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Determination of HLA Tissue Type According to the Etiology of Patients with Chronic Renal Failure

Kronik Böbrek Yetmezliği Hastalarının Etiyolojilerine Göre HLA Doku Tipinin Belirlenmesi

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ABSTRACT

Objective: Chronic kidney disease (CKD) is a prominent public health concern, is defined as functional and structural damage to the kidneys. This study aims to investigate the association between human leukocyte antigen (HLA) alleles individuals with CKD and the different etiological subgroups of disease.

Methods: Genomic DNA was obtained from peripheral blood samples of 1,079 patients with retrospective CKD and 1,111 healthy control individuals. HLA genotyping was conducted using the Luminex based low-resolution method. Allele frequency distributions were calculated with the help of Arlequin v3.11 population genetics statistics program and SPSS v23.0 program, and p<0.05 values were accepted as significant by chi-square tests.

Results: HLA A*02 (21.83%), B*35 (18.30%), DRB1*11 (21.41%) alleles were observed most frequently in individuals with CKD, respectively. In our study, B*08, B*49, B*50 alleles in the HLA B locus (p=0.002, p=0.012 p=0.009) and DRB1*03, *04 alleles in the HLA DRB1 locus (p<0.001, p<0.001) were found positively associated with CKD. A*02, A*11, A*74 alleles at the HLA A locus (p=0.003, p<0.001, p=0.009) and B*27, B*39, B* alleles at the HLA B locus 40, B*59 (p<0.001, p<0.001, p<0.001, p=0.009), DRB1*07, *08, *09, *13, *16 (p<0.001, p=0.012, p=0.007, p<0.001, p<0.001) alleles were determined as negatively associated with the disease. Among the etiological groups of CKD, cystic kidney disease (36.8%), hypertension (16.8%) and urological anomalies (16.6%) were negatively associated with the HLA-DR*13 allele.

Conclusions: Since CKD shows serious morbidity and mortality, this comprehensive study of HLA subgroups gave an explanatory idea about which alleles associated with the disease in terms of susceptibility and protection.

Keywords: Chronic Renal Insufficiency, DNA Probları, HLA, polymerase chain reaction-sequence-specific oligonucleotide probing

ÖZ

Amaç: Kronik böbrek hastalığı (KBH) belirgin bir halk sağlığı sorunu olup fonksiyonel ve yapısal böbrek hasarı ile tanımlanmaktadır. Bu çalışma, KBH olan bireylerde insan lökosit antijeni (HLA) allel dağılımının, hastalığın farklı etiyolojik alt gruplarıyla olan ilişkisini araştırmayı amaçlamaktadır.

Yöntemler: Retrospektif olarak incelenen, KBH tanılı 1079 hasta ve 1111 kontrol grubundan alınan periferik kan numunelerinden elde edilen DNA'lar, HLA tiplmesi için düşük çözünürlüklü Luminex yöntemi kullanılarak analiz edilmiştir. Allel frekans dağılımları, Arlequin v3.11 popülasyon genetiği istatistik programı ve SPSS v23.0 yazılımı ile hesaplanmış; p<0,05 olan değerler ki-kare testleri ile anlamlı kabul edilmiştir.

Bulgular: KBH'li bireylerde sırasıyla en yaygın HLA A*02 (%21,83), B*35 (%18,30), DRB1*11 (%21,41) alelleri gözlenmiştir. Araştırmamızda, HLA B lokusunda B*08, B*49, B*50 (p=0,002, p=0,012, p=0,009) ve HLA DRB1 lokusunda DRB1*03, DRB1*04 ve DRB1*15 (p<0,001, p<0,001, p=0,035) alelleri KBH ile pozitif ilişkili bulunmuşken, HLA A lokusunda A*02, A*11, A*74 (p=0,003, p<0,001, p=0,009) ve HLA B lokusunda B*27, B*39, B*40, B*59 (p<0,001, p<0,001, p<0,001, p=0,009) alelleri ve HLA DRB1 lokusunda DRB1*07, *08, *09, *13, *16 (p<0,001, p=0,012, p=0,007, p<0,001, p<0,001) alelleri ise hastalıkla negatif ilişkili olarak bulunmuştur.

Sonuçlar: KBH'nin ciddi sağlık sorunlarına ve ölüme yol açması nedeniyle, bu çalışmada hastalığa yatkınlık oluşturan ve hastalıktan koruyucu HLA alt grupları belirlenmiştir.

Anahtar kelimeler: Kronik Böbrek yetmezliği, DNA propları, HLA, polimeraz zincir reaksiyonu-diziye özgü oligonükleotid araştırması

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INTRODUCTION

Chronic kidney disease (CKD), which is characterized as a continuous and permanent damage of renal function, diagnosed with glomerular filtration rate (GFR) of 60 mL/min per 1.73m² and the constant development of kidney damage-related symptoms such as active urine sediments, proteinuria, structural abnormalities, histological damages or a background of renal transplantation that lasts for more than three months¹. CKD has historically been a global public health issue and represents a significant healthcare and fiscal strain, as it leads to a decreased GFR, which is commonly associated with a greater change of hospitalization, cognitive problems, cardiovascular events, and total mortality².

CKD represents a major public health issue both in Türkiye and worldwide, especially as its frequency is rising in Türkiye, count of individuals with end-stage kidney disease requiring kidney transplantation has grown during the previous two decades. CKD is caused by three major factors: Diabetic nephropathy, chronic glomerulonephritis, and hypertension³.

Genetic and non-genetic factors are thought to contribute to CKD. Numerous gene mutations that affect the progress of CKD have been found in most genetic investigations, though the consequences of these variants on kidney disease risk are fairly mild⁴. Many gene regions, including APOL1, MYH9, and UMOD, are nowadays implicated for the disease's development⁵.

Human leukocyte antigen (HLA) is one of the most significant genetic factors that causes CKD. The major histocompatibility complex (MHC), which is found on chromosome 6p21.3, encodes the HLA, the most polymorphic locus within the human genome. Numerous HLA genes encoded in the MHC are connected to an increased or decreased risk of renal failure⁶⁻⁹.

In our country, few regional studies have investigated the connection between HLA alleles and CKD. In our study, the objective was to examine the connection between HLA allele distribution and the etiological subtypes of the disease among 1,079 patients diagnosed with CKD and 1,111 healthy control individuals attending the Akdeniz University Hospital Organ Transplantation Center from across Türkiye.

MATERIALS and METHODS

Research Group and HLA Genotyping

The study, which was made retrospectively and supported by Akdeniz University Scientific Research Fund (Project:1176/2016) was approved by Akdeniz

University Clinical Research Ethical Committee (no:354, date 01.09.2015), was DNA isolated from peripheral blood samples of 1,079 patients diagnosed with CKD who applied to the outpatient clinics of Nephrology and Organ Transplantation Center between 2010-2015. As the control group, healthy bone marrow donors (1,111) of pediatric and adult bone marrow transplant patients without a history of CKD were selected.

All investigations were carried out in HLA Tissue Typing Laboratory of Akdeniz University Hospital, which is constantly going through the external quality control tests from the European Federation of Immunogenetics and the United Kingdom's National External Quality Assessment Service.

EZI advanced XL Workstation, employing magnetic bead technology for sample processing, was used to harvest genomic DNA from peripheral venous blood samples (Qiagen, Germany). Luminex technology was utilized for HLA genotyping in each participant using the polymerase chain reaction-SSO method (IMMUCOR-Lifecodes, Georgia).

Statistical Analysis

The allele frequencies (AF) of HLA alleles were calculated in patients with CKD and controls using the following formula: $AF (\%) = (n/2N) 100$, where n defined as the the number of alleles and N indicated as total amount of individuals. By using Arlequin v3.11 population genetics software, the frequencies of HLA class I and II alleles and haplotypes in all participants were performed¹⁰.

SPSS 23.0 software was used to conduct statistical analysis (SPSS Inc, Chicago IL, USA). Cross tabulation with corrected chi-square test or Fisher's exact test was utilized to assess the differences in allele percentages between CKD patients and the control group. The p-value was computed using the expected value in the statistical method. The chi-square test was applied directly when the expected value was grater than 5. The Fisher test was used when one of the predicted values was less than one. Correction chi-square tests were performed in other examples. The degree of the disease association to a specific allele was reflected using an odds ratio (OR) and 95% confidence intervals (CI). Statistical significance was prioritized at a level of $p < 0.05$.

RESULTS

Etiology of CKD Patients

As per Turkish Society of Nephrology (TSN) data from 2018, CKD cases in Türkiye were categorized into

9 subgroups based on the underlying etiologies. With the data from our study, we were able to create detailed subgroups of CKD patients¹¹.

Once patients were separated into 13 groups based on their initial illnesses, CKD arose approximately in 36.8% due to cystic kidney diseases. Following cystic kidney disease, the most frequent main disorders leading to CKD were hypertension (16.8%, n=181), urologic abnormalities (16.6%, n=179) and nephrolithiasis (6.5%, n=71) (Table 1).

HLA A, B, DRB1 AF of 1,079 patients diagnosed with CKD and 1,111 healthy bone marrow donors as the control group is given in Table 2.

When comparing the patient group and the healthy control group, HLA alleles with high allele frequency in the patient group were deemed positively associated with the disease, while HLA alleles with a higher allele frequency in the control group relative to the patient group were considered negatively associated.

In our study, according to the data obtained, HLA A*02 (21.83%), B*35 (18.30%), DRB1*11 (21.41%) alleles were observed most frequently in individuals with CKD,

Table 1. The proportion of core diseases among CKD patients

Etiology of CKD	(N)	(%)
Unknown etiology	70	6.5
Hypertension	181	16.8
Urologic abnormalities	179	16.6
Tubulointerstitial nephritis	21	1.9
Diabetic nephropathy	31	2.9
Diabetic nephropathy and hypertensive nephropathy	18	1.7
Nephrolithiasis	71	6.6
Cystic kidney disease	397	36.8
Familial mediterranean fever (FMF)	29	2.7
Glomerulonephritis	26	2.4
Amyloidosis	12	1.1
Congenital renal disease	20	1.9
Genetic disorder (Ankylosing spondylitis, Bardet Biedl syndrome, Bartter syndrome, Alport syndrome)	24	2.2
Total	1079	100

N: Patient number, CKD: Chronic kidney disease

Table 2. HLA-A, B, DRB1 allele frequencies in chronic kidney patients and control groups

HLA	CKD (2n=2158)	Control (2n=2222)	Statistical analysis	
	n-AF (%)	n-AF (%)	OR (95 %CI)	p
A* 01	225 (10.43)	207 (9.32)	1.133 (0.929- 1.382)	0.218 _a
A* 02	471 (21.83)	637 (28.67)	0.695 (0.605- 0.797)	0.003 _a
A* 03	263 (12.19)	245 (11.03)	1.120 (0.931-1.348)	0.230 _a
A* 08	-	1 (0.05)	-	-
A* 09	3 (0.14)	-	-	-
A* 10	1 (0.05)	-	-	-
A* 11	1 (0.05)	116 (5.22)	0.008 (0.001-0.060)	<0.001 _a
A* 13	1 (0.05)	-	-	-
A* 20	1 (0.05)	-	-	-
A* 22	-	1(0.05)	-	-
A* 23	85 (3.94)	70(3.15)	1.261(0.913-1.739)	0.158 _a
A* 24	362 (16.78)	334(15.03)	1.13 (0.96-1.340)	0.115 _a
A* 25	15 (0.70)	7(0.32)	2.215 (0.901-5.443)	0.075 _a
A* 26	126 (5.84)	154 (6.93)	0.833 (0.653-1.062)	0.140 _a
A* 27	1 (0.05)	-	-	-
A *28	2 (0.09)	1 (0.05)	2.060(0.186-22.738)	0.620 _b
A* 29	41(1.90)	37 (1.67)	1.144 (0.730-1.791)	0.557 _a
A* 30	78 (3.61)	75 (3.38)	1.074 (0.777-1.482)	0.667 _a
A* 31	38 (1.76)	28 (1.26)	1.405 (0.859-2.297)	0.174 _a
A* 32	98 (4.54)	108 (4.86)	0.931 (0.704-1.232)	0.618 _a

Table 2. Continued				
HLA	CKD (2n=2158)	Control (2n=2222)	Statistical analysis	
	n-AF (%)	n-AF (%)	OR (95 %CI)	p
A* 33	53 (2.46)	70 (3.15)	0.774 (0.539-1.112)	0.164 _a
A* 34	3 (0.14)	4 (0.18)	0.772 (0.173-3.453)	1.000 _b
A* 36	1 (0.05)	-	-	-
A* 39	1 (0.05)	3 (0.18)	0.343 (0.036-3.299)	0.625 _b
A* 66	13 (0.60)	6 (0.27)	2.238 (0.849-5.900)	0.094 _a
A* 68	91 (4.22)	99 (4.46)	0.944 (0.706-1.2639)	0.698 _a
A* 69	10 (0.46)	4 (0.18)	2.581 (0.808-8.243)	0.097 _a
A* 74	3 (0.14)	14 (0.63)	0.220 (0.063-0.756)	0.009 _a
A* 80	-	1 (0.05)	-	-
B* 05	2 (0.09)	-	-	-
B* 06	1 (0.05)	-	-	-
B* 07	88 (4.08)	114 (5.13)	0.786 (0.591-1.045)	0.097 _a
B* 08	95 (4.40)	60 (2.70)	1.659 (1.194-2.305)	0.002 _a
B* 11	1 (0.05)	-	-	-
B* 12	2 (0.09)	-	-	-
B* 13	65 (3.01)	84 (3.78)	0.790 (0.569-1.099)	0.161 _a
B* 14	48 (2.22)	34 (1.53)	1.464 (0.940-2.281)	0.090 _a
B* 15	86 (3.99)	99 (4.46)	0.621 (0.462-0.833)	0.001 _a
B* 18	138 (6.40)	129 (5.81)	1.108 (0.865-1.420)	0.415 _a
B* 21	3 (0.14)	4 (0.19)	0.772 (0.173-3.453)	1.000 _b
B* 22	1 (0.05)	2 (0.09)	0.515 (0.047-5.679)	1.000 _b
B* 25	-	1 (0.05)	-	-
B* 27	46 (2.13)	92 (4.18)	0.504 (0.352-0.722)	<0.001 _a
B* 33	1 (0.05)	-	-	-
B* 35	395 (18.30)	374 (16.83)	1.706 (0.606-0.823)	0.200
B* 36	-	1 (0.05)	-	-
B* 37	15 (0.70)	30 (1.35)	0.511 (0.274-.953)	0.032 _a
B* 38	108 (5.01)	83 (3.74)	1.358 (1.013-1.819)	0.040 _a
B* 39	19 (0.88)	72 (3.24)	0.265 (0.159-0.441)	<0.001 _a
B* 40	71 (3.29)	121 (5.45)	0.591 (0.438-0.797)	<0.001 _a
B* 41	52 (2.41)	42 (1.89)	1.282 (0.850-1.933)	0.236 _a
B* 42	1 (0.05)	1 (0.05)	1.030 (0.064-16.742)	1.000 _b
B* 44	154 (7.14)	182 (8.19)	0.861 (0.689-1.077)	0.190 _a
B* 45	6 (0.28)	1 (0.05)	6,192 (0.745-51.478)	0.066 _b
B* 46	3 (0.14)	-	-	-
B* 47	5 (0.23)	6 (0.27)	0.858 (0.261-2.815)	0.800 _a
B* 48	13 (0.60)	8 (0.36)	1.677 (0.694-4.055)	0.246 _a
B* 49	95 (4.40)	66 (2.97)	1.504 (1.093-2.071)	0.012 _a
B* 50	77 (3.57)	50 (2.25)	1.607 (1.120-2.306)	0.009 _a
B* 51	313 (14.50)	282 (12.69)	1.167 (0.982-1.388)	0.080 _a

Table 2. Continued

HLA	CKD (2n=2158)	Control (2n=2222)	Statistical analysis	
	n-AF (%)	n-AF (%)	OR (95 %CI)	p
B* 52	89 (4.12)	74 (3.33)	1.249 (0.912-1.710)	0.165 _a
B* 53	15 (0.70)	20 (0.90)	0.771 (0.394-1.509)	0.446 _a
B* 54	4 (0.19)	-	-	-
B* 55	71 (3.29)	60 (2.70)	1.226 (0.865-1.738)	0.252 _a
B* 56	5 (0.23)	10 (0.45)	0.514 (0.175-1.505)	0.216 _a
B* 57	36 (1.67)	41 (1.85)	0.902 (0.574-1.418)	0.656 _a
B* 58	24 (1.11)	25 (1.13)	0.988 (0.563-1.736)	0.967 _a
B* 59	2 (0.09)	12 (0.54)	0.171 (0.038-0.764)	0.009 _a
B* 60	-	4 (0.18)	-	-
DRB1* 01	112 (5.19)	90 (4.05)	1.297 (0.976-1.723)	0.072 _a
DRB1* 03	228 (10.57)	150 (6.75)	1.632 (1.136-2.024)	<0.001 _a
DRB1* 04	372 (17.24)	278 (12.51)	1.457 (1.231-1.723)	<0.001 _a
DRB1* 06	1 (0.05)	-	-	-
DRB1* 07	160 (7.41)	237 (10.67)	0.671 (0.544-0.827)	<0.001 _a
DRB1* 08	39 (1.81)	66 (2.97)	0.601 (0.403-0.897)	0.012 _a
DRB1* 09	10 (0.46)	27 (1.22)	0.378 (0.183-0.784)	0.007 _a
DRB1* 10	55 (2.55)	64 (2.88)	0.882 (0.612-1.271)	0.500 _a
DRB1* 11	462 (21.41)	438 (19.71)	1.110 (0.958-1.285)	0.165 _a
DRB1* 12	27 (1.25)	34 (1.53)	0.815 (0.490-1.356)	0.431 _a
DRB1* 13	214 (9.92)	350 (15.75)	0.589 (0.491-0.706)	<0.001 _a
DRB1* 14	153 (7.09)	146 (6.57)	1.085 (0.858-1.372)	0.496 _a
DRB1* 15	229 (10.61)	194 (8.73)	1.241 (1.015-1.517)	0.035 _a
DRB1* 16	88 (4.08)	147 (6.62)	0.600 (0.458-0.787)	<0.001 _a
DRB1* 18	3 (0.14)	-	-	-
DRB1* 21	1 (0.05)	-	-	-
DRB1* 23	1 (0.05)	-	-	-
DRB1* 45	1 (0.05)	-	-	-
DRB1* 51	-	1 (0.05)	-	-

*:p<0.05, **:p<0.001. HLA: Human leukocyte antigen, CKD: Chronic kidney disease, AF: Allele frequency, OR: odds ratio, CI: Confidence interval, 2n: each individual was represented by two codominant allelic data, _a: Pearson chi-square test, _b: Fisher's exact test

respectively. Although the HLA A*02 (p=0.003) allele was statistically significant when compared to the control group, no notable difference was observed for the B*35 (p=0.200) and DRB1*11 (p=0.165) alleles.

Alleles were positively associated with the disease at the HLA B locus B*08, B*49, B*50 [OR; 1.66 (95% CI; 1.19-2.30), p=0.002], [OR; 1.50 (95% CI; 1.09-2.07), p = 0.012], [OR; 1.60 (95% CI; 1.12-2.30), p=0.009] and at the HLA DRB1 locus DRB1*03, * 04, *15 (p =0.035) [OR; 1.63(95% CI; 0.13-2.03, p <0.001], [OR; 1.46 (95% CI; 1.23-1.72), p <0.001], [OR; 1.24 (95% CI; 1.01-1.51), p = 0.035], respectively.

In the HLA A locus, A*02, *11, *74 (p=0.003, p<0.001, p=0.009), and in the HLAB locus B* 27, B* 39, B* 40, B* 59 (p<0.001, p<0.001, p<0.001, p=0.009) alleles and DRB1*07, *08, *09, *13, *16 (p <0.001,) at the HLA DRB1 locus p=0.012, p=0.007, p<0.001, p<0.001) alleles were found to be negatively associated with the disease.

According to the data obtained in our study, the distribution of patients with CKD according to their known etiology is shown in Table 3.

DISCUSSION

It has been reported in publications that CKD is a common disease with high socio-economic cost in our country and in the world, and its incidence is increasing gradually.

Patients with end-stage renal disease (ESRD) necessarily need a kidney transplant to avoid uremia, a life-threatening condition¹². Nowadays, with the development of molecular techniques, it is becoming increasingly important to investigate the relationships between HLA, which is one of most genetically diverse

gene region in the human genome, and diseases¹³. Although there are various publications from previous studies regarding the association between many diseases and HLA in our region and country, there are only a limited number of studies that have investigated the relationship between CKD and HLA^{14,15}.

HLA polymorphism may be correlated with ESRD due to its association with the causes and progression of renal disease⁷. For example, HLA B*51 was linked to ESRD in Venezuelan and Brazilian subjects¹⁶, whereas A*26 has shown a protective effect against ESRD in Saudi Arabia¹⁷ and Türkiye¹⁴.

Table 3. Allele frequencies of HLA-A*, B*, DRB1* in chronic kidney patients and control group according to etiological subgroups					
Etiology of CKD	Gene/or locus	Patient	Control (2n=2222)	Statistical analysis	Etiology of CKD
	HLA	N-AF (%)	N-AF (%)	OR	p-value
Urological anomalies (2n=358)	A* 01	53(14.80)	207 (9.32)	1.69 (1.22-2.341)	0.001 _a *
	A* 02	75 (20.95)	637 (28.67)	0.65 (0.50-0.864)	0.002 _a *
	A* 11	30 (8.38)	116 (5.22)	1.66 (1.09-2.557)	0.016 _a *
	A* 26	12 (3.35)	154 (6.93)	0.46 (0.25-0.847)	0.010 _a *
	B* 08	22 (6.15)	60 (2.70)	2.35 (1.42-3.897)	0.001 _a **
	B* 40	7 (1.96)	121 (5.45)	0.34 (0.16-0.748)	0.005 _a *
	B* 44	17 (4.75)	182 (8.19)	0.55 (0.33-0.931)	0.023 _a *
	B* 45	2 (0.56)	1 (0.05)	12.47 (1.12-137.964)	0.008 _a *
	DRB1* 03	40 (11.17)	150 (6.75)	1.73 (1.20-2.511)	0.003 _a *
	DRB1* 13	35 (9.78)	350 (15.75)	0.53 (0.37-0.771)	0.001 _a *
Cystic kidney disease (2n=794)	A* 02	169 (21.29)	637 (28.67)	0.67 (0.55-0.816)	<0.001 _a **
	A* 11	67 (8.44)	116 (5.22)	1.67 (1.22-2.28)	0.001 _a *
	A* 23	42 (5.29)	70 (3.15)	1.71 (1.16-2.540)	0.006 _a *
	B* 13	17 (2.14)	84 (3.78)	0.55 (0.32-0.944)	0.028 _a *
	B* 27	14 (1.76)	92 (4.14)	0.41 (0.23-0.733)	0.002 _a *
	B* 39	4 (0.50)	72 (3.24)	0.15 (0.05-0.415)	<0.001 _a **
	B* 40	28 (3.53)	121 (5.45)	0.63 (0.41-0.965)	0.032 _a *
	B* 41	25 (3.15)	42 (1.89)	1.68 (1.02-2.787)	0.039 _a *
	B* 49	50 (6.30)	66 (2.97)	2.19 (1.50-3.200)	<0.001 _a **
	DRB1* 03	80 (10.08)	150 (6.75)	1.54 (1.16-2.057)	0.002 _a *
	DRB1* 04	140 (17.63)	278 (12.51)	1.49 (1.19-1.898)	<0.001 _a **
	DRB1* 07	52 (6.55)	237 (10.67)	0.58 (0.43-0.802)	0.001 _a *
	DRB1* 13	78 (9.82)	350 (15.75)	0.58 (0.44-0.756)	<0.001 _a **
Unknown (2n=140)	A* 02	19 (13.57)	637 (28.67)	0.39 (0.23-0.639)	<0.001 _a **
	A* 11	18 (12.86)	116 (5.22)	2.67 (1.57-4.476)	<0.001 _a **
	A* 30	10 (7.14)	75 (3.38)	2.20 (1.11-4.360)	0.020 _a *
	DRB1* 01	5 (3.57)	90 (4.05)	2.42 (1.32-4.456)	0.003 _a *
	DRB1* 04	26 (18.57)	278 (12.51)	1.59 (1.02-2.487)	0.038 _a *

Table 3 (continued)

Etiology of CKD	Gene/or locus	Patient	Control (2n=2222)	Statistical analysis	Etiology of CKD
	HLA	N-AF (%)	N-AF (%)	OR	p-value
Hypertensive Nephropathy (2n=362)	B* 39	2 (0.55)	72 (3.24)	0.16 (0.04-.674)	0.004 _a *
	DRB1* 01	25 (6.87)	90 (4.05)	1.57 (1.11-2.778)	0.015 _a *
	DRB1* 03	41 (11.26)	150 (6.75)	1.74 (1.22-2.541)	0.002 _a *
	DRB1* 13	37 (10.17)	350 (15.75)	0.60 (0.42-0.872)	0.006 _a *
	DRB1* 16	12 (3.30)	147 (6.62)	0.48 (0.26-0.881)	0.015 _a *
Diabetic nephropathy (2n=62)	A* 29	4 (6.45)	37 (1.67)	4.07 (1.40-11.803)	0.024 _b *
	A* 66	2 (3.23)	6 (0.27)	12.31 (2.43-62.254)	0.018 _b *
	B* 55	7 (11.29)	60 (2.70)	4.58 (2.00-10.489)	0.002 _b *
	DRB1* 03	12 (19.36)	150 (6.75)	3.31 (1.72-6.360)	0.001 _b **
	DRB1* 04	17 (27.42)	278 (12.51)	2.64 (1.49-4.680)	0.001 _a **
Diabetic nephropathy and Hypertensive Nephropathy (2n=36)	A* 11	6 (16.67)	116 (5.22)	3.63 (1.48-8.897)	0.011 _b *
	B* 42	1 (2.78)	1 (0.05)	63.45 (3.89-1035.056)	0.032 _b *
	B* 51	9 (25.00)	282 (12.69)	2.29 (1.06-4.926)	0.041 _b *
Nephrolithiasis (2n=142)	A* 26	3 (2.06)	154 (6.93)	0.29 (0.91-0.920)	0.025 _a *
	B* 18	18 (12.33)	129 (5.81)	2.35 (1.39-3.98)	0.001 _a *
	B* 40	2 (1.37)	121 (5.45)	0.24 (0.06-1.014)	0.036 _a *
	B* 50	8 (5.48)	50 (2.25)	2.59 (1.20-5.581)	0.021 _b *
	B* 52	12 (8.22)	74 (3.33)	2.67 (1.42-5.057)	0.004 _a *
	DRB1* 13	12 (8.22)	350 (15.75)	0.49 (0.27-0.902)	0.019 _a *
Familial mediterranean fever (2n=58)	B* 48	2 (3.45)	8 (0.36)	9.88 (2.05-47.605)	0.025 _b *
	B* 50	4 (6.90)	50 (2.25)	3.21 (1.12- 9.229)	0.046 _b *
Amyloidosis (2n=22)	B* 48	2 (8.33)	8(0.36)	25.15 (5.05-125.282)	0.005 _b *
	DRB1* 14	5 (20.8)	146 (6.57)	3.74 (1.37-10.165)	0.019 _b *
Genetic disorders (2n=48)	B* 52	5 (10.4)	74 (3.33)	3.37 (1.29-8.768)	0.024 _b *
	DRB1* 04	12 (25.0)	278 (12.51)	2.33 (1.19-4.534)	0.010 _a *
	DRB1* 15	9 (18.8)	194 (8.73)	2.41 (1.15-5.05)	0.034 _b *
Congenital kidney diseases (2n=48)	B* 07	6 (12.5)	114 (5.13)	2.64 (1.10-6.343)	0.038 _b *
	B* 38	10 (10)	83 (3.74)	2.99 (1.15-7.762)	0.036 _b *
Chronic glomerulonephritis (2n=52)	A* 24	19 (36.54)	334 (15.03)	3.25(1.82-5.791)	<0.001 _a ..
Tubulointerstitial nephritis (2n=42)	DRB1* 04	13 (30.95)	278 (12.51)	3.13 (1.61-6.102)	<0.001 _a *

*:p<0.05, **:p<0.001. HLA: Human leukocyte antigen, CKD: Chronic kidney disease, AF: Allele frequency, OR: odds ratio, CI: Confidence interval, 2n: each individual was represented by two codominant allelic data, _a: Pearson chi-square test, _b: Fisher's exact test

Our research was carried out to evaluate the etiological distribution and frequency of patients with CKD who applied to the Akdeniz University Hospital Organ Transplantation Center from all over our country, and to investigate the HLA allele distribution of their patients.

Etiological information about CKD in our country is being researched by the TSN. Especially in the last two decades, a relative change in the etiology of CKD has been reported by TSN. While the most important cause of CKD in the past was chronic glomerulonephritis, in a study by Suleymanlar et al.¹⁸ 2009, the leading etiologic cause was diabetes mellitus (35%), followed by hypertension (27%), glomerulonephritis (7%), polycystic kidney disease (7%), pyelonephritis (3%), amyloidosis (2%) and other causes. The primary disease is unknown in 15% of the patients.

In our study, cystic kidney disease 36.8%, hypertension 16.8%, urological anomalies 16.6% and nephrolithiasis 6.58% were found to be the most common etiological causes of 1079 chronic kidney failure patients who applied to the organ transplantation polyclinics of Akdeniz University Hospital Nephrology Department, respectively.

The literature suggests that the HLA system is linked to the development of various conditions, including autoimmune disorders, inflammatory bowel disease, allergies, and certain kidney diseases such as diabetic nephropathy, immunoglobulin A (IgA) nephropathy, and glomerulonephritis. These connections highlight the involvement of HLA in the pathogenesis of these diseases¹⁹. Detection and examination of HLA polymorphism are tissue typing tests that are critical not just for studies related to ESRD susceptibility but concerning the selection of tissue recipients and donors for tissue transplantation in. In 2014, Cao et al.¹² in his study, HLA A*24, B*55, B*54, B*40, DRB1*04 alleles were associated with ESRD in Asian countries. In another study, DRB1*11 and DRB1*03 alleles were identified as positively associated with CKD in individuals with ESRD, while the HLA DRB1*08 allele was found to be negatively associated with the disease¹⁹. Crispim et al.²⁰ They reported that the HLA A*78 and DRB1*11 alleles were at a high frequency in patients with ESRD, while the HLA B*14 allele was at a low frequency, but these values were not statistically significant. In Türkiye in 2010, Karahan et al.¹⁴ in his study on patient groups with CKD, the most prevalent HLA alleles were identified as HLA A*02 (43.8%), DRB1*11 (43.8%) and B*35 (32.4%).

In our study, additionally HLA A*02 (21.83%), B*35 (18.30%), DRB1*11 (21.41%) alleles were observed in individuals with CKD, respectively. B*08, B*49, B*50

(p=0.002, p=0.012, p=0.009) in the HLA B locus and DRB1*03, DRB1*04, DRB1*15 (p<0.001, p<0.001, p = 0.035) in the HLA DRB1 locus, respectively. While its alleles were positively associated with CKD, A*02, A*11, A*74 (p=0.003, p=0.001, p=0.009) in the HLA A locus and HLA B*27, B*39, B*40, B*59 (p=0.000, p<0.001, p<0.001, p=0.009) alleles and DRB1*07, *08, *09, *13, *16 (The p<0.001, p=0.012, p=0.007, p=0.001, p=0.001) alleles were found to be negatively associated with the disease.

In countries such as China, France, South America, and England, research has indicated that the HLA DR*03 allele is positively associated with the development of membranous and diabetic nephropathy, but has a protective effect in the development of idiopathic IgA nephropathy^{21,22}.

It has also been stated that HLA DRB1*03 and DRB1*11 are associated with diabetic nephropathy in the Egyptian population²³. While HLA DRB1*15 and DQB1*05 alleles are positively associated with ESRD due to type 2 diabetes in Mexico, HLA DRB1*04 has been found to be protective in the USA and Mexico^{24,25}. In 2009, Karahan et al.¹⁴ showed that HLA B*58 and HLA DRB1*03 alleles were positively associated with diabetic nephropathy and amyloidosis diseases.

In our study, the most common HLA alleles in the CKD group with diabetic nephropathy were found to be HLA A*02 (17.74%), B*35 (17.74%), DRB1*04 (27.42%). As a result of the statistical analysis, HLA A*29, A* 66, B* 55, DRB1* 03, DRB1*04 (p=0.024, p=0.018, p=0.002, p=0.001, p=0.001) respectively, who had alleles were positively associated with the disease. According to the literature, the HLA DRB1*03 (p=0.001) allele was found to be positively associated with diseases, while the negatively associated allele was not found.

HLA-DR4 has been linked to immune complex-mediated glomerulonephritis in studies carried out in populations from China, Italy, the United States, and different countries²⁶. In the Han population of China, the HLA DRB1*04 allele was observed with a high frequency in patients with IgA nephropathy²⁷. In a study carried out in 2016, nephrotic syndrome and DRB1*07, DQB1*02, alleles were found to be strongly associated, while DRB1*10, DQB1*05, DQB1*06 alleles were found to be protective against the disease²⁸. In our study, the most prevalent alleles in the HLA A group in patients in the glomerulonephritis group were found to be HLA A*24 (36.54%), B*35 (17.31%), DRB1*11 (30.77%).

As a result of the statistical analysis, the HLA A*24 ($p<0.001$) allele was identified as positively associated with the disease. We came to the conclusion that this different situation may be due to polymorphism in populations.

There are not many research in the literature investigating the association between hypertension and CKD. In the USA, in individuals with HLA B*35 and DRB1*03 alleles, AF were demonstrated to be high in individuals with CKD due to hypertensive nephropathy and were observed to be statistically significant²⁹. In our study, the most common alleles in patients in the hypertension group were HLA A*02 (24.73%), B*35 (20.06%), HLA DRB1*11 (19.23%). DRB1*01 ($p=0.015$) and DRB1*03 ($p=0.002$) alleles were found to be positively associated with the disease. B*39 ($p=0.004$), DRB1*13 ($p=0.006$), DRB1*16 ($p=0.015$) alleles were found to be negatively associated with the disease. Some of the researches were found to be compatible with the literature and some were not.

In one of the studies in China in which the relationship between PCD and HLA was investigated serologically in a family of 9 people, 4 of whom had a history of PCD, it was found that the HLA-A9-B22-HLA-DR5 haplotype was associated with PCD¹³.

