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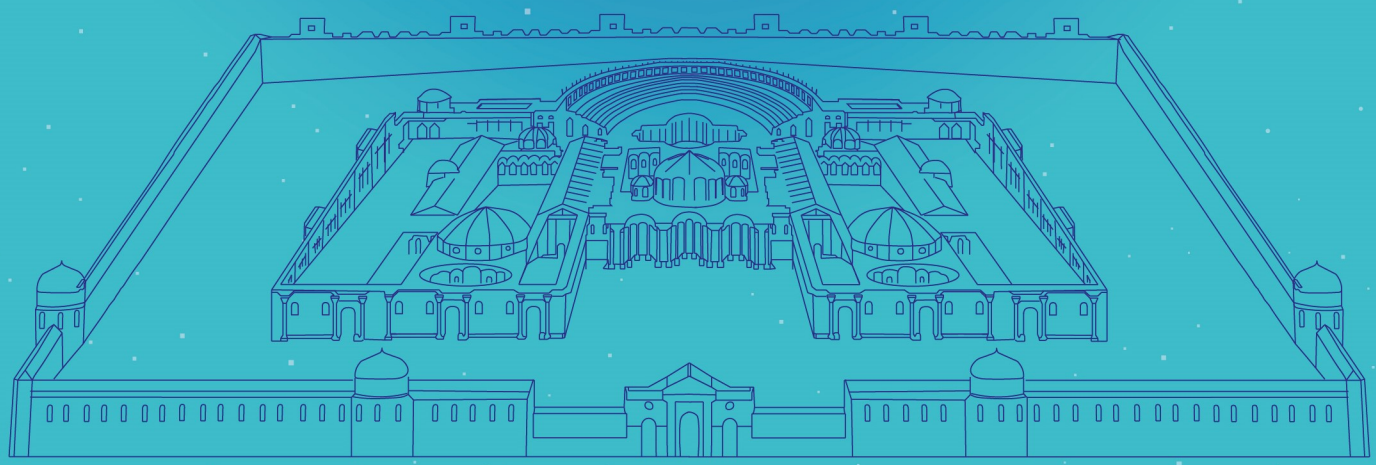

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Research Article | Araştırma Makalesi

ASSOCIATION OF N-TERMINAL PROHORMONE BRAIN NATRIURETIC PEPTIDE LEVEL AND ECHOCARDIOGRAPHIC LEFT VENTRICULAR SYSTOLIC OR DIASTOLIC DYSFUNCTION IN NON-ACUTE DYSPNEA

AKUT OLMAYAN DİSPNEDE N-TERMİNAL PROHORMON BEYİN NATRİÜRETİK PEPTİT SEVİYESİ İLE EKOKARDİYOGRAFİK SOL VENTRİKÜL SİSTOLİK VEYA DİYASTOLİK İŞLEV BOZUKLUĞU İLİŞKİSİ

Pelin Karaca Ozer[1*], Elif Ayduk Govdeli[1]

[1]Istanbul University, Istanbul Medical Faculty, Department of Cardiology, Istanbul, Türkiye.

ABSTRACT

Objective: The aim of the study was to evaluate serum N-terminal prohormone brain natriuretic peptide (NT-proBNP) level and evidence of left ventricular (LV) systolic dysfunction (SD) or diastolic dysfunction (DD) in non-obese patients with non-acute dyspnea.

Methods: This study retrospectively evaluated the serum NT-proBNP level and LV SD or DD from transthoracic echocardiography (TTE) in patients with non-acute dyspnea between October 2020 and October 2021. The normal limit for the serum NT-proBNP level (125 pg/ml) was used as the cut-off value.

Results: Ultimately, 435 patients were included in the study. In 61% of the patients (n=264), the NT-proBNP level was elevated (≥ 125 pg/ml). There was no evidence of SD or DD in 56% of the patients (n=147) with ≥ 125 pg/ml. The patients whose NT-proBNP ≥ 125 but who had no SD or DD had a significantly higher H2FPEF score ≥ 6, atrial fibrillation, malignancy, previous COVID-19, and need for hospitalization than the patients whose NT-proBNP < 125 and who had no SD or DD (13% vs. 4%; 5% vs. 1%; 16% vs. 9%; 29% vs. 5%; and 25% vs. 11%, respectively). An NT-proBNP value < 752.1 pg/ml excluded SD with 72.5% sensitivity and 83.1% specificity and < 350.3 pg/ml excluded DD with 71.3% sensitivity and 75.5% specificity.

Conclusion: A high NT-proBNP value does not always indicate SD or DD. NT-proBNP measurement may detect not only overt heart failure but also subclinical LV dysfunction in various clinical entities, in addition to adding prognostic significance in non-acute dyspnea.

Keywords: Dyspnea, heart failure, NT-proBNP

ÖZ

Amaç: Bu çalışmanın amacı, akut olmayan nefes darlığı şikayeti olan, obez olmayan hastalarda serum N-terminal prohormon beyin natriüretik peptit (NT-proBNP) düzeyinin ve sol ventrikül (SV) sistolik disfonksiyonunun (SD) veya diyastolik disfonksiyonunun (DD) kanıtlarını değerlendirmektir.

Yöntem: Bu çalışmada, Ekim 2020 ile Ekim 2021 arasında akut olmayan nefes darlığı şikayeti ile başvuran hastalarda transtorasik ekokardiyografi (TTE) ile değerlendirilen SV SD veya DD varlığı ile serum NT-proBNP düzeyi geriye dönük olarak değerlendirildi. Serum NT-proBNP düzeyi için normal sınır (125 pg/ml) cut-off değeri olarak kullanıldı.

Bulgular: Toplamda 435 hasta çalışmaya dahil edildi. Hastaların %61'inde (n=264), NT-proBNP düzeyi yüksekti (≥ 125 pg/ml). ≥ 125 pg/ml olan hastaların %56'sında (n=147) SD veya DD kanıtı yoktu. NT-proBNP'si artmış ancak SD veya DD'si olmayan hastalarda, H2FPEF skoru ≥ 6, atriyal fibrilasyon, malignite, önceki COVID-19 ve hastaneye yatış ihtiyacı olan hastalar, NT-proBNP'si normal olan ve SD veya DD olmayan hastalara göre anlamlı olarak daha yüksekti (sırasıyla %13'e karşı %4; %5'e karşı %1; %16'ya karşı %9; %29'a karşı %5; ve %25'e karşı %11). NT-proBNP değerinin < 752,1 pg/ml oluşu SD'u %72,5 duyarlılık ve %83,1 özgüllük ile ve < 350,3 pg/ml oluşu DD'u %71,3 duyarlılıkla ve %75,5 özgüllük ile dışladı.

Sonuç: Yüksek bir NT-proBNP değeri her zaman SD veya DD'yi göstermez. NT-proBNP ölçümü, akut olmayan dispnede prognostik önem eklemenin yanı sıra, çeşitli klinik durumlarda sadece aşikar kalp yetersizliğini değil, aynı zamanda subklinik SV disfonksiyonunu da saptayabilir.

Anahtar Kelimeler: Dispne, kalp yetersizliği, NT-proBNP

*** Corresponding author/İletişim kurulacak yazar:** Pelin Karaca Ozer; Istanbul University, Faculty of Medicine, Department of Cardiology. Topkapi Mahallesi, Turgut Ozal Millet Caddesi, 34093, Fatih/Istanbul, Türkiye.
Phone/Telefon: +90 (539) 717 05 01 **e-mail/e-posta:** pkaracaozer@gmail.com
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Introduction

Measurement of natriuretic peptides (NPs) is important for clinical decision-making when evaluating patients with suspected heart failure (HF).[1,2]

Studies of the role of NPs had shown that they are valuable in excluding the presence of HF due to their high sensitivity. However, the low specificity of the test that measures NPs limits its usefulness.[2,3] In addition, the lack of a precise reference range for the NP level indicative of HF creates difficulties in assessing the diagnostic utility of the test.[4]

Cut-off points for NPs have been recommended in guidelines, including the most recently published HF guidelines.[5,6] However, the frequent absence of echocardiographic HF with elevated brain NP (BNP) in outpatient clinics suggests that it is necessary to continue to evaluate the impact of clinical factors on decision cut-off points.

The diagnostic value of serum N-terminal prohormone brain natriuretic peptide (NT-proBNP), the decision cut-off points that maximize its diagnostic value, how it performs in daily practice in patients with non-acute dyspnea, and the interpretation of its high level in patients without HF are the areas of interest in this study.

Methods

This is a single-center, cross-sectional study conducted retrospectively on 435 non-obese patients who visited cardiology outpatient clinic between October 2020 and October 2021 with the complaint of dyspnea, whose NT-proBNP levels were measured and who underwent transthoracic echocardiography (TTE).

Patients with acute dyspnea and emergency presentations such as acute coronary syndrome, myocarditis, acute pulmonary embolism, and pneumonia; patients with explainable cardiac dyspnea such as moderate to severe valvular heart disease, cardiomyopathy; and patients with chronic renal failure and a body mass index (BMI) > 35 kg/m^2 were excluded from this study.

The study protocol was implemented according to the principles of the Declaration of Helsinki and was approved by the local Ethics Committee (approval number: 2067; 12/2021).

Conventional Echocardiographic Analysis

Examinations were performed using Vivid 7 (GE), IE33 (Philips), or Vivid T8 (GE) echo devices, with a middle-range frequency (3-8 MHz) broadband transducer to evaluate parasternal and apical images (2D, M-mode, Doppler echo), with the patient placed in the left lateral decubitus position. Images were obtained using the techniques recommended by the American Society of Echocardiography guidelines.[7]

LV systolic dysfunction (SD) was defined as LV ejection fraction (EF) < 50%. The presence or absence of diastolic dysfunction (DD) in patients with a normal LVEF are based on the assessment of four variables. These variables and their cutoff values include: Septal e' < 7 cm/sec or lateral e' < 10 cm/sec, average E/e' > 14, left atrial volume index (LAVI) > 34 mL/m^2, and peak TR velocity > 2.8 m/sec. DD is present if more than half of the available variables are abnormal (> 50% positive) according to the guidelines for the evaluation of LV diastolic function by TTE.[8]

Calculating H_2FPEF Score

(1) BMI > 30 kg/m^2 (H); (2) the use of ≥ 2 antihypertensive medications (H); (3) the presence of atrial fibrillation (AF) (F); (4) pulmonary hypertension defined as systolic pulmonary artery pressure (sPAP) > 35 mmHg (P); (5) an age > 60 years (E); and (6) elevated filling pressures evident from E/e' > 9 (F). The presence of paroxysmal or persistent AF yields 3 points, a BMI > 30 kg/m^2 yields 2 points, and all the other criteria listed above yield 1 point.[9]

Natriuretic Peptide Measurements

The same assay kits were used to measure each peptide in each patient. Specifically, NT-proBNP was measured using the Roche Elecys proBNP test (Roche Diagnostics, Indianapolis, IN). NT-proBNP levels were evaluated retrospectively from medical records.

Statistical Analysis

All statistical tests were conducted using the Statistical Package for the Social Sciences 26.0 for Windows (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to analyze the normality of the data. Continuous data are expressed as mean ± standard deviation (SD), and categorical data are expressed as percentages. A chi-square or Fisher's exact test was used to assess the differences in categorical variables between the groups. A Student's t-test or the Mann–Whitney U test was used to compare unpaired samples as needed. The relationships among the parameters were assessed using Pearson's or Spearman's correlation analysis according to the normality of the data. The primary analysis used ANOVA to compare all reported data for parametric variables, whereas the Kruskal–Wallis test was used for comparison among non-parametric variables between groups. Univariate and multivariate logistic regression analyses were used to identify the independent variables of hospitalization. The results of the univariate and multivariate regression analyses are presented as odds ratios with 95% confidence intervals (CIs). For the pro-BNP levels, receiver operating characteristic (ROC) curves were obtained, and the optimal values with the greatest total sensitivity and specificity in the exclusion of SD or DD were selected. Significance was assumed at a two-sided $p < 0.05$.

