19). Polycystic ovarian syndrome (PCOS) is primarily characterized by the presence of hyperandrogenism, hyperinsulinemia, obesity, and insulin resistance (IR). These manifestations are driven by many pathogenic pathways. A multitude of infrared markers have been assessed. The connecting peptide (Pep-C) is secreted in an equimolar ratio with insulin from pancreatic beta cells straight into the portal circulation. It plays a role in the folding, assembly, and processing of insulin. Additionally, it can serve as an alternate marker for insulin resistance (IR) and malfunction of beta cells. An increase in C-peptide levels indicates insulin resistance because it is considered important in the diagnosis for various reasons, including lack of metabolism in the liver and a longer period of work or life (5). In this study, it was found that magnesium levels were lower in the case group compared to the control group, and these results are consistent with Babapour et al. (20), The results also showed that magnesium levels in women with obesity, diabetes, and polycystic ovary syndrome were lower than those in thin women from the same group. This indicates the important role of magnesium in obesity and diabetes and its relationship with inflammatory conditions that can affect its levels in the blood. Furthermore, our understanding of the therapeutic potential of magnesium in treating endocrinological illnesses is constantly evolving. Rajeswari et al. (21) suggest that a deficiency in magnesium can lead to various biochemical issues associated with the reproductive abnormalities of PCOS. A separate investigation conducted by Rasoul et al. (22) reveals that individuals diagnosed with PCOS

exhibit significantly reduced levels of magnesium in their bloodstream. However, they also demonstrate elevated levels of magnesium in their urine when insulin is present. The findings of this randomized clinical trial indicate that administering a daily dose of 250 mg magnesium to women with PCOS did not yield substantial improvements in their hyperandrogenism, hirsutism, or sleep quality. The global prevalence of individuals consuming dietary supplements has been steadily increasing (21). However, it is important to note that many supplements may not confer any health benefits and could potentially have enduring adverse consequences (22). Consequently, it would be advantageous for study to examine the efficacy of dietary supplements in treating various conditions, such as PCOS (21, 22). In both the PCOS group and the control group, there was no correlation seen between the levels of magnesium in the serum and either C-peptide or body mass index (BMI). There is no statistical significance in magnesium levels, fat levels, insulin sensitivity, and sugar levels in women of reproductive age who suffer from polycystic ovary syndrome, and this is what we found Kaufmann and colleagues (14). This confirms the data we have found and the results shown Marj et al. (23) Magnesium supplements do not have a significant effect on lipid levels and blood sugar levels in women with polycystic ovary syndrome. On the contrary, according to other results, the levels of magnesium and C-peptide were under the curves of 0.803 and 0.875. By examining the deterministic values and the sensitivity and specificity ratios, this confirms Accuracy of results and levels reached in the research. A subsequent limited investigation revealed that even lean individuals with PCOS exhibit insulin resistance, so bolstering the concept that insulin resistance may be a core aspect of PCOS. Despite this finding, the existing diagnostic criteria for polycystic ovary syndrome (PCOS) continued to prioritize hyperandrogenism and ovarian morphology and function, while disregarding the clinical factors related to glucose metabolism. Consequently, there is a significant variation in metabolic diversity among the four phenotypes based on the diagnostic criteria established in Rotterdam. Within the subset of patients with phenotype A, insulin resistance (IR) is the most commonly proposed cause (24). According to recent estimates, 75% of women with polycystic ovarian syndrome exhibit insulin resistance as determined by the hyperinsulinemic-euglycemic clamp technique. Despite the considerable amount of research investigating the connection between obesity, decreased insulin sensitivity, the exact arrangement of this complex and mutually interdependent series of events, including hyperinsulinemia in women with polycystic ovarian syndrome, remains incomprehensible. The frequency of different assessments for insulin resistance (IR) and the absence of definitive evidence about the degree of IR in thin PCOS patients compared to age and BMI-matched controls (25) contribute to the increased level of uncertainty. In polycystic ovarian syndrome (PCOS), a hypothesis similar to that of type 2 diabetes suggests that the series of events begins with compensatory hyperinsulinemia in response to insulin resistance (IR), which temporarily maintains normal blood sugar levels. In individuals with a genetic predisposition, the presence of either a relative or absolute lack of insulin becomes evident as prediabetes or type 2 diabetes progresses (26). During this stage, a substantial part of the illness progression is marked by high levels of insulin in the blood, which directly and indirectly disturb ovarian function, while also affecting unexplained changes (9). Insulin can act as a co-gonadotrophin alongside luteinizing hormone in ovarian theca cells to promote the production of androgens. In addition, insulin inhibits pre-antral folliculation and supports the growth of follicles (27).

Patients with polycystic ovary syndrome (PCOS) showed a drop in serum magnesium levels and an increase in C-peptide levels. However, no statistically significant link was observed between these two variables. Further investigation and therapy are required to ascertain the role of magnesium and its correlation with PCOS, as a result of the reduced levels of magnesium in the serum of women with PCOS.

Ethical Statement

Ethical approval was received from the ethical and research committee of College of Medicine-Al-Nahrain University-Iraq. Informed consent was obtained from all participants (Decision No: 20211048, Date: 9/12/2021).

Conflict of interest

The authors declare there is no conflict of interest.

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Authors' contributions

Concept: N.S.H., W.T.A., E.A.A.K., Design: N.S.H., W.T.A., E.A.A.K., Data Collection or Processing: N.S.H., W.T.A., E.A.A.K., Analysis or Interpretation: N.S.H., W.T.A., E.A.A.K., Literature Search: N.S.H., W.T.A., E.A.A.K., Writing: N.S.H., W.T.A., E.A.A.K.

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Review Article

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Ways of microbial escape from the immune system: A brief overview

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Abstract

Viruses, bacteria and other microbes always try to disable our body system. Our immune system has a complex structure that continuously tries to defend our body against "foreign enemies". Microbes that infect other organisms encounter host immune responses and must overcome or evade innate and adaptive immune responses to successfully establish infection, providing fascinating insights into attack and escape. Many pathogens appear to have developed parallel pathways for escape, and several principles of escape are shared not only among members of the same genus but even among different organisms such as bacteria, viruses, fungi, and parasites. In this article, according to the recent findings, the unique escape strategies of microorganisms will be analyzed in more detail. Part of our immune system is responsible for clearing pathogens, either killing the pathogen directly or marking it for destruction by other immune cells. Therefore, it is possible to develop a new targeted therapy when the mechanism of microorganisms is fully understood. This review describes the mechanisms of interaction between the pathogen and its host in the context of infection.

Keywords: immune system, immune response, infection, innate immunity, adaptive immunity, phagocytosis

1. Introduction

Today, infectious diseases are one of the main causes of death worldwide and a great challenge for biomedical sciences (1). The main function of the immune system of humans and other animals is to protect against infection (2). This complex and coordinated system includes a set of cells, tissues and organs (3). Most of the diseases caused by the infection of bacteria, viruses, parasites and fungi can be classified into two categories of intracellular and extracellular pathogens according to the immunopathological point of view (4). In the face of infection, the innate immune system creates the host's first line of defense and thus plays an important role in early detection and then creating an inflammatory response to the invading pathogen (5). Antigen-presenting cells (APCs), which are equipped with receptors for microbial patterns, often provide the first response against a variety of pathogens (6). Professional phagocytes are recruited to the site of infection, where they form the host cell's first line of defense, tasked with engulfing and destroying pathogens. Therefore, bacteria must resist the bactericidal activity of professional phagocytes, including macrophages, to fight the host's immune system (7). Among other mechanisms of innate immunity against bacteria are complement activation and inflammatory response, and genetic modification of surface antigens is the main process used by bacteria to evade humoral immunity (8, 9). The adaptive immune system is responsible for eliminating pathogens in the final stage of infection and is also responsible for creating immunological memory (5). Bacteria and other pathogens can change chemical pathways in the body and secrete different virulence proteins, called effectors, to limit the host's defense mechanisms by disrupting host cell signaling pathways (8). Innate immunity against viruses is inhibition of infection and killing of infected cells by natural killer cells (NK cells), remaining viruses escape immune control by blockade of antigen presentation, cytokine escape, escape from NK cell activities, escape from apoptosis and antigenic changes (10). One of the general characteristics of proteins that help microorganisms to modulate immunity and actively evade host defenses is their structural and thus functional similarity to host proteins that they effectively mimic. In general, microorganisms create different mechanisms to block the cellular, humoral or systemic immune response (11). As a result, the immune escape of microorganisms from the host's immune system is a complex process that is created by a set of diverse interactions and strategies. Understanding the factors

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involved in this process can help us identify and develop ways to improve and strengthen the host's immune system against microorganisms. This study and research can ultimately help to improve the methods of prevention, diagnosis and treatment of infectious diseases and lead to the improvement of the health of the society and increase the immunity of our body. In the present study, we investigated the various escape routes of opportunistic pathogens from the host's immune system, in order to better understand how infectious diseases develop.

2. A common mechanism for bacterial escape

A common mechanism for bacterial escape from the immune system is their encapsulation, which is carried out by a number of extracellular bacteria that circulate systematically, for example, the capsule of *Streptococcus pneumonia (S. pneumonia)* remains far from access to antibodies and complementary substances and does not have the ability to be opsonized and phagocytized (12). A number of bacteria have the ability to repair and regenerate their cell membranes after the immune system attacks them. By manipulating APCs, these bacteria prevent their migration to the lymph nodes. As a result, cell-related antigens are not presented to cluster of differentiation (CD) 8+ T cells by major histocompatibility complex (MHC) and cell response is not activated (13). Some phagocytosed bacteria such as *Mycobacterium tuberculosis (M. tuberculosis)*, *Listeria monocytogenes (L. monocytogenes)*, *Shigella flexneri (S. flexneri)*, *Francisella tularensis (F. tularensis)* can enter from inside escape the phagosome and enter the cytoplasm. These bacteria prevent the maturation of the primary phagosome. Primary phagosomes initially lack the ability to kill bacteria, and if they merge with lysosomes, they mature and find the ability to produce acid and then kill bacteria. The primary phagosome is formed after the bacteria are engulfed by macrophages. The combination of endocytic vesicles with the early phagosome, as well as the separation of secretory vesicles from it, leads to the formation of the late phagosome. Some bacteria prevent acidification inside the phagosome by preventing the fusion of lysosomes with the phagosome. Then, using some enzymes and secretory systems, they lead to the lysis of the phagosome membrane and finally enter the host's cytoplasm (14). *Listeria* can hydrolyze the phagosome membrane by enzymes listeriolysin and phospholipase and escape to the cytoplasm (15). *Staphylococcus aureus (S. aureus)* is not an intracellular pathogen by nature, but to escape the host's immune system, it has acquired the ability to enter the host's cells. Another escape mechanism of *S. aureus* inside the phagosome is the production of ammonia, which deacidifies and neutralizes the acidic environment inside the phagolysosome (16).

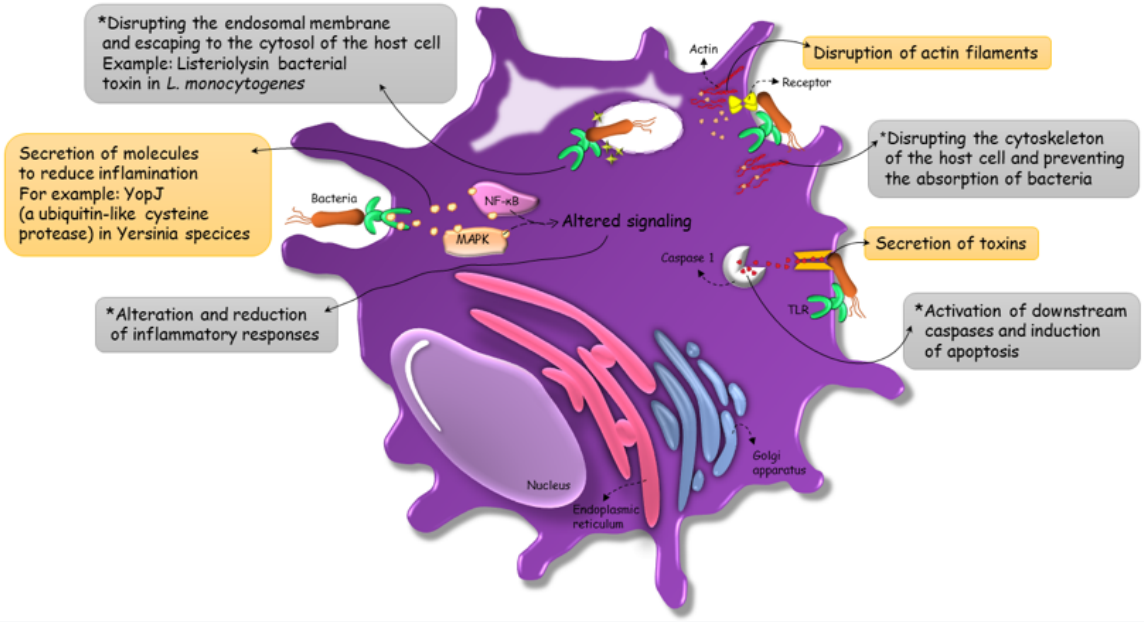

Fig. 1. Some bacterial escape routes from macrophage phagocytosis

NF-κB (Nuclear factor kappa-light-chain-enhancer of activated B cells), MAPK (A mitogen-activated protein kinase), TLR (Toll-like receptor)

In some cases, when *S. aureus* falls into the trap of immune phagocytic cells, reactive oxygen species (ROS) and reactive nitrogen species (RNS) mediators are released inside the phagolysosome. Bacteria neutralize their effect by producing and secreting protective enzymes such as catalase, superoxide dismutase (SOD) and peroxiredoxin (Prx). A number of bacteria can avoid being recognized by pattern recognition receptors (PRR) by making antigenic changes in the surface of flagellum, lipid A and peptidoglycan (17). *Yersinia* species target signaling pathways such as mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB). *Yersinia pestis (Y. pestis)* produces interleukin (IL) 10 by inhibiting Toll-like receptor (TLR) 2/6 signaling. IL-10 is a type of inhibitory cytokine that inhibits the immune response of the host (18, 19). *Salmonella* have a two-component sensor (PhoP, PhoQ). With these sensors, they can regulate their virulence genes (20). Salmonellas create structural changes in the lipid part of their membrane with the mechanisms of deacylation,

palmitylation and addition of amino arabinose. As a result, they create a negatively charged surface of the cell membrane due to the effect of positively charged cationic peptides (21, 22). They also reduce defensin and cathelicidin. Autophagy is one of the body's cell defense mechanisms where cytoplasmic compounds and whatever are inside the cell is broken down and destroyed by lysosomes (23). Autophagy is one of the defense mechanisms of body cells in which cytoplasmic compounds and everything inside the cell are broken down and destroyed by lysosomes. Microorganisms inside the cell are also destroyed by this mechanism, but some intracellular bacteria prevent the autophagy of the host cell by producing proteins in an irreversible way, so autophagy does not take place in the infected cell (24). Some microorganisms escape from the immune system by causing genetic changes and creating antigenic diversity. For example, *Neisseria* species (gonorrhea and meningitidis) have one of the most complete changes, and for this reason it has not been possible to design a vaccine (25) (Fig. 1).