In our study, in individuals with cystic kidney disease, HLA A*11 ($p=0.001$), A*23 ($p=0.006$), B*41 ($p=0.039$), B*49 ($p<0.001$), DRB1*03 ($p=0.002$), DRB1*04 ($p<0.001$), alleles were positively associated with the disease, HLA A*02 ($p<0.001$), B*13 ($p=0.028$), B*27 ($p=0.002$), B*39 ($p<0.001$), B*40 ($p=0.032$), DRB1*07 ($p=0.001$), DRB1*13 ($p<0.001$) allele was found to be identified as having a negative association with the disease.

In a thesis study carried out in KTU Health Sciences Institute Medical Biology Department in 2014, the most common HLA A*02 (33%), HLA B*35 (17.51%) and HLA DRB1*11 (17.51%) alleles have been determined. However, since these alleles are also common in the control group, no relationship was found between CKD and HLA alleles for HLA A*02 (30.54%), HLA B*35 (17.09%), HLA DRB1*11 (19.72%). In this study, the HLA A*25, A*69 and B*08 alleles were positively associated with diabetic nephropathy, while the HLA DRB1*03 allele was negatively associated with the disease. The data have been confirmed in the literature, with the HLA B*08 allele positively associated with glomerulonephritis, and the HLA DRB1*04 allele negatively associated. In addition, in patients with CKD due to hypertension from the study, no alleles could be detected, either positively or negatively associated with

the disease. In addition, the HLA A*25, A*26 and A*30 alleles were positively associated in PCKD, while the HLA DRB1*11 allele was negatively associated³⁰.

Other etiological groups of CKD in our study, on the other hand, were found to be positively associated with the HLA B*18 ($p=0.001$), B*50 ($p=0.021$), B*52 ($p=0.004$) alleles in the nephrolithiasis patient groups as a result of statistical analyzes.

HLA A*26 ($p=0.025$), B*40 ($p=0.036$), DRB1*13 ($p=0.019$) allele were found to be negatively associated with the disease. HLA B*48 ($p=0.005$) and DRB1*14 ($p=0.019$) alleles were found to be positively associated with the disease as a result of statistical analyzes performed in the patient group with CKD due to amyliodosis. As a result of the statistical analyses performed in the patient group with CKD due to congenital kidney damage, negatively associated allele with the disease was found. HLA B*07 ($p=0.038$), B*38 ($p=0.036$) were found to be positively associated. In individuals with CKD due to urological anomaly, according to statistical data, HLA A*01 ($p=0.001$), A*11 ($p=0.016$), B*08 ($p=0.001$), DRB1*03 ($p=0.003$) alleles positively associated with disease, HLA A*02 ($p=0.002$), A*26 ($p=0.010$), B*40 ($p=0.005$), B*44 ($p=0.023$), B*45 ($p=0.008$), DRB1*13 ($p=0.001$) alleles were found to be negatively associated with the disease.

According to statistical data, HLA A*24 ($p=0.002$), B*38 ($p=0.021$), DRB1*04 ($p<0.001$) alleles were found to be positively associated with the disease in individuals with CKD due to tubular interstitial nephritis.

In the patient group with CKD due to diabetes and hypertension, according to statistical data, HLA A*11 ($p=0.001$), A*42 ($p=0.032$), A*51 ($p=0.041$), allele positively associated with the disease found. In the group of patients with CKD caused by genetic disorders (Ankylosing Spondylitis, Bardet-Biedl Syndrome, Bartter Syndrome, Alport syndrome) a positive association was found with the HLA B*52 ($p=0.024$), DR*04 ($p=0.010$), and DR*15 ($p=0.034$) alleles.

According to statistical data, HLA B*48 ($p=0.025$), B*50 ($p=0.046$) alleles were found to be positively associated with the disease in patient groups with CKD due to Familial Mediterranean Fever. In the patient group with CKD of unknown etiology, according to statistical data, HLA A*11 ($p<0.001$), A*30 ($p=0.020$), DRB1*04 ($p=0.038$), DRB1*15 ($p=0.012$), alleles were positively associated with the disease. HLA A*02 ($p<0.001$), B*40 ($p=0.038$), DRB1*01 ($p=0.003$), DRB1*13 ($p=0.022$) allele were found to be negatively associated with the disease. Since there is no study in the literature investigating the association

between these etiologies and CKD, a comparison could not be made.

CONCLUSION

In all CKD patients in our study, B*08, B*49, B*50 in HLA*B locus and DRB1*03, DRB1*04, DRB1*15 alleles in HLA DRB1 locus were positively associated with CKD. HLA A*02, A*11, A*74 at HLA A locus, B*27, B*39, B*40, B*59 alleles at HLA B locus and DRB1*07, DRB1*08, DRB1*09, DRB1*13, DRB1*16 alleles at HLA DRB1 locus were found to be negatively associated with the disease.

When classified according to etiological distributions in the whole patient group, the HLA DR*13 allele was identified as having a negative association with in Hypertensive Nephropathy, Nephrolithiasis, cystic kidney disease, and Urological Anomalies subgroups and it is thought that it may be a protective allele in CKD.

Susceptible alleles can serve as important markers for risk classification. Additionally, in consanguineous kidney transplantation, avoiding these susceptible alleles when selecting optimal donors could significantly improve the extended survival of transplant recipients.

Ethics

Ethics Committee Approval: The study approved by Akdeniz University Clinical Research Ethical Committee (no:354, date 01.09.2015) and was conducted in full compliance with the Declaration of Helsinki.

Informed Consent: This study is retrospective.

Footnotes

Author Contributions

Surgical and Medical Practices: V.T.Y., H.K., B.A., Concept: B.K.T., F.U., V.T.Y., Y.K., H.K., B.A., Design: B.K.T., F.U., Data Collection and/or Processing: B.K.T., V.T.Y., H.K., B.A., Analysis and/or Interpretation: B.K.T., F.U., Y.K., Ş.D.A., N.S.E., H.S.A., Literature Search: B.K.T., Y.K., Ş.D.A., N.S.E., H.S.A., Writing: B.K.T., Y.K., S.D.A., N.S.E., H.S.A.

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Clinical Implications of Pan-Immune-inflammatory Values in Patients with Hypertrophic Cardiomyopathy

Hipertrofik Kardiyomiyopatili Hastalarda Pan-İmmün-inflamatuvar Değerlerin Klinik Etkileri

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ABSTRACT

Objective: Despite significant advances in understanding hypertrophic cardiomyopathy (HCM) in recent years, there is a need to improve risk stratification for patients at high risk of adverse outcomes. The relationship between inflammation and disease severity in HCM patients is known. Recently, a new inflammation parameter called the pan-immune inflammation value (PIV) has been introduced. However, the relationship between PIV and HCM has not yet been examined. Hence, we aim to investigate the effect of PIV on prognosis in a large series of HCM patients.

Methods: The study included 389 consecutive patients with HCM admitted to a tertiary care hospital between 2004 to 2021. The PIV for patients was calculated as: Neutrophil count x platelet count x monocyte count / lymphocyte count. The cohort was categorized into three groups according to PIV, and the association between these groups and long-term mortality was evaluated.

Results: Over an average follow-up period of 55.5±12.7 months, long-term mortality occurred in 47 out of 389 patients. Long-term mortality was recorded in 7 patients in tertile 1, 12 patients in tertile 2, and 28 patients in tertile 3. Multivariate regression analysis revealed that long-term all-cause mortality was 3.5 times higher in tertile 3 compared to tertile 1. The receiver-operating characteristic curve based on the PIV had 62% sensitivity and 65% specificity for long-term mortality.

Conclusions: High PIV levels may serve as a predictor of long-term mortality in patients with HCM. PIV could be a useful screening tool for identifying HCM patients at increased risk of adverse outcomes.

Keywords: Hypertrophic cardiomyopathy, pan-immune-inflammation value, systemic inflammatory markers, prognosis

ÖZ

Amaç: Son yıllarda hipertrofik kardiyomiyopati (HCM) konusunda önemli ilerlemeler kaydedilmiş olmasına rağmen, yüksek riskli hastalar için risk sınıflandırmasının iyileştirilmesine ihtiyaç vardır. HCM hastalarında inflamasyon ile hastalık şiddeti arasındaki ilişki bilinmektedir. Son zamanlarda, pan-immün inflamasyon değeri (PIV) adı verilen yeni bir inflamasyon parametresi tanımlanmıştır. Ancak, PIV ile HCM arasındaki ilişki henüz incelenmemiştir. Bu nedenle, geniş bir HCM hasta serisinde PIV'nin prognoz üzerindeki etkisini araştırmayı amaçlıyoruz.

Yöntemler: Çalışma, 2004 ile 2021 yılları arasında bir üçüncü basamak hastaneye kabul edilen ardışık 389 HCM hastasını içermektedir. Hastalar için PIV değeri şu şekilde hesaplandı: Nötrofil sayısı x trombosit sayısı x monosit sayısı / lenfosit sayısı. Kohort, PIV'ye göre üç gruba ayrıldı ve bu gruplar ile uzun dönem mortalite arasındaki ilişki değerlendirildi.

Bulgular: Ortalama 55,5±12,7 aylık bir takip süresi boyunca, 389 hastanın 47'sinde uzun dönem mortalite gözlemlendi. Uzun dönem mortalite, tertil 1'de 7 hastada, tertil 2'de 12 hastada ve tertil 3'te 28 hastada kaydedildi. Çok değişkenli regresyon analizi, tertil 3'te uzun dönem tüm nedenlere bağlı mortalitenin tertil 1'e göre 3,5 kat daha yüksek olduğunu ortaya koymuştur. PIV'ye dayalı alıcı çalışma karakteristiği eğrisi, uzun dönem mortalite için %62 duyarlılık ve %65 özgüllük göstermiştir.

Sonuçlar: Yüksek PIV seviyeleri, HCM hastalarında uzun dönem mortalitenin bir göstergesi olarak hizmet edebilir. PIV, HCM hastalarını olumsuz sonuçlar açısından artmış risk taşıyanlar olarak tanımlamak için yararlı bir tarama aracı olabilir.

Anahtar kelimeler: Hipertrofik kardiyomiyopati, pan-immün-inflamasyon değeri, sistemik inflamatuvar belirtiler, prognoz

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INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common genetic heart disorder, occurring in approximately 1 in 500 individuals in the general population¹. The primary pathological features of the myocardium in HCM include myocyte hypertrophy and disorganization, interstitial fibrosis, and small vessel disease^{2,3}. The disease consists of a broad spectrum ranging from asymptomatic to severely limiting symptoms with adverse clinical outcomes including severe heart failure, malignant arrhythmias, and cardiac death⁴⁻⁶. In spite of substantial progress in understanding the etiology, diagnosis, and treatment of HCM in recent years, the condition and its related complications remain a significant healthcare burden^{7,8}. Consequently, there is a need to assess the prognosis of HCM patients and improve risk stratification for those at high risk of adverse outcomes.

Recently, there has been increasing interest in evaluating the role of inflammation and oxidative stress in the pathogenesis and prognosis of HCM. Recently, the pan-immune inflammation value (PIV) has become a new, cost-effective, straightforward, and easily accessible marker based on inflammation and oxidative stress. PIV, initially considered an important prognostic marker in cancer patients⁹. However, the detailed relationship between HCM and PIV has not been extensively studied yet.

The purpose of this study was to examine the impact of PIV on the prognosis of a large cohort of HCM patients monitored at a tertiary care hospital.

MATERIALS and METHODS

We performed a retrospective analysis of 389 consecutive HCM patients who were monitored at our tertiary care center from 2004 to 2021. The clinical diagnosis of HCM is established through echocardiographic or cardiovascular magnetic resonance imaging, which shows a maximum end-diastolic wall thickness of ≥ 15 mm in any segment of the left ventricle (LV), provided there is no alternative cause for the hypertrophy¹⁰. Patients with left ventricular hypertrophy resulting from secondary causes, including hypertension, infiltrative diseases, and severe aortic stenosis, were excluded from the study. Additionally, to minimize bias, patients with a follow-up period of less than 5 years were excluded from the study.

Laboratory results including complete blood count, creatinine, urea, albumin, hormone levels, and glucose levels were assessed based on the first blood samples

collected at admission. The MIND-RAY BC-6800 from China was utilized for automated blood cell count, while the ARCHITECT PLUS CI-4100 from the USA was employed for biochemical parameters. The PIV for patients was calculated as: neutrophil count ($10^3/\mu\text{L}$) \times platelet count ($10^3/\mu\text{L}$) \times monocyte count ($10^3/\mu\text{L}$) / lymphocyte count ($10^3/\mu\text{L}$). All patients received transthoracic echocardiography carried out by a cardiovascular imaging expert using the Vivid 7 system (GE Vingmed Ultrasound AS, Horten, Norway). The modified Simpson method was used to evaluate left ventricular ejection fraction. Maximum LV wall thickness was determined as the largest wall thickness measured in parasternal long and short axis views. The peak instantaneous LV outflow gradient was measured using continuous wave Doppler. LV outflow obstruction was defined as a gradient measurement of 30 mm Hg either at rest or during exercise¹¹. The HCM risk-sudden cardiac risk model, was calculated according to the variables of the patients at the time of diagnosis¹². The study population was categorized into tertiles 1, 2, and 3 based on the baseline PIVs tertiles. Clinical characteristics, risk profiles, echocardiographic measurements and laboratory parameters of the patients were assessed from follow-up visits, patient records, and the electronic database. The study examined the association between these three groups and their respective outcomes. The study was approved by Health Sciences University Hamidiye Scientific Research Ethics Committee (decision no: 28/17, date: 30.12.2022) and was conducted in full compliance with the Declaration of Helsinki.

The main objective of this study was to assess long-term all-cause mortality. The long-term survival status of each patient was determined through the National Death Notification System.

Statistical Analysis

The study population was grouped into tertiles in terms of PIV calculated at admission. The baseline characteristics, laboratory variables, and echocardiographic parameters were compared across the groups.

The assessment of normality was conducted using the Kolmogorov-Smirnov test. Quantitative variables with skewed distributions were shown as median (interquartile range), while continuous variables were compared using the Kruskal-Wallis test. Normally distributed variables are presented as mean \pm standard deviation, and an independent samples t-test was used for comparisons. Categorical variables were presented as numbers and percentages. Analyses of categorical variables were

performed by Pearson’s chi-square test. Univariate and multivariate analyses, including Cox proportional hazards regression, were utilized to identify predictors of long-term mortality. Two Cox multivariable models were used: model 1, unadjusted; model 2, adjusted for age, gender, congestive heart failure, urea, albumin, glucose, total cholesterol and LV end-systolic diameter. The parameters used in model 2 were identified with a p-value<0.05 in multivariate Cox regression analysis. A two-tailed p-value of <0.05 was deemed statistically significant, and 95% confidence intervals were provided for all hazard ratios. Cut-off values for PIV and long-term mortality, demonstrating the highest sensitivity and specificity, were determined through non-parametric receiver-operating characteristics (ROC) curve analysis. Analyses were conducted using version 20.0 of the Statistical Package for the Social Sciences software (SPSS; IBM, Armonk, New York, USA).

RESULTS

During an average follow-up period of 55.5±12.7 months, 47 patients experienced long-term mortality. Non-ischemic heart failure was observed at a significantly higher in tertile 3 (p=0.089). Atrial fibrillation was observed in 30 patients in tertile 1, 39 patients in tertile 2, and 40 patients in tertile 3, with no significant difference

detected among the groups (p=0.301). In tertile 2, there was a higher proportion of male patients (p=0.087). Table 1 summarized the basic clinical characteristics of the patients according to their PIV.

The mean PIV value was 256 (184-307) in tertile 1, 490 (423-573) in tertile 2, and 1,001 (797-1309) in tertile 3 (p<0.001). While monocyte, neutrophil, and platelet counts were higher in tertile 3 compared to the other groups, lymphocyte values were lower. The tertile 3 had higher levels of glucose and total cholesterol compared to the other groups. LV end-systolic diameter was measured higher in tertile 3 compared to the other groups (p=0.049). The comparison of patients’ echocardiographic parameters and laboratory variables according to the PIV is presented in Table 2.

Long-term mortality was observed in 7 patients in tertile 1, 12 patients in tertile 2, and 28 patients in tertile 3. After adjusting for all covariates using multivariate regression analysis, long-term all-cause mortality was determined to be 3.5 times greater in tertile 3 compared to tertile 1. Table 3 shows Cox-regression models for the incidence of long-term mortality based on PIV. ROC analysis indicated that a PIV cut-off level of 624 predicted long-term mortality with 62% sensitivity and 65% specificity (Figure 1).

Table 1. Baseline clinical characteristics of hypertrophic cardiomyopathy patients according to pan-immuno-inflammation value.				
	Tertiles according to pan-immuno-inflammation value			
	Tertile 1 (n=130)	Tertile 2 (n=130)	Tertile 3 (n=129)	p-value
Age, years	61±14	60±14	58±14	0.179 ^a
Gender, male, n (%)	76 (58.5)	93 (71.)	84 (65.1)	0.087 ^b
Diabetes mellitus, %	25 (19.2)	17 (13.1)	21 (16.3)	0.401 ^b
Diabetes mellitus with insulin dependent, %	5 (3.8)	1 (0.8)	3 (2.3)	0.256 ^b
Hyperlipidemia, n (%)	19 (14.6)	11 (8.5)	22 (17.1)	0.111 ^b
Hypertension, %	54 (41.5)	58 (44.6)	50 (38.8)	0.633 ^b
Chronic obstructive pulmonary disease, %	4 (3.1)	7 (5.4)	8 (6.2)	0.480 ^b
Cerebrovascular accident, %	5 (3.8)	7 (5.4)	5 (3.9)	0.786 ^b
Atrial fibrillation, %	30 (23.1)	39 (30.0)	40 (31.0)	0.301 ^b
Coronary artery disease, %	45 (34.6)	44 (33.8)	43 (33.3)	0.976 ^b
Percutaneous coronary intervention, %	12 (9.2)	20 (15.4)	14 (10.9)	0.281 ^b
Coronary artery bypass grafting, %	9 (6.9)	7 (5.4)	10 (7.8)	0.741 ^b
Chronic kidney disease, %	8 (6.2)	12 (9.2)	11 (8.5)	0.631 ^b
Congestive heart failure, ischemic, %	7 (5.4)	9 (6.9)	9 (7.0)	0.838 ^b
Congestive heart failure, non-ischemic, %	8 (6.3)	11 (8.5)	18 (14.2)	0.089 ^b
Continuous variables are reported as median and interquartile range, Nominal variables reported as frequency (%)				
^a : Independent sample t-test, ^b : Pearson’s chi-square test				

Table 2. Laboratory variables and echocardiographic parameters of hypertrophic cardiomyopathy patients according to pan-immuno-inflammation value.

	Tertiles according to pan-immuno-inflammation value			
	Tertile 1, (n=130)	Tertile 2, (n=130)	Tertile 3, (n=129)	p-value
Laboratory variables				
Hb (g/dL)	13.5 (12.5-14.9)	13.7 (12.0-15.1)	13.5 (11.5-15.0)	0.750 ^a
Lymphocytes (10 ³ /μL)	2.4 (2.0-2.8)	2.0 (1.6-2.5)	1.4 (1.0-1.9)	<0.001 ^a
Monocytes (10 ³ /μL)	0.45 (0.38-0.54)	0.54 (0.44-0.65)	0.69 (0.51-0.88)	<0.001 ^a
Neutrophils (10 ³ /μL)	6.8 (5.9-7.4)	7.7 (6.9-9.1)	9.0 (7.4-11.0)	<0.001 ^a
Platelet count (10 ³ /μL)	208 (167-240)	231 (189-281)	260 (226-310)	<0.001 ^a
PIV	256 (184-307)	490 (423-573)	1001 (797-1309)	<0.001 ^a
Creatinine (mg/dL)	0.9 (0.8-1.0)	0.9 (0.8-1.0)	0.9 (0.8-1.0)	0.918 ^a
Urea (mg/dL)	25 (21-43)	24 (19-35)	25 (21-38)	0.083 ^a
AST (U/L)	25 (20-42)	23 (19-35)	24 (18-36)	0.242 ^a
ALT (U/L)	23 (17-39)	21 (15-30)	23 (17-34)	0.130 ^a
TSH (mIU/L)	1.5 (1.1-2.3)	1.4 (0.9-1.9)	1.5 (1.0-2.3)	0.258 ^a
Albumin (mg/dL)	4.2 (4.0-4.4)	4.1 (4.0-4.4)	4.1 (4.0-4.4)	0.095 ^a
Glucose (mg/dL)	98 (93-108)	97 (91-112)	102 (93-125)	0.062 ^a
HbA1c (%)	5.5 (5.4-5.9)	5.5 (5.4-5.7)	5.5 (5.4-6.0)	0.749 ^a
Uric acid (mg/dL)	7.7 (7.0-9.8)	7.7 (6.8-9.5)	7.6 (6.8-9.8)	0.578 ^a
Total cholesterol (mg/dL)	199 (169-222)	197 (160-217)	210 (176-225)	0.094 ^a
LDL (mg/dL)	120 ± 35	117 ± 37	122 ± 37	0.346 ^b
HDL (mg/dL)	38 (32-48)	39 (32-45)	38 (32-46)	0.787 ^a
Triglycerides (mg/dL)	141 (100-198)	146 (89-202)	157 (113-212)	0.172 ^a
Medical therapy				
Beta-blockers, %	111 (85.4)	111 (85.4)	114 (88.4)	0.721 ^c
Echocardiography parameters				
Ejection fraction, %	60 (55-60)	60 (55-60)	60 (55-60)	0.835 ^a
LVEDD, mm	46 (42-50)	46 (42-50)	46 (42-50)	0.998 ^a
LVESD, mm	27 (23-30)	27 (23-30)	28 (24-31)	0.049 ^a
Maximal wall thickness (mm)	18 (16-22)	18 (16-21)	18 (16-22)	0.906 ^a
LV outflow gradient (mmHg)	25 (21-32)	27 (21-33)	30 (22-34)	0.292 ^a
LA diameter (mm)	42 (36-49)	44 (37-50)	43 (38-49)	0.347 ^a
Syncope, %	10 (7.7)	11 (8.5)	19 (14.7)	0.124 ^c
Positive Family History (for SCD), %	7 (5.4)	8 (6.2)	13 (10.1)	0.293 ^c
Non-sustained VT	8 (6.2)	6 (4.6)	8 (6.2)	0.820 ^c
HCM Risk-SCD (%)	1.79 (1.21-2.43)	1.90 (1.27-2.79)	1.89 (1.30-2.95)	0.276 ^a

Hb: Hemoglobin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDL: Low density lipoprotein, HDL: High density lipoprotein, TSH: Thyroid stimulating hormone, PIV: Pan-immune-inflammatory value, LVEDD: Left ventricular end-diastolic diameter, LVESD: Left ventricular end-systolic diameter, LV: Left ventricle, LA: Left atrium, VT: Ventricular tachycardia, SCD: Sudden cardiac death, HCM: Hypertrophic cardiomyopathy, ^a: Kruskal-Wallis test, ^b: Independent sample t-test, ^c: Pearson's chi-square test

Table 3. Cox-regression models for long-term mortality incidence by pan-immuno-inflammation value.			
	Tertiles according to pan-immuno-inflammation value		
	Tertile 1, (n=130)	Tertile 2, (n=130)	Tertile 3, (n=129)
Long-term mortality			
Number of patients	7	12	28
Case rate, %	5.4	9.2	21.7
Long-term mortality, HR (95% CI)			
Model 1: unadjusted	1[Reference]	1.8 (1.2-6.8)	4.8 (2.4-11.2)
Model 2: adjusted for all covariates ^a	1[Reference]	1.4 (0.8- 4.1)	3.5 (2.0-6.9)
Two Cox multivariable models were used: model 1, unadjusted; model 2, adjusted for age, gender, congestive heart failure, urea, albumin, glucose, total cholesterol and left ventricle end-systolic diameter. The parameters used in model 2 were identified with a p value<0.05 in multivariate Cox regression analysis			
CI: Confidence interval, HR: Hazard ratio, ^a :Adjusted for age, gender, congestive heart failure, urea, albumin, glucose, total cholesterol and left ventricle end-systolic diameter			

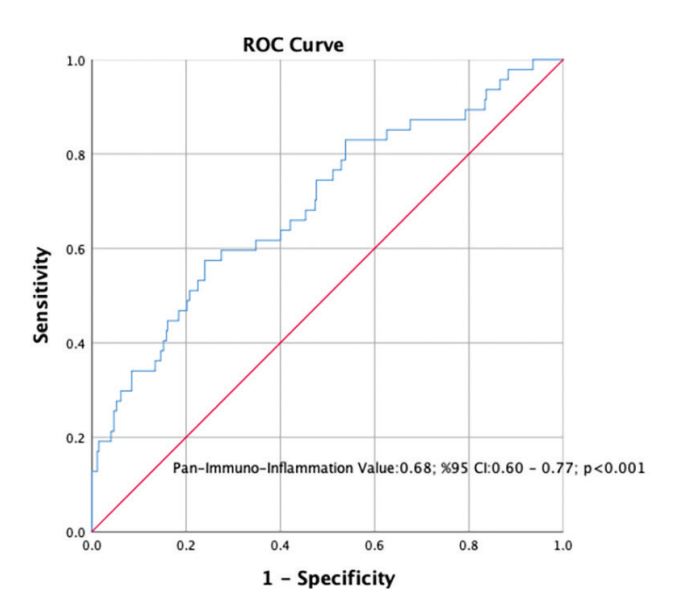


Figure 1. ROC curve analysis of pan-immuno-inflammation value to predict long-term mortality.
CI: Confidence interval, ROC: Receiver-operating characteristics

DISCUSSION

Our study demonstrated an association between PIV and adverse outcomes in patients with HCM. As far as we know, this is the first study to investigate the potential role of PIV in predicting the prognosis of patients with HCM.

Inflammation is a recognized risk factor for the onset and progression of numerous cardiovascular diseases. Systemic inflammation has been shown to be associated with parameters of disease severity and particularly

fibrosis in HCM patients¹³. The proposed mechanism suggests that sustained, low-grade myocardial inflammation triggers the invasion of inflammatory cells and fibroblasts, ultimately resulting in myocardial fibrosis^{14,15}. It has been reported that oxidative stress levels are elevated in patients with HCM due to left ventricular pressure overload¹⁶. Moreover, myocardial fibrosis is a significant determinant of sudden cardiac death, heart failure, and ventricular tachyarrhythmia¹⁷. Since myocardial fibrosis is a key factor in malignant arrhythmias and systolic heart failure in HCM, modifying the inflammatory cascade may help prevent cardiac death by reducing myocardial fibrosis.

Several researches have identified interstitial inflammation, endocardial inflammation, and immunocyte infiltration as the primary histopathological characteristics of HCM^{14,18}. Inflammatory parameters like TNF- α , MCP-1, and IL-6 are not typically accessible in routine clinical practice. Thus, there is a need for a clinically straightforward, cost-effective, and readily accessible method to evaluate inflammation and the resulting fibrosis. Parameters that can reflect inflammation and subsequent fibrosis may play a crucial role in the pathophysiology and prognosis of HCM. A recent study found that inflammation-indicating monocyte to high-density lipoprotein cholesterol ratio has a significant and independent prognostic value in HCM patients¹⁹. Additionally, the neutrophil-to-lymphocyte ratio has been identified as an independent risk factor for all-cause mortality in patients with HCM²⁰. Lymphocyte to monocyte ratio predicts all-cause mortality in HCM patients²¹. Additionally, elevated levels of high-sensitivity C-reactive protein (CRP) have been found to substantially increase the risk of adverse outcomes, indicating the prognostic significance of this inflammatory marker²².

The pan-immuno-inflammation value, which includes counts of neutrophils, platelets, monocytes, and lymphocytes, is an index used to assess the immune and inflammatory status of patients²³. PIV has the potential to offer a more comprehensive reflection of inflammation compared to other immune indicators like the neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and monocyte-to-lymphocyte ratio. Previous studies have indicated that PIV are linked to the prognosis of conditions such as myocardial infarction, hypertension and heart failure²⁴⁻²⁶. While the optimal cut-off value for PIV in patients with metastatic colorectal cancer is 390²³, another study evaluating frailty reported a cut-off value of 372, which is slightly lower than the value found in our study²⁷. Another study evaluating the prognosis in ST-elevation myocardial infarction patients found the PIV cut-off value to be 622.71, which is close to the value identified in our study²⁸.

Considering that PIV reflects the inflammatory status of patients, it can be thought that high PIV values indicate high inflammatory processes in HCM patients. Elevated inflammatory markers have the potential to indirectly indicate the intensity of fibrosis, the severity of disease, and ultimately mortality risk in HCM patients. Given that PIV is a cost-effective and readily available marker with significant prognostic value for patients with HCM, it could assist in identifying high-risk individuals who need closer monitoring. Thus, PIV could be a potential screening tool to identify HCM patients who are at increased risk for adverse outcomes. PIV, which has already been shown in the literature to reflect inflammation status, is supported by our current study as a reliable inflammatory parameter. In addition, the fact that high inflammatory indicators, as we know from the literature, have prognostic value in HCM patients and that PIV shows similar results emphasizes the importance of inflammation in the course of HCM. Therefore, it is believed that the predictive significance of PIV in patients with HCM may be attributed to the presence of cardiac inflammation and fibrosis.

Our study has limitations that must be acknowledged. First, since our study is a single-center, observational, retrospective analysis involving a relatively small patient cohort, there may be potential biases. Consequently, the generalizability of the data is restricted. Second, unfortunately, cardiovascular magnetic resonance imaging was not conducted on all participants in the study. Third, commonly used markers of inflammation such as plasma CRP, procalcitonin, and erythrocyte sedimentation rate could not be assessed, thus their relationship with PIV is lacking. Fourth, PIV levels were calculated only from blood samples collected during hospital admission.

CONCLUSION

This study demonstrated that high PIV levels have the potential to predict long-term mortality in patients with HCM. Thus, PIV could act as a useful screening tool for identifying HCM patients who are at increased risk of adverse outcomes.

Ethics

Ethics Committee Approval: The study was approved by Health Sciences University Hamidiye Scientific Research Ethics Committee (decision no: 28/17, date: 30.12.2022) and was conducted in full compliance with the Declaration of Helsinki.

Informed Consent: This study is retrospective.

Footnotes

Author Contributions

Surgical and Medical Practices: S.D., H.K., Concept: L.P., M.I.H., Design: T.C., M.I.H., Data Collection and/or Processing: S.D., H.K., Analysis and/or Interpretation: T.C., T.Ci., Literature Search: L.P., H.K., Writing: L.P., A.C.Y., T.Ci., M.I.H.

Conflict of Interest: The authors have no conflict of interest to declare.

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The Serum Biomarkers in Ulcerative Colitis

Ülseratif Kolitte Serum Biyobelirteçleri

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ABSTRACT

Objective: In this study, the aim was to evaluate the diagnostic effectiveness of more easily applicable and cost-effective serum biomarkers, such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), C-reactive protein (CRP) to albumin ratio (CAR), and CRP-to-lymphocyte ratio (CLR), instead of the endoscopic activity index (EAI) used to determine disease activation in ulcerative colitis (UC) patients.

Methods: Blood tests performed during the same period as colonoscopy were reviewed, and NLR, PLR, CAR, and CLR values were calculated. Based on the EAI score, patients with a score <4 were classified as having UC in remission, those with a score ≥4 as having active UC, and those with normal colonoscopy results as the control group.

Results: The study included 66 patients with active UC, 31 with UC in remission, and 99 controls. The CLR and CAR values of active and remission UC patients were found to be higher compared with the control group ($p<0.001$), while no significant difference was found between the groups in terms of PLR and NLR values ($p>0.05$). The AUC calculated for CLR in diagnosing active UC was significant ($p<0.001$), and the best cut-off value was determined as >1,75. For CAR, the best cut-off value was calculated as >0,11.

Conclusions: This study demonstrated that the CLR and CAR had high sensitivity and specificity for detecting UC activity, whereas the PLR and NLR had low diagnostic value.

Keywords: Ulcerative colitis, inflammation markers, colonoscopy, endoscopic activity index

ÖZ

Amaç: Bu çalışmada, ülseratif kolit (ÜK) hastalarında hastalık aktivasyonunu belirlemek için kullanılan endoskopik aktivite indeksi (EAI) yerine nötrofil-lenfosit oranı (NLO), trombosit-lenfosit oranı (PLO), C-reaktif protein (CRP) albümin oranı (CAO) ve CRP-lenfosit oranı (CLO) gibi daha kolay uygulanabilir ve maliyet etkin serum biyobelirteçlerinin tanısal etkinliğinin değerlendirilmesi amaçlanmıştır.

Yöntemler: Kolonoskopi ile aynı dönemde alınan kan testleri gözden geçirilmiş ve NLO, PLO, CAO ve CLO değerleri hesaplanmıştır. EAI skoruna göre, skoru <4 olan hastalar remisyonda ÜK, skoru ≥4 olanlar aktif ÜK ve kolonoskopi sonuçları normal olanlar kontrol grubu olarak sınıflandırılmıştır.

Bulgular: Çalışmaya 66 aktif ÜK, 31 remisyondaki ÜK ve 99 kontrol grubu katılımcısı dahil edilmiştir. Aktif ve remisyondaki ÜK hastalarının CLO ve CAO değerleri kontrol grubuna göre yüksek bulunurken ($p<0,001$), PLO ve NLO değerlerinde anlamlı fark bulunmadı ($p>0,05$). Aktif ÜK tanısında CLO için hesaplanan AUC anlamlıydı ($p<0,001$) ve en iyi cut-off >1,75 olarak belirlendi. CAO için en iyi cut-off >0,11 olarak hesaplanmıştır.

Sonuçlar: Bu çalışma, CLO ve CAO'nun ÜK aktivitesini saptamada yüksek duyarlılık ve özgüllüğe sahip olduğunu, PLO ve NLO'nun ise düşük tanısal değere sahip olduğunu göstermektedir.

Anahtar kelimeler: Ülseratif kolit, inflamasyon belirteçleri, kolonoskopi, endoskopik aktivite indeksi

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disease¹. During disease course, periods of relapse and remission are often observed. Disease activity was assessed using clinical symptoms and endoscopic findings. The Rachmilewitz Endoscopic Activity Index (EAI) is an index used to determine disease severity. Based on findings such as mucosal erythema, ulceration,

granularity, vascular pattern, and bleeding, the EAI is used to evaluate the remission and activation status of the disease². Early detection of disease activity and appropriate treatment are crucial for improving prognosis and quality of life^{3,4}.