Results

A total of 435 patients were included in this study, the mean age of whom was 58±9.7 years. Sixty-one percent

(n=264) of them had an NT-proBNP level ≥ 125 pg/ml and thirty- nine percent (n=171) of them had an NT-proBNP level < 125 pg/ml. There were no statistically significant differences between the two groups in terms of age, gender, and BMI.

DD and SD were more frequent in the patients with NT-proBNP ≥ 125 pg/ml (p < 0.001 for both). Of the 264 patients with NT-proBNP ≥ 125 pg/ml, 34% (89 patients) had DD and 11% (28) had an EF < 50%; while of the 171 patients with NT-proBNP < 125 pg/ml, only 10 patients (6%) had DD and only three patients (2%) had an EF < 50%. The flow-chart diagram of the study was shown in Figure 1.

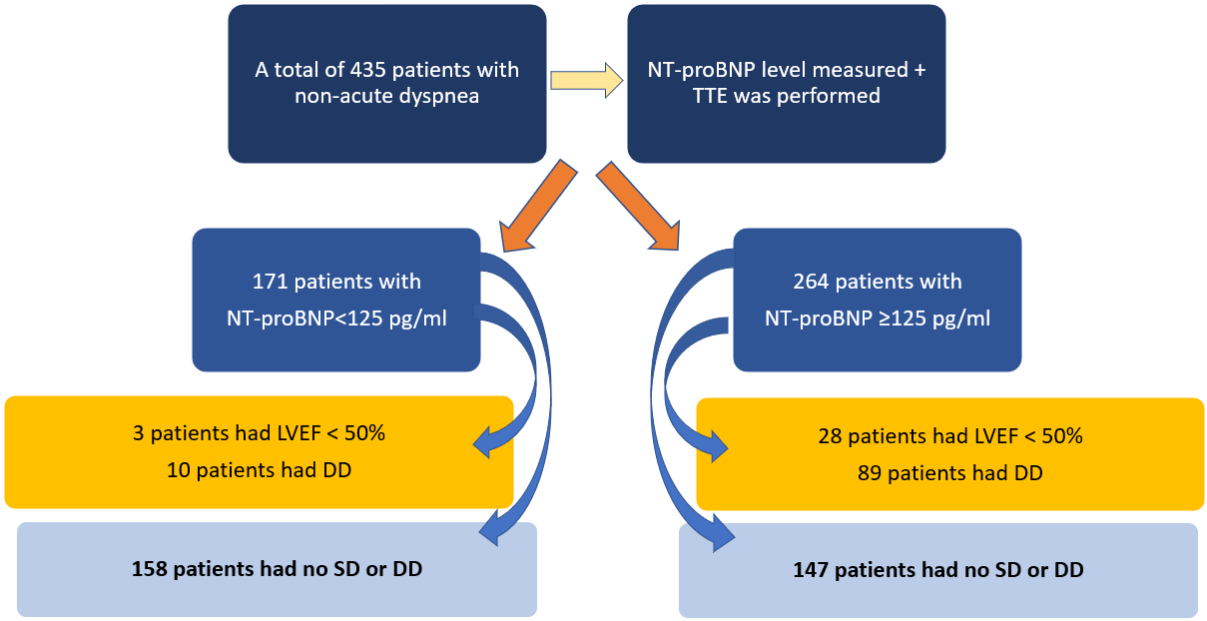

Figure 1. The flow-chart diagram of the study

Coronary artery disease (CAD), AF, recovery from coronavirus disease 2019 (COVID-19), and a need for hospitalization were more common in the patients with elevated NT-proBNP than in those without (36% vs. 18%, p = 0.002; 13% vs. 2%, p < 0.001; 26% vs. 6%, p < 0.001; and 31% vs. 11%, p < 0.001, respectively).

The demographic, clinical, and echocardiographic characteristics of the patients with NT-proBNP < 125 pg/ml and ≥ 125 pg/ml, as well as with SD and DD, are shown in Table 1.

There was no evidence of SD or DD with TTE in 158 (92%) of the patients with NT-proBNP < 125 pg/ml and in 147 (56%) of the patients with ≥ 125 pg/ml, for a total of 305 patients (70% of all the patients). The patients without SD or DD but with NT-proBNP ≥ 125 pg/ml had a significantly higher H2FPEF score ≥ 6, AF, malignancy, previous COVID-19 ailment, and need for hospitalization than those with NT-proBNP < 125 pg/ml (4% vs. 13%, p = 0.004; 1% vs. 5%, p = 0.013; 9% vs. 16%, p = 0.049; 5% vs. 29%, p < 0.001; and 11% vs. 25%, p = 0.002, respectively).

When the echocardiographic parameters were compared, in the patients without echocardiographic SD or DD but with NT-proBNP ≥ 125 pg/ml, the interventricular septal (IVS) thickness, LV mass index (LVMI), right ventricle (RV), right atrium (RA), LAVI, sPAP, and E/e' ratio were higher than in the patients with NT-proBNP < 125, and MAPSE was lower.

The demographic, clinical, and echocardiographic characteristics of the patients with NT-proBNP < 125 and ≥ 125 without SD or DD are shown in Table 2.

NT-proBNP was positively correlated with age, hs-troponin-T, sPAP, the H2FPEF score, LAVI, LVMI, the E/e' ratio, and the IVS thickness, but was negatively correlated with MAPSE, LV EF, and the E/A ratio (Table 3).

In the multivariate logistic regression analysis, the presence of AF (OR 3.247, 95% CI 1.127–9.352, p = 0.029) and the NT-proBNP level (OR 1.000, 95% CI 1.000–1.000, p = 0.006) were independent predictors of the need for hospitalization (Table 4).

An NT-proBNP level ≥ 125 pg/ml had a negative predictive value (NPV) of 98% and a positive predictive value (PPV) of 11% for SD, and an NPV of 94% and a PPV of 33% for DD.

ROC curve analysis was performed to show the specificity and sensitivity of NT-proBNP in excluding SD and DD.

An NT-proBNP value below 752.1 pg/ml excluded SD with 72.5% sensitivity and 83.1% specificity (AUC = 0.862, 95% CI = 0.812–0.912, p < 0.001) (Figure 2). The cut-off of 752.1 pg/ml for the NT-pro-BNP had a negative predictive value of 95.8% (95% CI = 93.57%–97.27%) for SD.

An NT-proBNP value below 350.3 pg/ml excluded DD with 71.3% sensitivity and 75.5% specificity (AUC = 0.809, 95% CI = 0.763–0.855, p < 0.001) (Figure 3). The cut-off of 350.3 pg/ml for the NT-proBNP had a negative predictive value of 88.85% (95% CI = 85.47%–91.52%) for DD.

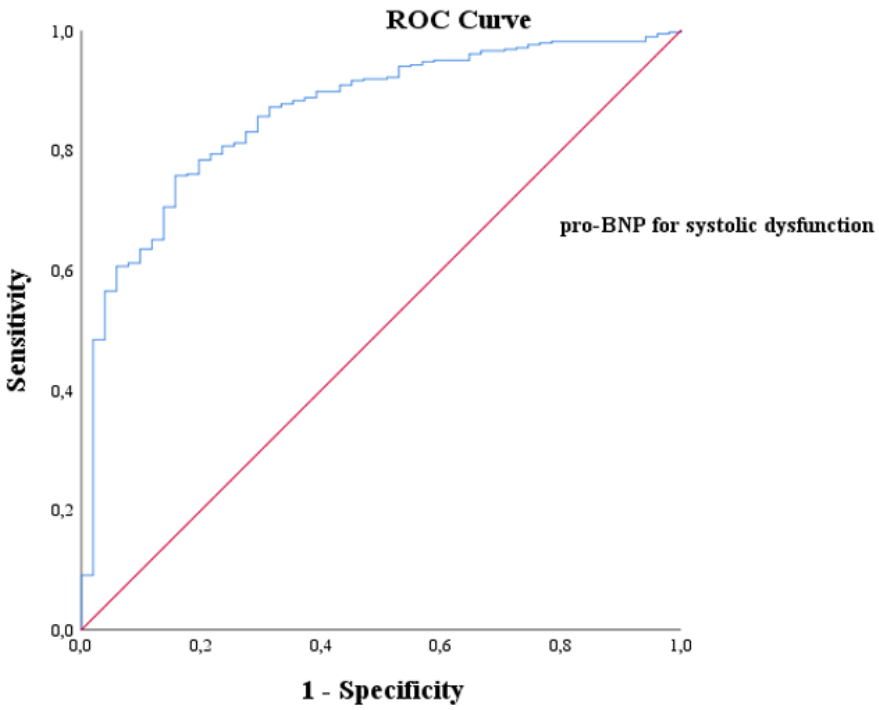

Figure 2. ROC curve analysis showing the specificity and sensitivity of the NT-proBNP in excluding systolic dysfunction

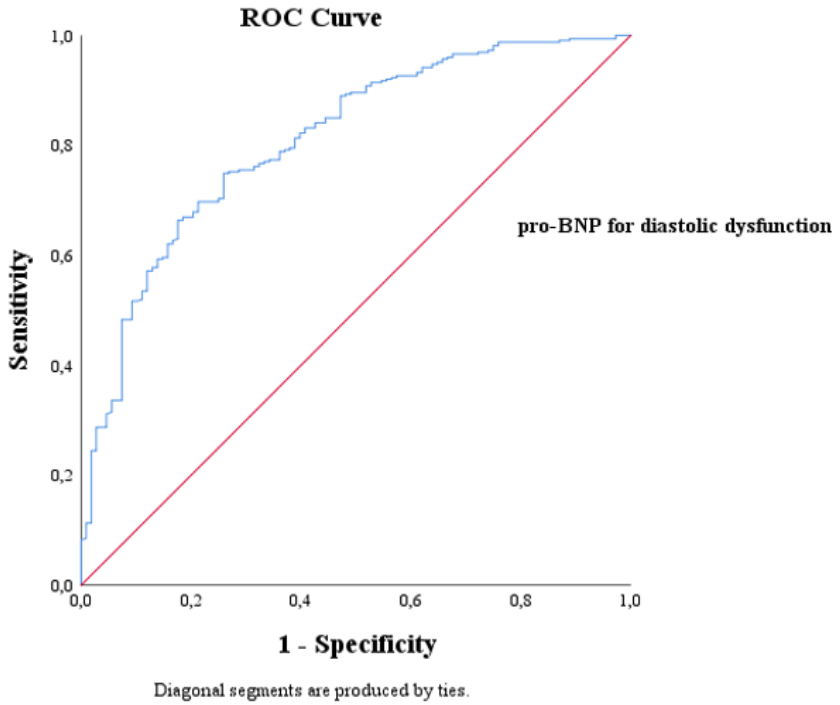

Figure 3. ROC curve analysis showing the specificity and sensitivity of the NT-proBNP in excluding diastolic dysfunction

Table 1. Demographic, clinical and echocardiographic characteristics of patients with NT-proBNP <125 and ≥125 pg/ml