2.1. Escape of extracellular and intracellular bacteria

In Table 1, the virulence of extracellular bacteria is related to some mechanisms that resist innate immunity. Bacteria with capsules rich in polysaccharides resist phagocytosis and, as a result, are more virulent than similar species without capsules (26). In contrast to intracellular bacteria, several strategies have been developed to resist removal by phagocytes. These strategies include:

•Inhibition of phagolysosome incorporation or escaping into the cytosol and thus hiding from the germicidal mechanisms of lysosomes.

•Direct inactivation or clearance of germicidal substances such as ROS. The outcome of infection with these organisms often depends on the interaction between the antimicrobial mechanisms of macrophages stimulated by T cells and the resistance of the microbe to killing. Resistance to phagocyte-mediated removal is also the reason why such bacteria tend to cause chronic infections that may last for years, often recur after complete treatment, and are difficult to eradicate (27-29).

Table 1. Comparison of immune evasion mechanisms of extracellular and intracellular bacteria with example

Extracellular bacteria		Intracellular bacteria	
Example	**Escape Mechanism**	**Example**	**Escape Mechanism**
Neisseria gonorrhoeae	Antigenic change	*Mycobacterium tuberculosis*	Inhibition of phagolysosome formation
Escherichia coli			
Salmonella typhimurium		*Legionella pneumophila*	
Many bacteria	Inhibition of complement		
Pneumococcus	Activation	*Listeria monocytogenes*	Deactivation of reactive oxygen and nitrogen species
Neisseria meningitidis		*Chlamydia trachomatis*	Distruption of the phagosome membrane and escape to the cytoplasm
Catalase positive bacteria (Many bacteria)	Purification of reactive oxygen species		

3. A common mechanism for fungi escape

C-type lectin receptor (CLR), which is a part of the PRR family, plays a major and outstanding role in the identification of fungi (30). According to a general classification, fungi evade the immune system in three ways:

a) Hiding: Chitin and beta-glucan (β-glucan) are components of the cell wall of fungi and are classified as pathogen-associated molecular pattern molecules (PAMPs), molecular patterns associated with pathogens and can be recognized by Dectin-1. Dectins are part of PRR, A number of fungi can hide from dectin-1 by changing the structure of these membrane molecules and cannot be phagocytosed (31). *Candida albicans (C. albicans)* can cover the beta-glucan surface by o-mannan and secrete them (32). *Aspergillus fumigatus (A. fumigatus)*, *Cryptococcus neoformans (C. neoformans)*, *C. albicans* and *Histoplasma capsulatum (H. capsulatum)* have the ability to produce biofilm. Dimorphic fungi such as *H. capsulatum* and *Paracoccidioides* can change from the β-glucan form to the α-glucan form and are therefore undetectable. Biofilms are microbial communities and are two billion years old. Biofilms adhere to surfaces and form a nearly impenetrable mass, thereby keeping fungi out of the reach of the host's immune system (33).

b) Control: Despite all this stealth, most of the time, a healthy immune system will eventually identify pathogens and initiate an appropriate response, at this time, it is the microorganism's turn to fight and control the immune response. *C. albicans* can express regulatory proteins on its surface to control the complement system (34). Complement regulatory proteins include C4b binding protein (C4BP) and factor H. *C. albicans* by expressing a ligand called phosphoglycerate mutase (PGM) 1 that interacts with complement regulatory proteins, inhibits complement regulatory activity and inhibits the immune response .In addition, This fungus can produce and secrete aspartic protease enzymes .Which are completed by the destruction of C3b, C4b and C5 elements (35, 36).

c) Attack: The last way to survive is to secrete toxins. At this stage, the goal of microorganisms is not to escape the immune system, but only to survive. These enzymatic mechanisms include SOD, catalases, glutathione peroxidases (GPX), and non-enzymatic types such as fungal melanin, mannitol, and trehalose, which are toxins against immune system elements (37). The table below shows the escape

mechanism of fungi, which is different from that of bacteria. Some common fungal examples are listed in Table 2.

Table 2. Escape mechanisms of fungi from the immune system

Example	Escape mechanism
Cryptococcus neoformans	Production of polysaccharide capsule to prevent phagocytosis and weaken opsonin effects of complement and antibody.
Candida albicans	Hiding glucan under mannan outer coat to avoid detection by Dectins
Histoplasma capsulatum	Preventing the formation of phagolysosomes and changing the natural path of maturation of phagosomes
Dermatophytes (Trichophyton, Microsporum, Epidermophyton)	Suppression of the host T-mediated response

Cytokine and chemokine responses of phagocytes to fungal stimuli are highly dependent (38). *In vitro*, it seems that TRL2 is the most important TLR for signaling responses to fungi (39). Some fungi are able to enter cells by using phagocyte receptors. This facilitates intracellular parasitism and inhibits mechanisms that are activated in response to opsonins, mannans, and beta-glucans (40-42). *H. capsulatum* entry into macrophages and neutrophils is mediated by the interaction between heat shock proteins 60 (HSP60) on the fungal cell surface and CD18 on the phagocytic cell surface. In contrast, although dendritic cells (DCs) express CD18, they instead utilize very late antigen-5 (VLA-5) to phagocytose *H. capsulatum*. *Blastomyces dermatitidis (B. dermatitidis)* uses a cell wall protein blastomyces adhesin-1 (BAD-1) to access macrophages via complement receptor 3 (CR3) and initiate an anti-inflammatory program that induces pathogen survival (43). *C. albicans* begins to express the yeast flavohemoglobin1 (YHB1) gene when exposed to nitric oxide (NO) produced by macrophages. The product of this gene is a flavoprotein that copes with the stress caused by RNS (44). Cytochrome C peroxidases reduce the effects of RNS against the fungus and force the host to produce less RNS (45). When some fungi enter the phagolysosome, by changing their metabolic patterns, they produce substances that are toxic to macrophages and induce them to undergo apoptosis (46). The immune response against fungi, which are mainly extracellular, is the responsibility of neutrophils, which are the first line of defense of innate immunity. A number of fungi damage the DNA of neutrophils by producing DNase enzymes and escape from them (47). Some fungi interact with their receptor on the surface of leukocytes by expressing a ligand called pH-regulated antigen 1 (Pra1) and prevent leukocytes from sticking to the surface of fungi (48). Typically, *Pneumocystis jirovecii (P. jirovecii)* takes over the human lung and adapts to live. In immunocompromised individuals, it can develop into a deadly pathogen and cause pneumonia in the correct circumstances. *P. jirovecii* frequently evades the host's immune system's destruction by switching to the major surface glycoprotein (MSG) antigen (49, 50). In contrast to other pathogenic fungi, *P. jirovecii* lacks chitin and glucan in some cell cycle stages, which may inhibit the host's innate and acquired immune responses (51) (Fig. 2).

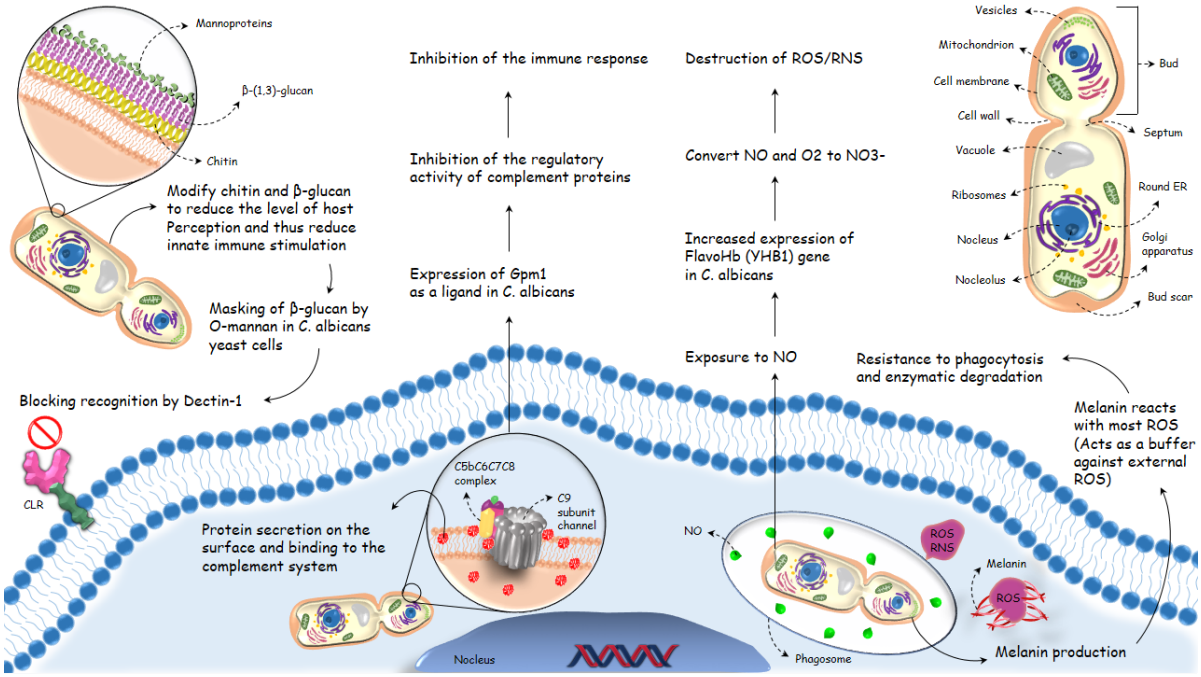

Fig. 2. A summary of anti-immune mechanisms of fungi
CLR (C-type lectin receptors), ROS (Reactive oxygen species), RNS (Reactive nitrogen species), PGM (phosphoglycerate mutase), ER (Endoplasmic reticulum), YHB1 (Yeast flavohemoglobin1), NO (Nitric oxide).

4. A common mechanism for virus to escape

Viruses are all obligate intracellular parasites and have various strategies to escape from the elements of the immune system (52), such as molecular mimicry of host cell surface receptors, complement inhibitors and leukocyte activity regulators. One of the important mechanisms that exists in viruses, bacteria and a number of parasites is mutation in genomic nucleotides, which leads to antigenic changes. This practice is known as antigenic variation, which misleads acquired immunity. In fact, it is a kind of bypass of humoral and cellular immunity. The most common antigenic changes in viruses are changes in surface glycoproteins (53). Mutations in RNA viruses are more than in DNA viruses. Among the types of mutations, we can mention the point mutation, which is more common in viruses. The reason is that the frequency of RNA replicase mutations is higher than that of DNA polymerase. Due to the fact that influenza viruses have several hosts of different species, they can perform genome rearrangement in the cells of these hosts and become recombinant which is different from their early ancestors (54). A number of viruses, such as Epstein-Barr virus (EBV) and Herpes simplex virus (HSV), can remain alive inside the host's target cells for years and even until the end of a person's life and start multiplying if optimal conditions are created (55). Some viruses can avoid the attack of the immune system by mimicking the proteins and receptors of the host cells. Given that NK cells have activated receptors, some viruses can bind to the inhibitory receptors of NK cells by producing specific bonds and preventing their activation (56). Viruses, like some bacteria and fungi, have protective mechanisms to counter the antimicrobial action of NO and free oxygen radicals of active macrophages. After entering the host cell, some viruses cause the expression of complement membrane regulating molecules such as decay-accelerating factor (DAF) and major capsid proteins (MCP) on the surface of the membrane of the infected host cell. By affecting macrophages, some viruses prevent the expression of endosomal TLRs or prevent the transmission of TLRs, interferon regulatory factor 3 (IRF3), NF-κB messages in the downstream areas (56-58). When HSVs enter the target cell, they inhibit apoptosis by inhibiting caspase 3, and as a result, they start multiplying easily inside the infected cell. There is a surface glycoprotein called C1 in HSV that binds to the C3b component and inhibits it (59). Some large DNA viruses can mimic cytokine molecules to bind to specific receptors on the target cell. This increase in the number of cytokines increases the replication of the virus and the immune response is inconsistent in this case. In some cases, when antiviral cytokines are produced, viruses block the receptors of those cytokines so that the antiviral immune response does not take place (60). RNA viruses that are located in the cytoplasm of host cells are recognized by cytoplasmic receptors such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) PRRs and an appropriate immune response is given, but these viruses make nucleotide changes in their genome in order to escape from this immune response. As a result, cytoplasmic receptors cannot recognize them (61) (Fig. 3).