Colonoscopy is the most important examination for evaluating disease activity in UC. However, this procedure is invasive, requires bowel preparation, and is not

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always easily performed on demand. Numerous serum biomarkers that are easy to implement, non-invasive, and inexpensive have been investigated as alternatives to endoscopic evaluation for determining disease activation in UC⁵. C-reactive protein (CRP) is a test used to assess the activation of UC; however, their sensitivity and specificity are not satisfactory⁶. Recently, various integrated indices, such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), CRP-to-albumin ratio (CAR), and CRP-to-lymphocyte ratio (CLR), have been utilized for the assessment of infectious illnesses⁷⁻¹⁰.

The aim of this study was to determine the diagnostic effectiveness of more easily applicable and low-cost serum biomarkers (NLR, PLR, CAR, CLR) as alternatives to EAI for detecting disease activation in UC.

MATERIALS and METHODS

For this retrospective study, patients aged over 18 years with UC who underwent colonoscopy at Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital between January 2022 and August 2024 were screened from the hospital information system. Patients diagnosed with UC through endoscopic and histopathological evaluations and assessments based on the Rachmilewitz EAI were included in the study. The control group consisted of consecutive patients without a diagnosis of UC who had normal colonoscopy findings.

The exclusion criteria were as follows: individuals with suspected UC, Crohn’s disease, patients who were not graded according to the Rachmilewitz EAI, individuals with solitary rectal ulcers, those with radiation colitis, patients with missing routine blood test results, individuals who had undergone total or subtotal colectomy, patients with inflammatory conditions unrelated to UC (trauma, liver cirrhosis, malignancy), acute and chronic renal failure, pregnancy, other autoimmune diseases (Behçet’s disease, psoriasis, rheumatoid arthritis), and those with active viral or bacterial infections that could potentially affect laboratory parameters were excluded from the study. The exclusion criteria were similar for the

control group and the UC group. Ethical approval was obtained from the Health Sciences University Türkiye, Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital, Non-interventional Clinical Research Ethics Committee (decision no: 2024-07/103, date: 25.07.2024). The Helsinki Declaration was waived for the requirement of written informed consent, as only medical data from the patients’ electronic records were extracted.

Data Collection

Blood tests performed concurrently with colonoscopy were included in the evaluation.

The following inflammatory indices were computed for analysis. The NLR was calculated by dividing the neutrophil count (10⁹/L) by the lymphocyte count (10⁹/L)⁷. The PLR was calculated by dividing the platelet count (10⁹/L) by the lymphocyte count (10⁹/L)⁸. The CAR was calculated by dividing the CRP levels (mg/L) by the albumin levels (g/L)⁹. CLR was calculated by dividing CRP levels (mg/L) by the lymphocyte count (10⁹/L)¹⁰. The activity of UC was assessed by scoring according to the Rachmilewitz EAI (Figure 1)¹¹.

Groups

According to the Rachmilewitz EAI, patients with a score <4/12 were classified as being in remission from UC, those with a score ≥4/12 were classified as having active UC, and individuals with normal colonoscopy results and no UC were designated as the control group.

Sample Size

For the sample size calculation, a significance level of 5% and statistical power of 80% were utilized in accordance with the retrospective study methodology. According to the literature, studies on biomarker ratios require a minimum of 30-50 participants in each group to detect medium effect sizes¹². This study included 66 participants in the active UC group, 31 in the remission group, and 99 in the control group, achieving a sufficient sample size to detect differences in biomarkers among the groups.

Endoscopic Activity	0	1	2	3
Mucosal Erosions	None	Mild	Moderate	Severe
Ulcers	None	Single-surfaced	Superficial, multiple	Deep, localized
Mucosal Hyperemia	None	Mild	Moderate	Severe
Loss of Vascular Pattern	None	Mild	Moderate	Severe
Hemorrhagic Mucosal Surface	None	Minimal	Moderate	Widespread

Figure 1. Rachmilewitz endoscopic activity index

Statistical Analysis

Descriptive statistics for continuous data included mean, standard deviation, median, minimum, maximum, and interquartile range with the 25th-75th percentile values, while counts and percentages were reported for categorical data. The Shapiro-Wilk test was used to assess the normality of the data distribution. Comparisons of continuous variables among patients in the active, remission, and control groups were performed using the Kruskal-Wallis variance analysis. The sources of the differences among the groups were examined using the Kruskal-Wallis multiple comparison test. For nominal variable group comparisons (in cross-tabulations), the chi-square test was utilized. The diagnostic performance of the CLR and CAR values was evaluated using the area under the receiver operating characteristic (ROC) curve (AUC). The optimal cut-off point was determined using Youden's index. The diagnostic accuracy metrics for the CLR and CAR values (sensitivity, specificity, positive predictive value, and negative predictive value) were assessed. IBM SPSS for Windows version 20.0 (SPSS Inc., Chicago, IL) was used for the analyses, with a significance level set at $p < 0.05$.

RESULTS

In total, 4,056 colonoscopy reports were screened, and 422 patients with UC were identified. Following the application of the exclusion criteria, 196 patients were enrolled in the study: 66 with active UC, 31 in remission, and 99 in the control group (Figure 2).

The distribution of sex among patients in the active, remission, and control groups did not reveal any meaningful distinction ($p > 0.05$) (Table 1).

In contrast, a notable variation in white blood cell (WBC) counts was observed between the groups ($p < 0.05$). Using the Kruskal-Wallis multiple comparison test, we observed that the WBC counts in the active UC group were significantly elevated compared with the control cohort, whereas no notable differences were found among the other group comparisons ($p > 0.05$). Moreover, no meaningful distinction was found in hemoglobin (HB) levels across the active, remission, and control groups ($p > 0.05$). No meaningful distinction was observed in the NLR values among the active, remission, and control groups ($p > 0.05$). The PLR values did not exhibit

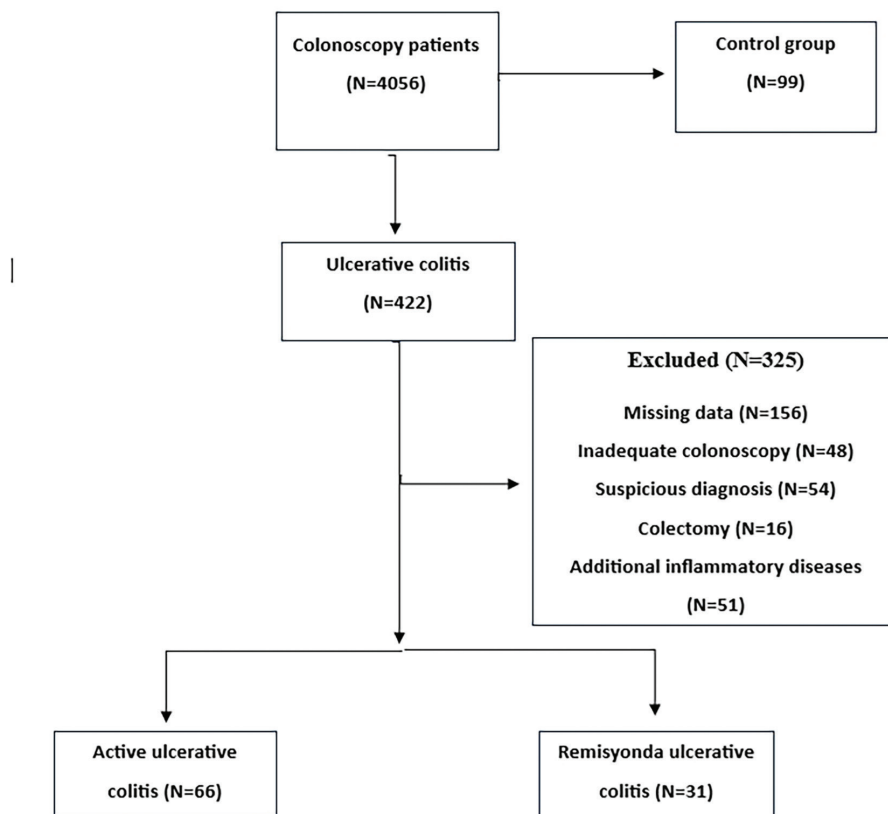


Figure 2. Flow chart of the study

significant variations between the active, remission, and control groups ($p>0.05$). However, a statistically significant distinction was detected in the CLR values among the active, remission, and control groups ($p<0.001$). The CLR values in the active and remission UC groups were significantly elevated compared with the control group. There was no noteworthy difference between the CLR values of the active and remission UC groups ($p>0.05$). Additionally, a meaningful distinction was observed in CAR values among patients across the three groups ($p<0.001$). Further analysis using the Kruskal-Wallis multiple comparison test revealed that the CAR values in the active and remission UC groups were significantly higher than those in the control group, with no meaningful distinction between the CAR values of the active and remission UC cohorts ($p>0.05$) (Table 2).

The AUC for CLR values in distinguishing active UC diagnosis was found to be significant ($p<0.001$), with

the cut-off point for CLR values established at >1.75 (Figure 3A) (Table 3).

Similarly, the calculated AUC for CAR values in differentiating active UC diagnosis was found to be significant ($p<0.001$). The cut-off threshold for CAR values was set to greater than 0.11 (Figure 3B) (Table 3).

The AUC calculated for CLR values in distinguishing remission UC was found to be significant ($p<0.01$). The cutoff threshold for CLR values was found to be greater than 1.28 (Table 4).

The AUC calculated for CAR values in distinguishing remission UC was found to be significant ($p<0.01$). The cut-off threshold for CAR values was set to greater than 0.07 (Table 4).

Table 1. Comparison of sex between the active, remission, and control groups

	Active UC (n=66)	Remission UC (n=31)	Control (n=99)	
	n (%)	n (%)	n (%)	
Sex				
Female	22 (33.3)	9 (29.0)	41 (41.4)	0.358 ^c
Male	44 (66.7)	22 (71.0)	58 (58.6)	

^c; Chi -Square test, UC; Ulcerative colitis

Table 2. Comparison of findings among patients in the active, remission, and control groups

	Active UC (n=66)	Remission UC (n=31)	Control (n=99)		
	Median (IQR)	Median (IQR)	Median (IQR)	p-value	Post hoc
WBC	7.69	6.71	6.64 (5.61-7.83)	0.012 ^k	a-b p=0.843
(10 ³ /μL)	(5.98-9.40)	(6.04-8.72)			a-c p=0.009
					b-c p=0.751
HB (g/dL)	14.1 (12.8-15.0)	14.2 (12.6-15.5)	14.0 (12.3-15.5)	0.968 ^k	
NLR	2.28 (1.73-3.10)	1.94 (1.66-2.55)	1.89 (1.46-2.57)	0.057 ^k	
PLR	140.25	127.50	127.71	0.054 ^k	
	(112.24-198.21)	(102.20-172.60)	(102.52-166.53)		
CLR	3.21 (1.36-6.25)	1.61 (0.83-3.0)	0.93 (0.37-1.44)	<0.001 ^k	a-b p=0.099
					a-c p<0.001
					b-c p=0.010
CAR	0.14 (0.06-0.26)	0.07 (0.03-0.20)	0.05 (0.02-0.07)	<0.001 ^k	a-b p=0.155
					a-c p<0.001
					b-c p=0.024

^k:Kruskal-Wallis test, data are presented as median (25%-75%), UC: Ulcerative colitis, a: Active ulcerative colitis, b: Remission ulcerative colitis, c: Control, WBC: White blood cell, HB: Hemoglobin, NLR: Neutrophil-lymphocyte ratio, PLR: Platelet lymphocyte ratio, CLR: C-reactive protein lymphocyte ratio, CAR: C-reactive protein albumin ratio, IQR: Interquartile range

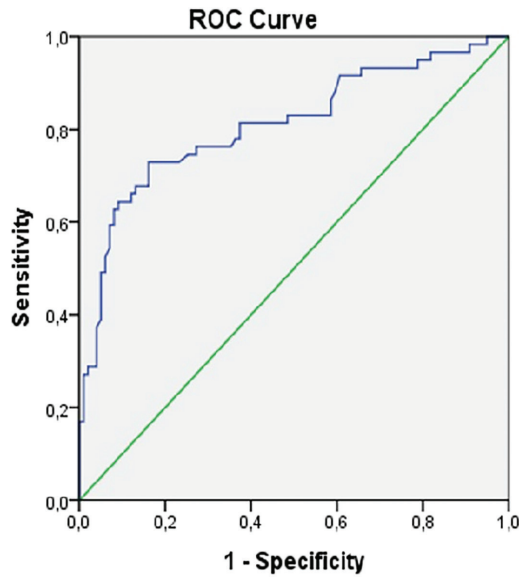


Figure 3A. Cut-off point for CLR values

CLR: C-reactive protein lymphocyte ratio, ROC: Receiver operating characteristic

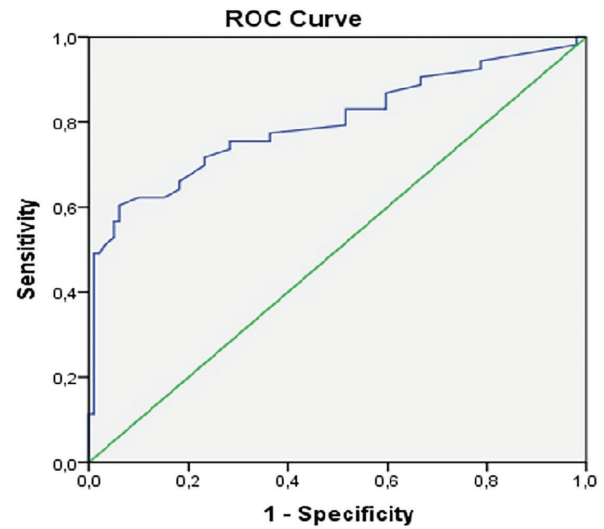


Figure 3B. Cut-off point for CAR values

CAR: CRP-to-albumin ratio, ROC: Receiver operating characteristic

Table 3. Diagnostic performance of CLR and CAR in the identification of active ulcerative colitis patients.

	AUC 95% CI	p-value	Cut-off	Sensitivity 95% CI	Specificity 95% CI	PPV 95% CI	NPV 95% CI
CLR	0.810	<0.001	>1.75	72.8%	83.8%	72.8%	83.8%
	0.735-0.885			60.4-82.5	75.3-89.8	65.1-79.5	76.9-89.0
CAR	0.798	<0.001	>0.11	60.3%	92.9%	82.0	81.4
	0.716-0.881			46.9-72.4	86.1-96.5	74.8-87.6	74.1-87.1

AUC: Area under the curve, PPV: Positive predictive value, NPV: Negative predictive value, CLR: C-reactive protein lymphocyte ratio, CAR: C-reactive protein albumin ratio, CI: Confidence interval

Table 4. Diagnostic performance of CLR and CAR values in distinguishing remission ulcerative colitis patients.

	AUC 95% CI	p-value	Cut-off 95% CI	Sensitivity 95% CI	Specificity 95% CI	PPV 95% CI	NPV 95% CI
CLR	0.698	<0.001	>1.28	65.5	68.6	38.0	87.2
	0.589-0.806			47.3-80.0	59.0-76.9	29.6-47.0	79.8-92.2
CAR	0.668	0.008	>0.07	55.5	76.7	39.4	86.3
	0.547-0.789			37.3-72.4	67.5-83.9	31.0-48.6	78.8-91.6

PPV: Positive predictive value, NPV: Negative predictive value, CLR: C-reactive protein lymphocyte ratio, CAR: C-reactive protein albumin ratio, CI: Confidence interval

DISCUSSION

Our study demonstrated that the serum biomarkers CLR and CAR had high sensitivity and specificity for detecting active UC according to the Rachmilewitz EAI, whereas the PLR and NLR values proved ineffective in determining the activation status. Additionally, we

identified a cut-off value for CLR of >1.75 and CAR of >0.11 for active UC.

Lymphocytes secrete cytokines and intestinal proteases, resulting in mucosal layer damage. These immune cells tend to accumulate in the inflamed region of the lamina propria^{13,14}. In individuals with

active inflammatory bowel disease (IBD), lymphocytes translocate from the peripheral circulation to inflamed intestinal tissues, thereby resulting in peripheral lymphopenia. Patients with IBD frequently exhibit increased levels of thrombopoietin and interleukin-6, both of which contribute to the maturation of megakaryocytes¹⁵. The platelets present in peripheral blood may be activated and exhibit spontaneous aggregation, as well as increased sensitivity to pro-aggregatory agents¹⁶. Therefore, peripheral thrombocytosis is frequently observed in patients with active UC¹⁷.

Although the precise mechanism linking CRP to disease activity remains somewhat ambiguous, multiple biologically plausible explanations have been proposed. CRP and albumin are widely used as markers of acute inflammation in clinical practice, with albumin also reflecting the nutritional status. Cytokines produced by inflammation may suppress albumin production in the liver¹⁸. Increased levels of cytokines as a result of inflammation may cause malnutrition¹⁹. This may explain why CAR better reflects disease activity in patients with longer disease durations.

Lin et al.²⁰ Found that CLR and CAR had high predictive accuracy for diagnosing severe UC, with AUC values of 0.732 and 0.714, respectively. The higher sensitivity of CLR (67%) compared with CAR suggests its broad applicability in screening for inflammation. Zhang et al.²¹ demonstrated that CAR had high specificity in diagnosing active UC according to the Rachmilewitz EAI, but its sensitivity was relatively low. Similar to our study, we found that the sensitivity of CLR (72.8%) for predicting active UC was higher than that of CAR (60.8%). In patients with active UC, the AUC values for CLR and CAR were 0.810 and 0.798, respectively. The cut-off values for active UC were >1.75 for CLR and >0.11 for CAR. The higher sensitivity of CLR in patients with active UC in our study indicates that CLR is more effective than CAR for detecting inflammation. However, although the specificity of CLR (83.8%) is not as high as that of CAR (92.9%), it is still quite robust. The difference in our cut-off values compared with Lin et al.²⁰ may be attributed to our comparison of CLR and CAR with the EA score instead of the Mayo Clinic score.

The presence of parameters more closely associated with chronicity, such as albumin, in the CAR calculation may have contributed to its slightly lower sensitivity in active inflammatory conditions compared with CLR.

In remission UC, the cutoff values for CLR and CAR (>1.28 and >0.07 , respectively) and their negative

predictive values (87.2% and 86.3%) indicate that these markers may serve as reliable indicators for ruling out remission.

In a study conducted by Feng et al.²², the PLR and NLR ratios in patients with active and remission UC were compared with those of CRP, ESR, and fecal calprotectin. The sensitivity of NLR in active UC was 78.8% with a specificity of 65%, whereas that of PLR was 58.3% with a specificity of 75%.

Samuel et al.²³ did not identify any relationship between PLR and NLR values and UC activation. Similarly, in our study, we did not find an association between NLR and PLR values and disease activation. The differing results compared with those of Feng et al.²² may be due to our comparison of the PLR and NLR values with the EAI.

The strengths of our study include its ability to be one of the rare studies to evaluate biomarkers in conjunction with the Rachmilewitz EAI in the Turkish population. Second, it provides a cost-effective and easily applicable option for disease activity assessment.

Study Limitations

The limitations of our study include its retrospective nature, which led to missing demographic data for some patients. Second, although the diagnosis of UC in our participants was confirmed through past pathology reports, there were deficiencies in pathological evaluations that could have added additional insight for determining disease remission alongside the EAI. Third, the limited number of patients in remission has restricted the statistical interpretation of this patient group.

CONCLUSION

Our study demonstrated that both CLR and CAR have high sensitivity and specificity for detecting UC activity. There is a need for further investigation into the dynamic changes in these inflammatory indices in relation to the activity and severity of UC. Additionally, we determined the cutoff values for active UC to be >1.75 for CLR and >0.11 for CAR. Similarly, our findings indicate that CLR has a higher sensitivity (72.8%) compared to CAR (60.8%) in predicting active UC. On the other hand, the PLR and NLR values were ineffective in indicating UC activation. Future studies should evaluate the relationship between these indices and UC in greater detail.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Health Sciences University Türkiye,

Abdurrahman Yurtaslan Ankara Oncology CPSU Non-Interventional Clinical Research Ethics Committee (decision No: 2024-07/103, date: 25.07.2024)

Informed Consent: The Helsinki Declaration was waived for the requirement of written informed consent, as only medical data from the patients' electronic records were extracted.

Footnotes

Author Contributions

Concept: S.S., M.K., Design: S.S., M.K., Data Collection and/or Processing: S.S., M.I.K., Analysis and/or Interpretation: S.D., M.I.K., Literature Search: S.S., M.I.K., Writing: S.S.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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Endoscopic Type 1 Tympanoplasty: Evaluation of Clinical Success and Hearing Improvement

Endoskopik Tip 1 Timpanoplasti: Klinik Başarı ve İşitme İyileşmesinin İncelenmesi

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ABSTRACT

Objective: This study aimed to assess the functional and clinical outcomes of endoscopic Type 1 tympanoplasty in patients with chronic otitis media and tympanic membrane perforations, focusing on hearing improvement and graft success rates.

Methods: This retrospective study included 46 patients with dry tympanic membrane perforations who underwent endoscopic Type 1 tympanoplasty. Audiometric data, including pure tone averages (PTA) and air-bone gap (ABG) measurements at four frequencies (500, 1000, 2000, and 4000 Hz), were collected preoperatively and postoperatively. Functional success was defined as a postoperative ABG of 20 dB, whereas the integrity of the graft determined clinical success.

Results: The mean preoperative ABG was 36.74±7.62 dB, which significantly improved to 15.05±3.82 dB postoperatively ($p<0.001$). The PTA exhibited an average gain of 28.17±6.87 dB for all frequencies. Functional success was achieved in 42 patients (91.30%), with 17 patients (36.96%) achieving normal hearing and 22 patients (47.83%) presenting with slight hearing loss postoperatively. Anatomical success was achieved in 42 patients (91.30%).

Conclusions: Endoscopic Type 1 tympanoplasty is a safe and effective surgical method for treating chronic otitis media, with high rates of both functional and clinical success, significant improvements in hearing outcomes, and minimal postoperative complications.

Keywords: Type 1 tympanoplasty, chronic otitis media, endoscopic approach, tragal perichondrium graft, hearing improvement, graft success

ÖZ

Amaç: Bu çalışma, kronik otitis media ve timpanik membran perforasyonu olan hastalarda endoskopik Tip 1 timpanoplastinin fonksiyonel ve klinik sonuçlarını, işitme iyileşmesi ve greft başarı oranlarına odaklanarak değerlendirmeyi amaçlamaktadır.

Yöntemler: Kuru timpanik membran perforasyonu bulunan 46 hastaya uygulanan endoskopik Tip 1 timpanoplastinin retrospektif analizi yapılmıştır. Saf ses ortalamaları (SSO) ve hava-kemik aralığı (HKA) ölçümleri de dahil olmak üzere dört frekansta (500, 1000, 2000 ve 4000 Hz) odyometrik veriler ameliyat öncesi ve sonrası toplanmıştır. Fonksiyonel başarı, ameliyat sonrası HKA'nin 20 dB'nin altında olması olarak tanımlanırken, klinik başarı greftin bütünlüğü ile değerlendirilmiştir.

Bulgular: Ameliyat öncesi ortalama HKA 36,74±7,62 dB iken, ameliyat sonrası bu değer anlamlı bir iyileşme göstererek 15,05±3,82 dB'ye düşmüştür ($p<0,005$). SSO'da tüm frekanslarda ortalama 28,17±6,87 dB kazanç sağlanmıştır. Fonksiyonel başarı 42 hastada (%91,30) elde edilmiş olup, 17 hasta (%36,96) normal işitmeye ulaşırken, 22 hasta (%47,83) ameliyat sonrası hafif işitme kaybı göstermiştir. Anatomik başarı, timpanik membran greftinin sağlam kalması ile belirlenmiş ve 42 hastada (%91,30) başarı elde edilmiştir.

Sonuçlar: Endoskopik Tip 1 timpanoplasti, kronik otitis media tedavisinde güvenli ve etkili bir cerrahi yöntem olup, yüksek oranda fonksiyonel ve klinik başarı, anlamlı işitme iyileşmeleri ve minimal postoperatif komplikasyonlar sağlamaktadır.

Anahtar kelimeler: Tip 1 timpanoplasti, kronik otitis media, endoskopik yaklaşım, tragal perikondrium grefti, işitme iyileşmesi, greft başarı

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INTRODUCTION

Chronic otitis media (COM) is a persistent middle ear inflammation that frequently leads to tympanic membrane (TM) perforation. This condition is associated with recurrent infections and hearing loss and can significantly affect quality of life^{1,2}. The surgical management of TM perforations aims to restore membrane integrity, improve hearing, and prevent further episodes of infection. Tympanoplasty, a well-established procedure, is the preferred method for achieving these outcomes by reconstructing the perforated membrane and often utilizing autologous grafts, such as the fascia, cartilage, or perichondrium³⁻⁷.

Traditional tympanoplasty approaches, such as retroauricular and endaural techniques, involve the use of a microscope, which offers a three-dimensional view of the surgical field and the ability to operate with both hands. However, these techniques may require larger incisions and are associated with certain limitations, particularly when addressing anteriorly located perforations. Over the last decade, there has been growing interest in minimally invasive approaches, including endoscopic tympanoplasty (ET)^{8,9}.

ET, introduced as a viable alternative to microscopic methods, offers several advantages, including enhanced visualization of hidden anatomical structures, such as the sinus tympani, anterior TM, and ossicular chain. Using angled endoscopes, surgeons can access regions of the middle ear that are difficult to visualize using traditional approaches. This minimally invasive technique allows for transcanal access, avoiding external incisions, and often results in shorter hospital stays and quicker recovery times. Despite these advantages, the clinical outcomes of ET, particularly hearing improvement and graft success, remain areas of ongoing study¹⁰⁻¹².

Our study aimed to evaluate the clinical success and hearing improvement of endoscopic type 1 tympanoplasty. By analyzing preoperative and postoperative audiometric outcomes and surgical success rates in a cohort, we aim to provide valuable insights into the efficacy of this minimally invasive technique in managing COM with TM perforation.

MATERIALS and METHODS

This Study

This single-center, retrospective study was conducted between January 2022 and June 2024 at the Otorhinolaryngology Department of Məlhəm Beynəlxalq Hospital, Baku, Azerbaijan. This study adhered to the

Declaration of Helsinki's highest ethical standards and principles. Informed consent was obtained from all participants, ensuring anonymity and confidentiality. The Ministry of Health of the Republic of Azerbaijan, State Advanced Training Institute for the doctors named after A. The Aliyev Local Ethics Committee approved this study (decision no: EaR. No: 1/09.02.2024-2, date: 09.02.2024). There are no conflicts of interest to declare, and the manuscript respects participant privacy and aligns with relevant ethical guidelines. Forty-six patients diagnosed with COM and TM perforation underwent endoscopic Type 1 tympanoplasty. All participants provided written informed consent for study participation. Patients were selected based on specific inclusion and exclusion criteria. Only patients with large external auditory canals, no history of previous revision surgery, and who did not require ossicular chain reconstruction were included. Patients underwent a thorough audiometric evaluation before surgery, followed by at least 3 months of postoperative follow-up audiometric testing.

Inclusion Criteria

- Patients with conductive hearing loss due to COM and TM perforation.
- No revision surgeries were performed before the study.
- Dry TM perforations for at least two months before surgery.
- Age > 18 years

Exclusion Criteria

- Patients requiring ossicular chain reconstruction.
- Patients with middle ear or mastoid pathology.
- Incomplete follow-up or inadequate preoperative data.
- Age <18

Surgical Procedure

Endoscopic Type 1 tympanoplasty was performed under general anesthesia. A 0-degree rigid endoscope (4 mm diameter) was used for transcanal approach in all patients. After preparing the ear, the perforation margins were freshened. The tragal perichondrium was harvested and used as the graft material. Grafting was performed using the over-underlay technique, and the graft was carefully positioned to cover the perforation. The tympanomeatal flap was replaced, and the ear canal was filled with Gelfoam and a hydrocortisone-Furacillin mixture gauze. All patients were discharged on the same

day after the procedure, and follow-up assessments were conducted at 2 and 4 months postoperatively, with audiometric testing repeated after 3 months.

Data Collection and Evaluation

Demographic data, including age, sex, and the affected ear, were collected from all 46 patients who underwent endoscopic Type 1 tympanoplasty. Preoperative evaluations included computed tomography to assess middle ear pathology and audiometric tests to determine baseline hearing levels. The audiometric assessment included pure-tone audiometry at four frequencies (0.5, 1, 2, and 4 kHz). Postoperative audiometric data were collected at least 3 months after surgery, and audiometry was performed using appropriate masking techniques. The pure-tone average (PTA) was calculated preoperatively and postoperatively across the same four frequencies for each patient. The American Speech-Language-Hearing Association classification system categorizes hearing loss¹³. Postoperative PTA of 25 decibel (dB) or lower indicated functional hearing improvement¹⁴. The air-bone gap (ABG) was calculated by subtracting the bone conduction (BC) thresholds from the air conduction thresholds at 500, 1000, 2000, and 4000 Hz, both preoperatively and postoperatively. The ABG gain, which represents hearing improvement, was determined as the difference between the preoperative and postoperative ABG values for each ear. Success was defined as a postoperative ABG of 20 dB, whereas an ABG >21 dB was classified as "not success."¹⁴⁻¹⁶. Anatomical success was assessed during follow-up examinations, focusing on the condition of the TM. Clinical success was defined as the graft remaining intact without any reperforation. The rate of TM preservation was calculated by observing patients during follow-up otoscopic examinations conducted at two and four months postoperatively^{17,18}. The clinical findings and audiometric outcomes assessed the surgery's functional and anatomical success.

Statistical Analysis

Descriptive statistics were used to summarize the clinical and audiometric data, including the mean and standard deviation for preoperative and postoperative PTAs and ABG. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess the normality of data distribution. Depending on the results, parametric or non-parametric tests (such as the paired t-test or Wilcoxon signed-rank test) were applied to compare preoperative and postoperative audiometric outcomes. A p-value 0.05 was considered statistically significant. All analyses were performed using SPSS version 24.0 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.).

RESULTS

In total, 46 patients underwent unilateral Type 1 tympanoplasty. Of these, 28 were female (60.87%), and 18 were male (39.13%) were male, with a mean age of 35.84 ± 10.34 years at the time of surgery. The mean follow-up period was 10.28 ± 3.46 months. Regarding comorbidities, 11 patients (23.91%) had hypertension, and two patients (4.35%) patients had diabetes mellitus. Most patients (86.96%) were non-smokers, while six patients (13.04%) were smokers. Surgery was performed on the right ear in 18 patients (39.13%) and the left ear in 28 (60.87%).

Audiometric Parameters

The preoperative ABG across four frequencies (500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz) showed significant improvement. The mean preoperative ABG values were 36.74 ± 7.62 dB at 500 Hz, 36.52 ± 7.66 dB at 1000 Hz, 39.57 ± 6.31 dB at 2000 Hz, and 34.46 ± 7.47 dB at 4000 Hz. Postoperatively, these values were reduced to 18.48 ± 9.06 dB at 500 Hz, 15.87 ± 4.98 dB at 1000 Hz, 18.80 ± 5.29 dB at 2000 Hz, and 13.26 ± 4.50 dB at 4000 Hz. The ABG gain, representing the improvement in hearing, ranged from 18.26 dB to 21.20 dB across the frequencies, with p-values of less than 0.005, indicating a statistically significant improvement (Table 1).

Functional and Clinical Out-comes

The functional success rate, defined as achieving a postoperative ABG of 20 dB, was observed in 42 of 46 patients (91.30%). In terms of hearing improvement, 39 patients (84.78%) achieved hearing success, which was defined as slight or normal hearing after surgery. Preoperatively, 43 patients (93.48%) had moderate hearing loss and three (6.52%) had mild hearing loss.

After surgery, 17 patients (36.96%) achieved normal hearing and 22 (47.83%) had slight hearing loss. Only four patients (8.70%) continued to experience mild hearing loss postoperatively. The results showed significant hearing improvements ($p < 0.001$), with a clear shift from moderate hearing loss to normal or slight hearing levels postoperatively (Table 2).

Anatomical Success

Anatomical success, defined by the integrity of the TM and successful grafting, was achieved in 42 out of 46 patients (91.30%). The graft remained intact in all patients during follow-up, indicating high clinical success.

Table 1. Frequency-specific hearing outcomes in endoscopic type I tympanoplasty.

Parameters	500 Hz	1000 Hz	2000 Hz	4000 Hz
Preop. ABG	36.74±7.62	36.52±7.66	39.57±6.31	34.46±7.47
Postop. ABG	18.48±9.06	15.87±4.98	18.80±5.29	13.26±4.50
ABG gain	18.26±7.24	20.65±8.60	20.76±7.96	21.20±7.61
Pre-Post. ABG	p<0.005	p<0.005	p<0.005	p<0.005
p-values	1.56154E-17	4.01789E-27	8.08495E-31	3.13625E-29

ABG: Air-bone gap.

Table 2. Comprehensive clinical and functional outcomes in endoscopic type I tympanoplasty.

Parameters		Total ear (±SD) (n=46)
Age		35.84±10.34
Follow-up periot (month)		10.28±3.46
Gender (n/%)	F	28 patients (60.87%)
	M	18 patients (39.13%)
Smoking (n/%)	Non-smoker	40 patients (86.96%)
	Smoker	6 patients (13.04%)
Comorbidity (n/%)	HT	11 patients (23.91%)
	DM	2 patients (4.35%)
Side (n/%)	Right	18 patients (39.13%)
	Left	28 patients (60.87%)
ABG	Mean (dB)	16.60±3.09
	Success (number of patients) (postop total ABG <20)	42 patients (91.30%)
PTA	Preoperative mean (dB)	46.72±6.10
	Postoperative mean (dB)	19.57±5.76
	Gain (total±SD)	27.15±6.09
	Pre-postoperative. PTA	p<0.005
	p-values	3.30701E-38
Hearing success (number of patients) (Postop "Slight hearing loss+normal herring")		39 patients (84.78%)
Preoperative	Moderate hearing loss	43 patients (93.48%)
	Mild hearing loss	3 patients (6.52%)
Postoperative	Slight hearing loss	22 patients (47.83%)
	Normal hearing	17 patients (36.96%)
	Mild hearing loss	4 patients (8.70%)
Clinical Success (number of patients)		42 patients (91.30%)
M: Male, F: Female, H: Hypertension, DM: Diabetes mellitus, dB: Desibel, SD: Standard deviation, PTA: Pure tone averages, ABG: Air-bone gap.		

DISCUSSION

Tympanoplasty is commonly performed in otologic surgery, and microscopic and endoscopic approaches offer unique advantages. Although microscopic techniques allow for a two-handed operation and a three-dimensional view, they are limited in visualizing

anterior perforations. In contrast, ET provides a broader field of view, especially with angled scopes, and improves access to difficult areas like the anterior TM, attic, and facial recess^{9,10,19}.