	Total Patients (n=435)	**NT-proBNP<125 (n=171, 39%)**	**NT-proBNP≥125 (n=264, 61%)**	**p-value**
Age (year)	58.02 ± 9.7	54.18 ± 12.3	59.13 ± 7.8	0.083
Gender, Male, n(%) Female, n(%)	220 (50.6 %) 215 (49.4 %)	83 (48.5 %) 88 (51.5 %)	137 (51.9 %) 127 (48.1 %)	0.494
HR (bpm)	76.24 ± 13.9	76.33 ± 14.4	76.31 ± 14.4	0.722
BMI (kg/m^2)	28 ± 4.8	27.32 ± 4.3	28.63 ± 4.7	0.463
LV EF <50%	51 (12%)	3 (2%)	28 (11%)	<0.001*
LV diastolic dys + (n,%) diastolic dys – (n,%)	108 (25%) 327 (75%)	10 (6%) 161 (94%)	89 (33%) 166 (67%)	<0.001*
H_2FPEF Score ≥ 6, n(%)	64 (16.7 %)	8 (4.7 %)	56 (21.2 %)	<0.001*
H_2FPEF Score	2 (0-9)	1 (0-5)	3 (0-9)	<0.001*
HT, n(%)	135 (31%)	43 (25%)	92 (35%)	0.077
DM, n(%)	91 (21%)	30 (18%)	61 (23%)	0.178
CAD, n(%)	126 (29%)	30 (18%)	96 (36%)	0.002*
COPD, n(%)	78 (18%)	27 (16%)	51 (19%)	0.236
AF, n(%)	36 (8%)	3 (2%)	33 (13%)	<0.001*
Malignancy, n(%)	50 (12%)	14 (8%)	36 (14%)	0.082
Previous COVID-19, n(%)	80 (18%)	11 (6%)	69 (26%)	<0.001*
Hospitalization, n(%)	100 (23%)	19 (11%)	81 (31%)	<0.001*
Laboratory Findings				
Hgb (gr/dl)	12.67 ± 2.1	13.29 ± 1.6	12.19 ± 2.2	<0.001*
Creatinine (mg/dl)	0.8 ± 0.2	0.77 ± 0.2	0.82 ± 0.2	0.174
Hs-troponin-T (pg/ml)	4.47 (3-588)	3 (3-104)	7.13 (3-588)	<0.001*
NT-proBNP (pg/ml)	133.5 (6.95-14364)	65.63 (6.95-123.8)	383 (127.5-14364)	<0.001*
Treatment				
Beta-blocker, n(%)	147 (34%)	31 (18%)	116 (44%)	<0.001*
CCB, n(%)	95 (22%)	29 (17%)	66 (25%)	0.102
ACE inh/ ARB, n(%)	144 (32%)	32 (19%)	112 (42%)	<0.001*
Diuretic, n(%)	107 (25%)	15 (8%)	92 (35%)	<0.001*
Statin, n(%)	125 (28%)	39 (22%)	86 (33%)	0.028*
Chemotherapy, n(%)	50 (12%)	14 (8%)	36 (14%)	0.082
Echocardiography				
LVEDV (ml)	97.54 ± 21.9	97.1 ± 19.7	97.91 ± 23.9	<0.001*
LVESV (ml)	35.82 ± 16.7	33.69 ± 12	37.65 ± 19.7	<0.001*
EF (%)	62.56 ± 9	64.67 ± 6.1	60.75 ± 10.7	<0.001*
LVEDD (mm)	45.8 ± 4.2	45.75 ± 3.9	45.84 ± 4.6	<0.001*
IVS (mm)	11.3 ± 2.1	10.9 ± 1.6	11.7 ± 2	<0.001*
LV mass index (gr/m^2)	124.76 ± 39.5	111.96 ± 28.23	135.73 ± 44.5	<0.001*
LA (mm)	36.9 ± 4.9	35.5 ± 4.4	38.2 ± 5.1	<0.001*
RV (mm)	26.8 ± 2.6	26.6 ± 2.7	26.9 ± 2.5	<0.001*
RA (mm)	32.5 ± 3.7	31.6 ± 3	33.3 ± 0.4	<0.001*
E/A ratio	0.94 ± 0.4	1.04 ± 0.4	0.86 ± 0.4	0.008*
E/e' ratio	9.62 ± 3.2	8.59 ± 2.3	10.51 ± 3.5	<0.001*
LAVI (ml/m^2)	24.54 ± 10.6	21.75 ± 9	26.93 ± 11.3	<0.001*
CO (L/min)	5.02 ± 1.5	5.17 ± 1.4	4.15 ± 1.6	0.202
sPAP (mmHg)	28.46 ± 7.7	26.5 ± 6.7	30.14 ± 8.1	<0.001*
TAPSE (mm)	21.83 ± 5	21.6 ± 5.4	22.02 ± 4.6	0.006*
MAPSE (mm)	14.12 ± 1.8	15.27 ± 1.9	13.98 ± 1.8	<0.001*

Abbreviations: HR: heart rate, BMI: body mass index, HT: hypertension, DM: diabetes mellitus, CAD: coronary artery disease, COPD: chronic obstructive pulmonary disease, AF: atrial fibrillation, NT-proBNP: N-termina prohormone brain natriuretic peptid, Hs-troponin-T: high sensitive troponin-T, Hgb: haemoglobin, CCB: calcium channel blockers, ACE: angiotensin converting enzyme, ARB: angiotensin receptor blocker, LV: left ventricular, LVEDV: left ventricular end-diastolic volume, LVESV: left ventricular end-systolic volume, LVEF: left ventricular ejection fraction, LVMI: left ventricular mass index, LVEDD: left ventricular end-diastolic diameter, IVS: interventricular septum, LAVI: left atrial volume index, E: early diastolic transmitral flow, A: late diastolic transmitral flow, e': early diastolic tissue velocity, CO: cardiac output, RV: right ventricular, RA: right atrial, TAPSE: tricuspid annular plane systolic excursion, MAPSE: mitral annular plane systolic excursion. sPAP: pulmonary artery systolic pressure.

Table 2. Demographic, clinical, and echocardiographic characteristics of patients with NT-proBNP <125 and ≥125 pg/ml without systolic or diastolic dysfunction

	Total Patients (n=305)	**NT-proBNP<125 (n=158)**	**NT-proBNP≥125 (n=147)**	**p-value**
Age (year)	56.21 ± 9.1	55.77 ± 12	56.69 ± 4.4	0.990
Gender, Male, n(%)	145 (48%)	77 (49%)	68 (46%)	0.662
Female, n(%)	164 (52%)	83 (51%)	81 (54%)	
HR (bpm)	76.09 ± 14.1	75.33 ± 13.7	77.14 ± 14.8	0.963
BMI (kg/m^2)	26.92 ± 4.8	26.26 ± 4	27.74 ± 4.9	0.202
H_2FPEF Score ≥ 6, n(%)	25 (8%)	6 (4%)	19 (13%)	0.004*
H_2FPEF Score	1 (0-8)	1 (0-7)	2 (0-8)	<0.001*
HT, n(%)	65 (21%)	32 (20%)	33 (22%)	0.896
DM, n(%)	40 (13%)	18 (12%)	22 (15%)	0.530
CAD, n(%)	52 (17%)	23 (14%)	29 (20%)	0.145
COPD, n(%)	49 (16%)	23 (14%)	26 (18%)	0.192
AF, n(%)	9 (3%)	1 (1%)	8 (5%)	0.013*
Malignancy, n(%)	38 (12%)	14 (9%)	24 (16%)	0.049*
Previous COVID-19, n(%)	51 (17%)	8 (5%)	43 (29%)	<0.001*
Hospitalization, n(%)	55 (18%)	18 (11%)	37 (25%)	0.002*
Laboratory Findings				
Hgb (gr/dl)	12.72 ± 2	13.18 ± 1.8	12.17 ± 2.1	<0.001*
Creatinine (mg/dl)	0.79 ± 0.2	0.78 ± 0.2	0.80 ± 0.2	0.373
Hs-troponin-T (pg/ml)	3.62 (3-588)	3 (3-406)	5.64 (3-588)	<0.001*
NT-proBNP (pg/ml)	118.45 (6.95-4178.6)	71.53 (6.95-124.70)	271.3 (127.5-4178.6)	<0.001*
Treatment				
Beta-blocker, n(%)	66 (21%)	20 (13%)	46 (31%)	<0.001*
CCB, n(%)	48 (16%)	23 (14%)	25 (17%)	0.309
ACE inh/ ARB, n(%)	59 (19%)	24 (15%)	35 (23%)	0.025*
Diuretic, n(%)	43 (14%)	12 (8%)	31 (21%)	0.001*
Statin, n(%)	55 (18%)	21 (13%)	34 (23%)	0.027*
Chemotherapy, n(%)	38 (12.3 %)	14 (9%)	24 (16%)	0.049*
Echocardiography				
LVEDV (ml)	94.31 ± 17.7	92.62 ± 17.5	95.74 ± 17.9	0.324
LVESV (ml)	32.47 ± 8.8	32.28 ± 10	32.83 ± 7.7	0.091
EF (%)	64.75 ± 4.8	65.09 ± 4.6	64.85 ± 5.0	0.098
LVEDD (mm)	45.21 ± 3.6	45.5 ± 3.6	44.86 ± 3.6	0.324
IVS (mm)	11.1 ± 2	10.8 ± 2	11.3 ± 2	<0.001*
LV mass index (gr/m^2)	116 ± 30	110.42 ± 27.9	122.65 ± 31.5	<0.001*
LA (mm)	36.1 ± 0.5	35.5 ± 0.5	36.8 ± 0.5	<0.001*
RV (mm)	26.6 ± 0.2	26.4 ± 0.3	26.8 ± 0.2	0.023*
RA (mm)	32.1 ± 0.3	31.5 ± 0.3	32.8 ± 0.3	0.023*
E/A ratio	0.98 ± 0.4	1.06 ± 0.4	0.89 ± 0.4	<0.001*
E/e' ratio	8.79 ± 2.2	8.31 ± 2	9.36 ± 2.2	0.017*
LAVI (ml/m^2)	23.29 ± 10.2	21.89 ± 9.1	24.96 ± 11.2	0.003*
CO (L/min)	5.1 ± 1.5	5.3 ± 1.5	4.94 ± 1.5	0.707
sPAP (mmHg)	26.67 ± 6	25.84 ± 5.8	27.65 ± 6.2	<0.001*
TAPSE (mm)	22.11 ± 4.8	21.93 ± 4.6	22.32 ± 5.2	0.302
MAPSE (mm)	14.95 ± 1.7	15.86 ± 1.6	14.68 ± 1.9	<0.001*

Abbreviations: HR: heart rate, BMI: body mass index, HT: hypertension, DM: diabetes mellitus, CAD: coronary artery disease, COPD: chronic obstructive pulmonary disease, AF: atrial fibrillation, NT-proBNP: N-termina prohormone brain natriuretic peptid, Hs-troponin-T: high sensitive troponin-T, Hgb: haemoglobin, CCB: calcium channel blockers, ACE: angiotensin converting enzyme, ARB: angiotensin receptor blocker, LV: left ventricular, LVEDV: left ventricular end-diastolic volume, LVESV: left ventricular end-systolic volume, LVEF: left ventricular ejection fraction, LVMI: left ventricular mass index, LVEDD: left ventricular end-diastolic diameter, IVS: interventricular septum, LAVI: left atrial volume index, E: early diastolic transmitral flow, A: late diastolic transmitral flow, e': early diastolic tissue velocity, CO: cardiac output, RV: right ventricular, RA: right atrial, TAPSE: tricuspid annular plane systolic excursion, MAPSE: mitral annular plane systolic excursion. sPAP: pulmonary artery systolic pressure