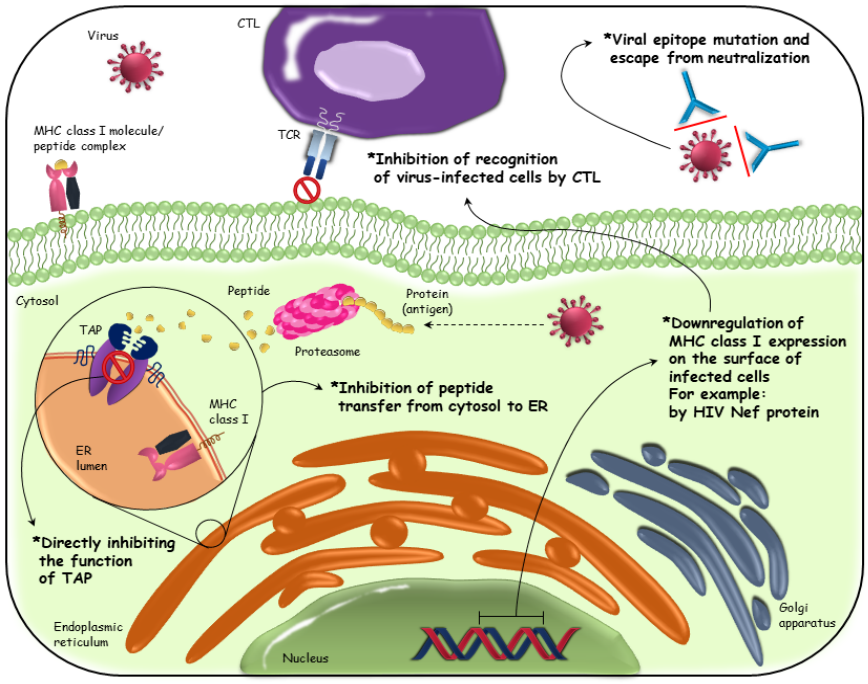

Fig. 3. An overview of some escape routes of viral pathogens from the immune system
CTL (Cytotoxic T lymphocyte), TCR (T-cell receptor), MHC (Major histocompatibility complex), TAP (Transporter associated with antigen processing), ER (Endoplasmic reticulum)

Human cytomegalovirus (HCMV), human immunodeficiency virus (HIV) and human T-lymphotropic virus type1 (HTLV-1) when releasing virus particles from inside the host cell, cause the production of complement system regulators such as DAF, CD59, MCP (62). These proteins prevent the activity of complement. The family of HSV are able to prevent the expression of cytokines interferon type I (IFN-I) and IFN-II, tumor necrosis factor (TNF) and interleukin-1 (IL-1) (63). There are viruses that, upon entering the host cell, force it to use less MHC molecules to escape from microorganisms NKs and cytotoxic T lymphocytes (CTLs) that evade the immune system, enter certain tissues or cells, and may remain in the host for months or years and this host acts as a healthy vector of chronic and latent infection (64) (Table 3).

Table 3. Escape mechanisms of viruses from the immune system

Examples	Virus family	Genome type	Escape Mechanism
HCMV	*Herpesviridae*	DNA	• Downregulation of host MHC class I expression • Inhibition of NK cell activation • Modulation of cytokine and chemokine signaling • Inhibition of apoptosis • Latency and reactivation
EBV	*Herpesviridae*	DNA	• Downregulation of MHC class I expression • Inhibition of antigen processing • Modulation of cytokine signaling • Induction of immune cell transformation • Evasion of NK cell recognition
Vaccinia virus (VACV or VV)	*Poxviridae*	DNA	• Inhibition of apoptosis • Suppression of inflammatory responses • Disruption of chemokine gradients • Interference with complement activation • Modulation of antigen presentation
HPV	*Papillomaviridae*	DNA	• Suppression of innate immune responses • Inhibition of antigen presentation • Modulation of apoptosis • Suppression of T cell function • Induction of immune tolerance
Influenza viruses	*Orthomyxoviridae*	RNA	• Antigenic variation • Inhibition of innate immune responses • Evasion of adaptive immune responses • Exploitation of immune privilege • Induction of immune exhaustion
HIV	*Retroviridae*	RNA	• Rapid genetic variation • Targeting and destroying CD4+ T cells • Inhibition of antigen presentation • Induction of immune exhaustion • Suppression of innate immune responses • Establishment of latent reservoirs
Coronaviruses (CoVs)	*Coronaviridae*	RNA	• Inhibition of IFN response • Disruption of antigen presentation • Induction of immune exhaustion • Evasion of antibody neutralization • Exploitation of immune privilege • Subversion of macrophage and dendritic cell function
Ebola virus (EBOV)	*Filoviridae*	RNA	• Impairment of the innate immune response • Disruption of antigen presentation • Induction of immune exhaustion • Evasion of antibody neutralization • Suppression of inflammatory responses • Induction of apoptosis in immune cells
Marburg virus disease	*Filoviridae*	RNA	• Suppression of the IFN response • Disruption of antigen presentation • Induction of immune exhaustion • Evasion of antibody neutralization • Suppression of inflammatory responses • Induction of apoptosis in immune cells

5. A common mechanism for parasite escape

Many parasites, particularly those that are diploid and have a life cycle involving a vertebrate host, have developed the ability to change their surface antigens as an immune evasion mechanism. This is a common strategy employed by various parasitic organisms to avoid recognition and elimination by the host's immune system (65). Mutations usually occur in regions that are targeted by antibodies and T cells. Protozoa are unicellular eukaryotic microorganisms belonging to the Protista family. The body wall is covered by a cell membrane. Its cytoplasm consists of ectoplasm and the endoplasm of the nucleus is usually single, but it may be two or more. Reproduction can be asexual such as binary fission, schizogony, endodygony or sexual. Most worms require more than 1 intermediate host to complete their life cycle. Worms, unlike protozoa, do not multiply in the human body, which leads to multiple infections (66). Protozoa hide from the immune system by producing resistant cysts. Some worms enter the intestinal tract and are out of reach. When the specific antibody recognizes *Entamoeba histolytica (E. histolytica)*'s antigenic coating and binds to it, the *Entamoeba trophozoites* (*E. trophozoites*) sheds the antigen in response to this action. In fact, a kind of skinning is done (67). Since the functional immune response against parasites is the responsibility of macrophages, here too will we observe the production of ROS and RNS. Some parasites prevent the production of cytokines or change the expression pattern of cytokines. For example, they switch from the IFN-γ form to the IL-10-producing form (68). In the *Leishmania* parasite, promastigote forms that are phagocytosed by neutrophils produce the lipophosphoglycan (LPG) molecule, which inhibits phagosome maturation. *Leishmania donovani (L. donovani)* prevents respiratory bursts by producing tartrate-resistant acid phosphatase (TRAP or TRAPase) (69). In the malaria parasite, sporozoites and merozoites are antigenically different. There is a latent state in all types of malaria. After the successful and complete treatment of malaria in the affected person, a form called hypnozoites remains. These forms are non-replicable and metabolically inactive. These forms can remain in the liver cells of the affected person for a long time, and during this time no humoral or cellular reaction is created against these hidden elements. In some situations, these insects become active and cause disease. To escape from Kupffer cells, liver merozoites enter vesicles called merosomes. In this case, the phagocytes located in the liver tissue can no longer respond to them (70, 71). About parasites that produce larvae. For example, schistosomes, larvalization renders the complement system and CTLs unable to recognize them. The larvalization of some parasites, such as schistosomes, leads to their non-recognition by MHCs and CTLs, and ultimately their escape from the host's immune system (72).

Table 4. Parasite escape mechanisms from the immune system

Classification	Examples	Escape mechanism
Protozoa	*Trypanosoma*	Antigenic change
	Plasmodium	
	Entamoeba	Antigen production and shedding
Metazoa	*Schistosomes*	Acquired resistance to complement
	Filaria (after lymphatic blockage)	Inhibition of host immune responses
	Toxoplasma gondii	

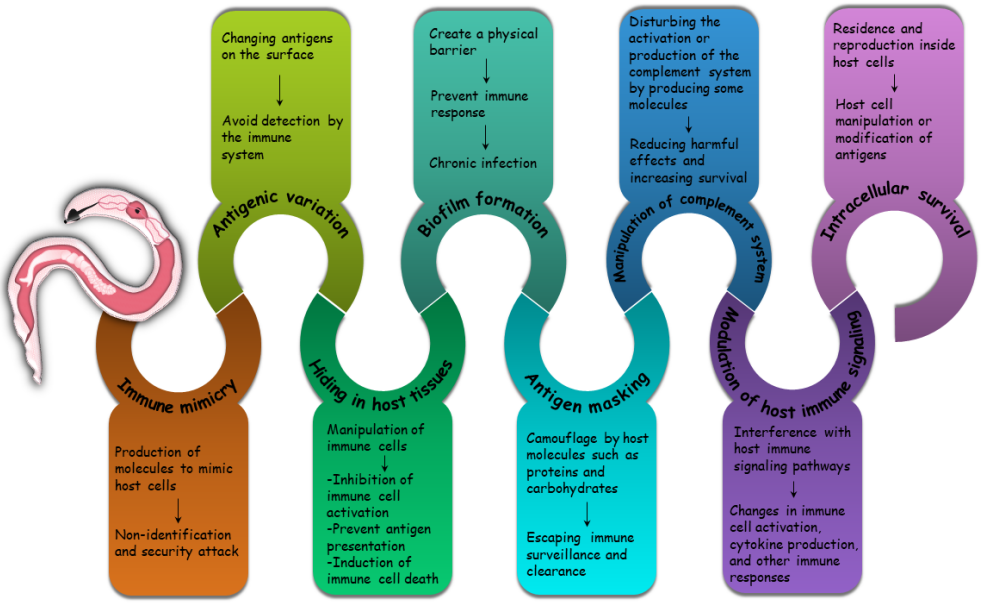

Fig. 4. A summary of immune evasion methods by pathogenic parasite

Table 4 shows the mechanisms of escape from the immune system. Parasites inhibit the host's immune responses through several mechanisms. These organisms reduce their immunogenicity and escape from the immune system by inhibiting the host's immune responses, antigen displacement, and acquired resistance to covalents, inhibiting the host's response to antigens. Some parasites, such as *Leishmania* and *Filariasis*, stimulate the development of regulatory T cells (Treg), which suppresses immune responses against the parasite and ultimately provides the continuation of the presence of the parasite (73). *Leishmania major* specifically inhibits the production of IL-12 by host macrophages, as a result, the production of IFN-ɣ by NK cells is inhibited and the differentiation and function of T helper type 1 (Th1) cells are also inhibited (74). In addition, it has been shown that *Leishmania major* actively induces Treg cells to produce IL-10, which suppresses the clearance of infection (75) (Fig. 4).

Technological advances in the last decade have facilitated studies on the mechanisms of interaction between the pathogen and its host in the context of infection. Each person's microbiota prevents the emergence and proliferation of pathogenic microorganisms as a strong and reliable mechanism. The immune system and the human physiological system, in general, are able to prevent the entry of microbial elements into the body, and in case of entry, they can give appropriate immune responses that cause their immediate removal, that is, in the shortest time, with the greatest response and the least damage to themselves. Microorganisms that enter certain tissues or cells and escape from the immune system may remain in the host for months or years, and the host acts as a healthy vector, and chronic infection remains latent. Part of our immune system is responsible for clearing pathogens, either killing the pathogen directly or marking it for destruction by other immune cells. The essay highlights the need of comprehending the immune system evasion methods by bacteria in order to design efficacious preventive and therapeutic approaches. By learning more about these escape techniques, scientists can pinpoint possible areas for intervention, such as creating vaccinations that specifically target conserved microbial components or creating medications that interfere with the ways in which bacteria evade the immune system. In conclusion, the paper emphasizes the variety and complexity of methods that microbes might evade the immune system. In addition to advancing our knowledge of host-pathogen interactions, more study in this area will make it easier to create cutting-edge strategies for fighting infectious diseases.

Conflict of interest

The authors declare that they have no competing interests.

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Authors' contributions

Concept: M.P., A.J.S, Design: M.P., Data Collection or Processing: A.J.S, Analysis or Interpretation: G.P., K.S., K.H.K., Literature Search: G.P., K.S., K.H.K., F.M., Writing: G.P., K.S., K.H.K., N.Y.

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Diving into the cellular puzzle: Exploring the connection between mitochondrial DNA depletion and prostate cancer development

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Abstract

Mitochondria, essential components of eukaryotic cells, play a central role in generating cellular energy, regulating metabolism, and facilitating cellular interactions. A distinguishing characteristic of mitochondria is their unique circular double-stranded DNA, known as mitochondrial DNA (mtDNA), essential for energy synthesis and overall mitochondrial function. MtDNA depletion is a notable decrease in mtDNA levels and is affected by a combination of genetic and environmental elements. Genetic contributors include inherited mutations, nuclear DNA changes that impact mitochondrial activity, and mutations within the D-loop region of mtDNA. Environmental factors encompass exposure to specific medications, oxidative stress, and insufficient nutrient consumption. Prostate cancer, a primary contributor to male cancer fatalities, has been associated with anomalies in mtDNA structure and function. The involvement of mitochondria in prostate cancer is intricate, influencing energy metabolism, the stability of the genome, and the emergence of aggressive, androgen-independent cancer variants. Notably, mtDNA depletion is implicated in the shift from androgen-sensitive to androgen-resistant prostate cancer, emphasizing its crucial role in disease advancement. Additionally, mtDNA depletion correlates with the development of a cancer stem cell-like phenotype, marked by increased tumor aggressiveness and heightened resistance to therapeutic agents. Research indicates that changes in mtDNA quantity are linked to the progression and prognosis of prostate cancer. Elevated levels of POLRMT, a nuclear enzyme essential for mtDNA synthesis, have been associated with prostate cancer proliferation, while diminished mtDNA levels are linked to increased invasiveness and a shift towards a mesenchymal cell state. In summary, grasping the intricate relationship between mtDNA depletion and prostate cancer is essential for formulating targeted treatment approaches and enhancing patient outcomes. This review article emphasizes the critical role of mtDNA in the advancement of prostate cancer and underscores its potential as a therapeutic focal point in addressing this widespread cancer.

Keywords: prostate cancer, androgen dependence, cancer stem cell, mitochondrial DNA depletion, mitochondrial DNA copy number

1. Introduction

Mitochondria, known as the power plant of eukaryotic cells, play a role in critical cellular activities as well as ATP production by oxidative phosphorylation. Mitochondria are involved in cellular energy production (1), metabolic regulation (2), regulation of reactive oxygen species (ROS) (3), apoptosis and cell fate (4), calcium homeostasis (5), cellular communication (6), and mitochondrial inheritance (7). In particular, mitochondria form a unique structure with mtDNA located in their matrix. The complex connection between mitochondria and mitochondrial DNA (mtDNA) highlights the essential functions that these organelles fulfil in both cellular energy production and genetic information transfer.