Despite its advantages, ET presents challenges such as a steep learning curve, single-hand operation, two-

dimensional view complicating depth perception, heat from light sources potentially posing risks, and smaller-diameter endoscopes limiting wide-angle views. However, these issues can be mitigated with experience and adjustments to light intensity^{20,21}.

Nevertheless, the endoscopic approach offers key benefits, including reduced operative time, improved cosmetic outcomes, faster recovery, and preservation of middle ear anatomy, leading to higher graft success and functional hearing improvement, with growing expertise further enhancing its efficacy in treating TM perforations²². ET outcomes have been well documented, particularly for large TM perforations. Choi et al.'s²² multicenter study in South Korea, involving 239 patients, reported a graft success rate of 86.2% for large or subtotal TM perforations, slightly lower than the 91.30% success rate achieved in our study. Notably, their results were influenced by the surgical technique, with the lateral underlay method showing superior outcomes ($p=0.027$), consistent with our focus on stable graft placement. Choi et al.²² also reported an average ABG improvement of 10.0 ± 0.6 dB in the graft success group, whereas our study showed a more significant ABG reduction from 36.74 ± 7.62 dB preoperatively to 15.05 ± 3.82 dB postoperatively ($p<0.005$), demonstrating the effectiveness of our ET approach. This finding could be attributed to the targeted methodology and surgical expertise of our cohort. Additionally, Choi et al.²² noted improvements in BC thresholds at 500 Hz ($p=0.028$), a finding mirrored by significant BC improvements in our study, highlighting ET's role in enhancing inner ear function. These comparisons show that while larger studies like Choi et al.'s²² validate ET broadly, our focused study aligns with and builds on these findings, underscoring ET's strong outcomes even in developing regions like Azerbaijan.

Sakagami et al.²³ conducted a study evaluating microscopic transcanal tympanoplasty and reported a perforation closure success rate of 76% and an improvement in the ABG of 11.8 dB postoperatively. In comparison, our endoscopic approach achieved a higher closure success rate of 91.30% and a more significant improvement in the ABG, with a mean reduction of over 18 dB. These results suggest that the endoscopic approach may provide superior outcomes in terms of both graft success and hearing improvement.

In the study by Usami et al.²⁴, the authors evaluated 22 patients who underwent endoscopic-assisted tympanoplasty, reporting a perforation closure success rate of 81.8% and an average improvement in the ABG of 14.8 dB. Our study demonstrated a higher perforation

closure success rate and a more significant improvement in hearing outcomes. Although both studies highlighted the efficacy of endoscopic techniques, our results suggest that the methods employed in our procedure may lead to enhanced anatomical success and better hearing improvement.

The outcomes of ET for large TM perforations have been explored in various studies^{6,8,11,12,22,25}, including those by Casas et al.²⁵, who reported a 94.4% graft success rate for subtotal and total perforations using a composite graft technique. This is notably higher than the success rate of 78.3% observed with classical underlay methods. Their study also highlighted that the mean postoperative ABG was reduced to 10 ± 10 dB, indicating significant hearing improvement. These findings resonate with our study's results, where we achieved a 91.30% graft success rate and improved the mean ABG from 36.74 ± 7.62 dB preoperatively to 15.05 ± 3.82 dB postoperatively ($p<0.005$), highlighting comparable effectiveness. The use of a composite graft to minimize the risk of anterior graft medialization, as described by Casas et al.²⁵, aligns with our focus on stable and precise graft placement. This approach reinforces the value of advanced grafting techniques for enhancing anatomical and functional outcomes. While Casas et al.²⁵ emphasized the stability provided by cartilage in subtotal and total perforations, our study underscores similar stability and effectiveness in achieving successful outcomes even with single-session ET. These comparative insights underline that ET, whether with composite or traditional grafts, can yield high success rates and significant audiometric gains, contributing valuable data to the growing evidence supporting ET's reliability.

The comparative analysis of grafting techniques is crucial for assessing ET outcomes. Lee et al.²⁶ conducted a retrospective study on Type I tympanoplasty in patients with severe middle ear granulation cases showed a 91% graft success rate of 91% for fascia, 100% for cartilage island grafts, and 100% for cartilage palisades. Our study's graft success rate of 91.30% was in close agreement with these outcomes, particularly for the cartilage-based techniques. Lee et al.²⁶ also reported significant improvements in PTA and ABG in most groups, except the cartilage palisade group. In contrast, our study showed a significant reduction in ABG from 36.74 ± 7.62 dB preoperatively to 15.05 ± 3.82 dB postoperatively ($p<0.001$), reinforcing the effectiveness of our endoscopic approach. These findings highlight that although different techniques yield different outcomes, our method is associated with consistent audiological and anatomical success.

Recent advancements in ear diseases have focused on developing innovative techniques and surgical approaches, particularly for treating COM sequelae, cochlear implant, tinnitus, etc.²⁷⁻²⁹. In tympanoplasty, contemporary innovations primarily involve optimizing grafting techniques during procedures performed with microscopic or endoscopic assistance^{7,30}. As an otolaryngologist practicing in a developing country, applying the well-established endoscopic tragal perichondrium graft technique can improve surgical success rates. This approach ensures reliable patient outcomes and contributes region-specific data to the existing otologic literature.

The main strength of our study lies in its focus on the endoscopic transcanal approach, which allowed for a minimally invasive procedure with high graft success rates (91.30%) and hearing improvement. Detailed preoperative and postoperative audiometric data confirmed the technique's efficacy. However, one limitation of the study is its retrospective design and relatively small sample size of 46 patients, which may limit the generalizability of the findings. Additionally, the study did not include a control group that used microscopic techniques, which could have provided a more direct comparison of outcomes.

This study is the first documented study on ET outcomes in Azerbaijan, and it makes a significant contribution to the regional otologic literature. Highlighting these findings underscores the importance of advancing surgical techniques and reporting outcomes in developing countries and enriching the global understanding of ET's efficacy.

CONCLUSION

This study demonstrated the efficacy and safety of unilateral Type I tympanoplasty using an endoscopic transcanal approach. The procedure led to significant hearing improvements, with a functional success rate of 91.30%, and clinical success, defined by intact grafts, was also 91.30%. Conclusion: Endoscopic type I tympanoplasty is a highly effective, minimally invasive treatment for COM with tympanic

Ethics

Ethics Committee Approval: The Aliyev Local Ethics Committee approved this study (decision no: EaR. No:1/09.02.2024-2, date: 09.02.2024).

Informed Consent: Informed consent was obtained from all participants, ensuring anonymity and confidentiality.

Footnotes

Author Contributions

Surgical and Medical Practices: A.A., Concept: A.A., Design: A.A., R.H., Data Collection and/or Processing: A.A., R.H., Analysis and/or Interpretation: A.A., R.H., Literature Search: A.A., R.H., Writing: A.A.

Conflict of Interest: The authors have no conflict of interest to declare.

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The Effect of Intradermal Injection with Two Different Injection Techniques on Pain and Functional Status in Patients with Chronic Nonspecific Neck Pain

Kronik Non-Spesifik Boyun Ağrılı Hastalarda İki Farklı Enjeksiyon Teknikle Uygulanan İntradermal Enjeksiyonun Ağrı ve Fonksiyonel Durum Üzerine Etkisi

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ABSTRACT

Objective: This study aimed to compare the effectiveness of different mesotherapy techniques applied at various dermal depths on pain, functional status, and quality of life in patients with non-specific neck pain.

Methods: A total of 43 patients who received mesotherapy for non-specific neck pain were retrospectively analyzed. Patients treated with the point-by-point technique (n=21) and the napaj technique (n=22) were recorded. A mixture of 2 mL lidocaine and tenoxicam was used as the mesotherapy solution. Pain was assessed using the visual analog scale (VAS), functional status was assessed using the neck disability index (NDI), and quality of life was assessed using the Nottingham health profile before (T0), after (T1), and 3 months after (T2).

Results: In both groups, which were homogeneous in terms of demographic data, statistically significant changes were observed in T1 and T2 values compared with T0 for all evaluation parameters (p=0.001). While both groups demonstrated improvement in all parameters, the posttreatment VAS and NDI score in the point-by-point group was slightly better than that in the napaj group (p=0.042), (p=0.043) but this difference disappeared in the 3-month evaluation.

Conclusions: Mesotherapy using a lidocaine-tenoxicam mixture with both the point-by-point and napaj techniques is an effective and safe treatment method for nonspecific neck pain. Both techniques were found to be effective in improving pain, functional status, and quality of life, but neither technique was found to be superior to the other.

Keywords: Intradermal injection, mesotherapy, neck pain, point by point, napaj

ÖZ

Amaç: Bu çalışmanın amacı, non-spesifik boyun ağrısı olan hastalarda, farklı dermal derinliklerde uygulanan farklı mezoterapi tekniklerinin ağrı, fonksiyonel durum ve yaşam kalitesi üzerindeki etkinliğini karşılaştırmaktır.

Yöntemler: Non-spesifik boyun ağrısı nedeniyle mezoterapi uygulanan 43 hasta retrospektif olarak incelendi. Teknik olarak Point by point (n=21) ve napaj (n=22) tekniği ile mezoterapi uygulanan hastalar kaydedildi. Mezoterapi solüsyonu olarak 2 mL lidokain ve tenoksikamdan hazırlanan karışım uygulandı. Hastaların ağrısı görsel analog skala (VAS), fonksiyonel durumu boyun engellilik indeksi (BDI) ve yaşam kalitesi Nottingham sağlık profili (NSP) kullanılarak tedavi öncesi (T0), tedavi sonrası (T1) ve tedaviden 3 ay sonra (T2) değerlendirildi.

Bulgular: Demografik veriler açısından homojen olan her iki grupta tüm değerlendirme parametrelerinde T0'a göre T1 ve T2 değerlerinde istatistiksel olarak anlamlı değişiklikler gözlemlendi (p=0.001). Her iki grupta tüm parametrelerde iyileşme gözükürken point by point grubunda tedaviden sonraki VAS (p= 0.042) ve BDI (p= 0.043) değeri napaj grubuna göre küçük bir farkla daha iyi olduğu fakat bu farkın 3. aydaki değerlendirmede ortadan kalktığı görüldü.

Sonuçlar: Point by point ve napaj tekniği ile lidokain ve tenoksikam karışımında oluşan mezoterapi uygulaması, non-spesifik boyun ağrılarında etkili ve güvenli bir tedavi yöntemidir. Her iki teknik ağrı, fonksiyonel durum ve yaşam kalitesi üzerine etkili ancak birbirlerine üstün bulunmadı.

Anahtar kelimeler: İntradermal enjeksiyon, mezoterapi, boyun ağrısı, point by point, napaj

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INTRODUCTION

According to the Guidelines on Neck Pain, neck pain is defined as an uncomfortable sensory and emotional experience connected to actual or potential damage to the tissues in the neck, extending from the superior nuchal line to the level of the scapula¹. Non-specific neck pain refers to discomfort occurring in the side and back areas of the neck, in the absence of neurological and specific pathologies, such as fractures, infections, inflammation, or cervical spondylosis². Neck pain affects between 10% and 21% of people annually, with approximately 5-10% of cases becoming chronic. It is one of the most common reasons for primary healthcare consultations and is the fourth leading cause of disability worldwide³⁻⁵. Neck pain has significant implications for treatment because it can reduce quality of life, decrease work productivity, limit daily activities, and increase healthcare costs⁴. The causes of neck pain are often multifactorial, predominantly arising from musculoskeletal issues. Risk factors include lack of physical activity, prolonged computer use, stress, and female sex, which involve both physical and psychological aspects^{4,5}.

Treatment for non-specific neck pain involves both pharmacological and nonpharmacological approaches. Non-pharmacological methods include education, physical therapy modalities, exercise, cognitive behavioral treatment, and cervical collar use. Pharmacological treatments often include pain relievers, muscle relaxants, steroids, narcotic analgesics, and antidepressants. Among the pharmacological treatments, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are most commonly prescribed for pain management. However, the use of these drugs, particularly in the elderly or in patients with comorbidities and multiple medications, can lead to significant complications, including drug interactions and life-threatening effects on the gastrointestinal, cardiovascular, and renal systems¹⁻³. To minimize such complications, local pharmacological therapies (e.g., interventional procedures, intra-articular or peri-articular injections) can be convenient alternatives^{1,2}. One of the effective and safe methods for treating localized musculoskeletal pain, including neck and lower back pain, is local intradermal therapy (mesotherapy)⁶.

Mesotherapy is a minimally invasive technique, involves injecting a mixture of compounds (such as herbal extracts, homeopathic agents, medications, vitamins, and other bioactive substances) intradermally or subcutaneously using very fine needles in small doses⁷. Initially described by Michel Pistor in 1958, monotherapy has become a widely known and practiced technique in various parts of the world for treating localized clinical conditions^{8,9}.

Clinical studies have shown that intradermal injections in monotherapy allow lower doses of drugs to remain in target tissues (skin, muscle, and joint) for longer periods than intramuscular injections. This method alters the absorption kinetics of the injected drug, thereby slowing its systemic absorption and enabling its local distribution in underlying tissues. The benefits of this technique include reduced drug doses, less frequent application, a lower risk of drug interactions in patients taking multiple medications, and the ability to combine monotherapy with other pharmacological and non-pharmacological approaches. These advantages have made monotherapy a widely used method worldwide⁸⁻¹⁰.

In pain monotherapy, a 4- mm needle (27-30 gauge) is typically used, and the technique is applied either by nappage or the point-by-point method. In the point-by-point technique, injections are made perpendicularly to the skin at a depth of 4 mm and spaced 1-2 cm apart. The nappage technique involves more superficial injections at a depth of 2 mm using a 4- mm needle, covering a larger area⁷. In local pain treatment, anesthetics, nonsteroidal anti-inflammatory drugs, and muscle relaxants are commonly injected intradermally either individually or as a mixture at very low doses⁹.

In our study, we aimed to retrospectively investigate the effectiveness of two different intradermal injection techniques, nappage and point-by-point injection, using a combination of local anesthetic and NSAIDs, on pain and functional status in patients with chronic nonspecific cervical pain, which is the first study of its kind in the literature.

MATERIALS and METHODS

Study Design

This study retrospectively evaluated the medical records of patients who presented with chronic non-specific neck pain and received monotherapy treatment at the Physical Medicine and Rehabilitation out-patient clinics of Istanbul Medeniyet University Goztepe Prof. Dr. Suleyman Yalcin City Hospital between January 2023 and September 2024.

The study included patients diagnosed with chronic nonspecific neck pain for at least 3 months, based on clinical and radiological assessments, with a Visual Analog Scale (VAS) score of 4 or higher. Patients with fractures, infections, inflammatory pain, cervical spondylosis, and radicular pain, as well as those who underwent neck surgery, were excluded from the study. Patients were randomly selected by considering inclusion and exclusion criteria. Participants were aged 18-65 years and of both

sexes, and they received monotherapy treatment using either nappage or point-by-point technique. This study was approved by the Clinical Research Ethics Committee of Istanbul Medeniyet University of Health Sciences Göztepe Training and Research Hospital (decision no: 2023/0813, date: 29.11.2023).

Mesotherapy Protocol

The monotherapy injection was performed using 2.5 mL sterile syringes (Meso-Relle, Biotekne, Serebellar reticular lesion, Italy) with a 32G x 4 mm needle. A total of 2 mL was prepared by diluting 1 mL of 2% lidocaine (2% Jetmonal, Adeka Pharma Co., Istanbul, Türkiye) was diluted with 1 mL of tenoxicam diluted in saline at a 3:1 ratio (Oksamen 20 mg/4 mL; Nobel Pharma Co., Istanbul, Türkiye). The injections were administered in four sessions, one week apart.

The application area was from the superior nuchal line and continued down to the level of the scapular spine (Figure 1). In patients treated with the point-by-point technique, 0.02 mL to 0.05 mL was injected at a depth of 4 mm, perpendicular to the skin, with intervals of 1-2 cm. In patients receiving the rappage technique, a more superficial injection was performed at a depth of 2 mm, applying light, continuous positive pressure on the piston, at a 45° angle to the skin, and delivering a drop (0.01-0.02 mL) of the solution to each area at short distances. The monotherapy sessions were conducted by an experienced physiatrist.



Figure 1: Injection site.

Clinical Evaluation

Demographic data, including age, height, weight, marital status, education status, employment status, and smoking habits, were recorded.

Pain was assessed using the VAS, the impact of neck pain on daily activities was evaluated using the neck disability index (NDI), and quality of life was assessed using the Nottingham Health Profile scales. Patients were evaluated by the same physiatrist (S.M) using the aforementioned scales before treatment (T0), one week post-treatment (T1), and three months after the final follow-up (T2).

VAS: This is a pain assessment tool. The scale ranges from a minimum of 0, indicating no pain, to a maximum of 10, representing the worst pain experienced by the patient¹¹.

NDI: This is a self-administered questionnaire that assesses how neck pain affects daily activities. The NDI consists of 10 sections, each exploring different aspects of daily life affected by pain (pain intensity; personal care; lifting; reading; headaches; concentration; work; driving; sleeping; recreation). Each section is scored from 0 to 5, where 0 indicates no difficulty or pain and 5 indicates an inability to perform the activity or debilitating pain. The total score ranged from 0 to 50, with higher scores indicating greater disability¹².

Nottingham Health Profile : The NHP is a general quality of life questionnaire that measures an individual's perceived health problems and the extent to which these problems affect routine daily activities. The first section, which assesses six dimensions of health, covers energy, pain, emotional reactions, sleep, social isolation, and physical activity. The second section evaluates areas such as work, housework, home life, sexual life, interests, hobbies, and holidays. Scores for each section ranged from 0 (worst health) to 100 (best health)¹³.

Statistical Analysis

Descriptive statistics for the measurements were calculated as mean, standard deviation, median, and 25th and 75th percentiles. The normal distribution of the measured variables was examined using the Shapiro-Wilk test. The relationships between categorical variables were assessed using the Pearson chi-square test. The Mann-Whitney U test was used to compare numerical characteristics between the two groups. Periodic comparisons within each group were performed using the Friedman test and post-hoc Dunn test. The correlation between age, BMI, and VAS scores was analyzed using the

Spearman rank correlation coefficient. A p-value of <0.05 was considered statistically significant. SPSS (ver. 29) was used for all analyses.

Power Analysis

Assessments in the study were conducted using VAS, NDI, and NHP scales. Among these scales, VAS scores were considered the primary out-come. Additionally, based on clinical knowledge and literature¹⁴, a change of 2 points in the VAS score was considered significant. According to this result, Cohen's standardized effect size was accepted as $d = 0.90$, and $\alpha = 5\%$ and prior power as 80% indicated that a sample size of at least 20 individuals would be sufficient.

RESULTS

A total of 43 patients were included in the study, with 21 receiving monotherapy using the point-by-point technique and 22 using the rappage technique. There were no significant differences between the

two groups in terms of age, BMI, duration of pain, or Beck Depression Scale scores (Table 1. When examining further demographic data of the patients, it was found that 72.1% (n=31) were married, 58.2% (n=25) had a high school education or higher, 53.5% (n=23) were actively working, 34.9% (n=15) were current smokers, and 69.8% (n=30) had no comorbidities. Additionally, 93.0% (n=40) of the patients were female. The analysis of marital status ($p=0.206$), education level ($p=0.614$), employment status ($p=0.887$), smoking ($p=0.206$), comorbidities ($p=0.273$), and gender ($p=0.578$) also showed no significant differences between the two groups. No side effects were observed other than pain during the injection.

Two groups were assessed before, after, and 3 months after the treatment. Significant improvements in VAS and NDI scores were observed in both groups post-treatment, and this improvement was maintained through the third month. When comparing the point-by-point group with the rappage group, the VAS and NDI scores were statistically higher in the rappage group in

Table 1. General characteristics at baseline.

	Treatment	N	Mean	SD	Percentiles			P*
					25 th	Median	75 th	
Age	Point-by-point	21	47.14	11.38	38.00	46.00	56.00	0.169
	Napaj	22	41.05	14.24	26.00	43.00	51.75	
BMI	Point-by-point	21	26.83	4.47	23.44	26.49	31.45	0.253
	Napaj	22	26.02	6.82	21.84	24.71	28.62	
Pain duration (years)	Point-by-point	20	5.90	5.20	2.25	5.00	7.50	0.929
	Napaj	22	6.50	5.65	2.00	5.00	9.25	
Beck depression scale sor	Point-by-point	21	12.90	8.67	4.50	10.00	19.00	0.473
	Napaj	22	14.64	8.53	6.75	15.00	20.50	

*Mann-Whitney U test (difference between two groups), $p < 0.05$ BMI: Body mass index. SD: standard deviation.

Table 2. VAS and Neck Disability Index comparison

	Point by point(n=21)					Napa j (n=22)					
	Mean	SD	Percentiles			Mean	SD	Percentiles			
			25 th	Median	75 th			25 th	Median	75 th	
VAS T0	7.9	1.67	8.0	8,00 ^a	9.0	7.73	1.42	7.0	8.00 ^a	9.0	0.529
VAS T1	3.0	2.07	2.0	3.00 ^b	4.0	4.14	1.93	2.75	4.00 ^b	5.25	0.042
VAS T2	3.43	2.58	1.5	4.00 ^b	6.0	4.55	2.96	2.5	4.50 ^b	8.0	0.182
P*	<0.001					<0.001					
NDI T0	17.48	7.61	11.5	15.00 ^a	23.5	20.82	8,37	15	20,00 ^a	26.25	0.189
NDI T1	9.43	5.24	5.0	9.00 ^b	13.0	13.41	6.75	8.75	14.00 ^b	18.25	0.043
NDI T2	8.29	5.66	3.0	9.00 ^b	13.5	11.77	6.58	6.0	10.50 ^b	18.0	0.082
P*	0.001					<0.001					

∴ $p < 0.05$, The Friedman test (comparison of periods). Periods that showed significant differences are symbolized with completely different letters (e.g., ^a and ^b). **: $p < 0.05$ Mann-Whitney U test (difference between two groups). SD: standard deviation.

the posttreatment assessment (p-values of 0.042 and 0.043 respectively). However, no significant differences were observed between the groups before treatment or at the 3-month follow-up (Table 2).

The six sub-parameters of the NPH along with the total scores for parts 1 and 2 were evaluated. Comparisons were made between and within groups. Regarding the total scores for part 1, no significant differences were observed

between the two groups at baseline, posttreatment, or at the three-month follow-up. However, it was noted that the total score significantly decreased post-treatment in both groups (p-values of 0.009 and 0.049, respectively), and this level was maintained at the 3-month follow-up (Table 3).

Within-group comparisons revealed a significant reduction in the pain subparameter for both groups in

Table 3. Nottingham Health Profile comparison in and between groups.

	Point by point (n=21)					Napa j (n=22)					
	Mean	SD	Percentiles			Mean	SD	Percentiles			
			25 th	Median	75 th			25 th	Median	75 th	
NHP T0 pain	54.19	33.39	22	52.00 ^a	91.5	56.36	26.1	31	59.00 ^a	80	0.874
NHP T1 pain	20.1	23.44	4.5	13.00 ^b	20.5	35.95	27.08	14.5	30.50 ^b	63.25	0.902
NHP T2 pain	26.1	27.44	0	13.00 ^b	52	30.05	25.18	9	29.00 ^b	50	0.990
P*	0.001					0.003					
NHP T0 emotional states	25.76	22.99	9.5	24.00 ^a	34.5	27.36	26.43	0	22	48.25	0.524
NHP T1 emotional state	13.52	17.85	0	9.00 ^b	21	25.91	26.73	0	22	36	0.275
NHP T2 emotional state	16.62	17.03	0	12.00 ^b	23.5	21.36	20.57	0	17.5	35.25	0.785
P*	0.013					0.528					
NHP T0 sleep	30.62	28.15	0	27.00 ^a	59	29.32	25.62	0	31.5	51.5	0.653
NPH T1 sleep	7.57	20.27	0	0.00 ^b	0	29.27	27.74	0	21.5	56.5	0.496
NHP T2 sleep	22.71	33.86	0	0.00 ^b	40	31.05	25.98	0	40	56	0.036
P*	0.001					0.886					
NHP T0 isolation	9.67	14.28	0	0	19.5	14.32	20,6	0	0	22.25	0.114
NHP T1 isolation	3.52	9.38	0	0	0	14.09	19.03	0	0	36.5	0.002
NHP T2 isolation	6.52	15.19	0	0	6.5	12.91	18.69	0	0	31.25	0.024
P*	0.296					0,169					
NHP T0 mobility	18.71	19.77	0	11	27	23.45	17.18	11	21.5	34.25	0.990
NHP T1 mobility	18.95	17.96	0	12	28.5	18.68	17.15	0	17.5	33	0.275
NHP T2 mobility	17.24	12.26	5	22	23	20.59	16.05	7.5	22	31.25	0.531
P*	0.839					0.905					
NHP T0 Energy	53.0	37.38	23	63	88	57.14	40.15	18	61.5	100	0.119
NHP T1 Energy	27.29	36.58	0	0	50	42.36	44.28	0	24	100	0.509
NHP T2 Energy	49.1	42.92	0	55	100	48.45	44.52	0	47	100	0.529
P*	0.338					0,070					
NHP T0= total 1	186.76	86.29	119.5	184.00 ^a	260.5	206.82	112.99	131.5	219.50 ^a	283.25	0.226
NHP T1 total1	90.9	93.17	33	70.00 ^b	123	166.5	135.47	62.25	115.00 ^b	310	0.199
NHP T2 total1	138.76	113.05	54.5	120.00 ^b	213	160.09	113.17	51.75	139.00 ^b	272.25	0.531
P*	0.009					0.049					
NHP T0 total2	1.95	1.6	1	2	3	2.32	1.89	0	2.5	4	0.496
NHP T1 total2	1.29	1.45	0	1	2	2.27	2.05	0	2	4	0.119
NHP T2 total2	1.14	1.15	0	1	2	1.77	1.45	0.75	1.5	3	0.146
P*	0.140					0.345					

*: p<0.05, The Friedman test (comparison of periods). Periods that showed significant differences are represented with completely different letters (e.g., ^a and ^b). **: p<0.05, Mann-Whitney U test (difference between two groups).

the post-treatment assessment compared with pre-treatment scores, and this improvement was maintained through the third month (point by point $p=0.001$, napaj $p=0.003$). Significant differences were noted in the emotional and sleep subparameters for the point-by-point technique, with p -values of 0.013 and 0.001, respectively, between the pre-treatment and post-treatment assessments. Comparisons of the treatments within themselves revealed no significant differences in other parameters ($p>0.05$).

When comparing the point-by-point technique to the rappage technique, the point-by-point technique demonstrated statistically significantly lower scores in the NPH sleep parameter at the 3-month assessment ($p=0.036$). Additionally, for the isolation parameter, the point-by-point technique showed statistically significantly lower scores than the rappage group both post-treatment and at the 3-month mark ($p=0.002$, $p=0.024$). No significant differences were found in the other sub-parameters between the groups ($p>0.05$). Furthermore, in the NPH total 2 scores, no significant differences were observed over time, either within or between the groups.

In our study, a total of 40 female and 3 male patients were included. For further evaluation, participants were divided into two groups based solely on their sex, independent of the treatments they received. The changes in the VAS scores over time were assessed. For female patients, the average VAS score before treatment was $7.80\pm1,539$, after treatment it was $3.60\pm2,122$, and at the 3-month follow-up, it was $4.13\pm2,848$. For male patients, the average VAS score before treatment was $8.00\pm1,732$, after treatment it was $3.33\pm1,155$, and at the 3-month follow-up, it was $2.33\pm1,528$. No significant differences were found between the two groups regarding VAS scores before treatment, after treatment, or at the 3-month follow-up ($p>0.05$).

When examining the correlation between patients' age and VAS scores, as well as between patients' BMI values and VAS scores in each period, no correlation was found at any assessment time ($p>0.05$).

Discussion

In this study, we compared the effects of monotherapy, which was applied using two different techniques, on pain, functional status, and quality of life in patients with chronic nonspecific neck pain. This study is the first to examine this specific topic. The current results showed that both techniques were effective in reducing pain and improving functional status. However, in the initial post-

treatment evaluation, the point-by-point technique was found to be statistically more effective than the rappage technique in terms of pain reduction and functional improvement. This difference was not observed in the 3-month follow-up evaluations.

Mesotherapy has been suggested as an ideal treatment for all localized musculoskeletal pain¹⁵. The analgesic mechanism of this treatment includes pharmacological effects, micro-traumatic effects induced by the needle and the injected solution, and endocrine neuro-immune reactions, defined under the concept of mesodermal modulation. In this concept, the dermis is considered a new target organ for analgesic effects^{16,17}. Recent studies have demonstrated the presence of dermis structures that can actively participate in pain modulation. The dermis, particularly glial cells, may be new potential targets for drugs administered through monotherapy¹⁸. These results suggest that the analgesic effect is not solely due to local pharmacological action but also involves complex interactions between the intradermal technique and dermal pain control systems. Therefore, dermal depth is crucial for the achievement of analgesic effect in monotherapy. The dermis thickness varies according to body location, sex, and age¹⁹. Based on these individual differences, standardizing intradermal injections may be difficult. In the early years of monotherapy, the depth of the injections was observationally defined based on the benefits obtained⁸. Mrejen D.²⁰ compared injections at depths of 4 and 10 mm and observed that substances injected at 10 mm diffused more quickly and entered the systemic circulation faster. Based on this study, injections should not exceed a depth of 4 mm. The optimal injection depth remains a research topic. The Italian Society of Mesotherapy recommends adjusting the needle angle to approximately 30°, depending on the dermis thickness^{8,9}. There are no randomized studies comparing the effectiveness of superficial versus deep dermal injections⁹. In our study, we utilized two injection techniques: the point-by-point technique (deep intradermal injection, 4 mm depth) and the rappage technique (superficial intradermal injection, 1-2 mm depth)⁷. Since we could not measure dermal thickness in this study, we followed the recommended injection techniques in the literature for these depths^{7,8,15}. Our study included 93% female and 7% male patients. The mean ages and BMIs were 47.14 and 26.83 for the point-by-point group and 41.05 and 26.02 for the rappage group, respectively. In the primary outcome measure, the VAS scores demonstrated pain reduction. There were no significant correlations between sex, age, BMI (factors that affect dermal thickness), and VAS scores ($p>0.05$). This result indicates that not only dermal depth but also several other factors, such as the needle and the administered drug, contribute to the analgesic effect.

Mesotherapy has been shown to result in prolonged drug diffusion into underlying tissues while maintaining tissue concentrations longer than intramuscular administration, with clinical outcomes comparable to other systemic routes (intravenous, intramuscular, oral)^{6,9,15,21,22}. In a study comparing doses of the same drug, patients receiving lower concentrations of monotherapy exhibited similar results to those receiving higher doses¹⁷. Additionally, studies comparing the use of multiple drugs in one syringe with the use of a single analgesic drug in monotherapy found similar clinical outcomes in patients with acute, subacute, or chronic pain¹⁶. Mesotherapy, even at minimal doses, is an economical treatment option due to its drug-saving effect. In our treatment, we used a solution consisting of 1 mL of 2% lidocaine and 1 mL of 2.5 mg of tenoxicam, which is only 12.5% of the NSAID dose. Analgesic effects were achieved at a low dose and volume (2 mL).

Mesotherapy is a safe method¹⁰. Systemic analgesics and anti-inflammatory drugs, which are frequently used for pain management, can cause life-threatening side effects, especially in elderly patients and those requiring polypharmacy²³. Mesotherapy is also an advantageous method for this purpose. The potential side effects of monotherapy include bleeding at the site of injection, pain, local reactions, nausea, vomiting, numbness, sweating, fatigue, and headache. These side effects are mild and transient and do not require additional treatment¹⁰. It is seen in the literature that different drug mixtures are used in monotherapy treatment^{21,24-26}. With monotherapy using multiple drug mixtures, the risk of drug interactions and local side effect increases. Studies have shown that monotherapy with a single drug is as effective in reducing pain as monotherapy with multiple drug mixtures²⁷⁻³⁰. Therefore, the use of multiple mixtures in monotherapy is not recommended¹⁶. In our study, no side effects other than pain were observed during the procedure. Because fewer injections are required and cause less pain, the point by point technique can be considered a more comfortable method for patients than the nappage technique.

Neck pain significantly affects daily quality of life³¹. Studies on monotherapy have shown positive effects on functional status and quality of life in patients with neck, low back, and knee pain^{24,29,32,33}. In this study, we observed improvements in NDI scores in both groups. However, the point-by-point group demonstrated statistically more significant improvements in the initial post-treatment assessment compared with the nappage group. In the Nottingham Health Profile sub-parameters assessing health status and quality of life, the point-by-point group

showed statistically better improvements although both groups exhibited clinical improvements. We conclude that this difference may be related to the small sample size.

Study Limitations

Our study was a retrospective study. Despite the power analysis determining the sample size, the small number of patients and the inability to objectively measure dermal depth were limitations. However, this pilot study provides useful data for calculating the statistical sample size required for future research on the most effective injection technique in monotherapy.

CONCLUSION

In conclusion, our study shows that monotherapy using a local anesthetic-NSAID cocktail injected at different dermal depths using different techniques is an effective, safe, economical, and well-tolerated method for treating non-specific neck pain. This treatment approach may be an alternative or complementary option to other treatment approaches for localized musculoskeletal pain. Both techniques were effective, but the point-by-point technique was more effective in the short term and provided a more comfortable injection experience for patients.

Ethics

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee of Istanbul Medeniyet University of Health Sciences Göztepe Training and Research Hospital (decision no: 2023/0813, date: 29.11.2023).

Informed Consent: Since this study was retrospective, patient consent was not required.

Footnote

Conflict of Interest: The authors have no conflict of interest to declare.

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Histopathological and Immunohistochemical Evaluation of Methotrexate-Induced Gonadal Damage in Rats: Role of SCF, mTOR, and SIRT-1

Sıçanlarda Metotreksata Bağlı Gonadal Hasarın Histopatolojik ve İmmünohistokimyasal Olarak Değerlendirilmesi: SCF, mTOR ve SIRT-1'in Rolü

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ABSTRACT

Objective: Methotrexate (MTX) is a highly effective chemotherapy for cancer. This drug has a gonadotoxic effect, mainly in the testes and ovaries. Our study used histopathological and immunohistochemical methods to assess the potential damage to testicular and ovarian tissue caused by MTX use.

Methods: Twenty-four Wistar albino rats, both male and female, were used in our study. Four sets of rats; control male, MTX male, control female, and MTX female were created. The male and female MTX-treated groups received a single intraperitoneal dose of 20 mg/kg MTX. The testes and ovaries of rats sacrificed under general anesthesia were extracted and histopathologically analyzed. In addition, the immunoreactivity intensities of stem cell factor (SCF), mechanistic target of rapamycin (mTOR), and SIRT-1 in both tissues were measured by immunohistochemistry.