Table 3. Correlation analysis of NT-proBNP with clinical, laboratory and echocardiographic parameters

	Variable	r	P
NT-proBNP	Age	0.200	<0.001*
	Hs-troponin-T	0.649	<0.001*
	Hgb	-0.357	0.385
	MAPSE	-0.317	<0.001*
	sPAP	0.475	<0.001*
	PA diameter	0.265	0.085
	H_2FPEF Score	0.405	<0.001*
	LVEF	-0.320	<0.001*
	LAVI	0.440	<0.001*
	LVMI	0.360	<0.001*
	E/A	-0.122	0.018*
	E/e'	0.414	<0.001*
	IVS thickness	0.306	<0.001*

Abbreviations: Hs-troponin-T: high sensitive troponin-T, Hgb: haemoglobin, LV: left ventricular, LVEF: left ventricular ejection fraction, LVMI: left ventricular mass index, IVS: interventricular septum, LAVI: left atrial volume index, E: early diastolic transmitral flow, A: late diastolic transmitral flow, e': early diastolic tissue velocity, MAPSE: mitral annular plane systolic excursion. sPAP: pulmonary artery systolic pressure, PA: pulmonary artery

Table 4. Multivariate logistic regression analysis of clinical and echocardiographic parameters predicting hospitalization

Variable	OR	95 % Confidence Interval	p-value
AF	3.247	1.127-9.352	0.029*
NT-proBNP	1.000	1.000-1.000	0.006*
LVEF	1.015	0.981-1.051	0.392
sPAP	0.987	0.953-1.022	0.453
CAD	0.958	0.914-1.004	0.070

Abbreviations: CAD: coronary artery disease, AF: atrial fibrillation, NT-proBNP: N-termina prohormone brain natriuretic peptid, LVEF: left ventricular ejection fraction, sPAP: pulmonary artery systolic pressure

Discussion

Of the 435 patients who were included in our study, most (61%, 264) has an NT-proBNP value higher than the cut-off, and 56% (147) of them had increased NT-proBNP levels but no evidence of SD or DD.

The patients without SD or DD but with NT-proBNP ≥ 125 pg/ml had a significantly higher H2FPEF score ≥ 6, AF, malignancy, previous COVID-19 ailment, and need for hospitalization than those with NT-pro-BNP < 125 pg/ml. Even though the EF was preserved and did not meet the criteria for DD according to the guidelines, we found that the IVS thickness, LVMI, RV, RA, LAVI, sPAP, and E/e' ratio were higher, and the MAPSE was lower, in the patients with a high NT-proBNP. These results show that a normal LVEF and the no DD may not necessarily mean complete echocardiographic normality.

Measurement of NPs and echocardiography are recommended in all patients with suspected chronic HF.[5,6] The upper limits of normality in the non-acute setting are 35 pg/mL for BNP and 125 pg/mL for NT-proBNP. In previous studies, the NPVs of the NP concentrations below the said thresholds ranged from 0.94 to 0.98.[9-12] In this study, the NPVs of < 125 pg/ml NT-proBNP were 98% for SD and 94% for DD. Apart from supporting the diagnosis of HF, high BNP concentrations also have prognostic significance.[13,14] In this study, the need for hospitalization for all causes increased significantly in patients with high BNP. It should be kept in mind that BNP may be elevated for cardiac reasons other than HF, or it can also be elevated for non-cardiovascular causes. These causes include AF, increasing age, and acute or chronic kidney disease. Conversely, NP concentrations may be disproportionately low in obese patients.[5,6,15] We therefore excluded patients with acute-chronic renal failure and BMI > 35 kg/m^2. In this study, the frequency of AF increased in the group without SD or DD but with high NT-proBNP. Since an H2FPEF score of ≥ 6 would most likely confirm the diagnosis of HF with preserved LVEF, a higher score in the group with high BNP but no DD in TTE is reasonable.

In addition, according to our data, the rate of diagnosis of malignancy and of chemotherapy treatment was significantly higher in the group without SD or DD but with high BNP. This makes sense given the cardiotoxic effects of chemotherapeutic agents.[16] In the absence of echocardiographic evidence of HF, elevated BNP appears to reflect subclinical myocardial involvement in this patient group, as shown in previous studies.

In fact, the most striking thing was that a high rate of 29% of the patients in the group with no evidence of HF but with high BNP had recovered from COVID-19. Among the frequent cases at our cardiology outpatient clinic are those of patients who complain of dyspnea and who had recovered from COVID-19 or are referred to us because of high BNP levels, or who request cardiac evaluation even if they are asymptomatic. In previous studies, COVID-19 was associated with subclinical myocardial involvement both during the active disease and after the patient's recovery. In one study, the NT-proBNP level was shown to be independently associated with in-hospital death rates in people with COVID-19 pneumonia and without HF, emphasizing its prognostic importance.[17] In a study that evaluated patients discharged from COVID-19, subclinical myocardial involvement with global longitudinal strain (GLS) was shown in both the left and right ventricles, but the BNP value was not mentioned.[18] Another study showed that the pro-BNP level was higher in patients with myocardial injury during hospitalization than in patients without myocardial injury, and that the patients with myocardial injury had impaired LV-GLS after recovery.[19] It is true that in our daily medical practice, there are many patients who had recovered from COVID-19 and are referred to us because of high pro-BNP, but we could not see any evidence of HF in the echocardiography. From this, it can be concluded that

COVID-19 may have a subclinical effect on the myocardium.

In the Breathing Not Properly trial, a cutoff of 100 pg/mL BNP was shown to have a sensitivity and specificity of 90% and 76%, respectively, for ruling out HF.[20] Similarly, the N-terminal Pro-BNP Investigation of Dyspnea in the Emergency department (PRIDE) study[21] and the International Collaborative of NT-pro-BNP (ICON) study demonstrated that the diagnostic performance of the measurement of NT-proBNP with a cut-off point of 300 pg/mL is similar to that with a BNP value of 100 pg/mL.[22] It should be noted that in this study, the cut-off values were found to be higher, especially for SD.

In this study, an NT-proBNP value below 752.1 pg/ml excluded SD with 72.5% sensitivity and 83.1% specificity, and a 752.1 pg/ml cut-off for pro-BNP had an NPV of 96% for SD. On the other hand, an NT-proBNP value below 350.3 pg/ml excluded DD with 71.3% sensitivity and 75.5% specificity, and a 350.3 pg/ml cut-off for pro-BNP had an NPV of 89% for DD.

This study showed that low pro-BNP values most likely exclude LV SD or DD in non-obese patients with non-acute dyspnea, but high values are not specific, so high cut-off values seem appropriate to use especially for SD. In addition, inthis study, the NT-proBNP cut-off value was higher than the NT-proBNP cut-off values in previous studies.

Morever, NT-proBNP was found to have prognostic significance and to be an important predictor of all-cause hospitalization. Patients who were undergoing chemotherapy and recovering from COVID-19 were frequently seen in the group with high BNP without echocardiographic HF. Although ongoing chemotherapy and recovery from COVID-19 are not considered among the causes of high BNP values in the guidelines, it should be emphasized that they characterize many cases at our cardiology outpatient clinic and are involved in many echocardiography indications in daily medical practice.

This study had limitations. First, it was retrospective, and the patients were not followed up. Also, strain measurement was not performed as an indicator of subclinical myocardial involvement, except when there was evidence of overt HF.

In conclusion, different diagnostic cut-off values of NT-proBNP for HF remain unclear. NP measurement may support the identification of patients with subclinical LV dysfunction, which will allow preventive measures to be taken to slow the progression of the condition to clinical HF. BNP concentrations should be interpreted in light of many clinical factors. Above all, this study clearly showed that NT-proBNP has prognostic value in presentations of non-acute dyspnea.

Compliance with Ethical Standards

The study was approved by the local Ethics Committee (approval number: 2067; 12/2021).

Conflict of Interest

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

Author Contribution

PKO: Study Conception; PKO, EAG: Study Design; PKO: Supervision; PKO, EAG: Materials; PKO, EAG: Data Collection and/or Processing; EAG: Statistical Analysis and/or Data Interpretation; PKO: Literature Review; PKO: Manuscript Preparation: PKO; and PKO, EAG: Critical Review

Financial Disclosure

None.

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Research Article | Araştırma Makalesi

EVALUATION OF THE SACRAL AND COCCYGEAL CURVATURES CALCULATED VIA COMPUTED TOMOGRAPHY IMAGES BASED ON GENDER

BİLGİSAYARLI TOMOGRAFİ GÖRÜNTÜLERİ ÜZERİNDEN HESAPLANAN OS SACRUM VE OS COCCYGİS EĞRİLİKLERİNİN CİNSİYETE GÖRE DEĞERLENDİRİLMESİ

Merve Nur Ozgen[1], Zulal Oner[2*], Mert Nahir[1], Serkan Oner[3]

[1]Tokat Gaziosmanpaşa University, Faculty of Medicine, Department of Anatomy, Tokat, Türkiye. [2]İzmir Bakırçay University, Faculty of Medicine, Department of Anatomy İzmir, Türkiye. [3]İzmir Bakırçay University, Faculty of Medicine, Department of Radiology, İzmir, Türkiye.

ABSTRACT

Objective: The skeletal structure has a significant role in the estimation of human gender. The os sacrum and os coccyx bones that constitute the pelvic skeleton are important in sex estimation due to their functional differences based on sex. In the present study, we aimed to determine the differences in os sacral and os coccygeal curvatures calculated with orthogonal plane computed tomography images based on gender.

Methods: Computed tomography images of 150 healthy individuals (75 females, 75 males) between the ages of 25-50 were used in the study. The computed tomography images were edited into a suitable format by the Horos software for measurement. Six sacral and coccygeal measurements, lumbosacral angle (LSA), sacral curvature (SC), sacral kyphosis (SK), sacrococcygeal angle (SCA), sacrococcygeal joint angle (SCJA), and coccygeal curvature (CC) were conducted on the sagittal image.

Results: The measurement results indicated that LSA and SCA values were higher in male subjects when compared to females, and SCJA values were higher in females when compared to males ($p \leq 0.05$). Quadratic Discriminant Analysis (QDA) results indicated that these parameters were 93.3% effective in estimating male gender, 85.3% effective in estimating female gender, with an overall estimation rate of 89.3%.

Conclusion: According to these results, it was concluded that the lumbosacral and sacrococcygeal joints appear flatter in men than in women. SC, SK and CC parameters did not show sexual dimorphism. Considering all the parameters we used, we achieved a high rate of gender discrimination.

Keywords: Computed tomography, gender prediction, discriminant analysis, coccyx, sacrum

ÖZ

Amaç: İnsanlarda cinsiyetin belirlenmesinde iskelet yapısı anahtar bir rol oynar. Pelvis iskeletini oluşturan os sacrum ve os coccygis, cinsiyete bağlı fonksiyonel farklılıklar nedeniyle cinsiyet tayini için önemli kemiklerdir. Bu çalışmada; ortogonal düzleme getirilmiş Bilgisayarlı Tomografi (BT) görüntüleri üzerinden hesaplanan os sacrum ve os coccygis eğriliklerinin cinsiyete göre farklılıklarını belirlemeyi amaçladık.