Cells structurally have more than one mitochondrion, depending on the cell type and energy requirements. Mitochondria have circular double-stranded DNA about 5µm length. Each mitochondrion contains 2-10 mtDNAs (8). Since the number of mtDNA per cell shows variation in tissue and different developmental stages, thousands of mtDNAs are reported to be present in an organism. ATP demand, nucleotide availability and replication origin regulation are among the factors that affect the number of mtDNAs in cells (9). The mtDNA encodes genes required for energy production and mitochondrial function. The size of mtDNA varies between different organisms, but is usually several kilobases in length. In humans, for example, mitochondrial genomes are approximately 16.569 base pairs long, contain no introns and carry two non-coding regions. Therefore, the mitochondrion is comprised of almost entirely of coding regions (8). MtDNA has a higher mutation rate compared to nuclear DNA. This is thought to be related to the generation of high levels of ROS during the energy production process in mitochondria, which lacks a DNA repair system (10).

Similarly, mtDNA is highly susceptible to oxidative damage due to the lack of histone proteins. Unlike nuclear DNA, which is packaged with histone proteins to form chromatin structure, mtDNA lacks histone proteins (11).

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Instead, it forms a nucleoid structure through the organization of a compact protein-DNA complex with the transcription factor TFAM (Mitochondrial transcription factor A) and proteins. The nucleoid structure regulates processes such as protection, replication and transcription of mtDNA. The nucleosome structure formed by histone proteins in nuclear DNA protects DNA against free radicals. At the same time, however, in the event of ROS-induced damage, nuclear DNA is compensated by various repair mechanisms and enzymes. However, since the nucleoid structure in mtDNA does not provide complete protection, it is usual for reactive oxygen species to reach mtDNA and cause damage. Furthermore, the limited DNA repair systems in the mitochondrial genome increase the sensitivity of mtDNA to ROS. This makes mtDNA vulnerable to oxidative damage and is associated with mitochondrial diseases (12).

Recently, mtDNA depletion, associated with nuclear DNA mutations, has attracted attention. MtDNA depletion is defined as a significant reduction or depletion of mtDNA content or copy number in mitochondria (13). This phenomenon is influenced by genetic and environmental factors. In terms of genetic factors, mtDNA depletion syndrome is associated with both the mitochondrial genome and the nuclear genome. Therefore, mtDNA depletion is associated with nuclear genes (14). In particular, mutations in nuclear genes involved in the mitochondrial nucleotide pool (TK2, SUCLA2, SUCLG1, RRM2B, DGUOK, MPV17, TYMP), mutations in nuclear genes involved in mtDNA replication (POLG, Twinkle, POLRTM, TFAM, TEFM) and mutations in the mitochondria-specific D-loop region explain the link between mtDNA depletion syndrome and nuclear genes (15-18). Not only are mitochondria critical for cellular function, but defects in mtDNA structure or function have been linked to many diseases, including cancer (19, 20).

Cancer and mtDNA depletion are associated in many aspects, such as energy metabolism, genomic instability, ROS production and tumor microenvironment (19). The study of mtDNA depletion is particularly relevant for prostate cancer, which is one of the leading causes of death after lung cancer in the male population and has limited therapeutic potential. Understanding the link between mtDNA depletion and prostate cancer will contribute to the development of new methods to investigate the complex molecular mechanisms behind the initiation and development of this common cancer.

2. Mitochondrial DNA depletion mechanisms

The leading causes of mitochondrial DNA depletion are genetic and environmental factors. Genetic factors are classified as hereditary, nuclear DNA mutations and *D-loop* region mutations (21-23). In addition, point mutations, large deletions or insertions, structural chromosomal changes in mtDNA can have critical consequences such as deletion, insertion or rearrangement of genes in mtDNA (24).

Individuals may inherit genetic mutations encoding enzymes required for mitochondrial DNA replication and repair. Thus, the appearance of defective proteins during mtDNA replication and repair may increase the susceptibility of individuals to mtDNA depletion (25). However, when mutations are evaluated in terms of nuclear genes, it is seen that the mitochondrial genome carries a gene anatomy different from but in touch with the nuclear genome. Therefore, mutations in nuclear DNA will affect mitochondrial function. The mitochondrial nucleotide mechanism requires a functional replication process and an adequate supply of the mitochondrial deoxyribonucleotides required for this process. In this context, defects in the mitochondrial nucleotide mechanism, related to nuclear genes will ultimately lead to diseases associated with mitochondrial depletion (22). *D-loop* mutations, which are highly prevalent in cancer cells, have a high mutation rate due to their triple helix structure and susceptibility to oxidative damage and constitute one of the main causes of mtDNA depletion. The *D-loop* region contains all the necessary materials for mitochondrial transcription and replication and is responsible for the regulation of this process. *D-loop* region mutations can alter the promoter sequences of mtDNA transcription modulators; therefore, due to the change in their protein binding affinity, faulty replication occurs, insufficient amounts of mtDNA are transferred into cells and mtDNA depletion results (23).

When mtDNA depletion is evaluated in terms of environmental factors, some chemotherapeutic agents or antiretroviral drugs, high production of ROS, inadequate intake of essential nutrients, ionizing radiation and environmental toxins can increase toxicity in mitochondria and results in mtDNA depletion (21). To understand the relationship between genetic and environmental factors, which are the leading causes of mtDNA depletion, a more comprehensive investigation is required.

3. The role of mitochondria in prostate cancer

According to the recent global cancer data conducted by the World Health Organization, prostate cancer has an increasing incidence in the male population and is the second leading cause of cancer-induced deaths after lung cancer. There are approximately 1.4 million new cases and approximately 10 million existing patients worldwide each year (26). Due to the heterogeneity of the disease and treatment response, asymptomatic or symptomatic, hormone naive or castration-resistant stages of prostate cancer, limited treatment modalities and drug resistance in the treatment of patients, it is necessary to investigate the cellular mechanisms underlying prostate cancer. In this respect, these findings will allow the development of new therapeutic targets for the treatment of prostate cancer (27-29).

Mitochondria, which contribute highly to cancer development are critical at the cellular level. In particular, defects in mtDNA, impaired electron transport system and increased ROS contribute to the carcinogenesis process. In

addition, as a result of mitochondrial dysfunction, epithelial-mesenchymal transition, an essential step in cancer progression, explains the critical role of mitochondria in cancer cells. In particular, dysfunction of mtDNA replication, repair or mito-dynamic processes as a result of nuclear DNA mutations or altered mitochondrial copy numbers results in impaired oxidative phosphorylation, increased production of ROS and metabolic reprogramming. As a result of mtDNA mutations that may occur due to aging are associated with the carcinogenesis process (30).

Loss of p53 function, changes in the glycolytic pathway, oxidative phosphorylation defects, increased survival proteins, and apoptotic resistance mechanisms that occur in the context of mitochondrial DNA depletion lead to androgen-independent, aggressive and invasive prostate cancer. A better understanding of these concepts will contribute to both the evaluation of prostate cancer in terms of mtDNA depletion and the development of new therapeutic targets in prostate cancer progression (31).

4. Mitochondrial DNA depletion affects androgen dependence in prostate cancer

The major regulator of prostate cancer is the androgen receptor. The process of carcinogenesis develops through increased activation of the androgen receptor-dependent signaling mechanism or through androgen-independent signaling (32, 33). Therefore, treatment is usually provided by androgen antagonists targeting the androgen receptor. The androgen receptor is targeted through medical or surgical castration. Although this method, called androgen deprivation therapy, has a positive effect on cancer patients at first, it limits the treatment due to the resistance that develops after 18-24 months. The most well-known agents in this part include various chemotherapeutic agents such as enzalutamide, abiraterone acetate, docetaxel, cabazitaxel, orteronel (34). Therefore, new combined treatment methods are being developed for androgen-dependent/independent prostate cancer (27, 28).

As it is known, mitochondria constitute the power plant of the cell. It plays an active role in apoptotic process, oxidative stress and intracellular signaling pathways. Therefore, any defect that may occur in mitochondria directly or indirectly affects intracellular systems. Mitochondria play an active role especially in cancer development. For example, free radicals, a by-product of oxidative phosphorylation, may contribute to cancer development due to DNA damage. In addition, impaired mitochondrial signaling may affect androgen-dependent/independent processes in prostate cancer. Apart from this, changes in the amount of mtDNA in cancer cells have been observed in studies (35-39).

Another constituent that can be targeted in this context is mitochondria, which have a critical role in the carcinogenesis process. The association of mtDNA depletion with prostate cancer as a result of the decrease in the number of copies of mitochondrial DNA has been stated in studies (40, 41).

In particular, it has been proven that the androgen-dependent stage of prostate cancer is the result of mutation-induced deletion or depletion of mtDNA in the transition to the androgen-independent stage. Loss of mtDNA causes LNCaP cells to grow faster, while pharmaceutical OXPHOS inhibition leads these cells to undergo apoptosis or necrosis. This suggests that some mitochondrial function protects LNCaP cells, maintaining ATP production and preventing diversion to glycolysis. The androgen receptor (AR) is involved in this process; AR reduces OXPHOS by crossing into mitochondria. Mitochondrial disruption increases the expression of AR, which in turn enhances its localization in mitochondria. Thus, cancer cells develop survival mechanisms by activating OXPHOS or AR signalling pathways. Therefore, the role of mtDNA depletion in the transition to the more aggressive androgen-independent stage of prostate cancer is inevitable (42-44).

In another proof of concept, the role of mtDNA depletion on mitochondrial function was evaluated by comparing mtDNA depletion in minimally invasive androgen-dependent and highly invasive androgen-independent cells. In androgen-independent cells, a higher decrease in *ND-6 (NADH-ubiquinone oxidoreductase chain 6 protein), D-Loop, Cox-I (cytochrome c oxidase subunit I), Cox-III (cytochrome oxidase III)* and mitochondrial membrane potential was observed compared to androgen-dependent cells. Furthermore, the study was elaborated in the context of migration rate and drug sensitivity. As predicted, the migration rate in androgen-independent cells and cell viability due to chemotherapeutic agent application to the cells were higher in androgen-independent cells. In this context, the effect on cell death was also examined and reported that when the *PARP-I(Poly [ADP-ribose] polymerase I)* gene was evaluated in terms of chemotherapeutic agent +/-, potential cell death was less in androgen-independent cells compared to androgen-dependent cells. This is attributed to the more invasive phenotype of androgen-independent cells (45).

5. Mitochondrial DNA depletion provides cancer stem cell phenotype

As is known, cancer stem cell phenotype emerges when normal stem cell proliferation and differentiation pathways are compromised. In this context, reduced mtDNA copy number is associated with cancer cell stemness through reprogrammed metabolism (46).

In a study, mtDNA depletion was directly induced in PC-3 cells, an aggressive and castration-resistant form of prostate cancer, by EtBr intervention. In these cells, decreased levels of *ND-4 (NADH-ubiquinone oxidoreductase chain 4), D-Loop, Cox-I (cytochrome c oxidase subunit I) and Cox-III (cytochrome oxidase III)* levels and immature mitochondrial morphology were observed. Accordingly, mitochondrial membrane potential and mito-dynamic induced gene levels

were decreased. Simultaneously, ATP production decreased in the cells, but the cells expressed high levels of Warburg effect-related genes. Likewise, cancer stem cell markers (Cd44, Aldh1a1 (Aldehyde Dehydrogenase 1 Family Member A1), α2β1 integrin, Met (*MET* Proto-Oncogene, Receptor Tyrosine Kinase), Cxcr4 (C-X-C chemokine receptor type 4), Cxcr8 (C-X-C Motif Chemokine Ligand 8), Mmp1 (Matrix metalloproteinase-1) and Dpp4 (Dipeptidyl peptidase-4) were expressed at high levels. However, the cells exhibited increased hypoxia and oxygen tension due to high invasiveness and metastatic potential. PC-3 cells with mtDNA depletion develop higher levels of resistance to docetaxel, an additional anti-mitotic cytotoxic chemotherapeutic agent, compared to wild-type PC-3 cells (46).

6. Mitochondrial DNA copy number is associated with prostate cancer

The mitochondrial genome contains a limited number of gene regions. In order to maintain its own replication and transcription cycle flawlessly, the relevant enzymes must be encoded from the nuclear genome. For example, *POLRMT (RNA polymerase mitochondrial)*, which plays a major role in mitochondrial replication, acts as a mitochondrial RNA polymerase and is encoded by the nuclear genome. Therefore, any defects in this gene will also affect mitochondrial function. For this purpose, tumor cells were obtained from individuals with castration-resistant prostate cancer and normal prostate epithelial tissues were also obtained from the patients to examine the correlation between these individuals. *POLRMT* expression level was found to be higher in tumor tissues compared to normal prostate epithelial tissues which was associated with poor survival. Likewise, *POLRMT* levels were found to be higher in the reference cell lines compared to primary prostate epithelial cells. When *POLRMT* was silenced by lentiviral *POLRMT* shRNAs, the cells became *POLRMT*-depleted. The cells showed mtDNA depletion for *POLRMT* encoded from the nuclear genome. In terms of mitochondrial function, this resulted in mitochondrial depolarization, oxidative stress, inhibition of mitochondrial complex-I and ATP depletion. Interestingly, *POLRMT* depletion inhibited prostate cancer cells proliferation, migration and survival while inducing apoptosis. Similarly, *in vivo* xenograft models resulted in a reduction in tumor volume and weight. The same results were verified by confirming the study with *POLRMT* inhibitor. Correspondingly, high-level expression of *POLRMT* increased pro-cancer activity in primary prostate cancer cells. The findings reveal that POLRMT contributes not only to mitochondrial functions but also to broader metabolic processes in prostate cancer cells. Understanding the interplay between mitochondrial health and cancer cell metabolism may aid in the development of novel therapeutic strategies. The fact that POLRMT is associated with poor prognosis offers the possibility to evaluate it as a biomarker for aggressive prostate cancer, which may benefit treatment selection. Furthermore, studying the molecular mechanisms by which POLRMT promotes cancer cell survival and proliferation will provide a deeper understanding of prostate cancer biology. Ultimately, the potential of POLRMT as a therapeutic target, its applicability in clinical applications, and the effect of combining POLRMT inhibitors with existing treatment modalities have the potential to improve treatment efficacy for castration-resistant prostate cancer (CRPC) patients (47).