Results: Johnsen's testicular biopsy score in the testicular seminiferous tubules was significantly lower in the MTX group than in the control group ($p<0.001$). The ovary showed substantial follicular degeneration ($p<0.05$), vascular congestion ($p<0.01$), and fibrosis ($p<0.001$). MTX reduced SCF immunoreactivity density in the testis and ovary ($p<0.05$). Furthermore, MTX reduced mTOR, a marker of autophagy, in the testis ($p<0.05$) and ovary ($p<0.001$) compared with the control. SIRT-1 intensity increased dramatically in the testis ($p<0.001$) and ovary ($p<0.01$) in the injured group, unlike the mTOR marker.

Conclusions: Our investigation revealed that the gonads incurred significant damage as a result of MTX. One vital option for reducing or eliminating this damage to the ovaries and testicles is the use of anti-oxidant-rich substances.

Keywords: Methotrexate, chemotherapy, testis, ovary

ÖZ

Amaç: Metotreksat (MTX) kanser olgularının önde gelen kemoterapötiklerinden biridir. Bu ilaç özellikle testis ve ovaryum üzerinde gonadotoksik bir etkiye sahiptir. Çalışmamızın amacı MTX kullanımına bağlı testis ve ovaryum dokusunda oluşabilecek olası hasarı histopatolojik ve immünohistokimyasal analizlerle araştırmaktır.

Yöntemler: Çalışmamız için 24 adet Wistar albino erkek ve dişi sıçanlar kullanıldı. Bu sıçanlar 4 farklı gruba ayrıldı. Bu gruplar; kontrol erkek, MTX erkek, kontrol dişi ve MTX dişi olarak isimlendirildi. MTX uygulanan erkek ve dişi grubuna 20 mg/kg MTX, tek doz ve intraperitoneal olarak uygulandı. Genel anestezi altında sakrifiye edilen sıçanların testis ve ovaryumları alınarak histopatolojik analizler için kullanıldı. Ayrıca her iki dokuda da kök hücre faktörü (SCF), rapamisin mekanistik hedefi (mTOR) ve SIRT-1 immünoaktivite yoğunluğu immünohistokimya ile değerlendirildi.

Bulgular: MTX grubunda testis seminifer tübülünde analizlenen Johnsen testis biyopsisi skoru kontrol grubuna kıyasla istatistiksel anlamda azalış gösterdi ($p<0.001$). Ovaryumda ise MTX tedavisi kontrol grubuna nazaran gözle görülür bir hasar meydana getirdi. Bu grupta foliküler dejenerasyon ($p<0.05$), damar konjesyonu ($p<0.01$) ve fibrozis ($p<0.001$) belirlendi. Hem testis hem de ovaryumda SCF immünoaktivite yoğunluğu MTX grubunda azalma gösterdi ($p<0.05$). Ayrıca otofaji ile ilişkili belirteçlerden mTOR kontrol grubuna nazaran MTX gruplarında testis ($p<0.05$) ve ovaryumda ($p<0.001$) anlamlı bir şekilde azaldı. SIRT-1 yoğunluğu ise mTOR belirtecinin aksine hasar grubunda testis ($p<0.001$) ve ovaryumda ($p<0.01$) anlamlı bir artış gösterdi.

Sonuçlar: Sonuç olarak, araştırmamızda MTX'in testis ve yumurtalık üzerindeki olası olumsuz etkilerini değerlendirmek adına histopatolojik ve immünohistokimyasal analizler gerçekleştirdik. Ve analizlerimiz MTX tedavisinin gonadlar üzerinde kayda değer bir hasar oluşturduğunu bize gösterdi. Testis ve yumurtalık üzerindeki bu hasarın azaltılması veya tamamen ortadan kaldırılması adına antioksidan içeriklerinin kullanımı oldukça önemli bir alternatif olacaktır.

Anahtar kelimeler: Metotreksat, kemoterapi, testis, ovaryum

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INTRODUCTION

Cancer is a growing global health concern, with an estimated 18.1 million new cases and 10 million cancer-related deaths worldwide in 2020^{1,2}. One of the most popular methods for treating tumors is the use of cancer-fighting drugs or chemotherapy. Many of these medications have negative effects and may permanently damage several tissues in the body and organs^{3,4}. Chemotherapy does not only target cancer cells but also affects healthy cells, causing gonadotoxicity, hepatotoxicity, cardiotoxicity, nephrotoxicity, neurotoxicity, and hematological system damage^{1,2}. Methotrexate (MTX), a key chemotherapeutic treatment for many kinds of cancer, is a folate inhibitor that swiftly attacks dividing cells. Numerous autoimmune conditions, including multiple sclerosis, scleroderma, rheumatoid arthritis, psoriasis, Crohn's disease, and systemic lupus erythematosus, are also dealt with effectively with MTX⁵. The pathophysiology of MTX-induced organ and tissue damage involves elevated levels of reactive oxygen species, oxidative stress, and inflammatory processes⁶. Testicular damage that could result in infertility is the most dangerous possible adverse effect of MTX⁷. MTX may alter the testicular microenvironment, reducing the number of spermatogonial stem cells essential for sperm production⁸. Prior studies have linked the use of MTX to damage the testicular seminiferous tubules, a fall in sperm count, sperm DNA damage, and decreased spermatogenesis⁹. Many chemotherapy medications, including MTX, which is highly effective in curing rapidly dividing neoplastic cells, have significant side effects on female gonads. Unfortunately, MTX has long-term effects on ovarian function. It causes amenorrhea and possibly menopause. The gonadotoxic effect of this drug is believed to cause infertility, especially in young patients, by reducing the number of ovarian follicles¹⁰. MTX, which is additionally frequently employed in ectopic and molar pregnancy treatments, boosts the amount of free oxygen radicals and pro-inflammatory compounds⁶. SCF is released by Sertoli cells in the testes, which stimulates germ cell growth. It stimulates oocyte development and is released by granulosa cells in the ovary. Testicular germ cells and mature oocytes in the ovary express high c-kit receptor expression¹¹. A well-known serine/threonine kinase protein, mechanistic target of rapamycin (mTOR) is specifically responsible for energy homeostasis, metabolism, protein synthesis, and cellular growth. Under specific circumstances, such as stress, low energy, or oxidative damage, mTOR is suppressed and autophagy is activated¹². SIRT-1 is a crucial autophagy regulator that promotes autophagy, particularly in stressful or energy-deficient situations. It maintains cell growth and autophagy in control of mTOR. In the case of

a lack of energy, SIRT-1 is active and initiates autophagy by deacetylating autophagy-initiating proteins, whereas mTOR is inhibited¹³. Although chemotherapy remains an effective treatment for various cancers, its adverse effects on the reproductive system are well documented. The link between SCF, mTOR, and SIRT-1 in the effects of MTX on the testes and ovaries has not received significant attention in the literature. The current research aimed to fill in information gaps regarding the influence of vital signaling pathways, such as SCF, mTOR, and SIRT-1, on the detrimental effects of MTX on reproductive organs. The present study aimed to investigate the histopathological and immunohistochemical effects of MTX toxicity on testes and ovary tissue in rats.

The Animals

Erciyes University Animal Experiments Local Ethics Committee Animal Experiments Local Ethics Committee approved our experimental guidelines (decision no: 24/057, date: 06.03.2024). Wistar albino adult male and female rats (n=24), aged 8-10 weeks, weighed 150-250 g, and were housed in standard accommodation under stress-free conditions (21°C; 12-12 cycles of light and dark). Animal experiments were performed according to the Animal Research: Reporting *In Vivo* Experiments guidelines¹⁴.

Study Design and Experimental Groups

The Wistar rats were randomly assigned to 4 groups: Control male (n=6), MTX male (MTX Male; n=6), control female (n=6), and MTX female (MTX Female; n=6). In the experiment, male and female Wistar rats not exposed to MTX were used as the control group. On the first day of the experiment, a single intraperitoneal dose of 20 mg/kg MTX (Koçak Farma, Türkiye) was administered to male and female rats receiving the treatment¹⁵. One day following the initial treatment, all animals were dissected under general anesthesia with xylazine (10 mg/kg body weight intraperitoneally) and ketamine (60 mg ketamine hydrochloride/kg body weight intraperitoneally). The testis and ovary tissue were taken for histopathologic and immunohistochemistry analyses.

Histopathological Evaluation

At the end of the experiment, tissue samples were collected and fixed in 10% formaldehyde. The testes and ovary tissue were then rinsed under tap water and placed through a series of increasing grades of alcohol. Blocks were formed by embedding them in paraffin after clearing with xylol. Hematoxylin&Eosin (H&E) and Masson's trichrome (MT) were used to stain 5-µm sections, which were then passed through an increasing alcohol series,

xylol, and a coverslip before being evaluated under a light microscope [Olympus BX51 (Olympus Corp., Tokyo, Japan)]. Testicular tissue stained with hematoxylin and eosin was analyzed and evaluated using standard light microscopy, scoring it according to Johnsen's criteria¹⁶. Five locations within each of the 10 seminiferous tubules in each section were sampled to calculate Johnsen's testicular biopsy score (JBTS). For each tubule, JBTS was estimated based on the total number of cells and maturation (Table 1). In addition, ovarian tissue damage was evaluated in terms of follicular degeneration, vascular congestion¹⁷, and fibrosis¹⁸. The assessment was graded semi-quantitatively on a scale of 0 to 3 (0: None, 1: Mild, 2: Moderate, 3: Severe) for each criterion.

Immunohistochemistry

SCF, mTOR, and SIRT-1 expression in testis and ovary tissue was demonstrated using immunohistochemistry. Immunohistochemical staining was performed using the avidin-biotin peroxidase assay. 5 µm sections from paraffin blocks were prepared on polylysine slides for staining. Sections were deparaffinized in xylol, maintained in each descending alcohol, and finally stored in distilled water to dehydrate them. Sections were boiled in a microwave oven at 600 W with 5% citrate buffer for antigen retrieval, washed with phosphate-buffered saline (PBS), and treated with 3% H₂O₂ to prevent endogenous peroxidase activity. The immunohistochemistry staining kit (Lab Vision™ UltraVision™ Large Volume Detection System: Anti-polyvalent, HRP, TA-125-HL) was used in the following steps, and the entire procedure was performed in a chamber that prevented the tissues from drying out. Block serum was applied to PBS-washed sections for 10 min at room temperature to ensure that the regions outside the anti-genic areas were covered. Subsequently, sections of mTOR (Cell Signaling Technology 7C10-1:100), SIRT-1 (NBPI 51641- 1:500), and SCF (Santa Cruz Biotechnology-1:450) primary anti-bodies were incubated at 4°C overnight. The sections were then

incubated with biotinylated secondary anti-bodies. After washing PBS, streptavidin-peroxidase complex was used. In the next step diaminobenzidine (Diaminobenzidine chromogen and substrate system, Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used to highlight the immunoreactivity. To enhance nuclear staining, Gill's hematoxylin was used as a counterstain on the sections. Under a light microscope, ovarian sections stained with immunohistochemistry were analyzed, and microscopic images of 10 randomly chosen areas were obtained. The immunoreactivity intensities of the markers identified in the photographs were assessed using ImageJ software. The ImageJ software's color threshold plugin was used to assess all immunoreactivity^{19,20}.

Statistical Analysis

Version 9 of Graph Pad Prism was used for all statistical analyses. The Shapiro-Wilk test was used to determine data distribution. For comparisons involving more than two groups, one-way analysis of variance (ANOVA) and the Kruskal-Wallis test were used. The Bonferroni test for one-way ANOVA and the Dunn test for the Kruskal-Wallis analysis both revealed significant post hoc comparisons of the variables. A p-value of less than 0.05 was considered statistically significant for all data.

RESULTS

Histopathological Results of the Testicular and Ovarian Tissues

The histomorphological evaluation of the testicular tissues of the control group revealed a normal structure in the cells of the spermatogenic series located on the basement membrane and seminiferous tubule outline structures. The number of spermatogenic series cells in the seminiferous tubules decreased and tubular vacuolization increased in the testicular tissues of the rats in the experimental group that administered MTX. Furthermore, the asymmetric seminiferous tubule

Table 1. Johnsen's scoring system was used for qualitative assessments of fertility and spermatogenesis.

Scores	Histological findings	Score	Histological findings
1	There are no visible germs or Sertoli cells. Atrophic tubules have been identified.	6	A few round spermatids were observed.
2	Only Sertoli cells are present; germ cells are absent.	7	Despite the absence of sperm, many rounds of spermatids were observed.
3	There are no primary spermatocytes. Germ cells were just as spermatogonium primary spermatocytes.	8	The sperm count is very low.
4	A few primordial spermatocytes were identified.	9	Although there are many sperm, they are not round, and the lumen lacks a regular form.
5	There are no and round spermatids. Too many primary spermatocytes were observed.	10	There are many sperm with consistently rounded edges in the lumen, indicating full spermatogenesis.

morphology and tubular architecture resulting in necrosis were noted. Additionally, each time we examined the testicular tissue stained with H&E-evaluated JBTS, we found that the MTX group had a considerably lower score than the control group ($p<0.001$). The interstitial connective tissue structure in the testicular tissues of the control group was within healthy limits after MT staining was used to identify possible variations in connective tissue. In the MTX-treated group, the interstitial connective tissue area of the testicular tissues did not change in this respect (Figure 1) (Table 2A). We examined the ovarian tissues of both control and MTX-treated mice under a light microscope. Various stages of follicle development were observed in the cortical layer of the ovary in the control group. The surrounding granulosa appeared healthy, and the oocytes were located near the center. There were many blood arteries and loose connective tissue in the medulla. Normal vascular structures were observed. The group that received MTX treatment had

unfavorable ovarian tissue. In this group, degenerative follicular structures were identified ($p<0.05$) (Figure 2 and Figure 3A). Furthermore, the vascular congestion structures were appealing ($p<0.01$) (Figure 2 and Figure 3B). Analyses of connective tissue formation in ovarian tissues were performed by examining sections stained with MT stain. There was an apparent increase in collagen in the medulla layer in the MTX group ($p<0.001$) (Figure 2 and Figure 3C) (Table 2B).

SCF Immunoreactivity of Testicular and Ovarian Tissue

When testicular tissue was examined for SCF immunoreactivity intensity, a high reactivity was found in the spermatogonium of the spermatogenic series cells of the seminiferous tubule in the control group. SCF immunoreactivity in the testicular tissues of the MTX-treated group dropped statistically substantially ($p<0.05$). We examined the intensity of SCF immunoreactivity in

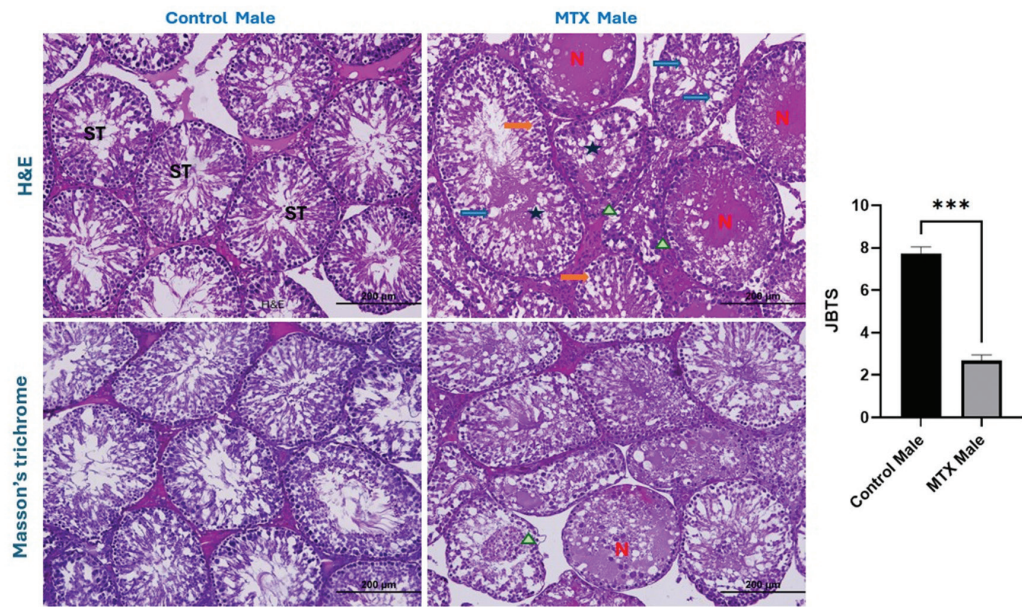


Figure 1. Light microscopic findings and JBTS of rat testis tissue. Control male group; ST: Seminiferous tubules. MTX Male group; N: Necrotic seminiferous tubules; orange arrow: descending spermatogenic lineage cells; blue arrow: tubular vacuolization; green triangle: Asymmetrical seminiferous tubule morphology. H&E: hematoxylin-eosin and MT staining (Olympus BX51, Tokyo, Japan. X20). JBTS graph of the experimental groups. Data are presented as mean ± standard deviation or median (min-max). *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$

JBTS: Johnsen's testicular biopsy score, MTX: Methotrexate

Table 2A. Testis JBTS statistics.			
Testis histoscore	Control male	MTX male	p-value
JBTS	8,000 (4,000-10.00)	3,000 (1,000-6,000)	<0,001
The Mann-Whitney U test was used to evaluate non-normal data distribution. Med. (min-max): The quartile value range is shown inside the lines of brackets, whereas the median value is outside.			
JBTS; Johnsen's testicular biopsy score, Med: Median, MTX: Methotrexate			

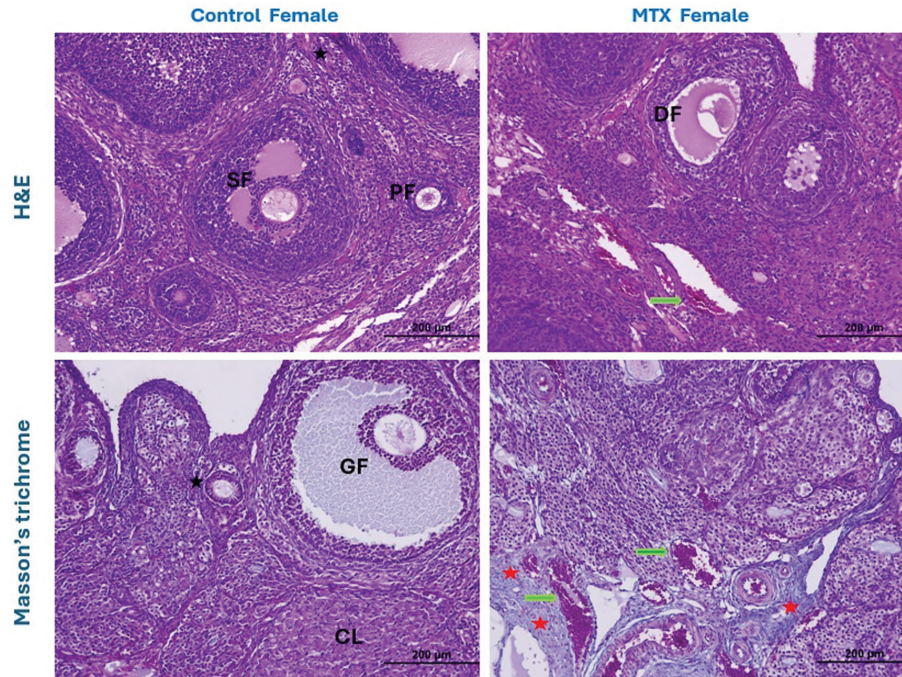


Figure 2. Light microscopic findings of the rat ovary tissue. Control Female group: Several developmental phases of folliculi are observed. Primordial follicles (*), primary follicles (PF), secondary follicles (SF), and Corpora lutea (CL) with large, weakly pigmented acidophilic cells, mature Graafian follicles (GF). MTX Female group: Degenerative follicles (DF). Green arrow, vascular congestion; red star, fibrosis. H&E: hematoxylin-eosin and Masson's trichrome staining (Olympus BX51, Tokyo, Japan. X20).

MTX: Methotrexate, H&E: Hematoxylin&Eosin.

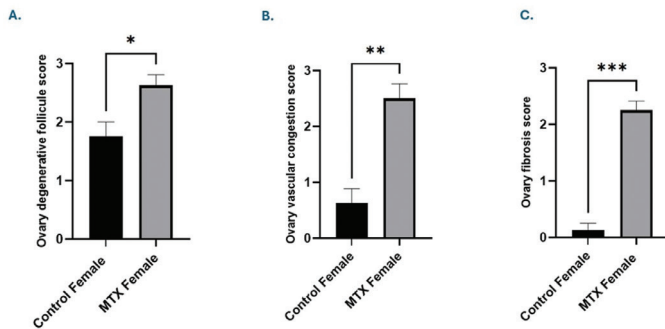


Figure 3. Histopathological findings of rat ovary tissue. Graph exhibiting degenerative follicles, vascular congestion, and fibrosis in the experimental groups. Data are presented as mean±standard deviation or median (min-max). *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

MTX: Methotrexate, Min-max: Minimum-maximum.

ovary tissue and found high expression levels in follicles at various stages of development, the corpus luteum, and in ovary stromal cells. In the ovarian tissue of the MTX-treated group, SCF expression was extremely low. Comparing this group with the control group, there was a statistically significant decrease in immunoreactivity ($p<0.05$) (Figure 4 and Table 3).

mTOR Immunoreactivity of Testicular and Ovarian Tissue

mTOR is expressed mostly in seminiferous tubule cells, interstitial connective tissue, and vessel walls in this region. Conversely, the group that received MTX treatment in testes showed diminished immunoreactivity intensity in the same area ($p<0.05$). When mTOR expression was measured in ovarian tissue, positivity was high in the corpus luteum and stroma of the control group. The granulosa layer of the follicles exhibited very little reactivity. In the MTX treatment group, mTOR immunoreactivity was considerably lower than that in the control group ($p<0.001$) (Figure 5 and Table 3).

SIRT-1 Immunoreactivity of Testicular and Ovarian Tissue

The SIRT-1 immunoreactivity intensity of testicular and ovarian tissue was also examined. The primary spermatocytes in the seminiferous tubules exhibit a very poor reaction in testicular tissue of the control group. All spermatogenic series cells, including primary spermatocytes, showed a significant increase in immunoreactivity intensity in response to MTX injury in the testicular tissue of the MTX-treated group, except

Table 2B. Ovary histoscore statistics.			
Ovary histoscore	Control female	MTX female	p-value
Degenerative follicle	2,000(1,000-3,000)	3,000(2,000-3,000)	0.039
Vascular congestion	0,5000(0.000-2,000)	3,000(1,000-3,000)	0.002
Fibrosis	0,000(0.000-1,000)	2,000(2,000-3,000)	<0.001
The Mann-Whitney U test was used to evaluate non-normal data distribution Med.(min-max): The quartile value range is shown inside the lines of brackets, whereas the median value is outside.			
Med: Median, Min-max: Minimum-maximum, MTX: Methotrexate.			

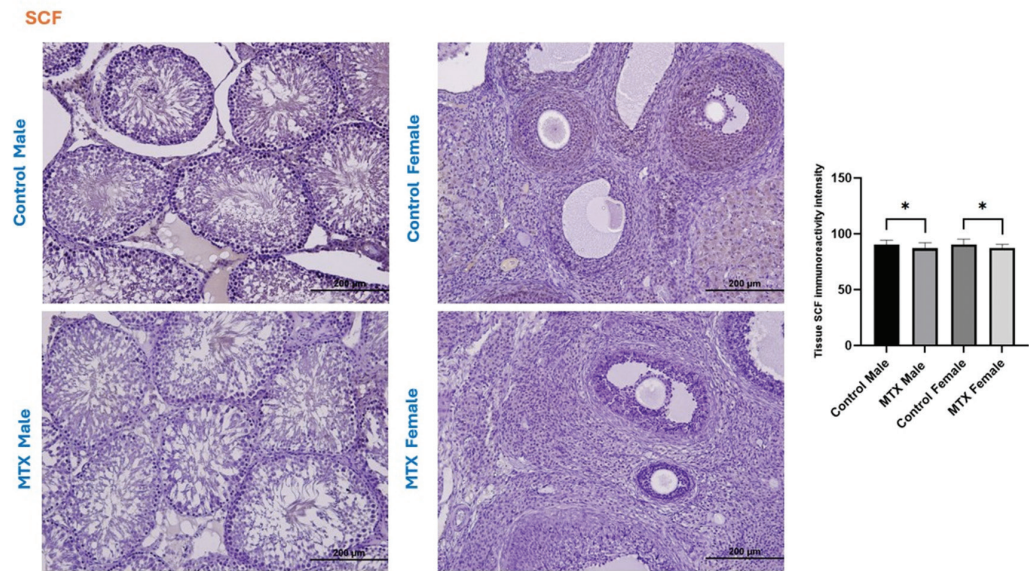


Figure 4. Immunohistochemical microscopic findings and graphs of SCF markers in testicular and ovarian tissues from the experimental groups. The brown areas indicate immunostaining. The slides were counterstained with hematoxylin. Data are presented as mean±standard deviation or median (min-max). *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. (Olympus BX51, Tokyo, Japan. X20).

SCF: Stem cell factor, Min-max: Minimum-maximum, MTX: Methotrexate.

for spermatogonium ($p<0.001$). In the granulosa cells of the follicles in the ovarian tissue of the control group, SIRT-1 was negative. The granulosa cells of the follicles, corpus luteum, and ovarian stroma, however, showed exceptionally high immunoreactivity intensity of this marker in the MTX group ($p<0.01$) (Figure 6 and Table 3).

DISCUSSION

MTX is an anti-metabolite with an extensive list of uses is MTX, which is used to treat autoimmune disorders and cancer. It is frequently selected to treat a wide range of conditions, especially because of the features that affect cell division and DNA synthesis. However, this effective medication may target the reproductive system. MTX's harmful effects of MTX on testicular and ovarian tissues can result in serious medical challenges because these tissues are composed of rapidly dividing cells⁵. Oxidative stress is a known adverse effect of MTX.

Its activation in the testes can lead to a rise in free radical generation, impairing spermatogenesis and ultimately resulting in infertility⁷. According to Felemban et al.²¹ MTX led to structural disruption and an atypical layout of the spermatogenesis cycle, resulting in the shedding of germ cells into the tubular lumen, severe degeneration in most seminiferous tubules, and significant decreases in Leydig cells and sperm. In this study, we found that MTX administration led to an erosion in the seminiferous tubule morphology and a decline in the spermatogenic series in the testicular tissue seminiferous tubules. Consistent with our findings, another MTX study that assessed testicular injury observed a substantial reduction in the JBTS score of the MTX-treated group compared with the control group²². In addition to all the previous studies, our findings suggest that the substantially decreased JBTS scores in the MTX-treated group indicate severe damage to the seminiferous tubules and serious degradation of the spermatogenesis process. The reduced number of

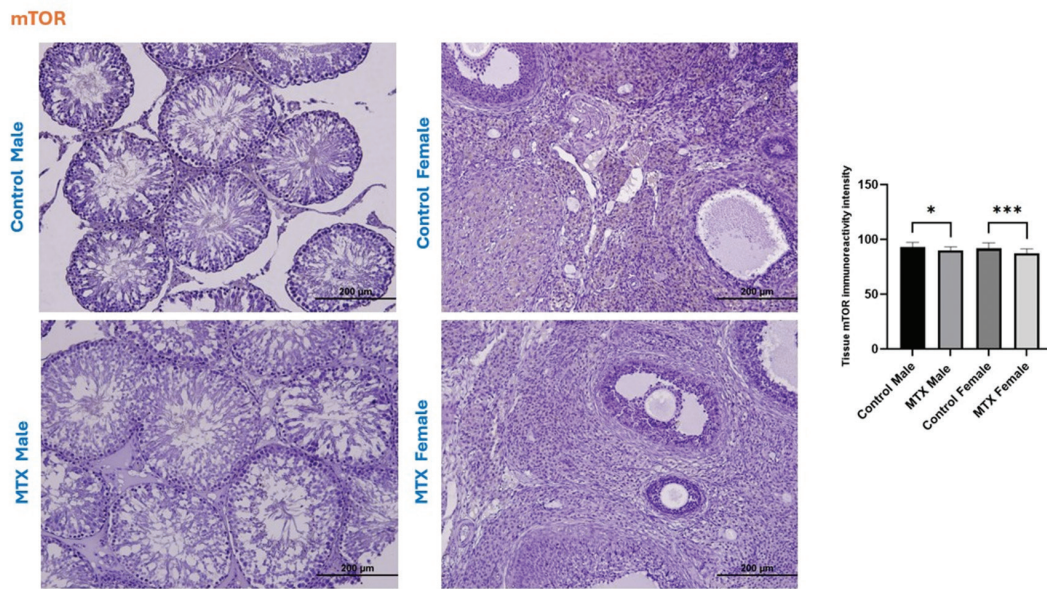


Figure 5. Immunohistochemical microscopic findings and graphs of mTOR markers in testicular and ovarian tissues from the experimental groups. The brown areas indicate immunostaining. The slides were counterstained with hematoxylin. Data are presented as mean \pm standard deviation or median (min-max). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. (Olympus BX51, Tokyo, Japan. X20).

MTX: Methotrexate, mTOR: Mechanistic target of rapamycin, Min-max: Minimum-maximum.

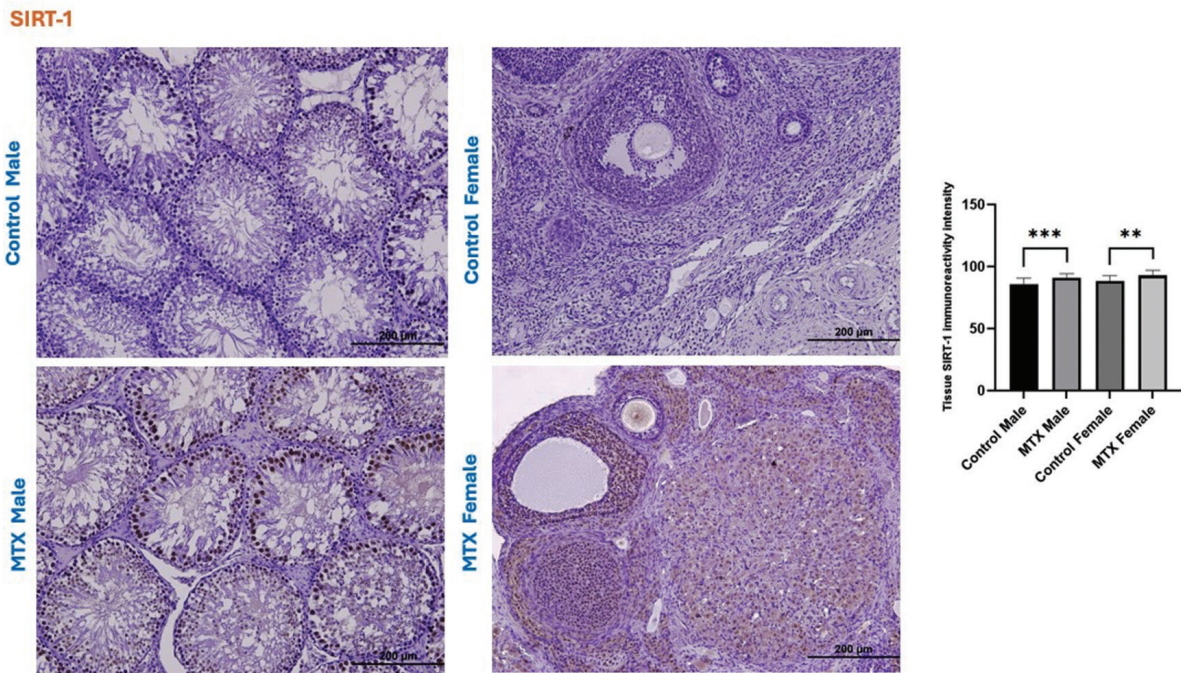


Figure 6. Immunohistochemical microscopic findings and graphs of SIRT-1 markers in testicular and ovarian tissues from the experimental groups. The brown areas indicate immunostaining. The slides were counterstained with hematoxylin. Data are presented as mean \pm standard deviation or median (min-max). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. (Olympus BX51, Tokyo, Japan. X20).

Min-max: Minimum-maximum, MTX: Methotrexate.