Yöntem: Çalışmada 25-50 yaş arası sağlıklı 150 bireye ait (75 Kadın, 75 Erkek) BT görüntüleri kullanıldı. Horos yazılımı ile BT görüntüleri ölçüm için uygun formatta düzenlendi. Sagittal görüntü üzerinde; os sacrum ve os coccygis üzerinden lumbosakral açı (LSA), sakral eğrilik (SE), sakral kifoz (SK), sakrokoksigeal açı (SKA), sakrokoksigeal eklem açısı (SKEA) ve koksigeal eğrilik (KE) olmak üzere 6 farklı ölçüm yapıldı.

Bulgular: Ölçüm sonuçlarına göre; LSA ve SKA değerleri, erkeklerde kadınlara göre yüksek, SKEA değeri de kadınlarda erkeklere göre yüksek olduğu tespit edildi ($p \leq 0,05$). QDA'ya göre tüm parametrelerden erkek bireyleri ayırt etme gücü %93,3, kadın bireyleri ayırt etme gücü %85,3 ve toplam ayırt etme gücü %89,3 bulundu.

Sonuç: Bu sonuçlara göre erkeklerde lumbosakral ve sakrokoksigeal eklemlerin kadınlara göre daha düz göründüğü sonucuna varıldı. SC, SK ve CC parametreleri cinsel dimorfizm göstermedi. Kullandığımız tüm parametreler dikkate alındığında yüksek oranda cinsiyet ayrımcılığı elde ettik.

Anahtar Kelimeler: Bilgisayarlı tomografi, cinsiyet tahmini, discriminant analizi, os coccygis, os sacrum

*** Corresponding author/İletişim kurulacak yazar:** Zulal Oner; Faculty of Medicine, Department of Anatomy, İzmir Bakırçay University, İzmir, 35665, Türkiye.
Phone/Telefon: +90 (232) 493 00 00-14154 **e-mail/e-posta:** zulal.oner@bakircay.edu.tr
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Introduction

It is necessary to know the ancestors, gender, age, and height of a human to define his/her biological identity in forensics and archeology.[1,2] However, major incidents such as air crashes, traffic accidents, and fire disasters might lead to problems in estimating the personal characteristics of individuals and their identities. Therefore, accuracy in sex estimation becomes highly significant in forensic and archaeological studies.[3] The pelvis and cranium bones, which were acknowledged as distinct dimorphic regions among other skeletal segments, can be widely used in sex estimation.[4,5] A plethora of studies was conducted on sex estimation based on almost all bones of the human skeleton and the accuracy of sex estimation was investigated across different populations.[6] Several studies reported that accuracy of sex estimation was 98% in skeletons with coxae and ossa cranii preserved together, 95% in skeletons with os coxae preserved alone, 90% in skeletons with ossa cranii preserved alone, and 80 to 90% in skeletons with both upper and lower extremity long bones preserved together.[7-10] The findings indicated that accurate results can be achieved even in cases where sex estimation is based only on certain bones of the skeleton. Methods such as DNA analysis, morphological, metric, geometric, morphometric, and probabilistic determination are used in sex estimation.[11] The highest accuracy is achieved via DNA analysis. However, it is still a technology that is difficult to access for developing and underdeveloped countries.[12,13] It is less complicated to evaluate and interpret the available numerical data using metric methods. Metric measurements obtained from Computed Tomography (CT) images can be used for reconstructive identification, comparative bone, and lesion identification.[14,15] CT is currently preferred as an effective method for sex estimation due to its sensitivity and due to being a low-cost, non-invasive, rapid, and reconstructive method.[11,16] It allows the researchers to re-orientate an image and constitutes new image series in different planes.

In the estimation of gender, the focus was primarily on the pelvis, which most evidently exhibited the difference between the genders, and the skull, where the size and morphological diversity were best represented.[17] The literature review revealed that the pelvis was a dimorphic region. It has been shown that the pelvis is the most reliable bone for sex determination.[4,18] The os sacrum and os coccyx in the pelvis are significant bones for gender estimation due to their functional differences.[19] The present study aims to estimate gender differences based on the sacrum and coccyx curvatures of healthy male and female individuals, calculated via the CT images, which were brought to the orthogonal plane.

Methods

The present study was approved by the Non-Interventional Clinical Research Ethics Committee, with decision number 6/3, on September 25th, 2019.

The CT images of individuals, aged between 25 and 50 years old, who were admitted to the Training and Research Hospital University, between January 2018 and June 2019 were reviewed. CT images of 110 female and 90 male subjects were randomly selected from the archive system, the Picture Archiving and Communication System (PACS). The subjects with significant degenerative diseases, bone pathologies, and history of surgery were not included in the study. As a result, CT images of 150 subjects, 75 females and 75 males, were selected for the present study.

All images were obtained using a 16-slice MDCT scanner (Aquilion 16; Toshiba Medical Systems, Tokyo, Japan) device. CT images with a slice thickness of 3 mm were obtained in the axial plane while the patients were in the supine position.

Computed Tomography

Computed Tomography is an imaging method that can show all tissues, especially bone tissues, with sharp boundaries. CT provides a three-dimensional view of the human skeleton with high bone resolution. CT allows us to measure virtual bones instead of dry bones. Since digital data is kept electronically in CT, it allows us to observe the images in detail anytime and anywhere, and to make accurate and reliable metric analysis. In addition, being re-measured and reinterpreted in data transfer between researchers increases estimation and reliability. Orientation are minor differences that may result from an individual's position on the CT device. Due to these position differences, some measurement errors due to orientation may occur during image analysis. These small errors can affect the measurement values. Therefore, in our study, all images were brought to the orthogonal plane to minimize measurement errors. In this way, it is possible to calculate the measured angles in a way that is less affected by the orientation.

Image Analysis

CT images in the PACS archive were saved in Digital Imaging and Communications in Medicine (DICOM) format and were transferred to Horos (Version 3.3, USA) personal workstation. Sagittal, transverse, and coronal image series were generated using the 3D Multiplanar Reconstruction (MPR) tool. The obtained images were brought to the standard bone dose. The images, focusing on the sacrum and the coccyx, with standard magnification, were adjusted orthogonally in three planes.

In obtaining the sagittal, transverse, and coronal image series, the sagittal images were aligned along the promontorium as the mid-axis, coronal images were aligned along the corpus vertebrae as the mid-axis, and the transverse images were aligned both along the processus spinosus of the vertebrae and the symphysis

pubis. Hence, all CT image series focusing on the sacrum with standard magnification were adjusted orthogonally in three planes (Figure 1).

The length and angle tools on the Horos Software were used to conduct measurements on the sagittal image series. The measurements for each of the six parameters were conducted at 3 separate times by the same researcher, to calculate the intra-observer reliability coefficient. 6 different curvature measurements were completed on the sagittal plane based on the parameter descriptions provided in Table 1: 1. Lumbosacral Angle (LSA), 2. Sacral Curvature (SC), 3. Sacral Kyphosis (SK), 4. Sacrococcygeal Angle (SCA), 5. Sacrococcygeal Joint Angle (SCJA), 6. Coccygeal Curvature (CC) (Figure 2).

Table 1. Sagittal plane measurement

Parameters	Details
Lumbosacral Angle (LSA)	The obtuse angle between the line that joins the midpoints of the upper and lower borders of the 5th lumbar vertebra and the line that joins the midpoints of the upper and lower borders of the 1st sacral vertebra
Sacral Curvature (SC)	The acute angle between the line that joins the midpoints of the upper and lower borders of the 1st sacral vertebra and the line that joins the midpoints of the upper and lower borders of the 5th sacral vertebra
Sacral Kyphosis (SK)	The acute angle between the line that joins the midpoints of the upper and lower borders of the 1st sacral vertebra and the line that joins the midpoints of the lower borders of the 2nd and 4th sacral vertebrae
Sacrococcygeal Angle (SCA)	The obtuse angle between the line that extends from the promontorium to the sacrococcygeal joint and the line that extends from the end of the last coccyx to the sacrococcygeal joint
Sacrococcygeal Joint Angle (SCJA)	The acute angle between the line that joins the midpoints of the upper and lower borders of the 5th sacral vertebra and the line that joins the midpoints of the upper and lower borders of the 1st coccygeal vertebra
Coccygeal Curvature (CC)	The acute angle between the line that joins the midpoints of the upper and lower borders of the 1st coccygeal vertebra and the line that joins the midpoints of the upper and lower borders of the last coccygeal vertebra