In another research corroborating this subject, altered mitochondrial genome content was associated found associated with poor pathology and prognosis of prostate cancer. Data from 115 prostate cancer tissues and normal adjacent prostate tissues were analyzed to determine the ratio of genomic to mitochondrial DNA content in the two tissues and to evaluate this ratio in terms of pathologic features and disease outcomes in prostate cancer tissues. Simultaneously, it was found that the amount of mtDNA was less in prostate cancer tissues compared to the genomic DNA ratio. However, high levels of mtDNA were detected in advanced cancer tissues. It was concluded that this situation was heterogeneous in terms of prostate cancer. The findings of this study reveal the complex relationship between mitochondrial DNA (mtDNA) content and prostate cancer pathology. Through comparative analysis of 115 prostate cancer and normal tissues, the study showed that mtDNA levels were lower in cancer tissues than in genetic DNA, but mtDNA levels increased in advanced cancers. This highlights the heterogeneous nature of prostate cancer and suggests that mtDNA changes may be linked to tumor aggressiveness. Changes in mtDNA levels have the potential to be used as a biomarker for disease prognosis. Furthermore, these findings highlight the importance of future research to better understand the role of mitochondrial dynamics in cancer development and progression. Overall, the results of the study suggest the need to customize treatment strategies based on metabolic profiles of prostate cancer (48).

The stage of prostate cancer is mainly determined by the Gleason score (49). In this study, depending on this score, tumor grade and mitochondrial genome content and related cancer survival pathways were investigated (50). In a study, in which mitochondrial DNA copy number was evaluated, especially in terms of *ND-1* gene, a lower copy number was detected in tumors with a high grade Gleason score compared to tumors with a low grade (50). The study was simultaneously confirmed in prostate cancer cell lines, and a lower copy number of the *ND-1* gene was detected in C4-2, PC-3 and DU-145 cells compared to androgen-dependent LNCaP cells. The effect of mitochondrial genome content on prostate cancer progression was examined and the mitochondrial genome was knocked out in LNCaP cells. By the FISH method, a lower mitochondrial genome content was detected in knockout cells compared to parental LNCaP cells. In addition, Erk subunits and Akt protein levels, which play a major role in cancer survival pathways, were evaluated and found to be activated in knockout LNCp0-8 cells. At the same time, the reduction of

mitochondrial genome content increased GTP-bound Ras protein levels (51).

7. Role of circulating mtDNA in prostate cancer

Circulating mtDNA is defined as 50-200 bp fragmented molecules released from the inter-mitochondrial space into the cytosol or bloodstream from damaged or apoptotic cells as a result of altered mitochondrial cycle, uncontrolled mitophagy (52). These molecules can be detected in blood samples and their levels can be determined. Circulating mtDNAs have biomarker potential. It is also used for prognostic purposes (53). Intracellular mtDNA can be released into circulation under various stress conditions such as cancer (54-56).

In recent studies, when prostate cancer patients treated with docetaxel were compared before and after treatment, it was found that mtDNA concentrations in serum were at high levels after treatment in the same individual (57). In the diagnosis of prostate cancer, one of the urogenital malignancies of circulating mtDNAs for diagnostic purposes, 79 bp and 220 bp fragments of mitochondria-specific 16S RNA were determined and detected at high levels in individuals with cancer (58, 59). This proves that circulating mtDNA is a biomarker. In addition, studies have shown that circulating mtDNA is not only specific to prostate cancer, but also associated with ovarian, breast, kidney and most other cancer types (59-61). When circulating mtDNA is evaluated from a prognostic point of view, in a study proving this context, out of 75 prostate cancer patients (14 individuals with benign prostatic hyperplasia), it was determined that individuals with poor prognosis had a high level of circulating mtDNA in plasma compared to individuals with benign prostatic hyperplasia (62).

Studies have shown that circulating mtDNAs are associated with other cancer types in terms of biomarker, diagnostic and prognostic aspects, as well as being directly linked to prostate cancer.

8. Conclusion

All in all, the intricate interplay between mtDNA depletion and prostate cancer development underscores the significance of mitochondrial function in disease progression. Genetic and environmental factors contribute to mtDNA depletion, impacting critical cellular processes involved in energy metabolism, genomic stability, and tumor microenvironment regulation. Prostate cancer, a leading cause of male cancer fatalities, exhibits alterations in mtDNA structure and function, influencing the transition from androgen-sensitive to androgen-resistant stages, as well as promoting aggressive cancer variants. Moreover, mtDNA depletion correlates with the emergence of a cancer stem cell-like phenotype, characterized by increased tumor aggressiveness and resistance to therapy. Understanding the role of mtDNA depletion in prostate cancer pathogenesis is pivotal for the development of targeted therapeutic strategies and improving patient outcomes. This comprehensive review highlights the essential role of mtDNA in prostate cancer progression and underscores its potential as a therapeutic target in combating this prevalent malignancy.

9. Future Perspectives

Future perspectives for this review entail multifaceted approaches spanning basic research, clinical translation, and therapeutic innovation. Firstly, a deeper understanding of the molecular mechanisms underlying mtDNA depletion in prostate cancer progression is imperative, requiring continued exploration of genetic, epigenetic, and environmental factors contributing to mitochondrial dysfunction. Integrating cutting-edge technologies such as single-cell sequencing and spatial transcriptomics will unveil the spatial and temporal dynamics of mtDNA alterations within the tumor microenvironment. Moreover, leveraging large-scale multi-omics datasets and artificial intelligence methodologies will facilitate the identification of novel biomarkers for early detection, risk stratification, and treatment response prediction in prostate cancer patients with mitochondrial aberrations. Translating these discoveries into clinical practice demands the development of non-invasive diagnostic assays and targeted therapeutic modalities tailored to individual patients based on their mitochondrial profiles. Furthermore, combination therapies synergizing mitochondrial-targeting agents with conventional treatments, immunotherapies, or hormone therapies hold promise for overcoming treatment resistance and improving long-term outcomes in prostate cancer. By embracing a multidisciplinary and collaborative approach, the integration of mitochondrial biology into prostate cancer research and clinical management will pave the way for precision medicine paradigms that optimize patient care and ultimately reduce the burden of this prevalent malignancy.

Conflict of interest

The authors declare that they have no conflict of interest.

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Pediatric anesthesia management in abdominal tumor with massive ascites

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Abstract

Pediatric anesthesia in a complex abdominal tumor with massive ascites is challenging both due to the abdominal compression effect and pediatric physiology. During perioperative preparation and surgery, anesthesia plays a crucial role. We present anesthesia management in pediatric abdominal tumors with massive ascites.

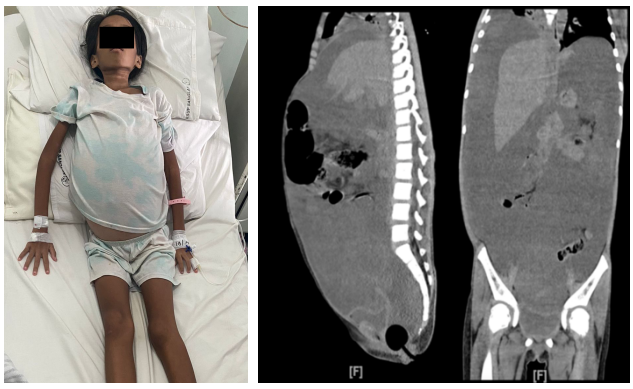
Keywords: pediatric anesthesia, anesthesia management, abdominal tumor, massive ascites

1. Introduction

Abdominal tumors in pediatric age have a wide range of differential diagnoses. These lesions may be classified as solid or cystic based on their site of origin. The most common intraabdominal solid tumor in children is neuroblastoma (30%). Intraabdominal cysts can be categorized based on their location in either the solid organs, retroperitoneum, mesentery, or omentum. Ovarian cysts are the most frequent of all. The symptoms in children can vary from abdominal distension, abdominal pain, palpable mass, and small bowel obstruction. Abdominal distension causes cardiopulmonary disturbance, especially in pediatric patients (1-3). The anesthesia goal in pediatric patients with high abdominal pressure is maintaining respiratory and cardiovascular reserve that has been compromised by abdominal pressure. Close monitoring for hemodynamic, intraoperative bleeding, hypoglycemia, hypercarbia, and hypothermia is mandatory. Meticulous patient preparation needs to be carried out to optimize intraoperative monitoring and minimize the risk of complications.

2. Case Presentation

We present a case report of a girl, 9 years old, with a large ovarian tumor and ascites, who will undergo tumor removal. The abdominal circumference was 82 cm, and the patient was in moderate respiratory distress, especially in a flat position. The patient was comfortable in a 45-degree position with slight right lateral decubitus, the patient's respiratory rate was 26 times per minute, and peripheral oxygen saturation was 92-96% with nasal oxygen supplementation of 4 liters per minute. The patient also had mild right pleural effusion. Examination of Congenital VACTREL (vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies, and limb abnormalities) was normal. The patient lost 8 kg within one one-month period and the patient's body weight was 27 kgs with height 138 cm and BMI 14.2 kg/m2. Her food intake was very limited due to , and she could only swallow fluid and soft food. The patient also complained that defecation and urination have been lessened for the past month but no vomiting history. The patient was moderately dehydrated on admission and already resuscitated with IV fluid. The laboratory revealed anemia with hemoglobin 9.6 g/dl and the rest of the laboratory test was within normal limits. In the abdominal CT Scan, a mass with a combination of lobulated cyst, septal, and solid components, pressing the rectosigmoid colon and bladder was found, which explained gastrointestinal and urology symptoms (Fig.1).



a **b**

Fig. 1. a. Patient's picture, b. Abdominal CT Scan

In the operating theatre, the patient's contact was adequate, and she felt comfortable with a 45° head-up position (Fowler position) (Fig.2). The patient was preoxygenated with a 10 lpm face mask for 10 minutes without positive pressured ventilation

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to avoid increasing the abdominal pressure during preoxygenation. We used fentanyl 3mcg/kgBW, ketamine 1 mg/kg, and sevoflurane for induction. We are using a titrated dose while carefully observing the hemodynamic response using arterial line monitoring. After ensuring adequate ventilation, a muscle relaxant using rocuronium 1.2 mg/kg was given. No cricoid pressure was used. After 1 minute, using a video laryngoscope and still in a Fowler position, the patient was intubated. Anesthesia maintenance using Sevoflurane 1-2 MAC, compressed air, oxygen, and intermittent fentanyl and rocuronium. We were using pressure control to avoid barotrauma due to the high pressure of abdominal compression in the supine position. The inspiratory pressure is increased from 15 (in the Fowler position) to 22 (in the supine position) to achieve a volume tidal of 5-6ml/kg, so we increase the respiratory rate to achieve the minute volume and normal CO2. The ETCO2 can be maintained within 38-40 during the surgery.

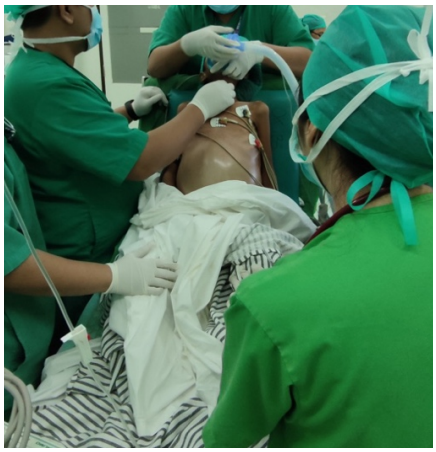

Fig 2. Fowler position during intubation

Intraoperative monitoring included 5 leads: ECG, non-invasive blood pressure (NIBP), Arterial line, blood oxygen saturation (SpO_2), and ETCO2. A central venous catheter was placed in the right jugular vein after intubation for fluid and emergency drug access. A nasogastric tube was also placed after intubation.

The patient was positioned slightly, head up 30 degrees, and the operation was started. The evacuation of 5 liters of ascite fluid was done slowly to maintain the hemodynamic. The tumor was inoperable, and the surgeon decided to do only left Salphingo-Oophorectecmy with bleeding of 200 ml. Fluid management using ringer lactate solution 450 ml and fluid warmer to maintain normothermia. Hemodynamic was stable, with no transfusion during the operation needed. The post-operative blood work showed that Hb was 10. After 3 hours of surgery, the patient was able to extubate and transferred to the pediatric intensive care for close monitoring. Post-operative pain management was using fentanyl continuous infusion 0.5mcg/kg/hour and paracetamol oral 10mg/kg every 6 hours.

3. Discussion

Perioperative anesthesia management in complex pediatric cases is one of the most challenging cases for anesthesiology. The inability to develop adequate compensation to overcome the anatomical or physiological changes caused by the diseases, made many pediatric patients come with distress symptoms. In this case, the massive and fast growth of the abdominal tumor causes distress, respiratory symptoms, dehydration, and gastrointestinal and urinary symptoms. The high abdominal pressure caused compression to the diaphragm lowered the patient's functional residual volume, and made them more prone to desaturation. The cardiac compression also can cause diastolic dysfunction, and the tumor/ ascites compression to the inferior vena cava will significantly reduce venous return. Pleural irritation and lymphatic blockage can also cause pleural effusion, which further reduces respiratory capacity. Anesthesia should also consider the metabolic effects of the cancer and chemotherapy effects, such as hypoalbuminemia, anemia, and electrolyte imbalance. [1,2,3] The use of invasive intraoperative monitoring is preferable for patient safety. The arterial line provides beat-to-beat hemodynamic information. Although the limited equipment in some hospitals may only use non-invasive hemodynamic monitoring, we recommend using it if available. The central access is also should be prepared if vasopressor and inotropic are needed to stabilize the hemodynamic. [4] Both intravenous and inhalation anesthesia can be used in this case. Ketamine may provide a more stable hemodynamic profile but may cause hypersalivation. Due to limited respiratory capacity, in a very severe case, we can consider the sleep non-apnea technique. In the case of obstructed gastrointestinal, we can use rapid sequence intubation (RSI) without positive pressure ventilation and using cricoid pressure to avoid aspiration. In this case, there is still good preservation of respiratory capacity, adequate fasting, and no significant abdominal obstruction, so we decided to use the sleep apnea intubation technique in a Fowler position with modified RSI. The monitoring of ETCO2 is also important to avoid hypercarbia, which can cause hemodynamic disturbance. The analgesia can be achieved using opioid, ketamine epidural, or caudal analgesia. The use of an epidural in high abdominal pressure should be done with caution due to the enlargement of the epidural venous plexus and increase the risk of epidural hematoma. Since the patient is a malnourished pediatric patient, there is also a high risk of hypoglycemia, a close monitoring for blood glucose is mandatory. [1,2,3,4]

Understanding the changes in pediatric physiology in a complex abdominal tumor with massive ascites case is mandatory. Close monitoring, especially during induction and ascites/ tumor evacuation, is the key point for successful anesthesia management.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient (patient's parents) consent forms. In the form, the patient's parents have given their consent for the patient's

images and other clinical information to be reported in the journal. The patient's parents understand that their names and initials will not be published, and due efforts will be made to conceal their identity.