Table 3. Statistics of immunohistochemistry findings.					
IHC marker	Control male	MTX male	Control female	MTX female	p-value
SCF	91.06 (82.19-96.86)	88.34 (73.45-96.71)	90.23 (79.29-98.59)	88.06 (80.49-92.31)	<0.001
mTOR	93.09±4.065	89.93±3.293	91.83± 5.036	87.45±4.036	<0.001
SIRT-1	87.44(70.41-93.35)	90.84 (84.79-98.17)	87.82 (81.51-97.81)	93.29 (81.51-99.59)	<0.001
IHC: Immunohistochemistry, SCF: Stem cell factor, mTOR: Mechanistic target of rapamycin, MTX: Methotrexate.					

spermatogonial stem cells and boosted vacuolization support the gonadotoxic effects of MTX in testicular tissue. Ovarian toxicity associated with MTX includes a reduction in the number of antral follicles, a decrease in anti-Müllerian hormone (AMH) levels, and ultimately a decrease in ovarian reserve²³. Kiremitli et al.²⁴ analyzed ovarian tissue following mtx-induced oxidative stress and revealed that the number of degenerative follicles and vascular congestion increased in the MTX group. In another study, MTX therapy in the ovary resulted in a substantial increase in fibrosis compared with the control group. This investigation confirmed our findings regarding MTX dosage and fibrosis²⁵. Increased follicular degeneration indicates that MTX causes significant stress and oxidative damage to cells that support oocyte formation. Increased vascular congestion and fibrosis imply that this process involves inflammatory components. These findings imply that MTX has a detrimental effect on ovarian reserve and can increase the risk of infertility in women. SCF, or Kit ligand, is the c-kit ligand for the tyrosine protein kinase receptor. SCF is mostly found in Sertoli cells in testes and is expressed exclusively in germ cells during development²⁶. In the fully developed ovary, c-kit is mostly expressed in oocytes, whereas granulosa cells release SCF, a well-known oocyte development booster²⁷. In a recent study, it was discovered that the amount of SCF protein decreased considerably in the MTX group compared with the control group²⁸. In the latest ovarian study, SCF immunoreactivity intensity was lower in the damaged group than in the control group²⁹. This research concluded that SCF immunoreactivity decreased in both testicular and ovarian tissue in the MTX treatment groups, which confirms previous findings. Sperm and oocyte damage results from MTX-induced inhibition of the folate pathway, which also affects DNA synthesis and cell proliferation. Stem cell factor (SCF) is essential for both the regeneration of damaged tissue and the defense of germ cells against such damage. If SCF production is elevated during MTX-induced cellular damage, germ cell regeneration can be enhanced. Autophagy is a well-known self-cannibalization system that adapts to changes in intracellular and extracellular environments during biosynthesis. On the other hand, unusual autophagy can be produced by specific atypical

stimuli and stresses, leading to damage³⁰. SIRT-1 is a type of sirtuin found in mammals. Sirtuins are nicotinamide adenine dinucleotide-dependent deacetylase that regulate cellular metabolism, proliferation, and genome stability. It affects various cellular processes, including cancer, aging, metabolic regulation, and differentiation. Additionally, it regulates autophagy by initiating the process³¹. The mTOR kinase is a well-known Ser/Thr protein kinase with a molecular weight of 290 kDa that regulates the cell energy status and metabolism³². mTOR is a crucial regulator of autophagy. SIRT-1 deficiency leads to enhanced mTOR signaling³³. SIRT-1 expression was investigated in a hamster study in which bisphenol caused testicular injury, and it was discovered that this marker was diminished in the damaged group³⁴. In another testicular study, contrary to our findings, SIRT-1 expression in radiation-induced testis decreased significantly³⁵. The results of these studies were reflected by a decrease in the level of this indicator in contrast with a increase in SIRT-1 immunoreactivity intensity in our MTX-treated testicular tissue. In an experiment where mTOR expression was assessed, it emerged that as the formaldehyde dose climbed in testicular tissue, mTOR immunopositivity was lessened compared with that in the control groups³⁶. In a rat study in which lead provoked testicular toxicity, mTOR levels were lower in the lead group than in the control group, which is consistent with our results. Despite the toxicity of lead, the anti-oxidant component used in the study increased the level of this protein to levels approximately comparable to those of the control group³⁷. Several studies have revealed that SIRT contributes to ovarian aging³⁸⁻⁴⁰. Transgenic mice with ovarian SIRT1 overexpression had lower mTOR levels and a higher ovarian survival rate⁴¹. In an ovarian examination, increasing amounts of Ti₃C₂ nanosheets progressively aggravated ovarian tissue damage, resulting in a steady decline in mTOR activity⁴². Onder et al.²⁹ analyzed the SIRT/mTOR pathway in non-ylphenol-induced ovarian damage. This study determined that SIRT immunoreactivity intensity increased while mTOR immunoreactivity intensity decreased in the NP-exposed group compared with the control group, which is in line with our results. All studies and our data indicate that MTX induces stress reactions in cells, which triggers

the autophagy system. Testicular and ovarian cells activate autophagy in response to MTX therapy. In this mechanism, mTOR inhibition and SIRT-1 activation disrupt spermatogenesis and oocyte development, resulting in germ cell loss and impaired reproductive function. This study confirmed that MTX has gonadotoxic effects on both the testes and ovaries. Impaired spermatogenesis in males and follicular degeneration in females suggest that MTX triggers infertility in young patients. Autophagy-regulating therapies should be investigated to minimize MTX-induced cellular stress and damage. Therapeutic techniques that specifically modify SIRT-1 may be beneficial for reproductive organ maintenance.

CONCLUSION

As a result, in our research, we evaluated the effects of MTX on the testicles and ovaries and performed histopathological and immunohistochemical analyses. This study demonstrated that MTX at a given dose caused significant damage to both testicular and ovarian tissues. In addition to histological evidence in the gonads, a decrease in SCF in the MTX treatment group, as well as immunoreactivity of SIRT-1 and mTOR expressions, which are autophagic pathways that develop by promoting another process, indicate damage. Unfortunately, the negative effects of chemotherapy on the reproductive system are visible. To minimize testicular and ovarian damage, a variety of anti-oxidant components are used. Our findings suggest that anti-oxidant therapies can be employed to lessen and prevent MTX's detrimental impact on the reproductive system. Potent anti-oxidants may be valuable to developing approaches that protect reproductive health, and they will probably be utilized in research examining their potential for reducing oxidative damage and cellular stress caused by MTX. Additionally, these anti-oxidants, along with SCF, may assist in managing autophagy processes through the SIRT-1 and mTOR signaling pathways.

Ethics

Ethics Committee Approval: Erciyes University Animal Experiments Local Ethics Committee Animal Experiments Local Ethics Committee approved our experimental guidelines (decision no: 24/057, date: 06.03.2024).

Informed Consent: Since this study was conducted on animals, patient consent was not required.

Footnotes

Author Contributions

Surgical and Medical Practices: B.Y., Ö.C.M., Concept: K.T.K., A.H.Y., Design: K.T.K., A.H.Y., Data Collection and/

or Processing: B.Y., Ö.C.M., Analysis and/or Interpretation: K.T.K., A.H.Y., Literature Search: K.T.K., Writing: K.T.K.

Conflict of Interest: The authors have no conflict of interest to declare.

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Three-Year Assault Cases Performed on the Emergency Trauma Center

Acil Travma Merkezine Başvuran Üç Yıllık Darp Olgularının Analizi

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ABSTRACT

Objective: This study aims to compare the results of cases with a history of assault presented to our emergency department over a period of three years with literature data and to share our experiences with colleagues.

Methods: Demographic characteristics, trauma findings, clinical conditions, surgical interventions, mortality, and outcomes of patients with a history of assault to the emergency department between 2020 and 2022 were retrospectively examined. The results were analyzed using the Number Cruncher Statistical System 2007 Statistical Software package program (Utah, USA).

Results: A total of 2900 patients, including 1850 males (63.79%) and 1050 females (36.21%), with an average age of 35.53±12.46 years, were included in the study. Most admissions (31.28%) occurred during the summer months and outside working hours. Males inflicted all injuries. The most common injuries occurred in the head-neck region (63.31%) and upper extremities (47.97%) in both sexes. Facial injuries were significantly more common in males ($p<0.05$), whereas spinal and pelvic injuries were common in females ($p<0.05$). Tenderness, edema, hematoma, abrasion, ecchymosis, and soft tissue laceration were the most frequently observed findings. Female patients were more likely to be discharged home ($p=0.0001$), whereas male patients had a higher hospitalization rate ($p=0.0001$). Life-threatening ailments were discovered in 23 patients (0.79%). Surgical intervention was performed in 50 patients (1.72%). No patient experienced fatal outcomes.

Conclusions: All assaults were committed by males. Males are more frequently and severely assaulted than females. The victims were mainly from young people, and assaults predominantly occurred during the summer months and outside working hours. Head-neck and extremity injuries were the most common. There were no fatal outcomes. Nine of the 10 patients were discharged to home from the emergency department.

Keywords: Assault, emergency, sex, education, age

ÖZ

Amaç: Bu çalışmanın amacı acil servisimize üç yıllık süre içinde başvuran darp öyküsü olan olguların sonuçlarını literatür verileri ile karşılaştırmak ve deneyimlerimizi meslektaşlarımızla paylaşmaktır.

Yöntemler: 2020-2022 yılları arasında 3. basamak bir hastane acil servisine saldırı öyküsü ile başvuran olguların demografik özellikleri, travma bulguları, klinik durumları, cerrahi müdahaleleri, mortaliteleri ve hasta sonuçları retrospektif olarak incelendi. Sonuçlar Number Cruncher Statistical System 2007 Statistical Software paket programı (Utah, ABD) kullanılarak analiz edilmiştir.

Bulgular: Yaş ortalaması 35,53±12,46 yıl olan 1850 erkek (%63,79) ve 1050 kadın (%36,21) olmak üzere toplam 2900 hasta çalışmaya dahil edildi. Başvuruların çoğunluğu (%31,28) yaz aylarında ve çalışma saatleri dışında gerçekleşmiştir. Tüm yaralanmalar erkekler tarafından gerçekleştirilmiştir. Her iki cinsiyette de en sık yaralanmalar baş-boyun bölgesinde (%63,31) ve üst ekstremitelerde (%47,97) meydana gelmiştir. Yüz yaralanmaları erkek hastalarda istatistiksel olarak daha sık görülürken ($p<0,05$), omurga ve pelvis yaralanmaları kadın hastalarda daha sıkı ($p<0,05$). Hassasiyet, ödem, hematoma, abrazyon, ekimoz ve yumuşak doku laserasyonu en sık gözlenen bulguları. Kadın hastaların eve taburcu edilme olasılığı daha yüksekken ($p=0,0001$), erkek hastaların hastaneye yatış oranı daha yüksekti ($p=0,0001$). Hayati tehlike 23 hastada (%0,79) tespit edildi ve 50 hastaya (%1,72) cerrahi müdahale uygulandı. Hiçbir hastada ölümcül sonuç görülmedi.

Sonuçlar: Çalışmamız, tüm saldırı olgularının erkekler tarafından gerçekleştirildiğini ve erkeklerin kadınlara kıyasla daha sık ve ağır saldırıya uğradığını ortaya koymuştur. Mağdurlar çoğunlukla genç yaş grubundandır ve saldırılar ağırlıklı olarak yaz aylarında ve mesai saatleri dışında gerçekleşmiştir. Saldırılarda en sık baş-boyun ve ekstremiteler yaralanmaları görülmüş, ölümler sonuçlanan olgu olmamış ve on hastadan dokuzu acil servisten eve taburcu edilmiştir.

Anahtar kelimeler: Saldırı, acil durum, cinsiyet, eğitim, yaş

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INTRODUCTION

The World Health Organization (WHO) defines violence as "events that involve the intentional use of physical force or power, threatened or actual, against oneself, another person, or a group or community, resulting in injury, death, psychological harm, poor development, or deprivation or with a high likelihood of occurrence". "Assault" is a form of interpersonal violence and ranks as the fourth leading cause of injury-related deaths¹. There was an observed increase in emergency department admissions due to intentional inflicted injuries related to domestic violence, friend violence, and, notably, violence against women. These events not only disrupt victims' sense of security and quality of life but also lead to psychological and emotional consequences for family members, friends, and the community at large^{2,3}. Materials such as fists, kicks, stones, weapons, and sticks are commonly used as attack tools to harm other objects⁴. Recognizing the significance of injuries in this context is crucial for evidence-based approaches to prevent death and disability and mitigate harm. These cases are assessed as "medico-legal cases" in emergency department admissions.

Globally, the mortality rate of assault cases varies, with males having double the mortality rate of females. One in three women aged above 15 years is reported to have been assaulted by an intimate partner⁵. More than 90% of interpersonal violence cases occur in low-and middle-income countries⁶. Assault cases tend to present more frequently to emergency departments during the summer months and outside working hours⁷. The head-neck regions are most commonly attacked, with bruises, swelling, fractures, contusions, soft tissue lacerations, and bleeding being the most prevalent findings⁸. The severity of an incident may not be directly proportional to the extent of the lesions⁹. Applying the advanced trauma life support algorithm is essential in patient management. Global reactions to such cases have been increasing in recent years, and these actions are raising awareness.

Emergency physicians play a significant role in the clinical evaluation and management of patients with a history of assault. The pattern of presentation of assaulted patients to the emergency department, their physical and psychological conditions, injury localisations and epidemiological characteristics of the victims help to understand and manage such incidents more accurately. The level of education and awareness of healthcare professionals also play a vital role in managing such cases¹⁰. It is of utmost importance for healthcare professionals, especially emergency physicians, who first encounter this patient group clearly and transparently

document injuries and treatment management, which is crucial to ensure that the rights of the parties involved are not violated¹¹. In this study, a retrospective evaluation was performed on the demographic characteristics, presentation patterns, trauma localizations, clinical conditions, treatments, and out-comes of patients with a history of assault admitted to the emergency department of tertiary hospital with a history of assault between 2020-2022. This study aimed to share our clinical experiences with emergency physicians and to raise awareness of assault both clinically and socially.

METHOD

Study and Location

Cases: Presented to the emergency department of tertiary hospital with a "history of assault" were retrospectively examined between January 01, 2020 and December 31, 2022. This study was approved by the H.S. Istanbul Medeniyet University Göztepe Training and Research Hospital Clinical Research Ethics Committee (decision no :2023/0219, date: 29.03.2023). The study was conducted in accordance with the ethical standards of the 1964 Helsinki Declaration (Appendix 3 of the Helsinki Declaration) and its subsequent amendments. The location of the survey, emergency department of tertiary hospital in Istanbul, is among the neighborhoods with the highest educational levels in the metropolitan city.

Data collection: Between the specified dates, cases with ICD codes W50 (assault by another person, striking, stomping, bending, biting) and W51 (assault by another person) were screened. Clinical and forensic case information found in the digital files of the cases were accepted as data. The data were recorded in an Excel file under main headings. After data collection, the data were transferred to an NCSS file for analysis.

Parameters screened: The data recorded for analysis in this study are as follows:

1. Demographic data of patients: age, sex, date, and time of admission
2. Glasgow Coma scale at the time of admission
3. Life-threatening situation
4. Localization of assault (including sub-regions in the head such as nasal, maxilla, mandible, zygoma, frontal, orbital, temporal, parietal, occipital, neck, and spinal injuries, thoracic injuries, abdominopelvic injuries, extremity injuries)
5. Physical examination findings related to the assault (tenderness, edema, hematoma, abrasion, ecchymosis, presence of cuts, fractures, lacerations, etc.)

6. Imaging results (X-ray, computed tomography)
7. Consultations requested.
8. Treatments performed (surgery, dressing, incision suturing, bone reduction, and splint application, prescription only, no treatment)
9. Outcomes (discharged home, outpatient follow-up, hospitalization, exists, treatment refusal, and unauthorized abandonment).

Inclusion criteria:

1. Patients aged 18 years who presented between January 01, 2020 and December 31, 2022
2. Patients diagnosed with a diagnosis entered digital files with codes W50 and W51.

Exclusion criteria:

1. Patients who were admitted to the emergency department with the request of "being assaulted" but were later identified as "victims of verbal violence only" during the investigation, and cases in which an entry-exit report under police supervision was requested due to an unspecified crime were excluded from the study.

2. During the three-year data screening phase, 3515 patient files were accessed. Of these, 2900 patients who satisfied the study criteria were recorded.

Statistical Analysis

In this study, statistical analyses were performed using the NCSS (Number Cruncher Statistical System) 2007 Statistical Software package program (Utah, USA). Descriptive statistical methods (mean, standard deviation) were employed to evaluate the data. Additionally, the Shapiro-Wilk normality test was used to assess the distribution of variables. For variables with a normal distribution, one-way analysis of variance was used for intergroup comparisons, Tukey's multiple comparison test for subgroup comparisons, and the chi-square test for comparisons of qualitative data. The results were evaluated at a significance level of $p < 0.05$.

RESULTS

Among the patients included in our study, 1850 (63.79%) were male and 1050 (36.21%) were female. The mean age of all patients was determined to be 35.53 ± 12.46 (minimum 18, maximum 92) years. The distribution of patients who presented in 2020, 2021, and 2023 was 29.00 (n=841), 33.62 (n=975), and 37.38 (n=1084), respectively.

The highest number of presentations occurred during the summer months (June, July, and August), at 31.28%. More than half of the patients (54.14%) were admitted to the emergency department pm 03.00.

When the region and localization of the trauma were examined, the head (63.31%) and upper extremities (47.97%) were most commonly injured, respectively. The maxilla (16.79%) within the head region showed the highest injury rate. The recorded patient findings included tenderness, edema, hematoma, abrasion, ecchymosis, and soft tissue laceration. Tenderness was the most frequently observed sign in the trauma region (62.45%). Among the 2900 patients included in the study, 23 (0.79%) were considered life-threatening, and 50 (1.72%) were hospitalized in surgical clinics (Table 1). Patients were predominantly consulted in the plastic surgery (8.31%) and orthopedic (7.48%) departments (Table 1).

Nasal ($p=0.0001$), mandibular ($p=0.045$), frontal ($p=0.0001$), orbital ($p=0.0001$), and temporal ($p=0.025$) trauma occurred at higher rates in male patients than in female patients. The incidence of spinal ($p=0.006$), pelvic ($p=0.0001$), lower extremity ($p=0.0001$), and upper extremity ($p=0.0001$) trauma was higher in female patients (Table 2). Surgical hospitalization and out-patient follow-up were higher in male patients, whereas the discharge rate in female patients was higher ($p=0.0001$) (Table 2). Tenderness ($p=0.001$) and ecchymosis ($p=0.0001$) were more prevalent in female patients, whereas laceration ($p=0.0001$), edema ($p=0.0001$), hematoma ($p=0.007$), and abrasion ($p=0.002$) were more prevalent in male patients (Table 2). Discharge rates were higher among patients arriving during the day ($p=0.0001$), whereas hospitalizations to surgical departments were higher in patients arriving more toward the evening and night ($p=0.02$). The mean age ratio was lower among patients arriving outside working hours ($p=0.0001$) (Table 3).

Patients who refused treatment and left the emergency department had a higher incidence of nasal ($p=0.0001$) and cervical ($p=0.011$) injuries. Patients with lower extremity and orbital injuries were more likely to be hospitalized and were treated ($p=0.0001$) (Table 4).

DISCUSSION

In Türkiye, the initial assessment of assault cases and the preparation of forensic reports are performed by emergency medicine physicians. Injuries can sometimes lead to dire consequences, including death. To reduce incidents of assault and their associated damages, the

Table 1. Distribution according to trauma location, symptoms, outcome, and ordered consultations.		
	n	%
Trauma localization		
Nasal	423	14.59
Maxilla	487	16.79
Mandible	242	8.34
Zygoma	217	7.48
Frontal	476	16.41
Orbit	417	14.38
Temporal	303	10.45
Parietal	279	9.62
Occipital	259	8.93
Thoracolumbar vertebrae	152	5.24
Cervical vertebrae	538	18.55
Thoracic	505	17.41
Abdomen	142	4.90
Pelvis	123	4.24
Upper extremity	1391	47.97
Lower extremity	632	21.79
Symptoms		
Vital danger	23	0.79
Sensitivity	1811	62.45
Edema	662	22.83
Hematoma	147	5.07
Abrasion	1519	52.38
Ecchymosis	1093	37.69
Incision	590	20.34
Outcome		
Outpatient discharge	2625	90.52
Hospitalization in surgical departments	50	1.72
Outpatient clinic control	187	6.45
Treatment rejection	21	0.72
Unauthorized abandonment	17	0.59
Consultations		
Neurosurgery	38	1.31
General surgery	20	0.69
Obstetrics and gynecology	23	0.79
Eye diseases	107	3.69
Plastic surgery	58	2.00
ENT	241	8.31
Orthopedics	217	7.48
Thoracic surgery	9	0.31
CVS	9	0.31
Neurology	4	0.14
Urology	7	0.24
Social services	5	0.17
ENT: Ears, nose, and throat, CVS: Cardiovascular surgery		

WHO, non-governmental organizations, and governments have been compelled to implement specific measures¹².

In developing countries, there is an increase in the number of various assault incidents worldwide. In Türkiye, articles have been published on this issue. In a study conducted by Seviner et al.¹³, in the southern part of Türkiye, in which 5870 forensic cases were analyzed, it was determined that the number of male cases was approximately twice that of female cases. In a study conducted by Kanal et al.¹⁴ in another province of Türkiye, the sex ratios were strikingly similar to the findings of this study. Our study was conducted in western Türkiye (Istanbul), and the sex ratios were similar to those observed in these two studies. According to data from the Turkish Statistical Institute, the education level in Istanbul is higher than in the other two locations where the studies were conducted. It was considered particularly interesting that there was no difference in sex ratios between the studies conducted in the three regions with different education levels. This observation suggests that assault incidents are a general issue in Türkiye, regardless of educational level. Our study, conducted in one Istanbul region, which has been officially recorded as the area with the highest education level, aligns with this trend. Despite being in a region with a high education level, assault incidents did not show significant differences between areas with lower educational levels.

The average age of assault victims is a significant factor in cases of assault. In our study, the mean age of assault victims presenting to our emergency department was similar to the results of four studies¹⁴⁻¹⁷ conducted in different regions of Türkiye. These results indicate that assault incidents in Türkiye predominantly affect younger age groups.

In the study by Bennet et al.¹⁸, although the sex ratios were slightly different, they favor males. The study suggests that the reason for the higher involvement of males in assault incidents is their increased risk of violence. There are also hypotheses that the male-favoring ratios result from better interhemispheric connections in females and deficits in the frontal cortex in males¹⁹. Fazel et al.²⁰ also emphasized the association between assault incidents and psychiatric disorders. In our study, all 2900 male and female victims were assaulted by males. There were no cases in which the perpetrator was female. Although our research did not specifically investigate the causes of these incidents, we believe that the dominant aggression observed in males cannot be solely attributed to structural and functional differences in the brain. We consider that factors such as family structure, the upbringing of children, the content

Table 2. Distribution of trauma sites according to sex.

	All patients n (%)	Male n (%)	Female n (%)	p+
Trauma localization				
Nasal	423 (14.59)	340 (18.38)	83 (7.90)	0.0001
Maxilla	487 (16.79)	326 (17.62)	161 (15.33)	0.113
Mandible	242 (8.34)	140 (7.57)	102 (9.71)	0.045
Zygoma	217 (7.48)	150 (8.11)	67 (6.38)	0.104
Frontal	476 (16.41)	350 (18.92)	126 (12.00)	0.0001
Orbit	417 (14.38)	298 (16.11)	119 (11.33)	0.0001
Temporal	303 (10.45)	211 (11.41)	92 (8.76)	0.025
Parietal	279 (9.62)	189 (10.22)	90 (8.57)	0.149
Occipital	259 (8.93)	173 (9.35)	86 (8.19)	0.292
Thoracolumbar vertebra	152 (5.25)	81 (4.39)	71 (6.77)	0.006
Cervical vertebrae	538 (18.55)	332 (17.95)	206 (19.62)	0.265
Thoracic	505 (17.41)	319 (17.24)	186 (17.71)	0.748
Abdomen	142 (4.90)	83 (4.49)	59 (5.62)	0.174
Pelvis	123 (4.24)	49 (2.65)	74 (7.05)	0.0001
Upper extremity	1,391 (47.97)	806 (43.57)	585 (55.71)	0.0001
Lower extremity	632 (21.79)	340 (18.38)	292 (27.81)	0.0001
Outcome				
Outpatient discharge	2625 (90.52)	1631 (88.16)	994 (94.67)	0.0001
Surgical departments	50 (1.72)	41 (2.22)	9 (0.86)	0.006
Outpatient clinic control	187 (6.45)	150 (8.11)	37 (3.52)	0.0001
Treatment rejection	21 (0.72)	17 (0.92)	4 (0.38)	0.122
Unauthorized abandonment	17 (0.59)	11 (0.59)	6 (0.57)	0.827
Signs of injury				
Tenderness	1811(62.45)	1114(60.22)	697(66.38)	0.001
Edema	662(22.83)	466(25.19)	196(18.67)	0.0001
Hematoma	147(5.07)	109(5.89)	38(3.62)	0.007
Abrasion	1519(52.38)	1009(54.54)	510(48.57)	0.002
Ecchymosis	1093(37.69)	578(31.24)	515(49.05)	0.0001
Laceration	590(20.34)	471(25.46)	119(11.33)	0.0001

+:Chi-square test

Table 3. Comparison of patient outcomes according to arrival time.

	All Hours	08:00-15:00	15:01-00:00	00:01-07:59	P
Age	35.53±12.46	37.53±12.50	35.42±12.66	33.56±11.54	0.0001*
Outcome					
Outpatient discharge	2625 (90.52)	650 (92.33)	1431 (91.15)	544 (86.90)	0.02+
Surgical departments	50 (1.72)	9 (1.28)	27 (1.72)	14 (2.24)	0.02+
Outpatient control	187 (6.45)	34 (4.83)	96 (6.11)	57 (9.11)	0.02+
Treatment rejection	21 (0.72)	4 (0.57)	11 (0.70)	6 (0.96)	0.02+
Vital danger	23 (0.79)	5 (0.71)	13 (0.83)	5 (0.80)	0.958+

*One-Way Analysis of Variance

	Discharge	Hospitalization in the surgical department	Outpatient control	Treatment rejection	Unauthorized abandonment	p-value
Nasal	325 (12.38)	5 (10.00)	84 (44.92)	7 (33.33)	2(11.76)	0.0001
Maxilla	432 (16.46)	14 (28.00)	33 (17.65)	7 (33.33)	1 (5.88)	0.035
Mandible	227 (8.65)	8 (16.00)	6 (3.21)	1 (4.76)	0 (0.00)	0.014
Zygoma	188 (7.16)	5 (10.00)	23 (12.30)	1 (4.76)	0 (0.00)	0.069
Frontal	432 (16.46)	11 (22.00)	23 (12.30)	5 (23.81)	5 (29.41)	0.173
Orbit	341 (12.99)	15 (30.00)	54 (28.88)	6 (28.57)	1 (5.88)	0.0001
Temporal	278 (10.59)	3 (6.00)	20 (10.70)	1 (4.76)	1 (5.88)	0.693
Parietal	256 (9.75)	4 (8.00)	16 (8.56)	2 (9.52)	1 (5.88)	0.949
Occipital	243 (9.26)	5 (10.00)	8 (4.28)	0 (0.00)	3 (17.65)	0.060
Toracolomber	133 (5.07)	5 (10.00)	13 (6.95)	1 (4.76)	0 (0.00)	0.343
Cervical	508 (19.35)	4 (8.00)	22 (11.76)	1 (4.76)	3 (17.65)	0.011
Thorax	460 (17.52)	7 (14.00)	31 (16.58)	4 (19.05)	3 (17.65)	0.968
Abdomen	121 (4.61)	5 (10.00)	12 (6.42)	3 (14.29)	1 (5.88)	0.085
Pelvics	110 (4.19)	3 (6.00)	9 (4.81)	1 (4.76)	0 (0.00)	0.859
Upper extremity	1.273 (48.50)	17 (34.00)	88 (47.06)	7 (33.33)	6 (35.29)	0.128
Lower extremity	563 (21.45)	24 (48.00)	37 (19.79)	4 (19.05)	4 (23.53)	0.0001

of the education provided, the social fabric of the society, the values and beliefs held, reward mechanisms, and the sanctity attributed to males contribute to these incidents.

The high prevalence of male violence is undoubtedly a global humanitarian issue that must be addressed. The fact that one in three women worldwide have been subjected to domestic violence by their partners confirms the severity of this problem.

As in the rest of the world, in Türkiye, the years 2020-2021 were marked by a unique sociological atmosphere due to the COVID-19 pandemic and the measures taken, including quarantine applications. During this period, there was a decrease in hospital admissions because of the risk of COVID-19 transmission, and hospital operations changed^{21,22}. In our study, the lowest number of forensic case reports occurred in 2020. We attribute this to the strict implementation of the COVID-19 pandemic restrictions in our country during that year. A similar result was demonstrated in a study by Kuitunen et al.²³. This is likely due to the first lockdown implemented in Türkiye on April 10-12, 2020. In the following two years, an increase in cases was observed. Díaz-Faes et al.²⁴ also noted that lockdowns and curfews decreased hospital admissions for forensic cases. However, it has become controversial whether restrictions on social life increase domestic violence incidents.

It is well-known that the number of trauma admissions to the emergency department increases during the summer months and prolonged vacations²⁵. In our study, when the seasonal distribution of assault cases was analyzed, it was observed that approximately one-third of all cases occurred during the summer months. Similar findings have been reported by Küçüker et al.²⁶, Altun et al.²⁷ also reported cases in different regions of Türkiye, indicating that forensic cases mainly occur during summer. The increase in forensic cases during the summer months in Türkiye can be attributed to the intensified workload in specific service sectors, particularly agriculture and livestock, during this period. Examining the hours of forensic case admissions have been a subject of interest in both our study and many others. In a study by Korkmaz et al.²⁸, who screened 47.758 cases, forensic case admissions mainly occurred during afternoon and night shifts. We conclude that factors such as fatigue at the end of the day, heavy traffic during rush hours in large cities leading to stress, higher alcohol consumption and its consequences during late-night hours, conflicts among fans attending late-night sports events, and relatively lower tolerance limits among young partners contribute to triggering assault incidents.

In cases of assault, the localization of injuries is crucial for assessing the victim's risk of mortality, organ damage/function loss, intention of the assailant and the degree of punishment they may face. Therefore, the first responding

physician must handle the situation exceptionally sensitively and maintain accurate records. In a study by Brink et al.²⁹, which analyzed 1481 cases, more than 95% of the victims were exposed to blunt trauma, and the trauma locations occurred in the craniofacial region in 69% of cases. In Payne-James and Dean's³⁰ study, craniofacial injuries were identified in at least half of the patients. Our study confirms the literature findings of a high incidence of craniofacial injuries, especially in the nasal, frontal, orbital, and maxillary regions. In attacks involving face-to-face violence, the craniofacial region is often the primary target. The higher incidence of nasal, maxillary, and orbital trauma in male patients than in female patients can be explained in this context. Conversely, women are more likely to find themselves in defensive positions, which may influence the localization of the impact they receive. Spinal injuries have been observed more frequently in women than in men. In our study, statistically significantly higher rates of spinal injuries were observed in female patients than in male patients. In a prospective study conducted in many different centers in Türkiye, examining approximately 150,000 cases, the most frequently identified injury regions were extremities, head-neck region, and abdomen³¹. We believe that the high frequency of extremity injuries, second only to head traumas, is mainly due to the use of extremities as both defensive and offensive tools.

It is well known that the mortality rate of male assault cases is higher. Data for approximately 25 years of assault cases in Scotland revealed that mortality is higher in men than in women³², no fatal outcomes were observed in our study. However, the proportion of male patients was significantly higher among those in whom life-threatening conditions were detected upon admission and hospitalization to surgical departments. The higher rate of outpatient discharge for female patients compared with male patients also indicates that males tend to sustain more serious injuries during assaults. Consistently, more severe injuries, such as cuts and fractures, were observed in male patients than in female patients.

Generally, nine out of every ten patients in our study were directly discharged from the hospital in a multicenter study conducted by Seviner et al.¹³, which involved forensic cases presenting to the emergency department; 56.8% of the cases were hospitalized, and 21.1% were observed to be in a life-threatening condition. In our study, the rate of life-threatening conditions was lower than that in this study (0.71%). A study in Brazil involving 62 centers and 4835 cases emphasized that

young males caused more severe and fatal injuries using cutting or stabbing tools and firearms³³. Some studies demonstrated the influence of sociocultural factors, societal patriarchal norms, and gender roles, suggesting that violence among men can lead to more dangerous consequences³⁴.

Since the injury locations are generally concentrated in the head and extremities, the need for consultation is usually in the otorhinolaryngology departments, plastic surgery, neurosurgery, and orthopedics. It is important to note that the mean age of patients consulted and hospitalized in surgical departments was higher than that of patients discharged as out-patients. There could be many reasons for this that we could not identify. One of the reasons may be that body tissues become more vulnerable with age, and accordingly, more injuries are sustained during impacts.

Study Limitations

Our study was designed retrospectively, and detailed data regarding the mechanisms of trauma formation could not be collected. The relationship between trauma and specific subgroups, such as the elderly population's abuse or the association with subgroups like partner violence, has not been investigated. Only adult patients (>16 years of age) were included, and incidents of abuse and violence in children were not analyzed. The primary cause of the assault could not be determined. This study is not multicenter and focuses on only one region of Istanbul, which is a metropolitan city.

CONCLUSION

In our study, the following findings were obtained: All assault incidents were committed by male individuals, with males being more frequently and severely assaulted than females. The victims were predominantly from the youth group. Assault incidents primarily occurred during the summer months and outside working hours. The head-neck region and extremities were the most injured areas during assaults. Assault incidents have increased over the years. Except for mortality, our findings demonstrated similarities with the literature. No fatalities were observed, and nine out of ten patients admitted to the emergency department were discharged home.

Ethics

Ethics Committee Approval: This study was approved by the H.S. Istanbul Medeniyet University Göztepe Training and Research Hospital Clinical Research Ethics Committee (decision no: 2023/0219, date: 29.03.2023).

Informed Consent: Presented to the emergency department of tertiary hospital with a "history of assault" were retrospectively examined between January 01, 2020 and December 31, 2022.

Footnotes

Author Contributions

Concept: M.Y.S., B.A., E.T., Design: M.Y.S., B.A., G.A.S., Data Collection and/or Processing: M.Y.S., B.A., V.C., E.T., Analysis and/or Interpretation: M.Y.S., K.A., B. A.C., N.S., Literature Search: K.A., B.A.C., N.S., Writing: M.Y.S., B.A., G.A.S.

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Perivascular Invasion: A Promising Prognostic Parameter for Breast Cancer

Perivasküler İnvazyon: Meme Kanserinde Umut Verici Prognostik Parametre

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ABSTRACT

Objective: Angiotropism/perivascular invasion (PVI) is an emerging topic in various types of cancer, with studies primarily focusing on melanoma. However, limited data are available on the significance of PVI in breast cancer. This study aimed to assess the prognostic significance of PVI in breast cancer and its correlation with traditional clinicopathological prognostic parameters.

Methods: A total of 150 patients with breast cancer diagnosed between July 2020 and January 2022 were included. Clinicopathological data were retrieved from the hospital records. The presence of PVI was evaluated on hematoxylin&eosin stained slides, and the association between PVI and clinicopathological parameters was statistically analyzed. A p-value of <0.05 was regarded as statistically significant.

Results: All patients were female. The mean age was 54.0±13.6 years (range 26-97 years). PVI was significantly more common in patients with ≥2.5 cm tumors and the absence of PVI showed a significant correlation with a lower histologic grade (p=0.004 and p=0.040, respectively). Lymphovascular invasion (LVI) and perineural invasion (PNI) were also significantly more frequent in tumors with PVI (p=0.001 and 0.02, respectively). There was a statistically significant association between the absence of both PVI and extranodal extension (ENE) (p=0.035).

Conclusions: The specific role of PVI in different types of cancer has not yet been clarified. Our findings showed that PVI was significantly associated with tumor size, histological grade, LVI, PNI, and ENE, all of which are well-known negative prognostic factors in breast cancer. The presence of PVI is a promising topic in breast cancer research, and the PVI status in pathology reports may help oncologists perform better risk assessments for patients with breast carcinoma.

Keywords: Breast, cancer, perivascular invasion, angiotropism, prognosis

ÖZ

Amaç: Anjiyotropizm/perivasküler invazyon (PVI), çeşitli kanser türlerinde yeni ortaya çıkan bir konudur ve çalışmalar çoğunlukla melanoma odaklanmaktadır. Ancak meme kanserinde PVI'nin önemi hakkında sınırlı miktarda veri bulunmaktadır. Bu çalışmada PVI'nin meme kanserinde prognostik önemini ve geleneksel klinikopatolojik prognostik parametrelerle ilişkisini değerlendirmeyi amaçladık.