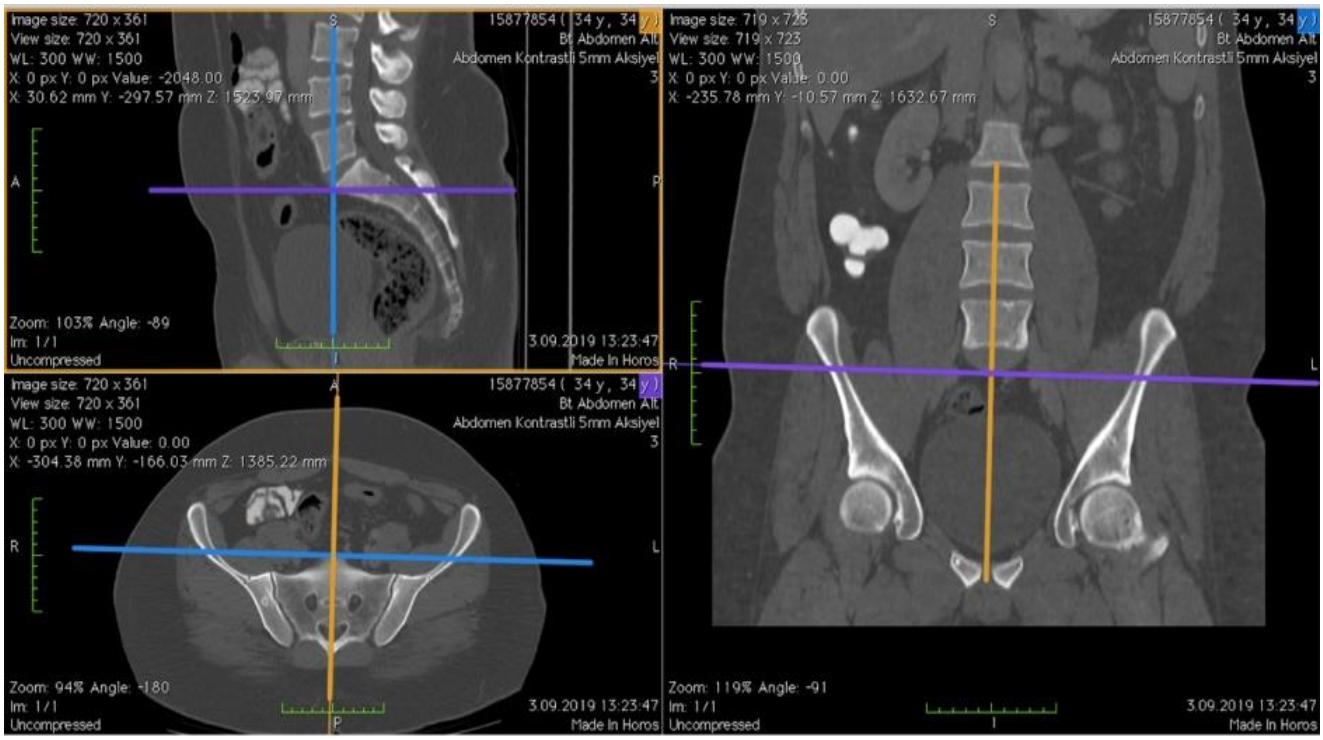

Figure 1. CT Images on the 3D Orthogonal Plane in Horos Software

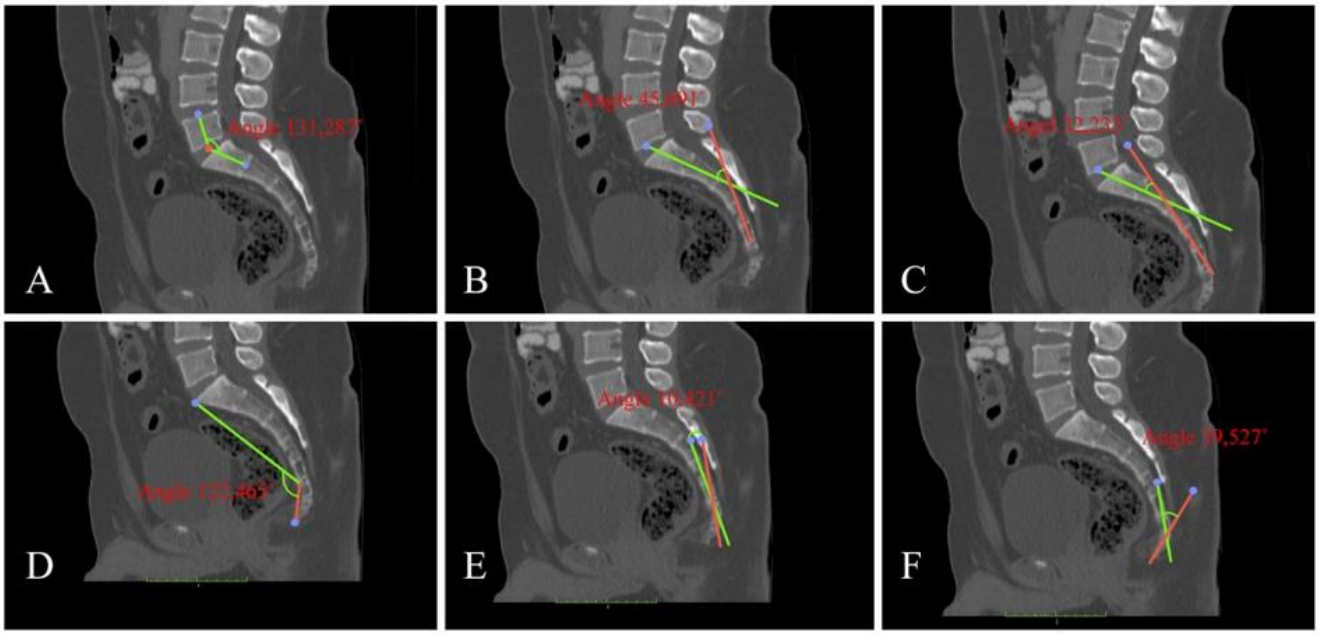

Figure 2. A: Measurement of Lumbosacral Angle, B: Measurement of Sacral Curvature, C: Measurement of Sacral Kyphosis, D: Measurement of Sacrococcygeal Angle, E: Measurement of Sacrococcygeal Joint Angle, F: Measurement of Coccygeal Curvature

Statistical Analysis

Descriptive analyses were conducted to obtain information on the characteristics of the study groups. Normality was tested with the Anderson-Darling test, which was applied for each data set. Two Sample t-test was used to study the relationship between the normally distributed data for the male and female subjects, and the Mann-Whitney U test was used to analyze the data that did not exhibit normal distribution ($p \leq 0.05$). Quadratic Discriminant Analysis (QDA) was used to analyze sex estimation. Minitab 17 software was used for the QDA analysis.

Reliability

Intraclass correlation coefficient (ICC) was used to calculate intra-observer reliability. Reliability value ranges between 0 and 1, with values closer to 1 representing stronger reliability.[20] In this study, the ICC value for intra-observer reliability was found as 0.98 for all quantitative measures. Such ICC value indicated high reproducibility.

Results

The mean ages of the 75 female and 75 male subjects, who were included in the study, were 39.2±7.2 and 40.8±6.2, respectively ($p>0.05$).

The obtained data sets were tested for normality with the Anderson-Darling test and it was concluded that the data were not normally distributed. The Mann-Whitney U test results revealed a statistically significant difference between genders based on the parameters, LSA, SCA, and SCJA ($p \leq 0.05$). It was found that LSA and SCA measurements were higher in male subjects when compared to those of female subjects. As a result of LSA measurements, it was found that the os sacrum is located more posteriorly in women than in men compared to the 5th lumbar vertebra. With the decrease in SCA value measured between os sacrum and os coccygis, os sacrum and os coccygis are positioned more backwards. Therefore, as a result of SCA measurements, it was found that the os sacrum and os coccygis were located behind in women and more in front in men. The SCJA values were larger in female subjects when compared to male subjects. Accordingly, it was found that the position of the os coccygis relative to the 5th sacral vertebra was lower in women than in men. There was no statistically significant difference between genders based on the parameters SC, SK, and CC ($p>0.05$) (Table 2).

Table 2. The comparison of the mean values of the parameters that did not exhibit normal distribution

	Female Median° (Min-Max)	Male Median° (Min-Max)	P
LSA	131.76 (114.17-152.52)	133.69 (118.31-156.54)	**≤0.05**
SC	50.95 (24.70-83.33)	51.08 (20.91-81.78)	0.37
SK	26.15 (9.96-53.55)	28.20 (10.42-60.90)	0.14
SCA	105.15 (73.75-143.59)	110.13 (85.54-142.29)	**≤0.05**
SCJA	17.16 (4.11-52.39)	11.19 (1.93-57.34)	**≤0.05**
CC	45.36 (2.49-94.90)	43.04 (10.78-86.88)	0.12

LSA: Lumbosacral Angle, SC: Sacral Curvature, SK: Sacral Kyphosis, SCA: Sacrococcygeal Angle, SCJA: Sacrococcygeal Joint Angle, CC: Coccygeal Curvature

In the QDA performed with six different parameters, when the average of all values in terms of gender is examined; in all parameters, the power to distinguish male individuals was 93.3%, the power to distinguish female individuals was 85.3%, and the total discrimination power was 89.3%. In other words, the adopted method in the present study was accurate in the sex estimation of 70 out of 75 male subjects and 64 out of 75 female subjects.

Discussion

As a hypothesis, in this study, we planned to reveal the differences between the sexes of the sacrum and coccyx curvatures, which are included in the pelvis skeleton, and it was determined that these bones showed dimorphism with a high accuracy rate. Studies investigating the morphometric measurements of the os sacrum in literature were commonly performed using dry bone, direct radiography, and CT methods.[15,21-23] In the literature, there is rare information about whether the images are brought to the orthogonal plane in studies using direct radiography and CT methods. In this study, the measurement technique used by Oner et al. and Turan et al.[24,25] were used. Measurements were made on CT images brought to the orthogonal plane so that the images were not affected by the orientation. However, it should be kept in mind that although CT provides multiparametric and realistic data in osteometric measurements, the sagittal measurements we use in particular can be used in centers working with post-mortem imaging.

A plethora of studies in literature conducted lumbosacral angle (LSA) measurements using the Ferguson Technique. In this technique, the acute angle between the line drawn along the upper-end plane of the sacral base and the horizontal line along the corpus of the 1st sacral vertebra is measured.[26-29] Okpala et al.[30] conducted radiological studies using the Ferguson Technique on 274 and Oyakhire et al.[27] on 220 black subjects and reported that the LSA value did not have a significant difference between the genders. The findings of the present study indicated that the LSA value was higher in male subjects compared to that of female subjects. It is the backward position of the sacrum relative to the 5th lumbar vertebra in the LSA measurement. Accordingly, as the LSA value decreases, the sacrum is positioned more backwards than the 5th lumbar vertebra. For this reason, the sacrum of women is located further back than men. Such finding is dissimilar to the findings in the literature and the dissimilarity is based on the difference in measurement methods, body position, gender, race, and working conditions, which affect LSA values. Our study revealed that LSA is one of the parameters that can be used to differentiate gender. In addition, LSA is the angle that gives normal lordosis to the waist. When examined clinically, it is thought that the decrease in LSA value affects the flattening of the lumbar lordosis in individuals. According to, low or high angulation of LSA is likely to be associated with low back pain.

Trinh et al.[31] conducted a radiological study on 40 subjects and obtained sacral curvature (SC) measurements based on the angle between the lines passing through the 1st and 5th sacral vertebrae in the sagittal plane and the lateral edges of the corpus. Trinh suggested that it was not possible to estimate gender between the males and females based on the SC measurements. However, the corpus of a vertebra increases from top to bottom. Therefore, the margin of error in the measurement increases. Woon et al.[32] conducted a retrospective study with 112 subjects and adopted a similar measurement approach with the present study, using pelvis CT images. Their findings indicated that there was no significant difference between the genders in SC value. Such findings were parallel with the data obtained in our study.

Wang et al.[26] conducted a study with 120 subjects and reported that measuring the kyphotic deformity in the sacral segment by the Cobb method was more effective in defining the sacral kyphosis (SK) value. Erbek[33] adopted the same method and established that SK values in healthy subjects did not exhibit a significant difference based on gender. We determined no statistically significant difference for the SK values in the present study.

Yoon et al.[34] conducted a study with 606 subjects and found that there were no significant differences in sacrococcygeal angle (SCA) values based on gender. In their study, they measured the SCA as the angle between a line from the midpoint of the S1 superior endplate to the midpoint of the S5 inferior endplate and a line from the midpoint of the S5 inferior endplate to the tip of the last coccygeal segment. Unlike other studies, we based the promontory in the measurement of SCA. Because we think that this measurement is more practical and can be adapted to dry bone measurements. In our study, os sacrum and os coccygis are positioned more backwards with the decrease in SCA value measured between os sacrum and os coccygis. Therefore, it was found that os sacrum and os coccygis are located more backwards in women and more anteriorly in men. Such finding indicated that SCA was a significant parameter in the estimation of gender. When examined clinically, we can emphasize that the SCA value is especially important in women in terms of delivery. We think that the data obtained as a result of the study will contribute to basic and clinical research on os sacrum in the future.

Woon et al.[32] conducted a study with 112 European subjects and stated that the sacrococcygeal joint angle (SCJA) values did not have a significant difference based on gender. In the study of Marwan et al.[35] with 202 Arab adults, they reported that os coccygis had a more ventral angle in women based on morphometric measurements. Also, the fact that men have longer and straighter coccyges and sacrums than women indicates a possible higher risk of coccidinia in women. In our study, when the SCJA value was examined, it was found that it was wider in female individuals than in male individuals.

Accordingly, the position of the os coccygis relative to the 5th sacral vertebra was found to be more dorsally in women than in men. Thus, the present study showed that the SCJA value is a significant parameter in estimating gender, despite the other findings in the literature. We argue that such difference might be due to population and environmental differences.

Kim and Suk[36] stated that the intercoccygeal angle is a useful radiological measurement in accurately assessing the increasing angular deformity of the coccyx. The coccygeal curvature (CC) value in the present study was named as the intercoccygeal angle in the literature. Kim and Suk[36] examined the CC values from the radiography of 20 Korean adult subjects and were not able to comment on sexual dimorphism due to insufficient data. Another larger CT study evaluated the CC values of 92 subjects, and it was reported that os coccyx tended to be slightly flatter in females.[37] However, the present study established no significant difference in CC values based on gender. However, the increased intercoccygeal angle can be considered as a possible cause of idiopathic coccygodynia. Insufficient sample size of the present study, different population and environmental factors might yield different findings.