Conflict of interest

Nothing to declare.

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None to declare.

Authors' contributions

Concept: P.K., S.P.S., B.J., I.W.S., Design: P.K., S.P.S., B.J., I.W.S., Data Collection or Processing: P.K., S.P.S., B.J., I.W.S., Analysis or Interpretation: P.K., S.P.S., B.J., I.W.S., Literature Search: P.K., S.P.S., B.J., I.W.S., Writing: P.K., S.P.S., B.J., I.W.S.

Ethical Statement

The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

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Case Report

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Ingestion of voice prosthesis in patient with total laryngectomy: A case Report

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Abstract

Granulomatous diseases, foreign bodies, trauma, surgical procedures, and the use of high pressure intubation tubes are the most common causes of acquired benign tracheoesophageal fistula (TEF). More frequent use of invasive procedures such as intubation, tracheostomy and mechanical ventilation increases the frequency of benign tracheoesophageal fistula development, especially in intensive care patients. In this case report, the patient with a history of total laryngectomy operation, aspirated the audio device secondary to the use of excessive force and the connection of the device to the gastrointestinal system due to the existing tracheoesophageal shunt.

Keywords: voice prosthesis, total laryngectomy, tracheotomy, tracheoesophageal fistula

1. Introduction

Although acquired Tracheoesophageal Fistula (TEF) is a rare condition, it is known that mechanical ventilator sources can occur in intubated or tracheostomy patients in intensive care units. In addition to the 50% cause of acquired TEF, malignancy is the most common cause of cuff-related tracheal tube damage (1,2). In addition, iatrogenic injuries, esophageal/tracheal surgery history, foreign body or caustic substance exposure, and permanent tracheoesophageal stents are among other causes (2). Clinical symptoms in acquired TEF are usually manifested by respiratory symptoms. Typical symptoms include cough, recurrent lung infections, increased tracheal secretion, cuff leakage, aspiration of gastric contents through the tracheal tube, the air in the nasogastric tube, or abdominal distention (3,4). It is difficult to diagnose TEF due to benign causes since it does not have specific symptoms and is rare. Therefore, it may be present for a long time without being diagnosed, resulting in significant morbidity and mortality (5-8).

Early diagnosis is very important to protect the patient from potentially fatal pulmonary complications. The target treatment is to complete the repair without recurrence, but the treatment varies depending on the clinical condition of the patient, etiology, and the structure of the fistula [2]. In this case report, the migration of the sound device he used after TEF to the digestive system without any respiratory symptoms, which developed in our patient with a history of total laryngectomy operation, is described.

2. Case Report

A 50-year-old female patient, who underwent total laryngectomy for recurrent laryngeal carcinoma, was applied to the emergency service after realizing that there was no sound device after intensive use (Fig. 1). In the patient's anamnesis, it was learned that there were no respiratory system symptoms, and the active complaint was only epigastric fullness. The patient's vital signs were stable at admission. In physical examination; It was observed that there was no discharge from the tracheostomy and there was no stridor, ral, roncus and wheezing in both lungs. Other system examinations were normal. The patient was first evaluated by the otolaryngology department and no foreign body was found in the examination from the tracheostomy to the carina. Afterward, a chest X-ray and then thoracoabdominal computed tomography (CT) were taken to the patient due to the suspicion of aspiration of the sound device to the respiratory or gastrointestinal tract. According to the CT interpretation, bilateral airways were intact, tracheoesophageal fistula developed, there was no foreign body in this region, and a foreign body compatible with the sound device was detected in the third part of the duodenum (Fig. 2;4). Endoscopy was not considered due to the absence of any intestinal obstruction and the patient was followed up.

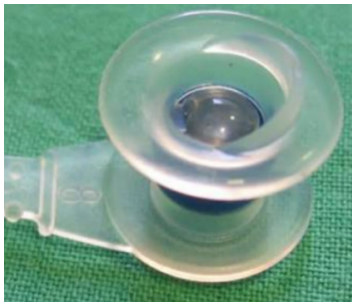

Fig. 1. Voice prosthesis

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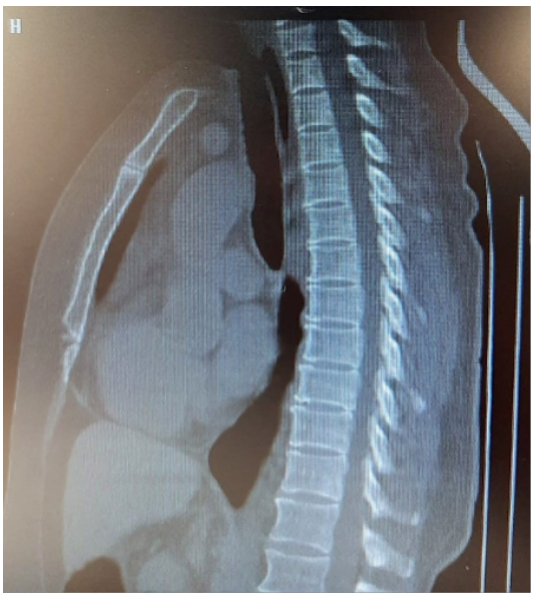

Fig. 2. Tracheoesophageal fistula

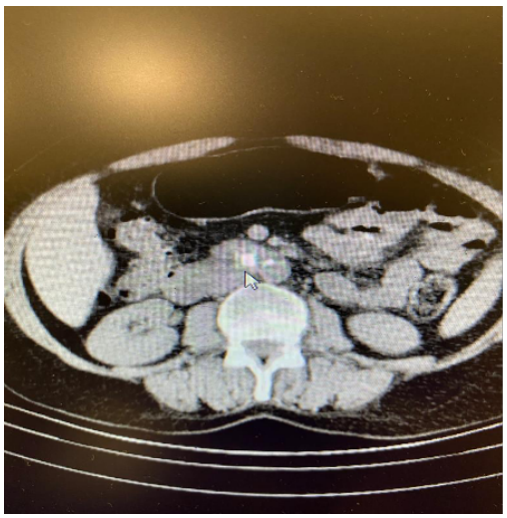

Fig. 3. Foreign body in the 3rd continent of the duodenum (horizontal)

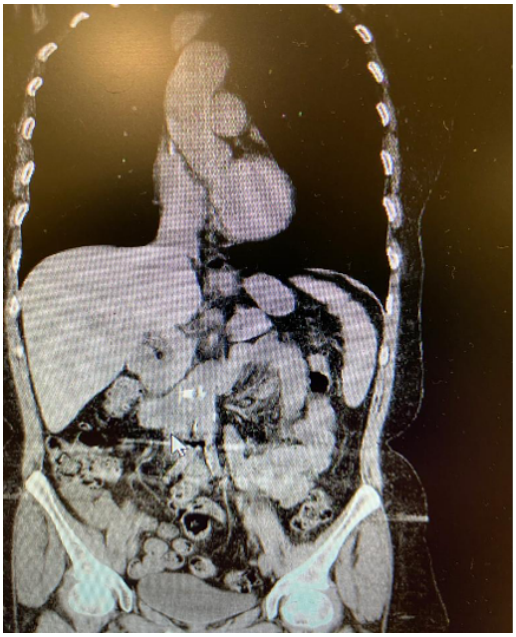

Fig. 4. Duodenum 3rd continent foreign body (axial)

In the follow-up, the patient did not have acute abdomen and ileus, oral intake was started gradually and a daily standing abdominal X-ray was taken (Fig.5). On the fifth day, a control abdominal computed tomography was performed on the patient who did not detect any foreign body in the standing direct abdominal X-ray. No foreign body was detected in the control tomography, and the patient was discharged after receiving the recommendations of the otolaryngology and thoracic surgery departments.

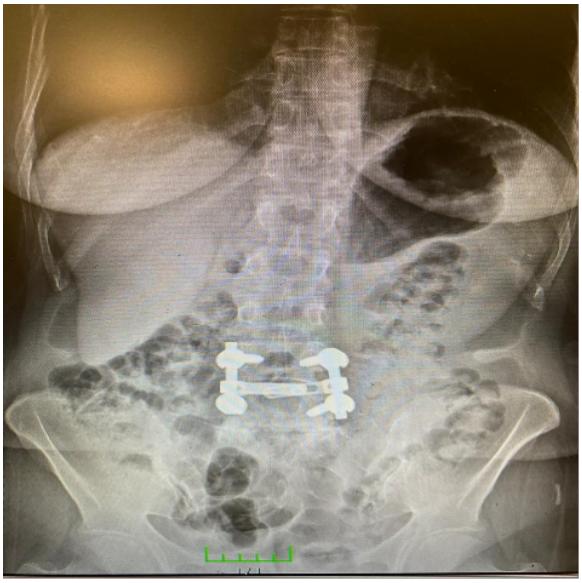

Fig. 5. Direct abdominal radiography before discharge

3. Discussion

TEF; it is a rare complication of various diseases of the esophagus and trachea, and most TEFs in adults have locally advanced esophageal or bronchogenic malignancies. However, TEF that develops for benign reasons may remain undiagnosed for years because of its rarity and lack of specific symptoms (5).

More than 75% of non-malignant acquired TEF is observed as a result of cuff-related trauma due to prolonged mechanical ventilation. It has been determined that erosion occurs between the trachea and the esophagus at a rate of 0.3-3% in mechanically ventilated patients. However, it is thought that tracheostomy does not reduce the risk of TEF due to mechanical ventilation (9-11). TEF caused by trauma to the posterior tracheal wall and esophagus is one of the late (> 7 days) complications of tracheotomy and its incidence is less than 1%. Fistulas seen in the early postoperative period are a result of iatrogenic injury during application (3,4,12). TEF that develops in the late period develops secondary to movement or opening of the tube, sound device, or tracheostomy cannula due to tracheal necrosis caused by excessive neck flexion, rigid aspiration, or high cuff pressure (3).

For TEF, post-feeding cough due to aspiration and persistent pulmonary infection are typical symptoms. At the same time, increased tracheal secretion, aspiration of gastric contents from the tracheal tube, rapidly developing abdominal

distension, or rhythmic filling of the nasogastric tube with a ventilator should be warning signs for TEF (13-15). Our patient was diagnosed with TEF after a total laryngectomy due to laryngeal cancer. Although primary tracheoesophageal fistula was opened in patients with advanced-stage laryngeal tumors who underwent total laryngectomy, TEF developed in this case due to acquired causes. The diagnosis of TEF can be made by high-resolution computed tomography, esophagography, or endoscopy/bronchoscopy. However, endoscopic/bronchoscopic methods give us the best information and help determine the surgical approach by providing information about the size and location of the fistula (16). Although non-surgical treatments such as an esophageal stent, tracheal endoprosthesis, bronchoscopic fibrin glue, and hyperbaric oxygen therapy are used in the treatment of TEF, the gold standard treatment method is surgical repair (2). The first step for each patient; Stabilizing the patient should be to separate the fistula from the airway to protect the lung from contamination. Gastrointestinal system complications (ileus, foreign body reaction, etc.) due to a migrated foreign body were not observed in our patient. No life-threatening complications were encountered during close follow-up, and TEF surgery was planned after the patient stabilized.

Acquired TEF is important, especially in patients with tracheostomy, as it causes life-threatening complications. If a foreign body is detected in the gastrointestinal tract in a patient using a sound device after total laryngectomy, TEF should be considered first.

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Authors' contributions

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Ethical Statement

This study was not required ethics approval.

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Innocent Adult-onset type II citrullinemia complicating pregnancy

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Abstract

Type II citrullinemia (CTLN2) is a rare autosomal recessive disorder characterized by hyperammonaemic encephalopathy, resulting from mutations in the SLC25A13 gene. It has age-related clinical manifestations, including neonatal intrahepatic cholestasis and adult-onset presentations. This case report presented a pregnant woman diagnosed with innocent CTLN2**.** A 22-year-old pregnant woman was diagnosed with CTLN2 after presenting with mental fog, agitation, and elevated ammonia levels. Her pregnancy was terminated, and she subsequently had three more successful pregnancies, during which she was treated with sodium benzoate, L-Arginine, and a protein-poor diet. Her symptoms and liver function tests improved with treatment. This was the first case report of a pregnant woman with CTLN2. Timely diagnosis and appropriate management strategies are crucial for both maternal and fetal health in cases of liver disease during pregnancy. CTLN2 should be considered in the differential diagnosis of coma in pregnancy, particularly when elevated liver enzymes and hyperammonaemia are present.

Keywords: citrullineamia, coma, L-arginine, pregnancy

1. Introduction

Citrin deficiency has two main age-related clinical manifestations neonatal intrahepatic cholestasis (NICCD), presenting in the first year of life, and type II citrullinemia in adulthood (CTLN2)(1). CTLN2 is a rare autosomal recessive adult-onset disorder characterized by episodes of hyperammonemic encephalopathy. It results from mutations in the *SLC25A13*, which is localized on chromosome 7q21.3 and encodes the liver-specific isoform of the mitochondrial aspartate–glutamate carrier (AGC2), also known as citrin (2). It is mostly developed by patients aged between 11 and 79 years. Unlike other types of citrullinemia, in citrullinemia, no primary defect has been found within the argininosuccinate synthetase (ASS) locus. Patients with CTLN2 present with various neurologic manifestations resembling those of hepatic encephalopathy closely (3). CTLN2 can cause premature death, and it manifests with frequent attacks of hyperammonaemia, neuropsychiatric symptoms, liver steatosis, and brain oedema (4).