Yöntemler: Temmuz 2020-Ocak 2022 tarihleri arasında meme kanseri tanısı alan toplam 150 hasta dahil edildi. Klinikopatolojik veriler hastane kayıtlarından elde edildi. Hematoksilen-eozin boyalı lamlarda PVI varlığı değerlendirildi ve PVI ile klinikopatolojik parametreler arasındaki ilişki istatistiksel olarak analiz edildi. İstatistiksel anlamlılık olarak p değerinin <0,05 olduğu kabul edildi.

Bulgular: Hastaların tamamı kadındı. Ortalama yaş 54.0±13,6 (dağılım 26-97) idi. PVI, 2,5 cm'den büyük tümörleri olan hastalarda anlamlı derecede daha yaygındı ve PVI yokluğu anlamlı derecede daha düşük histolojik derece ile ilişkiliydi (sırasıyla p=0,004 ve 0,040). Lenfovasküler invazyon (LVI) ve perinöral invazyon (PNI)da PVI'li tümörlerde anlamlı olarak daha sık görüldü (sırasıyla p=0,001 ve 0,02). PVI ile ektranodal uzanım (ENU) yokluğu arasında istatistiksel olarak anlamlı ilişki vardı (p=0,035).

Sonuçlar: PVI'nin çeşitli kanser tiplerindeki spesifik rolü henüz netleştirilmemiştir. Ancak bulgularımız, PVI'nin, meme kanserinde iyi bilinen negatif prognostik faktörler olan tümör boyutu, histolojik derece, LVI, PNI ve ENU ile anlamlı şekilde ilişkili olduğunu göstermektedir. Bu nedenle PVI varlığı meme kanseri açısından ümit verici bir konudur ve patoloji raporunda PVI durumunun yer alması onkoloğa meme kanserine olan hastalarda daha iyi risk değerlendirmesi yapmada yardımcı olabilir.

Anahtar kelimeler: Meme, kanser, perivasküler invazyon, anjiyotropizm, prognoz

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INTRODUCTION

The passive spread of tumor cells through vascular structures is considered most common mechanism of metastasis in patients with cancer. Additionally, tumor cells can actively disseminate along neural structures and the abluminal surface of vascular spaces¹. Pericytic angiotropism, also known as perivascular invasion (PVI), was first described in melanomas². The term describes the movement of tumor cells along the external surface of blood vessels. Some studies have identified this mechanism as being associated with an elevated risk of local recurrence and metastasis in melanoma^{3,4}. The concept of "PVI" is now being increasingly investigated in other tumors, including pancreatic and prostatic adenocarcinomas, well-differentiated liposarcomas, endometrial and tubo-ovarian carcinosarcomas, and sarcomatoid carcinoma of the vulva⁵⁻⁸.

Breast cancer is the primary cause of cancer-related mortality in women⁹. When examining the relationship between PVI and breast cancer is poorly understood. A previous study reported that PVI was correlated with infiltrative tumor behavior and occult lymph node involvement in invasive lobular breast carcinoma¹⁰. Additionally, periarterial and perivenous invasion in invasive breast carcinoma of the no special type (NST) was significantly correlated with lymph node metastasis, as demonstrated in a study by Shioya et al¹¹.

Investigating the relationship between vascular structures and tumor cells, specifically cancerous invasion around vessels, could provide a new method for detecting LVI and linking it to lymph node metastases. Therefore, our study focused on detecting PVI in breast cancer and examining its association with key clinicopathological prognostic factors, as well as LVI and lymph node metastasis.

MATERIALS and METHODS

Ethical Approval

This retrospective study protocol and informed consent form were approved by the Clinical Research Ethics Committee of Başakşehir Çam and Sakura City Hospital (decision no: 8, date:31.03.2021). This study was conducted in compliance with the 2013 Declaration of Helsinki. Patients provided informed consent for the use of histopathological images. This study was conducted according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines¹².

Patient Selection

In total, 178 consecutive mastectomy specimens from patients who underwent breast cancer surgery were retrospectively evaluated in the Department of Pathology at our institution between July 2020 and January 2022. The assessment of the required sample size was based on the total patient population who received treatment in the defined period. Follow-up intervals were documented for the overall survival analysis. Twelve patients were lost to follow-up; therefore, phone calls were organized to collect missing follow-up data for these individuals.

Clinical Information and Histopathological Evaluation

The clinical and histopathological parameters, encompassing age, multifocality, tumor size, histologic type and grade, presence of LVI, presence of perineural invasion (PNI), tumor stage (pT), nodal status (pN), presence of extranodal extension (ENE), and receptor status [estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2) status, and Ki67 labeling index] through immunohistochemical staining, was retrieved from the hospital's digital medical record system and corresponding pathology reports.

Patients' ages were categorized into two groups: <50 years and ≥50 years old. Tumor sizes were grouped as <2.5 cm and ≥2.5 cm. The histological types were invasive carcinoma of NST, invasive lobular carcinoma, and others. Histologic grading was performed using the Nottingham histologic score, which was classified as Grade 1, 2, or 3¹³. The presence or absence of multifocality, LVI, PNI, and ENE was noted. The stages for pT and pN were determined based on both pathological reports and clinical information. pTs were classified into stages 1, 2, 3, and 4, whereas nodal stages (pN) were categorized as 0, 1, 2, or 3, in accordance with the guidelines established by the College of American Pathologists (CAP)¹³.

Immunohistochemical assessment of ER, PgR, and HER2 was carried out in line with the CAP/American Society of Clinical Oncology (ASCO) Guidelines^{13,14}. HER2 positivity was determined by the presence of complete and circumferential immunohistochemical expression in >10% of tumor cells. Equivocal HER2 positivity was further analyzed by silver-enhanced in situ hybridization in accordance with the ASCO/CAP guidelines¹³. The proportion of tumor cells positive for Ki67 was assessed in hot spots, with a cut-off value of 30% based on the latest international consensus study¹⁵. Tumors were classified into five molecular based on the St Gallen International Expert Consensus 2013: Luminal A (ER and PgR-positive, HER2-negative, and Ki67 ≤14%); luminal B1 (ER-positive,

HER2-negative, and either PgR<10% or negative or Ki67 >14%); luminal B2 (ER-positive, HER2-overexpressed or amplified, any PgR, any Ki67); HER2-positive (non-luminal) (HER2-overexpressed or amplified, ER-negative and PgR-negative) and triple-negative breast cancer (ER-negative and PgR-negative, HER2-negative)¹⁶.

Evaluation of PVI

PVI was defined as tumor cells encircling the outer walls of blood vessels or lymphatics, within 1-2 mm of the leading edge of the primary tumor, as previously described¹⁷. The presence of PVI at the edges or periphery of the tumor was evaluated by using hematoxylin&eosin (H&E) staining alone. All tumor slides, including those stained with H&E, were reviewed for the presence of PVI.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 22.0 (Chicago, IL). Distributional characteristics, numerical parametric data as mean±standard deviation, whereas median and interquartile range were used to describe non-parametric data. Categorical data are expressed as frequencies. The comparison of categorical variables was carried out using Pearson’s chi-square test, Fisher’s exact test, and the Freeman-Halton test. The t-test was used to analyze independent numerical variables with parametric data, whereas the Mann-Whitney U test was used for non-parametric data. A complete-case analysis strategy was used to address missing data. Multivariate logistic regression was applied to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between clinical and pathologic parameters and presence of PVI. Statistical significance was set at a p-value threshold of <0.05.

RESULTS

The study involved 150 patients in total. Twenty-eight patients who received neoadjuvant chemotherapy were excluded from the study. Sentinel lymph node dissection was performed in all patients, and axillary lymph node dissection was performed in 64 patients based on the results of frozen section analysis or clinical considerations.

The study included female patients with an average age of 54.0±13.6 years (range: 26-97 years). Among the patients, 66% (n=84) were ≥50 years old. Tumors were multifocal in 27% (n=40) of the patients. The mean diameter of the tumor was 2.7±1.9 cm (ranging from 0.2 to 15 cm), and 54% (n=81) of the patients had a tumor diameter of <2.5 cm.

The most common histologic type was invasive carcinoma of NST (78%, n=117), followed by invasive lobular carcinoma (9%, n=13), and other histologic subtypes (13%, n=20) [apocrine carcinoma (n=1), mucinous carcinoma (n=5), tubular carcinoma (n=3), cribriform carcinoma (n=2), micropapillary carcinoma (n=1), solid papillary carcinoma with invasion (n=2), invasive carcinoma with mixed ductal and lobular features (n=6)].

Table 1. Demographic and clinicopathological characteristics of all patients.		
		Patient count (n=150)
Age	<50 years old	66 (44%)
	≥50 years old	84 (66%)
Multifocality	Present	40 (27%)
	Absent	110 (73%)
Tumor size	<2.5 cm	81 (54%)
	≥2.5 cm	69 (46%)
Histologic type	Invasive carcinoma of the NST	117 (78%)
	Invasive lobular carcinoma	13 (9%)
	Others	20 (13%)
Histologic grade	1	22 (15%)
	2	78 (52%)
	3	50 (33%)
Lymphovascular invasion	Present	73 (49%)
	Absent	77 (51%)
Perineural invasion	Present	56 (37%)
	Absent	91 (63%)
Tumor stage (pT)	1	53 (35%)
	2	79 (53%)
	3	10 (7%)
	4	8 (5%)
Nodal stage (pN)	0	75 (50%)
	1	53 (36%)
	2	11 (7%)
	3	11 (7%)
Extranodal extension	Present	50 (33%)
	Absent	100 (67%)
Molecular subtypes	Luminal A	34 (23%)
	Luminal B1	75 (50%)
	Luminal B2	23 (15%)
	HER2 (+) nonluminal	5 (3%)
	Triple negative	13 (9%)
NST: No special type, HER2: Human epidermal growth factor receptor 2		

The most common histologic grade of the tumors was Grade 2 (52%, n=78). LVI was present in 49% (n=73) of the patients, whereas PNI was detected in 37% (n=56). Metastatic nodal disease (pN1, pN2, and pN3) was observed in 50% (n=75) of the patients. Among the molecular subtypes, the most common was the luminal B1 subtype, accounting for 50% (n=75) of the patients. The demographic and clinicopathological data for all patients are provided in Table 1.

PVI was detected in 30% (n=45) of the patients. Among these, PVI involved capillaries in 47% (n=21) of the patients, while 53% (n=24) showed involvement of medium- to large-caliber vessels. The histopathological appearance of PVI in invasive carcinoma of NST is shown in Figure 1.

PVI was significantly more common in patients with tumors ≥ 2.5 cm (p=0.004). A significant statistical relationship was observed between the absence of PVI and histologic grade I tumors (p=0.040). Furthermore, the presence of LVI and PNI was significantly more common in tumors with PVI (p=0.001 and p=0.020, respectively).

The absence of PVI was significantly more common in tumors without ENE (p=0.035). No statistically significant correlation was observed between PVI and molecular subtypes (p>0.05). The results of the multivariate logistic regression analysis indicated that patients with LVI had a threefold higher risk of PVI (OR=3.226, 95% CI=1.308–7.957, p=0.011).

Table 2 presents the univariate and binary logistic regression analyses of clinicopathological parameters in relation to PVI status.

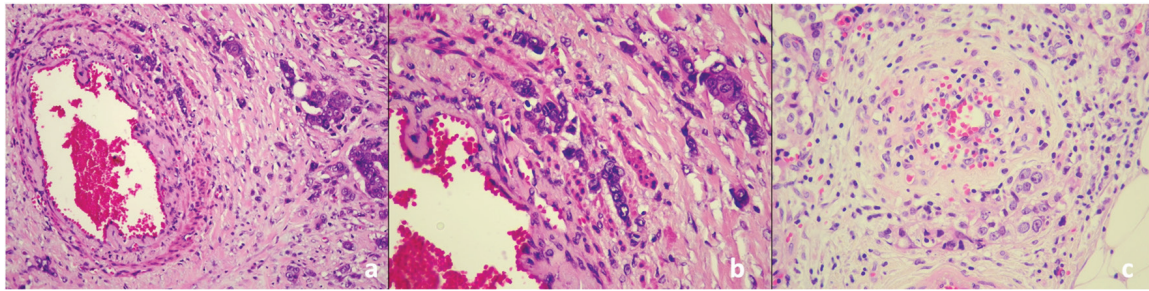


Figure 1. Perivascular invasion and infiltration of tumor cells surrounding vascular structures. a: Invasive carcinoma of no special type (H&E, x200), b: Invasive carcinoma of no special type (H&E, x400), c: Invasive carcinoma of no special type (H&E, x400).

H&E: Hematoxylin&eosin

Table 2. Univariate and binary logistic regression analyses of clinicopathological parameters according to perivascular invasion status.

Patient and tumor characteristics		Perivascular invasion		Univariate analysis	Binary logistic regression analysis	
		Present (n=45)	Absent (n=105)	p-value	p-value	Odds ratio
Tumor size (cm)	<2.5	16 (11%)	65 (43%)	0.004 ^{1*}	-	
	≥ 2.5	29 (19%)	40 (27%)			
Histologic grade	1	3 (2%)	19 (13%)	0.040 ^{2*}	0.443 ³	1.096
	2	30 (20%)	48 (32%)			
	3	12 (8%)	38 (25%)			
Lymphovascular invasion	Present	32 (21%)	41 (27%)	0.001 ^{1*}	0.011 ^{3*}	3.226
	Absent	13 (9%)	64 (43%)			
Perineural invasion	Present	23 (15%)	34 (23%)	0.020 ^{1*}	0.092 ³	0.471
	Absent	22 (15%)	71 (47%)			
Extranodal extension	Present	21 (14%)	29 (19%)	0.035 ^{*1}	0.332 ³	0.645
	Absent	24 (16%)	76 (51%)			

*p< 0.05, ¹: Pearson's chi-square test, ²: Fisher's freeman's Halton test, ³: Binary logistic regression analysis.

Follow-up

The average follow-up time was 28.0 ± 6.2 months, with a median of 27 months (ranging from 2 to 39 months) for overall survival. Eight patients died because of the disease. None of these patients had PVI; however, they presented with other negative prognostic indicators, including advanced histologic grade, later-stage tumor, LVI, and metastatic nodal disease.

DISCUSSION

This study examined the presence and prognostic value of PVI in patients with breast cancer. In addition to the strong association between PVI and LVI, we observed a correlation between PVI and other poor prognostic factors, including higher histological grade, PNI, and ENE. The main limitations of this study include the limited sample size and the short follow-up duration, which hindered a thorough survival analysis.

In our study, we observed that larger tumors more frequently exhibited PVI. This may be attributed to the irregular, infiltrative growth pattern, which is typical of larger malignant tumors. Igawa et al. recently reported that PVI was associated with infiltrative tumor growth in invasive lobular carcinoma of the breast¹⁰. Additionally, a study on gastric cancer found cancer cells in the periarterial tissues of patients with advanced gastric cancer patients¹⁸.

In the current study, a significant relationship was observed between tumor grade and PVI. Although the majority of our patients had grade 2 tumors, PVI was significantly less common in those with lower grades. There are currently no studies in the literature that examined the relationship between PVI and tumor grade, and our findings contribute to the existing literature in this regard. While this observation warrants verification in larger study groups, it likely reflects the epithelial-to-mesenchymal transition process, as high-grade tumors tend to migrate more easily to perivascular areas due to the loss of adhesion molecules.

Shioya et al.¹¹ demonstrated that PVI could be highly sensitive and prevalent (95.5%) in invasive carcinoma of NST with lymph node metastases. In their study, the authors also reported that PVI was observed in 75.4% of tumors without lymph node metastasis. The authors concluded that lymphatic invasion could occur prior to lymph node metastasis. A study on PVI in invasive lobular carcinoma found that PVI is associated with lymph node metastasis in the absence of lymphadenopathy, which can be challenging to detect even with imaging techniques¹⁰. In light of the existing literature, our findings

also suggest a strong relationship between PVI and LVI. We observed PVI in half of the patients with lymph node metastasis, which is consistent with the expectations based on prior research. However, our study did not support the hypothesis that PVI occurs before lymph node metastasis, as we found a PVI rate of only 13% in patients without lymph node metastasis, a rate lower than that reported in the literature.

PVI appears to be a consequence of talk between tumor cells and blood vessels, potentially representing an early phase of LVI, as the significant association between PVI and LVI persisted in multivariate analysis. However, further studies, particularly those using in vitro techniques, are necessary to test these theories.

In addition to LVI, PNI was found to be correlated with PVI. Fedda et al. reported PVI in a patient with recurrent acantholytic squamous cell carcinoma of the scalp, which was characterized by poor differentiation and significant PNI¹⁹. They stated that the concept of PVI is similar to that of PNI, which is consistent with our findings.

The association between ENE and poor prognosis in breast cancer has been demonstrated in various studies^{20,21}. Although the data on this issue remain conflicting, most a negative prognostic effect of ENE in patients with breast cancer. We found that the absence of ENE was significantly associated with the absence of PVI. As far as we know, our study is the first to reveal this association, which may be particularly important for exploring different mechanisms of metastasis to lymph nodes. We believe this aspect warrants further investigation.

The molecular subtypes of breast cancer are an evolving topic that provides crucial insights into the mechanisms underlying the disease. Each tumor type has a distinct prognosis and various heterogeneous features²². In a recent study, patients with triple-negative breast cancer exhibited higher microvascular density; however, this finding did not directly support the presence of PVI²³. In our study, PVI was not found to be specific to any molecular subtype of breast cancer, likely due to tumor heterogeneity.

Although the mechanism of PVI can be described as the movement of tumor cells along the abluminal side of blood vessels, there is a lack of standardized definitions for evaluating tumor cells associated with vessels with varying wall thicknesses²⁴. PVI can affect capillaries and larger vessels. Although most blood vessels affected by PVI in melanoma are typically capillaries, carcinoma often involves medium- to large-caliber vessels^{25,26}. In our

study of breast carcinomas, we observed slightly higher involvement of larger caliber vessels compared with capillaries.

CONCLUSION

In conclusion, our study provides new insights into the relationship between PVI and clinicopathological features of breast cancer. Our findings suggest that the presence of PVI may raise the suspicion for the existence of LVI and/or PNI in breast carcinomas, particularly in large or high-grade tumors. The incorporation of PVI status into pathology reports could enhance oncologists' understanding and improve prognostic predictions. Tumor spread is a critical concern in breast cancer, and we believe our findings contribute valuable insights into this important topic.

Ethics

Ethics Committee Approval: This retrospective study protocol and informed consent form were approved by the Clinical Research Ethics Committee of Başakşehir Çam and Sakura City Hospital (decision no: 8, date:31.03.2021).

Informed Consent: This study was conducted in compliance with the 2013 Declaration of Helsinki. Patients provided informed consent for the use of histopathological images.

Footnotes

Author Contributions

Surgical and Medical Practices: BÇ.G., T.S.A., H.E., E.Ş., B.P., Concept: BÇ.G., T.S.A., B.P., Design: BÇ.G., T.S.A., B.P., Data Collection and/or Processing: BÇ.G., T.S.A., H.E., H.İ.Ö., E.Ş., Analysis and/or Interpretation: BÇ.G., T.S.A., H.E., H.İ.Ö., Literature Search: BÇ.G., T.S.A., H.İ.Ö., B.P., Writing: BÇ.G., T.S.A., B.P.

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Letter to the Editor Regarding Manuscript on "Clinical Characteristics of Children with Acute Post-Streptococcal Glomerulonephritis and the Re-Evaluation of Patients with Artificial Intelligence"

"Akut Post-Streptokokal Glomerülonefritli Çocukların Klinik Özellikleri ve Hastaların Yapay Zeka ile Yeniden Değerlendirilmesi" konulu makaleyle ilgili Editöre Mektup

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Keywords: Post-Streptococcal, glomerulonephritis, re-evaluation, artificial intelligence

Anahtar kelimeler: Post-streptokokal, glomerülonefrit, yeniden değerlendirme, yapay zeka

Dear Editor,

The publication on "Clinical Characteristics of Children with Acute Post-Streptococcal Glomerulonephritis (APSGN) and the Re-Evaluation of Patients with Artificial Intelligence" ¹. The study on the use of ChatGPT 3.5 for APSGN patient follow-up is a fascinating example of the fusion of clinical medicine with artificial intelligence (AI). However, the information is worth analyzing critically, especially in light of the methodology and limited sample size. There are questions regarding the generalizability of the data given that only 11 patients were included. Robust statistical analysis that would have provided weight to the results and clarified whether the accuracy of ChatGPT's responses connected with certain patient outcomes or demographics appears to be missing from the study. In addition, it seems that ChatGPT queries are only asking about known information, not about the intricacies of specific cases or clinical circumstances that

require real-time decision-making. The practical usability of AI in dynamic clinical situations is questioned by this shortcoming.

Furthermore, although the researchers claimed that every response pertaining to APSGN was accurate, the criteria used to determine response accuracy. Researchers that employ subjective evaluation in place of objective grading risk creating bias and cast doubt on the validity of their findings. More openness is also needed when it comes to clinical traits extracted from patient data and the choice of whether to offer treatment suggestions based on these traits. What precise criteria or clinical parameters were applied by the researchers to inform the AI model? In addition, were there any differences in the treatment choices made by the physicians and the AI's suggestions, and if so, how were they resolved?

This study provides opportunities for further research, especially in the area of larger dataset exploration, which

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may enhance the validity of AI applications in clinical settings. Longitudinal studies including several health facilities could shed light on the predictive power of AI across a range of demographics. In addition, future research should investigate whether AI can interpret real-time patient reactions or more complex clinical data to enable real-time treatment adjustments. Furthermore, incorporating AI into clinical decision support systems may significantly improve patient care.

In terms of novelty, future research might focus on creating specialized AI systems designed to more accurately analyze complicated cases of APSGN or other related nephropathies. It may be possible to identify trends that human clinicians may not always notice by integrating machine learning models that adjust in response to real-time patient data. Additionally,

research could examine how AI can be integrated into interdisciplinary teams with the goal of utilizing both the analytical powers of AI and the practical experience of healthcare experts. In conclusion, this partnership might improve patient outcomes and simplify follow-up for APSGN and related disorders.

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Response to the Editor Regarding Our Manuscript on "Clinical Characteristics of Children with Acute Post-streptococcal Glomerulonephritis and Re-evaluation of Patients with Artificial Intelligence"

"Akut Post-Streptokokal Glomerülonefritli Çocukların Klinik Özellikleri ve Hastaların Yapay Zeka ile Yeniden Değerlendirilmesi" Konulu Makalemize İlişkin Editöre Mektuba Yanıt

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Keywords: Acute postinfection glomerulonephritis, artificial intelligence, ChatGPT

Anahtar kelimeler: Akut postenfeksiyon glomerülonefrit, yapay zeka, ChatGPT

Dear Editor,

We would like to thank the readers for their interest in our article¹. Our responses to their questions regarding our manuscript² are provided below.

Our study is a preliminary study examining the usability of an artificial intelligence (AI) model for the diagnosis and treatment of acute poststreptococcal glomerulonephritis (APSGN) in children. The most recent data of the last 11 patients who were followed up and treated in our department between September 2023 and March 2024 were shared with AI. The results obtained with this limited number of patients are intended to shed light on large-scale studies in the future.

The 12 questions directed at AI regarding APSGN are presented in Table 1. The AI answers were evaluated by two pediatric nephrologists with 5 and 16 years

of experience in the field. To make the evaluation transparent, the answers given by AI are shared with the reader in Supplemental File 1. These answers are reproduced in Supplemental File 1. Thus, the answers provided by AI can be easily reviewed by anyone.

In our study, patient data were retrospectively analyzed using the AI model after the treatment processes of our patients were completed. No support was received from the AI in the diagnosis and treatment management of the patients. When the clinical and laboratory data of the patients at the time of initial presentation were summarized to AI, the concordance of the diagnosis and treatment recommendations with the physicians was examined. As an example for readers, the clinical and laboratory data of the first three patients referred to AI, as well as AI's responses in terms of diagnosis and treatment, are presented in Table 3. Data on the other

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patients are also shared with the reader as Supplemental File 2. For maximum clarity, these data are presented in Supplemental File 2.

In conclusion, the current study highlights the potential of AI for the diagnosis and treatment of APSGN in children. The findings from our limited patient data may pave the way for more comprehensive future research. In addition, the integration of AI into clinical decision-making processes could significantly improve patient care. Feedback from our readers is invaluable in advancing our work in this field. Thank you for your interest.

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Supplemental File 1. Questions directed at AI regarding APSGN and answers provided by AI

1. The definition of Acute Post-Streptococcal Glomerulonephritis

ChatGPT

Acute post-streptococcal glomerulonephritis (APSGN) is a kidney condition that occurs following infection with certain strains of Streptococcus bacteria, most commonly after a throat infection (pharyngitis) or skin infection (impetigo). In this condition, the immune response to the bacterial infection leads to inflammation of the glomeruli, which are the tiny filtering units in the kidneys.

The symptoms of APSGN include the following:

- Swelling (edema) particularly in the face and around the eyes
- Elevated blood pressure
- Hematuria (blood in the urine)
- Proteinuria (excess protein in the urine)
- Reduced urine output

APSGN typically develops 1-3 weeks after the initial streptococcal infection. Although it can occur at any age, it is more common in children. Most cases resolve with appropriate management, although some individuals may experience complications.

2. The epidemiology of APSGN

ChatGPT

The epidemiology of APSGN includes the following key points:

1. Incidence: APSGN is most commonly observed in children aged 2-12 years. The incidence of streptococcal infections is higher in developing countries where streptococcal infections are more prevalent due to factors like overcrowding and limited access to healthcare.

2. Gender: Males are more frequently affected than females, with a male-to-female ratio of approximately

3. Seasonality: This condition often occurs in colder months, which is associated with a higher incidence of streptococcal throat infections during this time.

4. Geographical Distribution: APSGN is more common in areas where streptococcal infections (especially group A beta-hemolytic streptococci) are endemic. In developed countries, the incidence of COVID-19 has decreased significantly because of improved living conditions and antibiotic use.

5. Relation to Infection: APSGN typically follows upper respiratory tract infection (pharyngitis) or skin infection (impetigo) caused by specific Streptococcus nephritogenic strains. The risk of developing APSGN increases after infection.

6. Recurrent Cases: Most APSGN cases are acute and resolve completely. However, some individuals may experience recurrent episodes, particularly if they have repeated infections with streptococci strains.

7. Outcome: Most children recover fully with appropriate treatment, but a small percentage may develop long-term complications, such as chronic kidney disease, particularly if there is a delay in diagnosis or treatment.

Overall, although APSGN is a significant public health concern in certain populations, advances in hygiene and medical care have reduced its prevalence in many regions.

3. The pathophysiology of APSGN

ChatGPT

The pathophysiology of APSGN involves a complex interplay between the immune response to streptococcal infection and subsequent inflammation of the glomeruli in the kidneys. Here is an overview of the key mechanisms involved:

1. Infection: APSGN typically follows a preceding infection, most commonly with nephritogenic group A beta-hemolytic streptococci. This condition can occur after pharyngitis (throat infection) or skin infection (impetigo).

2. Immune Response: After infection, the body mounts an immune response to clear the streptococcal bacteria. This response involves the production of antibodies against streptococcal antigens, particularly M protein and other streptococcal components.

3. Formation of Immune Complexes: Some of these antibodies form immune complexes with streptococcal antigens. These immune complexes can deposit in the glomeruli, which are the small filtering units of the kidneys.

4. Inflammation: The deposition of immune complexes in the glomeruli activates the complement system, which enhances the clearance of pathogens. This activation leads to inflammation, resulting in the recruitment of immune cells, such as neutrophils and macrophages, to the site.

5. Glomerular Damage: The inflammatory response causes damage to glomerular endothelial cells and podocytes (the cells that line the glomeruli). This damage disrupts the filtration barrier, leading to leakage of red blood cells (hematuria) and protein (proteinuria) leakage into the urine.

6. Clinical Manifestations: As a result of glomerular inflammation and damage, clinical symptoms such as edema (swelling), hypertension (high blood pressure), and changes in urine output and composition occur.

7. Resolution or Chronicity: In most cases, the inflammatory process resolves over time, leading to a return to normal kidney function. However, in some cases, particularly when significant or repeated damage occurs, individuals may develop chronic kidney disease.

APSGN is primarily caused by an inappropriate immune response to a preceding streptococcal infection, leading to glomerular inflammation and damage. The condition is usually self-limiting, and most patients recover completely with appropriate management.

4. What is the relationship between APSGN and the complement system?

ChatGPT

The relationship between APSGN and the complement system is crucial for understanding the pathophysiology of this disease. Here is how they are interconnected:

1. Immune Complex Formation: In APSGN, the immune response to streptococcal infection leads to the formation of immune complexes. These complexes consist of streptococcal antigens bound to antibodies produced by the body in response to infection.

2. Complement Activation: The deposited immune complexes in the glomeruli activate the complement system, which plays a key role in enhancing the inflammatory response and clearing pathogens. This activation can occur primarily through the classical pathway, although alternative and lectin pathways may also be involved.

3. Inflammation: The complement system generates various active components (such as C3a and C5a) that promote inflammation. These components attract immune cells (like neutrophils and macrophages) to the site of deposition in the glomeruli, leading to an inflammatory response.

4. Glomerular Injury: The influx of immune cells and the release of pro-inflammatory cytokines and other mediators contribute to glomerular injury. This results in increased permeability of the glomerular filtration barrier, causing hematuria (blood in urine) and proteinuria (excess protein in urine).

5. Complement Levels in APSGN: In APSGN, particularly during the acute phase, complement levels may be altered. Specifically, levels of C3 (a key complement component) are often decreased due to its consumption as it is activated in response to immune complexes. In contrast, C4 levels typically remain normal.

6. Clinical Implications: Measuring complement levels, especially C3, can help diagnose APSGN and differentiate it from other causes of glomerulonephritis. A low C3 level supports the diagnosis of APSGN, whereas persistently low C3 levels may indicate other pathologies.

In summary, the complement system plays a significant role in the pathophysiology of APSGN by mediating the inflammatory response to immune complex deposition in the glomeruli, leading to kidney injury and the clinical manifestations of the disease.

5. The clinical manifestations of APSGN

ChatGPT

The clinical manifestations of APSGN can vary in severity and presentation, but common symptoms include the following:

1. Edema: Swelling, particularly noticeable in the face (especially around the eyes) and in the lower extremities (ankles and feet) due to fluid retention.

2. Hypertension: Elevated blood pressure is common and may be significant in some cases, resulting from fluid overload and changes in kidney function.

3. Hematuria: The presence of blood in urine, which may cause urine to appear pink or cola-colored. This is due to red blood cell leakage from inflamed glomeruli.

4. Proteinuria: Urine protein levels increase, which can lead to foamy urine due to excess protein.

5. Decreased Urine Out-put: A reduction in urine production, often referred to as oliguria.

6. Fatigue: A general feeling of tiredness or malaise related to fluid overload and the body's response to inflammation.

7. Flank Pain: Some patients may experience discomfort or pain in the lower back or sides where the kidneys are located.

8 Other Symptoms: Depending on the severity of the condition, some patients may also exhibit nausea, vomiting, or headaches.

Time Course: Symptoms of APSGN typically develop 1–3 weeks after the initial streptococcal infection. The condition is often self-limiting, and most children recover fully with appropriate management.

Diagnosis: Diagnosis is typically confirmed through laboratory tests, including urinalysis (showing hematuria and proteinuria), blood tests (to check for elevated creatinine levels and complement levels), and throat or skin cultures to identify the preceding streptococcal infection.

Overall, the clinical manifestations of APSGN result from the inflammatory processes affecting the glomeruli in response to the immune reaction following streptococcal infection.

6. How is APSGN diagnosed?

ChatGPT

The diagnosis of APSGN involves a combination of clinical evaluation, laboratory tests, and imaging studies. Here, the diagnosis is typically made as follows:

1. Clinical History and Physical Examination

- **History of Recent Infection:** The clinician will inquire about a recent history of streptococcal infection, such as pharyngitis or impetigo, usually occurring 1–3 weeks before the onset of symptoms.

- **Symptoms:** The presence of symptoms like edema, hematuria, proteinuria, and hypertension, will be assessed.

- **Physical examination:** Examination for signs of fluid retention (e.g., swelling), hypertension, and general well-being.

2. Laboratory Tests

- **Urinalysis:** This is a key diagnostic test for APSGN. Typical findings include the following:
 - **Hematuria:** blood in urine, which may cause it to appear pink or cola-colored.
 - **Proteinuria:** Urine protein levels increase, often resulting in foamy urine.
 - **Red Blood Cell Casts:** May be present in urine, indicating glomerular damage.
- **Blood Tests:**
 - **Serum creatinine levels:** Elevated levels indicate impaired kidney function.
 - **Blood Urea Nitrogen (BUN):** May also be elevated, reflecting reduced kidney function.
 - **Complement Levels:** C3 levels may be decreased by complement consumption during inflammation, whereas C4 levels usually remain normal.
- **Antistreptolysin O (ASO) Titer:** This test measures the presence of antibodies against streptolysin O, a toxin produced by streptococci. Elevated ASO titers suggest recent streptococcal infection.
- **Anti-DNase B Antibody Test:** This test measures antibodies against DNase B, another streptococcal enzyme. Elevated levels can also indicate recent infection.

3. Additional Testing (if necessary)

- **Throat or Skin Culture:** To identify the specific strain of streptococcus causing the infection although this is not always necessary.
- **Imaging studies:** Although not routinely required, ultrasound may be used in cases of severe kidney dysfunction or to rule out other conditions.

4. Differential Diagnosis

- The clinician will also consider and rule out other causes of glomerulonephritis or nephrotic syndrome, such as systemic lupus erythematosus, IgA nephropathy, and vasculitis.

Summary

In summary, the diagnosis of APSGN is based on a combination of clinical history, physical examination, laboratory tests (urinalysis and blood tests), and sometimes culture or imaging. The characteristic findings of hematuria, proteinuria, elevated serum creatinine, and decreased complement levels (especially C3) support the diagnosis of APSGN following a recent streptococcal infection.

7. What are the indications for renal biopsy in APSGN?

ChatGPT

Renal biopsy is generally not the first-line diagnostic tool for APSGN, as the diagnosis is typically made based on clinical history, symptoms, and laboratory findings. However, there are specific indications for renal biopsy in patients with APSGN. These include:

Indications for Renal Biopsy

1. **Atypical Presentation:** If the clinical presentation does not align with classic APSGN (e.g., absence of a recent streptococcal infection, unusual age of onset, or atypical symptoms), biopsy may help clarify the diagnosis.
2. **Persistent or Worsening Renal Dysfunction:** If a patient experiences significant and persistent renal impairment (elevated serum creatinine) beyond the typical course of APSGN or has worsening kidney function despite conservative management, a biopsy may be warranted to assess for other underlying renal pathologies.