A comparison of the findings in our study and the findings in the literature revealed differences in metric values and statistical results. We argue that such differences stem from the differences in measurement, racial diversities, genetic and socioeconomic differences. Furthermore, the present study conducted measurements by bringing the CT images to the orthogonal plane to minimize the margin of error and to obtain more reliable results. Hence, such an approach is the most significant factor that differentiates our study from other studies. In three-dimensional structures, the angle value differs based on the plane. Our study presents a different approach than other studies in literature, through bringing the images to the orthogonal plane and enabling more reliable measurements.

Conclusion

The measurements conducted on the os sacrum and os coccyx curvatures revealed that the LSA and SCA values were higher in male subjects when compared to female subjects, and the SCJA value was higher in females when compared to males. According to these results, it can be said that the lumbosacral and sacrococcygeal joints appear flatter in men than in women. SC, SK and CC parameters did not show sexual dimorphism. The QDA, which was performed with six different parameters, showed 93.3% discrimination power for males, 85.3% for females and 89.3% for total discrimination power. Although this article provides measurements that reveal the sex differences of the sacrum and coccyx, it may contribute to the units working with post-mortem imaging in terms of the method used.

Compliance with Ethical Standards

Our study was evaluated by Karabuk University Non-Interventional Clinical Research Ethics Committee and approval was obtained with the decision number 6/3 dated 25.09.2019.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contribution

MNO: Project development, data collection, manuscript writing; ZO: Manuscript writing and editing; MN: Manuscript writing and editing; SO: Manuscript writing and data analysis

Financial Disclosure

None.

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Araştırma Makalesi | Research Article

ELEKTİF KORONER ARTER BYPASS CERRAHİSİNDE TRANEKSAMİK ASİT UYGULAMA TEKNİĞİNİN ETKİNLİĞİ

TRANEXAMIC ACID IN ELECTIVE CORONARY ARTERY BYPASS SURGERY: EFFECTIVENESS OF APPLICATION TECHNIQUE

Cenk İndelen[1*]

[1]Amerikan Hastanesi, Kalp Cerrahisi Departmanı, İstanbul, Türkiye.

ÖZ

Amaç: Bu çalışmanın amacı, elektif koroner arter bypass cerrahisi geçiren hastalarda traneksamik asitin kardiyopulmoner bypass öncesi ve sonrası uygulanmaya başlanmasının etkilerini değerlendirmektir.

Yöntem: Ocak 2002 ile Aralık 2007 yılları arasında 3729 izole elektif koroner arter bypass greft operasyonu geçiren hastaların rutin klinik takip arşiv kayıtları retrospektif olarak incelendi. Örneklem büyüklüğü her grupta 100 hasta olacak şekilde oluşturuldu. Grup 1, traneksamik asit verilmemiş kontrol grubudur. Grup 2, cerrahi insizyondan 20 dakika önce traneksamik asit 30 mg/kg yavaş bolus dozunda uygulanmış ve kardiyopulmoner bypass sonunda, protamin verilmesi bittikten 10 dakika sonra 10 mg/kg dozunda traneksamik asit başlanmış ve 4 saat boyunca infüzyona devam edilmiş gruptur. Grup 3, kardiyopulmoner bypass sonunda, protamin verilmesi bittikten 10 dakika sonra traneksamik asit 30 mg/kg yavaş bolus dozunda uygulanmış ve devamında 10 mg/kg dozunda başlanmış ve 4 saat boyunca infüzyona devam edilmiş gruptur. İstatistiksel olarak ANOVA, post hoc Tukey HSD, Pearson chi kare, Fisher's exact yöntemleri ile analizler yapıldı.

Bulgular: Traneksamik asit uygulama farklılığının postoperatif kanama miktarına anlamlı etkisi olmadığı görüldü. Yoğun bakım kalış süresini etkilemediği belirlendi. Kardiyopulmoner bypass sonrası traneksamik asit uygulamasının eritrosit transfüzyonunda olumlu anlamlı etkisi olmadığı görüldü. Ancak inme istatistiksel anlamlı olarak Grup 3'te daha fazla bulundu.

Sonuç: Traneksamik asit uygulama ve dozları ile ilgili ortak bir klinik yaklaşım yoktur. Kılavuzlar transfüzyon miktarının azaltılması ve "Hasta Kan Yönetimi" uygulamaları için traneksamik asit kullanımını önermektedir. İnme komplikasyonu tedirgin edicidir.

Anahtar Kelimeler: Traneksamik asit, antifibrinolitik, kan kaybı azaltılması

ABSTRACT

Objective: The aim of this study is to evaluate the effects of initiating the administration of tranexamic acid before and after cardiopulmonary bypass in patients undergoing elective coronary artery bypass surgery.

Methods: Records of 3729 isolated elective coronary artery bypass graft operations between January 2002 and December 2007 were retrospectively reviewed. The sample size was created to include 100 patients in each group. Group 1 is the control group that did not receive tranexamic acid. Group 2 is the group in which tranexamic acid was administered at a slow bolus dose of 30 mg/kg 20 minutes before the surgical incision, and at the end of cardiopulmonary bypass, 10 mg/kg tranexamic acid was started 10 minutes after the end of protamine administration and the infusion was continued for 4 hours. Group 3 is the group in which tranexamic acid was administered at a slow bolus dose of 30 mg/kg 10 minutes after the end of protamine administration at the end of cardiopulmonary bypass, followed by a dose of 10 mg/kg and continued infusion for 4 hours. Statistically, ANOVA analysis, post hoc Tukey HSD, Pearson Chi-square, and Fisher's exact methods were used.

Results: It was observed that the difference in tranexamic acid administration did not have a significant effect on the amount of postoperative bleeding. It was determined that it did not affect the length of stay in the intensive care unit. It was observed that tranexamic acid administration after cardiopulmonary bypass had no positive and significant effect on erythrocyte transfusion. However, stroke, which is a serious complication, was statistically higher in Group 3.

Conclusion: There is no common clinical approach regarding the administration and doses of tranexamic acid. Guidelines recommend the use of tranexamic acid for reducing the amount of blood transfused and for "patient blood management" applications. The complications of stroke are troubling.

Keywords: Tranexamic acid, antifibrinolytic, blood loss reduction

***İletişim kurulacak yazar/Corresponding author:** Cenk İndelen; Amerikan Hastanesi, Kalp Cerrahisi Departmanı, İstanbul, Türkiye.
Telefon/Phone: +90 (532) 447 54 13 **e-posta/e-mail:** cindelen@gmail.com
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Giriş

Kardiyovasküler cerrahide postoperatif kanama, mortalite ve morbidite ile ilişkilidir. Kardiyovasküler cerrahi sonrası kanama revizyonuna alınan hastaların yarısında cerrahi kanama odağı bulunamaz. Bu hastalarda koagülopatiye bağlı sızıntı (*using*) kanama olmaktadır.[1] Postoperatif kanamalar multifaktöriyeldir. Kardiyovasküler cerrahi sonrası kardiyopulmoner bypassın (KPB) olumsuz etkisi ile kanamaya ciddi bir eğilim oluşur.[1] KPB sistemik enflamatuvar yanıtı, fibrinolitik sistemi ve kompleman aktivasyonunu tetikler. Platelet adezyon ve agregasyonu bozulur. Ayrıca hipotermi koagülatif enzim çalışmasını yavaşlatır, faktörlerinin inhibisyonuna yol açar.[2] Traneksamik asit *(trans-4-aminometil siklohexan-1-karboksi asit,* ***TXA***) sentetik lizin analoğudur, plazminojenin kompetatif inhibitörüdür. TXA, plazminojen molekülleri üzerindeki lizin bağlanan bölgeleri geri dönüşümlü bloke ederek fibrinolizisi engeller. Yarılanma ömrü 120 dakikadır. İdrardan değişmeden ıtrah edilir. TXA kanama/pıhtılaşma bozukluklarının tedavisi ve postoperatif hastalarda kan kaybını azaltmak için uzun yıllardır yaygın olarak kullanılmaktadır.[3] Ayrıca, antienflamatuar etkilidir; sitokin salımı ve enflamatuar hücrelerin migrasyonunu engeller.[4]

Kan bileşenlerinin temininde günümüzde yaşanılan zorluklar, yüksek maliyetler ve kalite standartları nedeniyle dünyada "Hasta Kan Yönetimi" (HKY) birçok merkezde uygulanmaktadır. Farmakolojik ajanların kullanımı HKY prosedürleri içinde önemini giderek arttıran bir yöntemdir.[5] TXA, kardiyak prosedürler sırasında kan kaybını azaltmak için kılavuzlarda Class I, Level A önerilmektedir.[6]

Bu çalışmanın amacı, elektif koroner arter bypass cerrahisi geçiren hastalarda TXA'nın KPB öncesi ve sonrası uygulanmaya başlanmasının etkilerini değerlendirmektir. Birincil güvenlilik son noktası, ameliyat sonrası erken miyokart enfarktüsü, inme, tromboemboli (arteryal/venüz) olayları değerlendirmektir. Birincil etkinlik son nokta, eritrosit süspansiyonu transfüzyonu miktarı ve yoğun bakım (YB) kalış süresine etkiyi ölçmektir.

Yöntem

Ocak 2002 ile Aralık 2007 yılları arasında İsviçre Hastanesinde yapılan 3729 izole elektif koroner arter bypass greft operasyonu verileri retrospektif olarak incelendi. Bu çalışmada değerlendirilen her iki rutin klinik uygulama yönteminin verileri bu kayıtlardan geriye dönük olarak sağlanmıştır.[7] Kardiyak anestezistlerin TXA kullanımı için farklı klinik yaklaşımları vardı. Genellikle nadir kan grubuna sahip, preoperatif hazırlanan kan bileşeni sayısı daha az olan ve donör sıkıntısı yaşanan hastalarda TXA uygulanmaktaydı. Genellikle, bu üç kritere göre anestezistler TXA kullanımına ameliyathanede karar veriyordu. Klinik pratikte uygulandığı için bir randomize yaklaşım yoktu. Bu nedenlerle, bu çalışma için TXA ile ilgili bir uygulama protokolü oluşturulmadı. Seçili örneklem büyüklüğü ve etki büyüklüğü ile 0,05 hata payında üç grup için ANOVA analizine uygun olarak güç analizi yapılmış ve power değeri 0,839 olarak hesaplanmıştır (effect size=0,155; critical F=1,842). Güç analizi hesaplamasına göre 100 hastadan oluşan 3 grup, toplamda da 300 hasta çalışmaya dahil edildi. Çalışma için başhekimlikten izin alındı. Retrospektif çalışma olması nedeni ile hasta onayına gerek olmadı. Helsinki Deklarasyonu ve T.C. Sağlık Bakanlığı tarafından düzenlenen etik yönetmeliklerin ilgili hükümlerine uyulmuştur.

Grup 1, TXA verilmemiş kontrol grubudur. Grup 2, cerrahi insizyondan 20 dakika önce TXA 30 mg/kg yavaş bolus dozunda uygulanmış ve kardiyopulmoner bypass sonunda, protamin verilmesi bittikten 10 dakika sonra 10 mg/kg dozunda TXA başlanmış ve 4 saat boyunca infüzyona devam edilmiş gruptur. Grup 3, kardiyopulmoner bypass sonunda, protamin verilmesi bittikten 10 dakika sonra TXA 30 mg/kg yavaş bolus dozunda uygulanmış ve devamında 10 mg/kg dozunda başlanmış ve 4 saat boyunca infüzyona devam edilmiş gruptur.