CTLN2 patients usually present with hyperammonaemia and citrullinemia. Hyperammonaemia develops in patients with liver disease or if the urea cycle cannot control the ammonia load (5). Pregnancy itself is an important risk factor for this disease because it creates protein load. A spontaneous attack during pregnancy can cause diagnostic confusion with many other serious diseases and lead to overtreatment.

CTLN2 is also characterized by an unexplained liver-specific reduction in argininosuccinate synthetase, which is a key enzyme in the urea cycle, leading to decreased activity (6). The purpose of the urea cycle is to remove ammonia produced by deamination reactions in the mitochondrial matrix and to convert it to urea, which is excreted to achieve detoxification. Ammonia is very toxic to tissues, especially to neuronal tissues including the brain, and its accumulation can result in brain edema and neuropsychiatric symptoms (7).

According to detailed literature review results, type II citrullinemia cases diagnosed during pregnancy have not been previously reported in the literature. In this case report, a case with previously undiagnosed type II citrullinemia, who had an attack due to pregnancy and presented with coma, was presented, and the approach to such rare cases during pregnancy was summarized.

2. Case Report

A 38-year-old pregnant woman (Gravida 3, parity 2) was admitted for further evaluation of maternal citrullinemia type II to the high-risk pregnancy unit. The patients gave informed consent.

In 2006 (at the age of 22), she was referred to the current clinic for further evaluation of new-onset mental fog and agitation in the 16th gestational weeks of pregnancy. Sudden blurring of consciousness occurred after the patient was admitted to the hospital. In the physical examination, blood pressure was found to be 100/60 mmHg, and the patient was unconscious. In the laboratory investigation, she was negative for toxicology, serology (Hepatitis C Virus, Hepatitis B Virus, and human immunodeficiency virus, etc), autoimmunity, hormonal, and imaging studies, while the levels of aspartate

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aminotransferase (AST), alanine aminotransferase (ALT) and ammonia were elevated. The patient was intubated and referred to another hospital with the presumed diagnosis of liver failure. She was diagnosed with type 2 citrullinemia (CTLN2) there. Her first pregnancy was terminated due to poor health and a life-threatening condition. After pregnancy termination, the patient was administered L-Arginine and sodium benzoate and a protein-poor diet. With this treatment, liver function tests and general condition improved

Based on abdominopelvic sonography findings in 2012, all findings were normal except for mild hepatomegaly. The patient stopped the treatment in 2013 due to the absence of complaints, and the drugs she used caused nausea and fatigue.

Mild hepatomegaly was found on abdominal ultrasonography again in her second pregnancy in 2015. AST was found to be 18 IU/l, ALT to 21 IU/ L, and ammonia to 93.8 mc/l, and vitamin B12 replacement was started due to low serum vitamin B12 (95 pg/mL). At the 33rd gestational week of the patient, ammonia was found as 60.8 pg/mL, ALT as 78 IU/ L and AST: 68 IU/L. Treatment with sodium benzoate and a protein-poor diet was initiated. The patient delivered a 2850 g healthy male baby with APGAR 9 at the 1st minute and 10 at the 5th minute by normal vaginal mode at the 38th gestational week. Postpartum ammonia and liver function tests were within normal limits.

The patient had her third pregnancy under L Arginine and sodium benzoate treatment in 2017 and gave birth by normal vaginal route without any antenatal or postnatal complications. The patient did not receive postpartum sodium benzoate and L arginine treatment.

The patient was admitted to the current clinic at the 26th week of gestation in her fourth pregnancy in November 2022. She was 38 years old when presenting to the current clinic. In the examinations of the patient, ammonia was 58. 7 mc/L, AST 598 IU/L, and AST: 758 IU/L. Serologic and autoimmunity studies were all negative. The patient was consulted with the gastroenterology department, and treatment with sodium benzoate, L-arginine, and ursodeoxycholic acid (total daily dose of 750 mg) was initiated. Liver function tests and ammonia levels improved with treatment The patient gave birth to a healthy male baby with a birth weight of 3240 g, Apgar score 6 at 1 min. and 8 at 5 min by vaginal route spontaneously at 38 weeks of gestation. There were no postpartum maternal and neonatal complications. Postpartum ammonia (42.9 μmol/L (range 6-47)) and liver function tests (ALT 19 IU/L (range, 0-45), AST 19 IU/L (range 0-35)) were within normal limits.

3. Discussion

Adult-onset CTLN2 is known to be one form of citrin deficiency in urea cycle enzymes and results from mutations in the citrin gene. The other manifestations of CTLN2 include disorientation, abnormal behaviour, seizures, coma, and brain oedema (8). Citrin deficiency was described for the first time in Japan. However, subsequent cases were identified in Korea, Israel, Czech, China, England, and the United States, suggesting that citrin deficiency may be seen worldwide.

Presentation of CTLN2 is sudden and usually between 20 and 50 years of age. Our patient was diagnosed with CTLN2 when she was 22 years old. The diagnosis of citrin deficiency is established in a person with characteristic findings, including increased blood or plasma concentration of ammonia (hyperammonaemia). In our case, the level of ammonia was elevated. In one study investigating Type II (adult onset) citrullinemia, four of 10 patients had hyperammonaemia (9).

Urea cycle enzyme disorders result in the accumulation of toxic levels of ammonia in the blood due to deficient or absent enzymes involved in the urea cycle. Liver transplantation is considered an ideal treatment for these disorders, as the liver is the main site of urea cycle activity. However, conservative treatments such as a low-protein diet, dietary arginine supplementation, and medication can provide temporary relief by reducing ammonia levels. These treatments include sodium citrate, sodium benzoate, lactulose, non-absorbed antimicrobial agents, and branched-chain amino acid infusion. These treatments do not address the underlying enzyme deficiency and are only temporary solutions. For severe cases, liver transplantation may be necessary (10).

Recent studies have highlighted the importance of diet in CTLN2. It was demonstrated that a Medium-chain triglycerides supplement with a low–low-carbohydrate formula supplementation has a revolutionary effect on the treatment of CTLN2. It promptly decreases blood ammonia levels in six patients and improves hepatic enzyme expression and steatosis in one patient after long-term treatment (11). Our patient received a protein-poor diet, and her symptoms resolved over time.

Liver diseases during pregnancy pose a significant clinical challenge because they may affect both the mother and unborn baby. Abnormal liver tests are encountered in 3% to 5% of pregnancies due to numerous potential causes, and clinical symptoms range from self-limiting to rapidly fatal. In our case, the patient had mild hepatomegaly with elevated levels of AST and ALT that were returned to normal values with medical therapy. Timely diagnosis of liver disease during pregnancy is of great importance in order to establish appropriate management strategies. CTLN2 should be kept in mind in the differential diagnosis of liver diseases, especially in the presence of elevated liver enzymes and hyperammonaemia. To the best of our knowledge, this is the first case reported in the literature on a pregnant woman with CTLN2.

Informed consent

Informed consent was obtained for publication of this case report.

Conflict of interest
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Authors' contributions
Concept: M.A., Design: M.A., Data Collection or Processing: M.A., E.S.G.G., Analysis or Interpretation: M.A., S.G., E.S.G.G., Literature Search: M.A., S.G., Writing: M.A., S.G.

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Management of intracranial angiosarcoma metastasis to the right lateral ventricle choroid plexus by radiotherapy: A case report

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Abstract

Angiosarcomas are a type of rare malignant endothelial tumor. While its most common metastasis site is the scalp, cerebral metastases are rare. A 46-year-old woman came with a headache. A cranial magnetic resonance imaging (MRI) showed a hemorrhagic lesion within the right lateral choroid plexus and T1 contrast-enhancing lesions in the falx cerebri and right frontal dura. We used mini-craniotomy and neuronavigation to remove a purple-colored extradural lesion in the right frontal region. Histopathological analysis later confirmed the diagnosis of angiosarcoma. Cardiac MRI demonstrated the presence of a left atrial mass, which led to the decision to operate on the patient by the cardiovascular surgery team. After the surgery, the patient underwent radiotherapy and chemotherapy, as there was no cranial metastasis necessitating surgical resection. The cranial metastases responded to the treatment on follow-up, and there was no evidence of recurrence on the three-year follow-up. Although they are uncommon, pathology that indicates the presence of angiosarcoma requires cardiac screening. These patients may not require aggressive surgery as they respond well to adjuvant therapies.

Keywords: angiosarcoma, metastasis, radiotherapy, case report

1. Introduction

Cerebral metastases of angiosarcomas are a rare but very severe clinical situation, which confirms the malignant nature of these vascular tumors. Angiosarcomas, which develop from endothelial cells, are capable of forming brain metastases, resulting in substantial neurological sequelae and complicated treatment strategies (1). Brain metastases from angiosarcomas are a harbinger of a dismal clinical course, reflecting the aggressive behavior of the tumor and the challenges of managing metastatic disease in the central nervous system (2). Despite the advances in diagnostic and therapeutic approaches, treating metastatic cerebral angiosarcoma poses great challenges, requires a multidisciplinary approach, and emphasizes the need for further studies on targeted therapies and prognostic markers to improve the patient's outcome. We present a case that underscores the importance of the multidisciplinary approach to cerebral metastases of cardiac angiosarcomas.

2. Case Presentation

A 46-year-old female was referred to us from the emergency department. She has had episodes of headaches for the last three months that have become more severe recently. Computed tomography (CT) imaging revealed a hemorrhagic mass within the right ventricle (Fig. 1). Subsequent MRI imaging revealed multiple lesions in the falx cerebri, right frontal dura, and choroid plexus of the right lateral ventricle that were T1-contrast enhancing (Fig. 1).

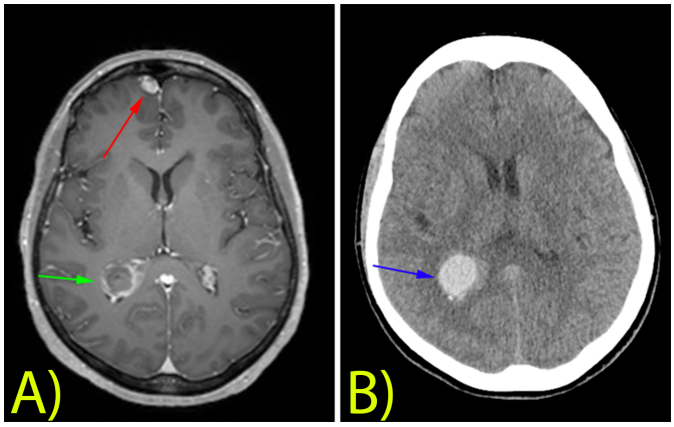

Fig. 1. T1 Contrast enhancing sequence(A), right atrial peripherally contrast-enhancing lesion is shown with the green arrow, while right frontal metastasis is shown with red arrow. On CT imaging (B), note the hemorrhagic lesion in the right ventricle, shown with a blue arrow

The patient was awake with no neurological symptoms. Apart from the headache, there were no notable features. She had no medical history, no tobacco use, and no history of contraceptive use.

Since there was no mass effect and no neurological symptoms, a biopsy from the right frontal dura was planned. The biopsy was obtained using neuronavigation and a mini-craniotomy. The lesion was extradurally located, purple-ish in color, and soft in nature. Postoperatively, the patient was

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discharged without complications.

On morphological analysis, the tumor exhibited atypical epithelioid cell nodules with eosinophilic cytoplasm on a highly inflammatory background. Immunohistochemical analysis revealed the tumor cell expression of endothelial markers CD31, CD34, and also ERG. The morphological findings and the immunophenotypic profile supported a diagnosis of grade 2 angiosarcoma.

We obtained a cardiac MRI scan, revealing a left atrial mass, which was removed by the cardiovascular surgery team, and the patient was discharged without complications.

The patient was discussed on our multidisciplinary tumor board. Since there was no mass effect of the right lateral ventricular metastasis, close follow-up and radiotherapy followed by chemotherapy using paclitaxel were planned. The patient received ten fractions of radiotherapy followed by 18 months of paclitaxel therapy and is disease-free after three years of follow-up (Fig. 2).

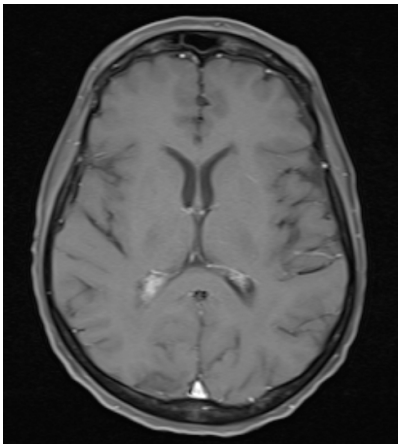

Fig. 2. T1 Contrast-enhancing sequence showing no recurrent lesions

3. Discussion

Intracerebral metastases originating from angiosarcomas are rare but emphasize the malignant nature of these vascular tumors and their ability to cause devastating neurologic sequelae (3). Approaches to the management of intracerebral angiosarcoma metastases usually include surgical resection, radiotherapy, and chemotherapy (4). Nevertheless, the effectiveness of such treatments is not high enough, which demonstrates that angiosarcomas are highly aggressive and do not respond to regular therapies. The prognosis of patients with cerebral metastases is still unfavorable (3), which emphasizes the urgent need for new therapeutic strategies and targeted therapies.

Molecular biology and immunotherapy have recently made great strides (5,6) and could provide new opportunities for treatment, thereby making the research into the pathogenesis of angiosarcoma even more critical. In addition, the importance of a multidisciplinary team must be considered as interdisciplinary care, including neurosurgeons, medical oncologists, radiologists, and pathologists, which is critical for the best patient outcomes.

To sum up, the treatment of intracranial angiosarcoma metastasis should be individualized according to the patient's clinical picture and the nature of the metastatic lesions. Our case and the recent literature case highlight the effectiveness of the multidisciplinary approach, including radiotherapy and chemotherapy. These cases add to the increasing evidence for the requirement of personalized treatment regimens and underscore the need for further research to formulate more conclusive treatment recommendations for this uncommon clinical entity.

Conflict of interest

The authors declare that they have no competing interests.