3. Severe or Unusual Clinical Features: If the patient presents with severe symptoms, such as significant hypertension, severe edema, or nephrotic syndrome (massive proteinuria), renal biopsy may help determine the underlying cause.

4. Failure to Respond to Treatment: If a patient with APSGN does not show expected improvement in symptoms or laboratory findings (e.g., persistent proteinuria or hematuria) after appropriate treatment, biopsy can help identify alternative diagnoses or complications.

5. Consideration of Other Causes: In cases with suspicion of other forms of glomerulonephritis (e.g., IgA nephropathy, membranoproliferative glomerulonephritis, or systemic diseases like lupus), biopsy may be needed to differentiate these conditions from APSGN.

6. Recurrent Episodes: If a patient experiences recurrent episodes of glomerulonephritis, biopsy may help to identify the underlying etiology.

Conclusion

Although renal biopsy is not routinely performed for APSGN, it can be indicated in specific circumstances where the clinical picture is atypical or when there is concern for other renal conditions. The decision to perform a biopsy should be based on a thorough clinical evaluation and discussion with the patient or his/her caregiver.

The treatment of APSGN

ChatGPT

The treatment of APSGN is primarily supportive, focusing on managing symptoms and complications. Here, we provide an overview of the treatment approach:

1. Supportive Care

- **Monitoring:** Regular monitoring of blood pressure, urine output, and kidney function (serum creatinine and electrolytes) is essential.
- **Rest:** Bed rest may be recommended in patients with significant edema or hypertension.

2. Management of Hypertension

- **Antihypertensive Medications:** If the patient presents with hypertension, medications such as diuretics, ACE inhibitors, or other antihypertensives may be administered to help control blood pressure.

3. Diuretics

- **Fluid Management:** Diuretics (e.g., furosemide) may be prescribed to help manage fluid overload and edema, thereby promoting diuresis (increased urine production).

4. Nutritional Support

- **Sodium and Fluid Restriction:** A low-sodium diet and possibly fluid restriction may be advised to help manage edema and hypertension.

5. Treating Infections

- **Antibiotics:** Although APSGN itself does not require antibiotic treatment, appropriate antibiotics (such as penicillin or amoxicillin) should be administered if there is an active streptococcal infection (e.g., a persistent throat infection), appropriate antibiotics (such as penicillin or amoxicillin) should be administered to clear the infection and prevent recurrence.

6. Management of Complications

- **Electrolyte Imbalances:** Monitoring and correcting electrolyte imbalances (such as hyperkalemia) may be necessary.

- **Severe Cases:** In rare cases where significant kidney dysfunction or complications arise (such as acute kidney injury), more intensive treatment, such as dialysis, may be required.

7. Follow-Up

- **Long-Term Monitoring:** Patients should be followed up regularly to monitor kidney function and any lingering symptoms. Most patients recover completely, but a small percentage may develop chronic kidney disease.

Conclusion

The treatment of APSGN is generally supportive, focusing on managing symptoms, controlling blood pressure, and ensuring fluid balance. Most patients respond well to treatment and experience full recovery. The prognosis is generally good, particularly in children, with complete resolution of symptoms and renal function in most cases.

9. What drugs are available for the treatment of APSGN?

ChatGPT

The treatment of APSGN is primarily supportive, but certain medications may be used to manage symptoms and complications. Here, we present a list of drugs that can be used for the treatment of APSGN:

1. Antihypertensive Medications

- **Diuretics:**

- Furosemide (Lasix): Used to manage fluid overload and edema by promoting diuresis.

- **ACE Inhibitors:**

- Enalapril, Lisinopril: May be used to control hypertension and reduce proteinuria.

- **Calcium Channel Blockers:**

- Amlodipine, Diltiazem: Can also be used for hypertensive management.

2. Corticosteroids

- **Prednisone:** In certain cases with severe or persistent inflammation, corticosteroids may be prescribed to reduce inflammation. This is not common in uncomplicated APSGN.

3. Antibiotics

- **Penicillin or azoxycillin:** If there is a persistent streptococcal infection (e.g., throat infection), antibiotics should be used to relieve the infection and prevent further complications. These agents are not used to treat APSGN directly but to address the underlying infection that precipitated the condition.

4. Electrolyte Management

- **Potassium Binders:**

- **Sodium Polystyrene Sulfonate (Kayexalate):** If hyperkalemia (high potassium levels) occurs, potassium binders may be used.

- **Calcium Gluconate:** Used in severe hyperkalemia to stabilize cardiac membranes.

5. Phosphate Binders

If renal impairment leads to elevated phosphate levels, phosphate binders may be used to manage hyperphosphatemia.

Conclusion

The primary approach in the management of APSGN focuses on supportive care, including hypertension and fluid overload. The medications listed above may be used as needed to alleviate symptoms and complications associated

with the condition. It is important to tailor treatment to individual patient needs and monitor progress closely. Most patients with APSGN recover fully with appropriate management.

10. Other immunosuppressive agents other than steroids in APSGN

ChatGPT

In APSGN, supportive care is the primary treatment, and corticosteroids are typically the main immunosuppressive therapy used in cases of severe inflammation or persistent symptoms. However, other immunosuppressive agents are generally not indicated for routine management of APSGN because the condition is usually self-limiting and resolves with supportive care.

However, in certain atypical cases or when severe or resistant cases need to be managed, additional immunosuppressive agents may be considered. These might include the following:

1. Cyclophosphamide

- An alkylating agent that suppresses the immune response may be used in severe cases in which steroid therapy is inadequate or in cases with complications.

2. Azathioprine

- Immunosuppressive drugs that inhibit the proliferation of immune cells. It may be considered in patients with significant kidney involvement or when there is suspicion of another underlying glomerulonephritis.

3. Mycophenolate Mofetil (MMF)

- A more selective immunosuppressive agent that inhibits lymphocyte proliferation. It may be used in patients with persistent disease, although it is more common in patients with chronic or progressive glomerulonephritis.

4. Rituximab

- A monoclonal antibody targeting CD20 in B cells, which is used primarily in cases of autoimmune diseases or resistant cases of glomerulonephritis. Its use in APSGN is not standard but may be considered in exceptional circumstances.

When to Consider Additional Immunosuppressive Agents

The use of these agents is not standard for APSGN and is typically only considered in specific situations, such as the following:

- Severe, persistent symptoms or significant renal impairment.
- Atypical presentation or rapid deterioration in kidney function.
- Diagnosis of a different underlying condition that may warrant immunosuppression.

Conclusion

In routine cases of APSGN, the emphasis is on supportive management, and additional immunosuppressive therapy is rarely required. If there are complexities in the clinical scenario, consultation with a nephrologist is essential to tailor the management appropriately.

11. How should clinical and laboratory findings be monitored during follow-up of APSGN?

ChatGPT

Monitoring clinical and laboratory findings during follow-up of APSGN is crucial for assessing recovery and identifying potential complications. Here, we describe how to approach this follow-up care:

1. Clinical Monitoring

- **Symptom assessment:** The symptoms are regularly evaluated for resolution, such as
 - **Edema:** Monitor for reduction in swelling, particularly in the face and extremities.
 - **Hypertension:** Monitor blood pressure regularly; hypertension may require control.
 - **Urine changes:** Urine appearance, frequency, and output should be assessed.
- **General health:** The overall well-being of the patient is evaluated, with consideration of signs of fatigue or malaise.

2. Laboratory Monitoring

• Urinalysis:

Hematuria and proteinuria: Urine urine should be monitored for the presence of blood and protein. Although hematuria may take weeks to resolve, significant proteinuria should decrease over time.

Urine microscopy: examination for red blood cells, white blood cells, and casts.

• Serum Tests:

- **Serum Creatinine:** Monitor kidney function by measuring serum creatinine levels at regular intervals. An initial rise may occur, but the levels should normalize as recovery progresses.

- **Electrolytes:** Regular check for electrolyte imbalances, particularly potassium, sodium, and phosphate, particularly if there are concerns about renal function.

- **Complement Levels:** Measure C3 levels to assess recovery; C3 should return to normal as the condition resolves.

- **Antistreptolysin O (ASO) Titer:** If needed, repeat testing to check for the resolution of streptococcal infection, although this is not routinely required in all cases.

3. Frequency of Monitoring

- **Initial Follow-Up:** Follow-up visits should be scheduled within 1-2 weeks after the initial diagnosis to assess symptom resolution and to perform repeat laboratory tests.

- **Subsequent Follow-Ups:** Depending on the clinical status, follow-up visits may be extended every 4–6 weeks, gradually extending to longer intervals as recovery occurs.

4. Long-Term Monitoring

- **Continued Assessment:** Even after recovery, some patients may require long-term follow-up for potential complications or recurrent issues, particularly if they have concerns about chronic kidney disease or other underlying conditions.

Conclusion

Regular clinical evaluations and laboratory tests are essential during follow-up of patients with APSGN to ensure full recovery and to identify potential complications early. Collaboration with a healthcare provider specializing in nephrology may be beneficial for managing ongoing care and monitoring. Most patients with APSGN experience complete resolution of symptoms and renal function with appropriate monitoring and support.

12. The prognosis of APSGN

ChatGPT

The prognosis of APSGN is generally favorable, particularly in children. Here are key points regarding the prognosis:

1. Complete Recovery

- **High recovery rate:** Most patients, especially children, experience complete recovery of kidney function and resolution of symptoms. Most cases of APSGN are self-limiting.

2. Time Frame for Recovery

- **Symptom Resolution:** Symptoms such as edema, hypertension, hematuria, and proteinuria typically begin to improve within weeks.

- **Renal Function:** Serum creatinine levels usually normalize within a few weeks to months after the acute phase of the illness.

3. Recurrence

- **Uncommon:** APSGN recurrence after the initial episode is rare. However, patients who experience recurrent infections caused by nephritogenic strains of streptococcus may be at risk of additional episodes.

4. Long-Term Complications

- **Chronic Kidney Disease (CKD):** While most patients recover fully, a small percentage may develop long-term complications, such as CKD. This is more common in adults than in children and typically occurs in cases with more severe initial presentations or in those with pre-existing renal conditions.

5. Factors Influencing Prognosis

- **Age:** Younger patients, especially children, tend to have a better prognosis than adults.
- **Severity of Initial Presentation:** Patients with severe hypertension, significant renal impairment, or persistent symptoms may have a less favorable prognosis and require closer monitoring.
- **Timeliness of Management:** Early recognition and appropriate management can improve outcomes.

Conclusion

In summary, the prognosis of APSGN is generally excellent, and most patients recover completely without long-term complications. However, individual outcomes can vary based on factors such as age, illness severity, and underlying health conditions. Regular follow-up and monitoring are essential for ensuring complete recovery and addressing any potential complications.

Supplemental File 2. Clinical and laboratory characteristics of Case 4-11 referred to AI and diagnosis and treatment recommendations by AI

Case 4

5-year-old girl: A patient presents to the emergency department with bloody urine. It was learned that she had fever and sore throat 10 days ago, was diagnosed with tonsillitis, started on antibiotics at the family health center, but did not continue treatment after 2 days when her symptoms improved. On examination, her blood pressure was measured at 86/64 mm Hg (39th and 89th percentiles, respectively). Laboratory findings showed serum creatinine 0.5 mg/dL, serum albumin 3.6 g/L, Na 139 mmol/L, K 5 mmol/L, Ca 9.1 mg/dL, and P 4.3 mg/dL. Urinalysis revealed a red blood cell count of 168/HPF and spot urine protein/creatinine ratio of 2.4 mg/mg. The complement C3 level was low at 0.26 g/L, and the ASO level was high at 1023 IU/mL. The throat culture shows growth of Group A beta-hemolytic streptococcus 1 day later.

What is the patient's diagnosis? What medications should be initiated for treatment? What points should be noted during follow-up?

ChatGPT Response

Based on the clinical information, the diagnosis of this patient was acute poststreptococcal glomerulonephritis (APSGN). Here is what should be done in terms of treatment and follow-up:

1. Treatment:

- Antibiotic treatment to eradicate group A beta-hemolytic streptococcal infection is essential. Penicillin or another appropriate antibiotic targeting streptococcus should be administered.
- Management of hyperkalemia is essential. Given the high serum potassium level, measures to lower potassium levels should be initiated promptly, such as dietary restriction of potassium and possibly administration of potassium-lowering medications like loop diuretics and potassium binders. Inhaled salbutamol and insulin can also be administered to selected patients.
- Supportive care to manage symptoms and complications. This step may include rest, fluid management, and monitoring for hypertension or fluid overload.

2. Follow-up:

- The close monitoring of clinical and laboratory parameters during the follow-up period.
- Regular assessment of blood pressure to detect hypertension, which can develop or worsen during the course of APSGN.
- Monitoring urine output and urine characteristics, including hematuria and proteinuria, to evaluate kidney function and recovery.
- Periodic measurement of serum creatinine and complement levels to assess renal function and resolution of the inflammatory process.
- Education and counseling for the patient and family regarding the importance of completing antibiotic treatment, adhering to follow-up appointments, and maintaining a healthy lifestyle.

Additional points to consider during follow-up:

- **Long-term sequelae:** Although APSGN generally has a good prognosis, some patients may develop complications, such as persistent hypertension or chronic kidney disease. Long-term follow-up is important to monitor the development of these complications and provide appropriate management.
- **Education:** It is essential to educate patients and caregivers about the signs and symptoms of complications and the importance of seeking medical attention if complications occur.

- **Psychosocial support:** Chronic illness can have a significant impact on children and their families. Providing psychosocial support and resources may help cope with the diagnosis and associated challenges.
- **Prevention:** The importance of preventive measures, such as good hygiene practices, to reduce the risk of recurrent streptococcal infections, which can help prevent future episodes of APSGN.

Case 5

12.5-year-old boy: He presented with bloody urine. It was learned that he had an upper respiratory tract infection about 15 days ago. On examination, his blood pressure was 120/80 mm Hg (95th and 97th percentiles, respectively). Laboratory findings showed serum creatinine (0.88 mg/dL, serum albumin (4 g/L, Na 142 mmol/L, K 4.7 mmol/L, Ca 9.3 mg/dL, and P (4.7 mg/dL. Urinalysis revealed a red blood cell count of 177/HPF and a spot urine protein/creatinine ratio of 1.2 mg/mg. The complement C3 level was 0.78 g/L (normal range 0.9 – 1.2), and the ASO level was 1320 IU/mL (elevated, normal <150). The throat culture revealed no growth.

What is the patient's diagnosis? What medications should be initiated for treatment? What points should be noted during follow-up?

ChatGPT Response

Based on the clinical information, the diagnosis of this patient was likely acute poststreptococcal glomerulonephritis (APSGN). Here is what should be done in terms of treatment and follow-up:

1. Diagnosis:

- **Acute Poststreptococcal Glomerulonephritis (APSGN):** This condition is suggested by a history of recent upper respiratory tract infection, high ASO titers, slightly low serum C3 levels, presence of hematuria and proteinuria, and hypertension.

2. Treatment:

- **Antibiotics:** Although the throat culture result was negative, the high ASO titer suggests recent streptococcal infection. However, antibiotics are not considered if the culture is negative.
- **Management of hypertension:** Blood pressure control is essential in the presence of hypertension. Depending on the severity of hypertension, antihypertensive medications, such as angiotensin-converting enzyme inhibitors (ACE inhibitors) or angiotensin receptor blockers (ARBs), may be administered.
- **Supportive care:** Supportive measures include rest, fluid balance, and monitoring for signs of fluid overload or electrolyte imbalance.

3. Follow-up:

- **Blood pressure monitoring:** Regular blood pressure monitoring is essential for assessing response to treatment and preventing complications associated with hypertension.
- **Renal function monitoring:** Periodic assessment of renal function, including serum creatinine and urine output, is necessary to evaluate renal function and recovery.
- **Follow-up urine analysis:** Urine analysis for hematuria and proteinuria can help assess the resolution of renal inflammation and injury.
- **Serum complement levels:** Although slightly low, serum complement levels may normalize as the inflammatory process resolves. Periodic monitoring of complement levels can provide additional information about disease activity and response to treatment.
- **Education and support:** Provide education to the patient and family about the condition, treatment plan, and importance of adherence to follow-up appointments. Emphasizing the signs and symptoms of complications and encouraging early reporting of any concerns or changes in health status.

Case 6

7.3-year-old boy: He presented with bloody urine. It was learned that he had an upper respiratory tract infection about 7 days ago. On examination, his blood pressure was 110/60 mm Hg (78th and 44th percentiles, respectively). Laboratory findings showed serum creatinine (0.79 mg/dL, serum albumin (4 g/L, Na 139 mmol/L, K 4.6 mmol/L, Ca 8.9 mg/dL, and P (4.6 mg/dL. Urinalysis revealed a red blood cell count of 897/HPF and a spot urine protein/creatinine ratio of 3.19 mg/mg. The complement C3 level was 0.55 g/L (low) and the ASO level was 788 IU/mL (high). The throat culture shows the growth of group A beta-hemolytic streptococcus.

What is the patient's diagnosis? What medications should be initiated for treatment? What points should be noted during follow-up?

ChatGPT Response

Based on the clinical information, the diagnosis of this patient was acute poststreptococcal glomerulonephritis (APSGN). Here is what should be done in terms of treatment and follow-up:

1. Treatment:

- Antibiotic treatment to eradicate group A beta-hemolytic streptococcal infection is essential. Penicillin or another appropriate antibiotic targeting streptococcus should be administered.
- Supportive care to manage symptoms and complications. This step may include rest, fluid management, and monitoring for hypertension or fluid overload.

2. Follow-up:

- The close monitoring of clinical and laboratory parameters during the follow-up period.
- Regular assessment of blood pressure to detect hypertension, which can develop or worsen during the course of APSGN.
- Monitoring urine output and urine characteristics, including hematuria and proteinuria, to evaluate kidney function and recovery.
- Periodic measurement of serum creatinine and complement levels to assess renal function and resolution of the inflammatory process.
- Education and counseling for the patient and family regarding the importance of completing antibiotic treatment, adhering to follow-up appointments, and maintaining a healthy lifestyle.

Additional points to consider during follow-up:

- **Long-term sequelae:** Although APSGN generally has a good prognosis, some patients may develop complications, such as persistent hypertension or chronic kidney disease. Long-term follow-up is important to monitor the development of these complications and provide appropriate management.
- **Education:** It is essential to educate patients and caregivers about the signs and symptoms of complications and the importance of seeking medical attention if complications occur.
- **Psychosocial support:** Chronic illness can have a significant impact on children and their families. Providing psychosocial support and resources may help cope with the diagnosis and associated challenges.
- **Prevention:** The importance of preventive measures, such as good hygiene practices, to reduce the risk of recurrent streptococcal infections, which can help prevent future episodes of APSGN.

Case 7

8.9-year-old boy: He presented with bloody urine. It was learned that he had an upper respiratory tract infection about 7 days ago. On examination, his blood pressure was 100/60 mm Hg (58th and 53rd percentiles, respectively). Laboratory findings showed serum creatinine (1.84 mg/dL, serum albumin (3.4 g/L, Na 135 mmol/L, K 5.2 mmol/L, Ca 9.1 mg/dL, and P (5.9 mg/dL. Urinalysis revealed a red blood cell count of 31,71/HPF and a spot urine protein:creatinine ratio of 4.32 mg/mg. The complement C3 level was 0.77 g/L (low) and the ASO level was 638 IU/mL (high). The throat culture revealed no growth.

What is the patient's diagnosis? What medications should be initiated for treatment? What points should be noted during follow-up?

ChatGPT Response

Based on the clinical information, the diagnosis of this patient was acute poststreptococcal glomerulonephritis (APSGN). Here is what should be done in terms of treatment and follow-up:

1. Treatment:

- **Antibiotics:** Although the throat culture result was negative, the high ASO titer suggests recent streptococcal infection. However, antibiotics are not considered if the culture is negative.
- Management of hyperkalemia is essential. Given the high serum potassium level, measures to lower potassium levels should be initiated promptly, such as dietary restriction of potassium and possibly administration of potassium-lowering medications like loop diuretics and potassium binders. Inhaled salbutamol and insulin can also be administered to selected patients.
- Supportive care to manage symptoms and complications. This step may include rest, fluid management, and monitoring for hypertension or fluid overload.

2. Follow-up:

- The close monitoring of clinical and laboratory parameters during the follow-up period.
- Regular assessment of blood pressure to detect hypertension, which can develop or worsen during the course of APSGN.
- Monitoring urine output and urine characteristics, including hematuria and proteinuria, to evaluate kidney function and recovery.
- Periodic measurement of serum creatinine and complement levels to assess renal function and resolution of the inflammatory process.
- Education and counseling for the patient and family regarding the importance of completing antibiotic treatment, adhering to follow-up appointments, and maintaining a healthy lifestyle.

Additional points to consider during follow-up:

- **Long-term sequelae:** Although APSGN generally has a good prognosis, some patients may develop complications, such as persistent hypertension or chronic kidney disease. Long-term follow-up is important to monitor the development of these complications and provide appropriate management.
- **Education:** It is essential to educate patients and caregivers about the signs and symptoms of complications and the importance of seeking medical attention if complications occur.
- **Psychosocial support:** Chronic illness can have a significant impact on children and their families. Providing psychosocial support and resources may help cope with the diagnosis and associated challenges.
- **Prevention:** The importance of preventive measures, such as good hygiene practices, to reduce the risk of recurrent streptococcal infections, which can help prevent future episodes of APSGN.

Case 8

16.3-year-old boy: This patient presented with a sore throat, weakness, and mild fever for the past week. On examination, his blood pressure was 120/80 mm Hg (70th and 91st percentiles, respectively). Laboratory findings showed serum creatinine 2.02 mg/dL, serum albumin 4.1 g/L, Na 143 mmol/L, K 4.6 mmol/L, Ca 8.9 mg/dL, and P 3.8 mg/dL. Urinalysis revealed a red blood cell count of 10/HPF and a spot urine protein:creatinine ratio of 0.23 mg/mg. The complement C3 level was 1.17 g/L, and the ASO level was 488 IU/mL. The throat culture shows the growth of group A beta-hemolytic streptococcus.

What is the patient's diagnosis? What medications should be initiated for treatment? What points should be noted during follow-up?

ChatGPT Response

Based on the information provided, the diagnosis of the 16.3-year-old male patient was acute poststreptococcal glomerulonephritis (APSGN). This diagnosis is supported by a history of upper respiratory tract infection caused by beta-hemolytic streptococcus, elevated ASO (anti-streptolysin O) titer, normal serum C3 levels, high serum creatinine levels, hematuria, and proteinuria.

Treatment for APSGN typically involves the following processes:

- 1. Supportive care:** This includes rest, adequate hydration, and symptomatic management of any associated symptoms.
- 2. Blood pressure monitoring:** Although the blood pressure is normal in this case, regular monitoring is important to detect any potential changes, especially if hypertension develops.
- 3. Antibiotics:** Antibiotics may be prescribed to eradicate streptococcal infection and prevent further complications. Penicillin or other appropriate antibiotics are typically used.

In follow-up:

1. Regular monitoring of renal function, including serum creatinine levels, is essential for assessing renal function and monitoring for any worsening of the condition.
2. Monitoring of blood pressure, urine protein:creatinine ratio, and urine sediment should be performed to assess response to treatment and monitor for any complications.
3. Close communication with the nephrologist is important for ongoing management and treatment adjustment.
4. Education and support for the patient and family regarding medication adherence, follow-up appointments, and lifestyle modifications are important for long-term management.

Given the potential for complications, such as hypertension and renal impairment, regular follow-up with a nephrologist is crucial to ensure appropriate management and monitoring of APSGN.

Case 9

11.2-year-old girl: The patient presented with eyelid and leg edema. It was learned that she had an upper respiratory tract infection about 10 days ago. On examination, her blood pressure was 100/60 mm Hg (49th and 47th percentiles, respectively). Laboratory findings showed serum creatinine (0.68 mg/dL, serum albumin (4.1 g/L, Na 140 mmol/L, K 4 mmol/L, Ca 9.2 mg/dL, and P (4.3 mg/dL. Urinalysis revealed a red blood cell count of 17/HPF and a spot urine protein:creatinine ratio of 0.19 mg/mg. The complement C3 level was 0.35 g/L, and the ASO level was 730 IU/mL. The throat culture revealed no growth.

What is the patient's diagnosis? What medications should be initiated for treatment? What points should be noted during follow-up?

ChatGPT Response

Based on the information provided, the diagnosis of the 11.2-year-old female patient was acute poststreptococcal glomerulonephritis (APSGN). This diagnosis is supported by a history of upper respiratory tract infection, significantly elevated ASO (anti-streptolysin O) titer, markedly low serum C3 levels, and hematuria.

Treatment and management of APSGN in this case would involve the following

:1. Supportive care: This includes managing edema and addressing any associated symptoms, such as fatigue and malaise. Dietary modification is recommended to restrict salt intake and manage fluid retention.

2. Blood pressure monitoring: Although the blood pressure was normal in this case, regular monitoring was essential to detect any potential changes, as hypertension can develop in some cases of APSGN.

3. Antibiotics: Although the throat culture result was negative, the high ASO titer suggests recent streptococcal infection. However, antibiotics are not considered if the culture is negative.

Drug therapy:

- In the absence of hypertension or significant proteinuria, specific drug therapy may not be required. However, if hypertension develops or significant proteinuria and declining kidney function occur, ACE inhibitors (ACEi) or angiotensin II receptor blockers (ARBs) may be considered to help manage blood pressure and reduce proteinuria.

In follow-up:

1. Regular monitoring of renal function, including serum creatinine and urine sediment, is essential for assessing renal function and monitoring for any worsening of the condition.

2. Monitoring of blood pressure and urine protein:creatinine ratio should be performed to assess response to treatment and monitor for any complications.

3. Close communication with a nephrologist or pediatrician is important for ongoing management and treatment adjustment, as needed.

4. Education and support for the patient and family regarding medication adherence, follow-up appointments, and lifestyle modifications are important for long-term management.

Given the potential for complications, such as hypertension and renal impairment, regular follow-up with healthcare providers is crucial to ensure appropriate management and monitoring of APSGN.

Case 10

6-year-old boy: He presented with foot swelling. It was learned that he had an upper respiratory tract infection 9 days ago. On examination, his blood pressure was 160/100 mm Hg (above 99th percentile for both). Laboratory findings showed serum creatinine (0.87 mg/dL, serum albumin (3.1 g/L, Na 134 mmol/L, K 5.5 mmol/L, Ca 8.6 mg/dL, and P 5.8 mg/dL. Urinalysis revealed a red blood cell count of 141/HPF and a spot urine protein/creatinine ratio of 2.39 mg/mg. The complement C3 level was 0.24 g/L, and the ASO level was 578 IU/mL. The throat culture revealed no growth.

What is the patient's diagnosis? What medications should be initiated for treatment? What points should be noted during follow-up?

Based on the clinical information, the diagnosis of this patient was likely acute poststreptococcal glomerulonephritis (APSGN). Here is what should be done in terms of treatment and follow-up:

1. Diagnosis:

- **Acute Poststreptococcal Glomerulonephritis (APSGN):** This condition is suggested by a history of recent upper respiratory tract infection, high ASO titers, slightly low serum C3 levels, presence of hematuria and proteinuria, and hypertension.

2. Treatment:

- **Antibiotics:** Although the throat culture result was negative, the high ASO titer suggests recent streptococcal infection. However, antibiotics are not considered if the culture is negative.

- **Management of hyperkalemia:** Given the high serum potassium level, measures to lower potassium levels should be initiated promptly, such as dietary restriction of potassium and possibly administration of potassium-lowering medications like loop diuretics and potassium binders. Inhaled salbutamol and insulin can also be administered to selected patients.

- **Management of hypertension:** Blood pressure control is essential in the presence of hypertension. Depending on the severity of hypertension, antihypertensive medications, such as angiotensin-converting enzyme inhibitors (ACE inhibitors) or angiotensin receptor blockers (ARBs), may be administered.

- **Supportive care:** Supportive measures include rest, fluid balance, and monitoring for signs of fluid overload or electrolyte imbalance.

3. Follow-up:

- **Blood pressure monitoring:** Regular blood pressure monitoring is essential for assessing response to treatment and preventing complications associated with hypertension.

- **Renal function monitoring:** Periodic assessment of renal function, including serum creatinine and urine output, is necessary to evaluate renal function and recovery.

- **Follow-up urine analysis:** Urine analysis for hematuria and proteinuria can help assess the resolution of renal inflammation and injury.

- **Serum complement levels:** Although slightly low, serum complement levels may normalize as the inflammatory process resolves. Periodic monitoring of complement levels can provide additional information about disease activity and response to treatment.

- **Education and support:** Provide education to the patient and family about the condition, treatment plan, and importance of adherence to follow-up appointments. Emphasizing the signs and symptoms of complications and encouraging early reporting of any concerns or changes in health status.

Case 11

5-year-old boy: He presented with bloody urine. It was learned that he had an upper respiratory tract infection about 10 days ago. On examination, his blood pressure was 84/62 mm Hg (38th and 87th percentiles, respectively). Laboratory findings showed serum creatinine (0.50 mg/dL, serum albumin (3.6 g/L, Na 139 mmol/L, K 5 mmol/L, Ca 9.1 mg/dL, and P (4.3 mg/dL. Urinalysis revealed a red blood cell count of 168/HPF and a spot urine protein/creatinine ratio of 2.40 mg/mg. The complement C3 level was 0.26 g/L (low) and the ASO level was 1023 IU/mL (high). The throat culture shows Group A beta-hemolytic streptococcus.

What is the patient's diagnosis? What medications should be initiated for treatment? What points should be noted during follow-up?

ChatGPT Response

Based on the clinical information, the diagnosis of this patient was acute poststreptococcal glomerulonephritis (APSGN). Here is what should be done in terms of treatment and follow-up:

1. Treatment:

- Antibiotic treatment to eradicate group A beta-hemolytic streptococcal infection is essential. Penicillin or another appropriate antibiotic targeting streptococcus should be administered.
- Management of hyperkalemia is essential. Given the high serum potassium level, measures to lower potassium levels should be initiated promptly, such as dietary restriction of potassium and possibly administration of potassium-lowering medications like loop diuretics and potassium binders. Inhaled salbutamol and insulin can also be administered to selected patients.
- Supportive care to manage symptoms and complications. This step may include rest, fluid management, and monitoring for hypertension or fluid overload.

2. Follow-up:

- The close monitoring of clinical and laboratory parameters during the follow-up period.
- Regular assessment of blood pressure to detect hypertension, which can develop or worsen during the course of APSGN.
- Monitoring urine output and urine characteristics, including hematuria and proteinuria, to evaluate kidney function and recovery.
- Periodic measurement of serum creatinine and complement levels to assess renal function and resolution of the inflammatory process.
- Education and counseling for the patient and family regarding the importance of completing antibiotic treatment, adhering to follow-up appointments, and maintaining a healthy lifestyle.

Additional points to consider during follow-up:

- **Long-term sequelae:** Although APSGN generally has a good prognosis, some patients may develop complications, such as persistent hypertension or chronic kidney disease. Long-term follow-up is important to monitor the development of these complications and provide appropriate management.
- **Education:** It is essential to educate patients and caregivers about the signs and symptoms of complications and the importance of seeking medical attention if complications occur.
- **Psychosocial support:** Chronic illness can have a significant impact on children and their families. Providing psychosocial support and resources may help cope with the diagnosis and associated challenges.
- **Prevention:** The importance of preventive measures, such as good hygiene practices, to reduce the risk of recurrent streptococcal infections, which can help prevent future episodes of APSGN.



Letter to the Editor Regarding on the Manuscript "Characteristics of Posterior Ethmoidal Artery and its Relationship with Anterior Ethmoidal Artery and Skull Base on CT Scan"

Bilgisayarlı Tomografide Posterior Etmoidal Arterin Özellikleri ve Anterior Etmoidal Arter ile Kafa tabanı ile ilişkisi" Başlıklı Makaleyle İlgili Editöre Mektup

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Keywords: Ethmoid artery, skull base, CT scan, Vietnam

Anahtar kelimeler: Etmoid arter, kafatası tabanı, BT taraması, Vietnam

Dear Editor,

The research published by Tran Phan and Dang¹ on the anatomical features of the posterior ethmoid artery (PEA) and the distance from PEA to the skull base and anterior ethmoid artery in Vietnamese is interesting. Tran Phan and Dang¹ fortunately presented a few important study limitations. We now present another worthy one. Certain brain vessel variants and sino-nasal anatomic variations were found to be significantly more prevalent in some demographic groups and considerably vary compared to another when geographical and ethnic variations present enhancing the need for ethnicity-specific data^{2,3}. Vietnam is an Asian country featured by its prominent ethnic diversity, and different ethnic classes contribute to the rich tapestry of the Vietnam community. The major ethnic groups recruit Kinh, Thái, Tay, Mường, Khmer, Hmong, Nùng, Hoa, Dao, and other minorities⁴. Tran Phan and Dang¹ regrettably did not consider the ethnic disparities in their studied Vietnamese population. Hence, this

limitation might further corrode the applicability of the study findings in endoscopic transnasal and skull base surgeries.

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Response to Letter to the Editor on the Manuscript "Posterior Ethmoidal Artery and its Relationship with Anterior Ethmoidal Artery and Skull Base on CT Scan"

"Bilgisayarlı Tomografide Posterior Etmoidal Arterin Özellikleri ve Anterior Etmoidal Arter ile Kafa Tabanı ile İlişkisi" konulu makale için Yazılan Editöre Mektuba Yanıt

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Keywords: ethmoidal artery, anterior ethmoidal artery, skull base, CT scan

Anahtar kelimeler: Etmoid arter, kafatası tabanı, BT taraması, Vietnam

Dear Editor,

I am very grateful for the comments of authors¹.

Our study also has some limitations, as mentioned in the Introduction. Our study was conducted at Ho Chi Minh City Ear, Nose, and Throat (ENT) Hospital, which is the largest ENT hospital in the South of Vietnam².

In Vietnam, there are a total of 54 ethnic groups, but the majority are the Kinh (85.32%). All patients in our study were Kinh. All 54 ethnic groups in Vietnam are Asian, especially Southeast Asian³.

The studies you mentioned are all anatomical studies on different races, such as the white, black, and yellow races^{4,5}.

In our study, we also did not say that this was a representative study of Vietnam; it was only a study conducted at the Ho Chi Minh City ENT Hospital and medical school, Vietnam National University HCM City².

In the future, we hope to conduct further research on ethnic minorities in Vietnam to determine if there are any differences.

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