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Authors' contributions

Concept: H.B., T.Y., Design: H.B., T.Y., Data Collection or Processing: B.B.A., Analysis or Interpretation: B.B.A., M.S.B., Literature Search: B.B.A., M.S.B., Writing: B.B.A., M.S.B.

Ethical Statement

The need for ethics approval was waived as this is a case report.

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Laparoscopic peritoneal biopsy in the diagnosis of pediatric primary tuberculous peritonitis

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Abstract

This study aimed to share our experiences with children who have primary tuberculous peritonitis, which is difficult to diagnose, who underwent diagnostic laparoscopy. The study included two boys (8 and 9 years of age) and two girls (10 and 14 years of age). Three of the patients had complaints of abdominal pain for 1-2 months, and one patient had a mass lesion in the epigastric region for 3 months. All patients' tuberculosis skin tests were negative. Acid-fast bacilli were not detected in throat, sputum, or tissue cultures. Peritoneal thickening was detected on abdominal computed tomography in all patients. Laparoscopic peritoneal biopsy was performed in all patients. Pathology results revealed chronic granulomatous inflammation in the patients. During postoperative follow-up, subileus findings were observed in one patient, and proximal jejunal perforation developed in one patient. Diagnostic laparoscopy is thought to be a useful diagnostic tool for both sampling and morphological identification of lesions in tuberculous peritonitis.

Keywords: child, tuberculous peritonitis, laparoscopy, diagnosis

1. Introduction

The incidence of tuberculosis ranges between 18 and 43 people per 100000 population, depending on regional socioeconomic characteristics (1). Tuberculous peritonitis accounts for 0.1-0.7% of all tuberculosis cases (1). In children, 3.5% of tuberculous peritonitis cases are accompanied by pulmonary tuberculosis (2). Although it is associated with cirrhosis and HIV/AIDS in adults, conditions such as diabetes mellitus and hematological diseases that cause immunodeficiency are reported to be predisposing factors for tuberculous peritonitis in children (3). Therefore, the diagnosis and treatment of primary tuberculous peritonitis in children requires a multidisciplinary approach (4).

Mortality can reach 60% in adults with tuberculous peritonitis if diagnosis and medical treatment are delayed (5). Because tuberculous peritonitis causes atypical clinical findings such as acute abdomen, intestinal obstruction, mass, and massive ascites, it is more challenging to diagnose than pulmonary tuberculosis (6). This is mainly attributed to the difficulty in obtaining specific findings in radiological, biochemical, histopathological, and microbiological diagnosis (7). In addition, medical control of the ongoing active inflammation in patients with tuberculous peritonitis can be both slow and problematic (4). Therefore, considering the possible complications at the diagnosis stage, the role of surgical interventions remains controversial (4).

This study aimed to contribute to the related literature by sharing our clinical experience with pediatric patients who underwent laparoscopic peritoneal biopsy during the diagnosis of primary tuberculous peritonitis.

2. Case Report

The study included two boys (8 and 9 years of age) and two girls (10 and 14 years of age). Three of the patients had a history of abdominal pain for approximately 1-2 months, and one patient had a palpable mass lesion and weight loss in the epigastric region for 3 months. QuantiFERON tuberculosis tests were positive in all patients, while tuberculin skin tests were negative and throat and sputum cultures yielded no acid-fast bacilli. Plasma immunoglobulin values were within normal limits and viral disease panel tests were also negative. No signs of pulmonary or solid organ tuberculosis were detected on abdominopelvic ultrasound or chest computed tomography (CT). On abdominal CT, peritoneal thickening was observed in all four patients, while diffuse fluid was present in only one patient (Fig. 1).

All patients underwent diagnostic laparoscopy. During the procedure, diffuse granulomatous lesions on the parietal and visceral peritoneal surfaces and intraperitoneal fluid were observed in all four patients. Laparoscopic peritoneal biopsy and intraperitoneal fluid sampling were performed from the parietal peritoneal area (Fig. 2). The pathology results indicated chronic granulomatous inflammation in three patients and necrotizing granulomatous inflammation in one patient (Fig. 3). Acid-fast bacilli were not detected in tissue cultures of the biopsy samples. Elevated adenosine deaminase

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level (75 U/L) was detected in the peritoneal fluid of one patient.

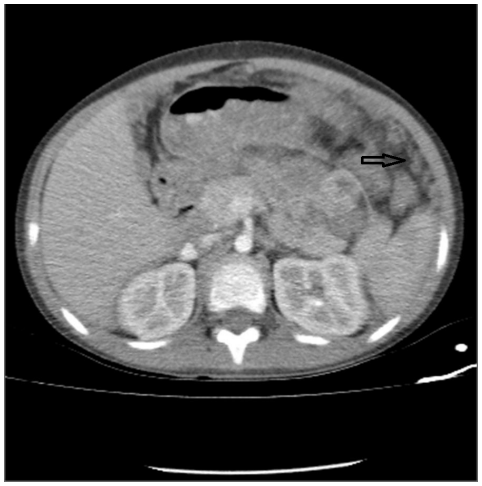

Fig. 1. Peritoneal thickening was observed in nine years old/boy patient

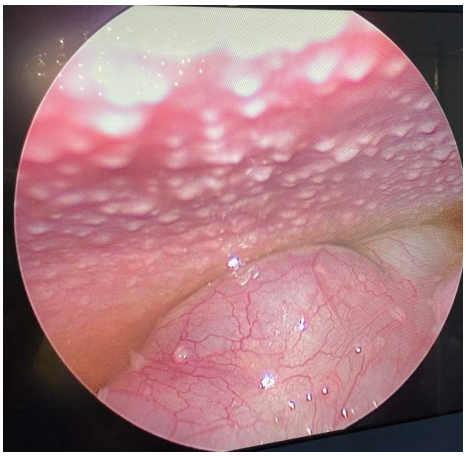

Fig. 2. Diagnostic laparoscopy image of 10 years old/girl patient

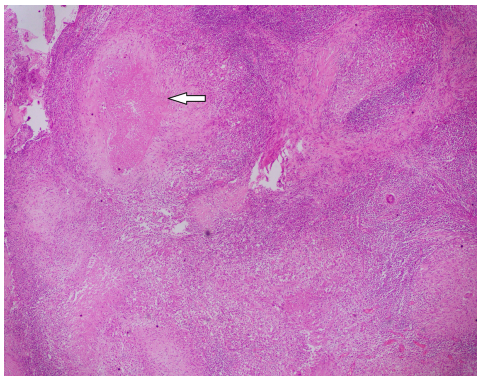

Fig. 3. Necrotizing granulomatous inflammation detected in laparoscopic biopsy material in a 14 years old/girl patient

All of the patients received quadruple antituberculous therapy consisting of isoniazid, ethambutol, pyrazinamide, and rifampicin (8). Postoperatively, one patient was followed up for a week due to signs of subileus. One patient presented with an acute abdomen and underwent an emergency laparotomy upon detecting signs of intestinal perforation. Examination revealed four perforation sites in the proximal jejunal segment, and primary repair was performed. The patients' mean postoperative follow-up time was 6.66±2.33 months. None of the patients died during follow-up.

3. Discussion

Despite advances in the treatment of tuberculosis, the diagnosis of primary peritoneal tuberculosis in children remains problematic due to the difficulty of detecting specific diagnostic findings (9). In addition, none of the diagnostic tools are sufficient on their own (10). The active inflammatory process in the peritoneal cavity of patients with tuberculous peritonitis also makes diagnosis and treatment prone to complications. For this reason, diagnostic laparoscopy procedures have notable advantages, such as enabling the identification of lesions in the intraperitoneal cavity and providing additional samples for diagnosis.

In children, extrapulmonary tuberculosis accounts for 0.3% of all tuberculosis cases (11). Lymphadenopathies are the most common extrapulmonary involvement of tuberculosis because it can spread via the lymphogenous route (12). Although 11% of abdominal tuberculosis is seen in the ileocecal area, it can also involve the liver and spleen (13). Primary tuberculous peritonitis is a clinical condition rarely seen in children (14). Children with tuberculous peritonitis generally have nonspecific findings such as abdominal pain, fever, and weight loss (14). Patients in this study had complaints of abdominal pain, a mass lesion in the epigastric region, and weight loss for several months. In addition, signs of the acute abdomen, such as intestinal obstruction and perforation, may occur along with increased inflammation in late-diagnosed cases of abdominal tuberculosis (15). In a study conducted in South Africa, Saczek et al. reported that 10% of tuberculous peritonitis cases were diagnosed by laparotomy after the patient presented with the clinical picture of an acute abdomen (16).

Aldriwesh et al. suggested that diagnostic methods with proven efficacy in pulmonary tuberculosis have low sensitivity in peritoneal tuberculosis (17). They reported that this caused delayed treatment and a resulting increase in morbidity among patients with extrapulmonary tuberculosis (17). Tuberculin skin tests are a diagnostic test in which an induration 10 mm in diameter occurs on the skin as a result of excessive immunological response, indicating infection with *Mycobacterium tuberculosis*. The positivity rate is up to 90% in patients with pulmonary tuberculosis (18). However, its sensitivity decreases in cases of comorbidities associated with immunodeficiency and in cases of miliary tuberculosis (19). In cases of extrapulmonary tuberculosis, the sensitivity of the tuberculin skin test decreases to 60% (19). Consistent with this, tuberculin skin tests were negative in all patients in this study. The QuantiFERON tuberculosis test, which is used as an alternative to tuberculin skin tests, measures the levels of interferon-alpha in response to mycobacterium antigens and is reported to have 70% sensitivity and 90% specificity (20). However, factors associated with bacterial load, such as the site

of involvement and the degree of inflammation, are reported to impact the sensitivity of the test (20). For this reason, it is thought that the tuberculosis quantiFERON test alone may not be sufficient to diagnose tuberculosis peritonitis. In all four patients in this study, QuantiFERON tuberculosis test was positive. These results suggest that the QuantiFERON tuberculosis test may aid the diagnosis of early tuberculous peritonitis at a stage when inflammation is milder and complications have not yet developed.

Adenosine deaminase is produced by T-lymphocytes against mycobacterium antigens. An adenosine deaminase level above 30 U/L in the peritoneal fluid was reported to have 96% sensitivity for abdominal tuberculosis (21). However, it was also reported that diseases affecting the immune system, such as congenital diseases, malignancy, and viral infections, may cause false-negative results (22). In one of the patients in this study, adenosine deaminase in the peritoneal fluid sample was found to be elevated, at 75 U/L. However, when evaluating this result, it is necessary to take into account the low bacterial load in extrapulmonary tuberculosis (22). Another diagnostic technique, polymerase chain reaction (PCR) test, has a reported sensitivity of 40% in the diagnosis of tuberculosis due to immunological reasons (23). Considering the cost of tuberculosis PCR test and its reduced sensitivity at low bacterial loads, its place in the diagnosis of extrapulmonary tuberculosis continues to be a subject of debate (23).

Regarding radiological diagnostic methods, while chest X-rays are within normal limits in 36% of pulmonary tuberculosis cases, nonspecific findings are obtained in 80% of abdominal tuberculosis cases on ultrasound and computed tomography (24). In our study, direct abdominal and chest radiographs were evaluated as within normal limits, while computed tomography revealed an edematous appearance suggestive of inflammation in the parietal peritoneum in all four patients. In diagnostic laparoscopy, diffuse granulomatous lesions were detected on the parietal and visceral peritoneum in all patients. This suggests that computed tomography findings of inflammation in the parietal peritoneum should be considered in the diagnosis, treatment, and follow-up of abdominal tuberculosis.

Culture remains the gold standard in the diagnosis of tuberculosis (25). However, culture positivity is low, with reported rates of 18% among all tuberculosis cases and 8.3% in patients with extrapulmonary tuberculosis. Another limitation of diagnostic cultures is that results are obtained after a period of 4-6 weeks (26). In tuberculosis, histological examination of tissue samples may reveal chronic granulomatous inflammation with and without caseating necrosis. However, the specificity of this type of inflammation to tuberculosis is reported to be 43% (26). A more sensitive diagnosis in histological examination requires the detection of the tuberculosis bacillus (acid-fast bacilli) (27). However, the histological identification of tuberculosis bacilli also involves drawbacks such as difficult detection because of the low bacterial load in the tissue and the cost of the dyes used (28). Although there was no growth in the tissue cultures of the patients in the study, findings suggestive of chronic granulomatous inflammation were detected upon examination of the tissue samples. Krishnan et al. reported that diagnostic laparoscopy procedures can be performed with low morbidity and mortality in patients with tuberculous peritonitis (29). Laparoscopy procedures in patients with tuberculous peritonitis offer a less invasive method of tissue sampling and obtaining morphological information about lesions in the intraperitoneal area (29). In the present study, diagnostic laparoscopy was used for the initial diagnosis. However, all patients responded to medical treatment and their clinical complaints regressed during follow-up, so control laparoscopy was not performed. In the literature, it is reported that the main determinant of postoperative complications in these patients is the severity of the ongoing inflammatory process (29). One patient in our study exhibited signs of partial ileus after laparoscopy, while intestinal perforation occurred in another patient. When all these results are evaluated together, it is seen that laparoscopy in patients with tuberculous peritonitis has advantages in terms of tissue sampling and determining the extent of intraperitoneal lesions. However, the low bacterial load in extrapulmonary tuberculosis is associated with disadvantages such as the low positivity of tissue cultures and low sensitivity of histological evaluation (29). In addition these results suggest that multiple diagnostic methods should be evaluated together when diagnosing tuberculous peritonitis.

Laparoscopic peritoneal biopsy is an effective diagnostic tool in patients with tuberculous peritonitis. In cases of tuberculous peritonitis, laparoscopy comes to the fore especially in the morphological identification of lesions. However, laparoscopy has limitations in the diagnosis of extrapulmonary tuberculosis, similar to microbiological, histopathological, and radiological methods. Nevertheless, due to the advantages it provides in terms of morphological characterization and tissue sampling, we think it is a useful diagnostic procedure for identifying latent infections both during diagnosis and treatment follow-up.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: C.İ., E.Y., Design: C.İ., E.Y., Data Collection or Processing: C.İ., E.Y., Ş.Ç., Analysis or Interpretation: C.İ, H.T., Literature Search: C.İ., E.Y., H.T. A.N., Writing: C.İ., E.Y., H.T., A.N.

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