Aşağıda değerlendirilen tüm veriler bir çalışma amacı ile kayıt edilmemiş olan rutin hasta klinik takip arşiv kayıtlardan geriye dönük olarak sağlanmıştır:

Preoperatif yaş, ağırlık, hemoglobin, platelet, üre, kreatinin, serum glutamik oxaloasetik transaminaz (SGOT), serum glutamik pirüvik transaminaz (SGPT) değerleri ile hastaların komorbiditelerinin (Diyabetes Mellitus-DM, Hipertansiyon-HT, Kronik Obstrüktif Akciğer Hastalığı-KOAH, Periferik Arter Hastalığı-PAH) olup olmaması.

Peroperatif yapılan bypass greft sayısı, çapraz klemp (KK) süresi, kariyopulmoner bypass (KPB) süresi, ameliyat süresi, peroperatif minimum ve maksimum hasta ısıları, peroperatif minimum ve maksimum hemoglobin değerleri.

Postoperatif yoğun bakıma geliş hemoglobin, 4. saat hemoglobin, 12. saat hemoglobin, 24. saat hemoglobin değerleri.

Sonlanım noktaları:

1. Yoğun bakıma geliş ve 4, 12, 24. saatlerdeki kanama(drenaj) miktarı, 24. saat sonunda total kan bileşeni transfüzyon miktarı ve yoğun bakım kalış süreleri.
2. Postoperatif entübasyon ve yoğun bakım kalış süreleri, toplam allojenik eritrosit süspansiyonu transfüzyon miktarları.
3. Postoperatif miyokard enfarktüsü, inme ve tromboemboli sıklığı.

Acil, redo, konkominant cerrahi, miniinvaziv cerrahi, antiagregan/antikoagülan alan hastalar ve off pomp bypass hastaları çalışmaya dahil edilmemiştir.

İstatistiksel Yöntemler

Sürekli sayısal değere sahip değişkenlerin incelenen üç farklı grup açısından istatistiksel olarak farklılaşıp farklılaşmadığı tek-yönlü "*ANOVA*" analizi ile değerlendirilmiştir. Farklılığın hangi gruplar arasında oluştuğunun tespiti için grupların eşit sayıda gözlem birimine sahip olmasından ötürü post hoc analizlerden "*Tukey HSD*" testi kullanılmıştır. Kategorik değişkenlerin

ilgili üç gruba göre dağılımları ise "Ki-*kare*" ve "*Fisher's exact*" testleri ile incelenmiştir. İstatistiksel olarak anlamlı farklılık için p<0,05 alınmıştır. Tüm istatistikler SPSS 23.0 yazılımı ile hesaplandı (SPSS, Inc., Chicago, IL, ABD). Örneklem büyüklükleri G*Power 3.1 programı kullanılarak hesaplanmıştır.

Bulgular

Gruplar arasında preoperatif ortalama yaş, ağırlık, hemoglobin üre, kreatinin, SGOT ve SGPT değerleri arasında istatistiksel anlamlı farklılık saptanmadı. Sadece platelet değerleri gruplar arasında istatistiksel anlamlı farklılık göstermektedir (p=0,002). Tukey HSD post hoc analizine göre farklılığın platelet için 1. ve 3. gruplar arasında oluştuğu görülmüştür. Platelet değeri anlamlı şekilde düşük bulunmuştur (Tablo 1). Preoperatif kategorik değişkenler diabetes mellitus, hipertansiyon, kronik obstrüktif akciğer hastalığı ve periferik arter hastalığı Pearson Ki-kare testine göre anlamlı farklılık göstermemektedir (Tablo 2).

Gruplar arasında bypass-greft sayısı, çapraz klemp ve kardiyopulmoner bypass süreleri, peroperatif minimum hasta ısısı, peroperatif minimum hemoglobin değeri, yoğun bakıma geldikten sonra 4. ve 12. saatlerdeki hemoglobin değerleri, yoğun bakımdaki 4., 12. ve 24. saatlerdeki drenaj/kanama miktarları arasında istatistiksel anlamlı fark saptanmamıştır. Tukey HSD post hoc analizine göre peroperatif maksimum hasta ısısı 2. ve 3. gruplar arasında (p=0,006), peroperatif maksimum hemoglobin değerleri 1. ve 3. gruplar arasında (p=0,001), yoğun bakım 24. saat hemoglobin değerleri hem 1. ve 3. gruplar hem de 2. ve 3. gruplar arasında (p=0,001) farklılık göstermiştir.

Birincil etkinlik son noktası olarak postoperatif eritrosit süspansiyonu transfüzyon miktarı, Tukey HSD post hoc analizine göre 1. ve 3. gruplar arasında anlamlı olarak farklıdır (p=0,043). Grup 3 eritrosit süspansiyonu transfüzyon miktarı azdır.

CABG x greft sayıları, kross klemp süreleri, kardiyopulmoner bypass süreleri, ameliyat süreleri, peroperatif minimum hasta ısıları, peroperatif minimum Hgb değerleri, yoğun bakım 4. ve 12. saat Hgb değerleri, yoğun bakım geliş, 4-12-24. saatlerdeki drenaj miktarları, yoğun bakım kalış süreleri bakımından gruplar arasında istatistiksel anlamlı farklılık bulunmadı. Peroperatif maksimum hasta ısısı (Grup 2 ile 3 arasında), peroperatif maksimum Hgb (Grup 1 ile 3 arasında), yoğun bakım geliş Hgb (Grup 1 ile 3. arasında), 24. saat Hgb değeri (Grup 1 ile 3 arasında), postoperatif transfüzyon (Her üç grup arasında) istatistiksel olarak anlamlı farklılık bulundu (Tablo 3).

Birincil güvenlilik son noktası değerlendirmesi için; myokart enfarktüsü, imne ve tromboemboli (venöz/arteryal) değişkenlerinde hücrelerde beş birimin altında gözlemin olmasından ötürü Fisher's exact test kullanılmıştır. Gruplar arasında istatistiksel olarak anlamlı fark sadece 3. grupta "inme" değişkeninde görülmüştür. (p=0,048) (Tablo 4).

Tablo 1. Grupların preoperative demografik özellikler bakımından tek-yönlü varyans analizi ile karşılaştırılması

	Grup 1 (n=100) Ort±SS	Grup 2 (n=100) Ort±SS	Grup 3 (n=100) Ort±SS	p/MD
Yaş	58,61±13,57	60,46±14,18	61,53±14,53	0,333
Ağırlık	80,63±11,65	79,49±12,81	81,32±13,13	0,579
Hemoglobin	12,98±1,35	12,89±1,55	12,57±1,54	0,144
Platelet	272790±136048,95	246790±132212	341831±170136,59	**0,002** **MD: (1-3=-69041; p=0,003)**
Üre	36,82±15,82	40,51±18,01	36,96±17,04	0,221
Kreatinin	1,30±0,27	1,17±0,36	1,23±0,36	0,510
SGOT	29,49±16,92	32,05±14,38	33,66±13,78	0,144
SGPT	33,04±17,42	35,96±17,23	38,11±14,39	0,090

SGOT: Serum glutamik oxaloasetik transaminaz, SGPT: Serum glutamik pirüvik transaminaz
Ort: Ortalama, SS: Standart sapma, MD: Mean difference (ortalamalar arası fark)

Tablo 2. Grupların preoperatif komorbiditeler bakımından Ki-kare testi ile karşılaştırılması

	Grup 1 (n=100) %	Grup 2 (n=100) %	Grup 3 (n=100) %	Pearson Ki-kare	p
Diabetes Mellitus	45	46	42	3,838	0,147
Hipertansiyon	50	33	41	4,792	0,091
KOAH	36	28	26	2,78	0,249
PAH	27	23	31	2,379	0,304

KOAH: Kronik obstrüktif akciğer hastalığı, PAH: Periferik arter hastalığı

Tablo 3. Peroperatif ve postoperatif sonuçlar

	Grup 1 (n=100) Ort±SS	Grup 2 (n=100) Ort±SS	Grup 3 (n=100) Ort±SS	p/MD
CABG x greft sayısı	2,77±1,32	2,83±1,18	2,78±1,1	0,933
KK süresi (dk)	51,32±30,40	49,60±27,70	50,78±23,64	0,902
KPB süresi (dk)	94,47±58,55	92,55±47,26	93,73±49,19	0,966
Ameliyat süresi (dk)	192,51±87,96	193±69,73	199,80±67,31	0,764
Peroperatif minimum hasta ısısı (C')	32,25±1,41	31,49±1,37	31,34±2,03	0,376
Peroperatif maksimum hasta ısısı (C')	37,01±1,12	37,30±0,63	36,91±0,81	**0,006** **MD: (2-3=-0,390; p= 0,005)**
Peroperatif minimum Hgb	8,82±1,16	8,59±1,38	8,84±0,97	0,246
Peroperatif maksimum Hgb	11,27±1,69	11,25±1,77	10,53±1,11	**0,001** **MD: (1-3=2,196; p=0,003)**
YB geliş Hgb	10,61±1,74	10,39±1,55	10,04±1,31	**0,033** **MD: (1-3=0,568; p=0,026)**
YB 4. saat Hgb	10,41±1,34	10,12±1,26	10,96±1,42	0,553
YB 12. saat Hgb	10,14±1,35	9,72±1,26	9,88±1,22	0,072
YB 24. saat Hgb	9,74±1,23	9,41±1,03	10,156±0,99	**0,001** **MD: (1-3=-0,443; p=0,012)** **MD: (2-3=-0,746; p<0,001)**
YB geliş drenaj (ml)	41,79±9,66	32,04±7,10	43,76±10,53	0,062
YB 4. saat drenaj(ml)	194,42±103,48	226,50±117,94	207,44±105,69	0,097
YB 12. saat drenaj (ml)	305,01±153,98	347,12±163,42	319,80±143,23	0,148
YB 24. saat drenaj (ml)	403,11±207,78	456,50±208,97	450,99±210,72	0,149
Postoperatif transfüzyon (U)	0,98±1,31	0,87±0,90	0,61±0,93	**0,043** **MD: (1-3=-0,370; p=0,038)**
YB kalış süresi (saat)	46,46±33,51	39,93±38,64	41,58±35,80	0,283

CABG: Koroner arter bypass cerrahisi, dk: dakika, C': Santigrad, Hgb: Hemoglobin, ml: mililitre, YB: Yoğun bakım, U: Ünite
Ort: Ortalama, SS: Standart sapma, MD: Mean difference (ortalamalar arası fark)

Tablo 4. Birincil güvenlilik son noktası sonuçları

	Grup 1 (n=100) %	Grup 2 (n=100) %	Grup 3 (n=100) %	Pearson Ki-kare	p
Myokart enfarktüsü	0	3	4	2,877	0,173
İnme	1	2	7	5,353	**0,048**
Tromboemboli	0	1	0	1,83	0,331

Tartışma

Kardiyovasküler cerrahide kanamanın mortalite, morbidite, hastane ve yoğun bakım kalış süreleri ve maliyete olumsuz etkileri uzun yıllardır bilinmektedir. Kanama miktarını azaltmak için "*gentle surgery*" altın kuraldır. Fakat postoperatif kanama için yeniden ameliyata alınan hastalarda "*using/sızıntı*" şeklindeki kanamalar görülmektedir.[1] Postoperatif dönemde "sızıntı" şeklindeki kanamaları azaltmak için tüm yaş gruplarında yaygın olarak TXA kullanılmaktadır.[8,9] Kanamaların nedenleri, hastanın soğukluğu, heparin/protamin nötralizasyonunun tam yapılmaması, kardiyopulmoner bypassa bağlı kompleman ve enflamatuvar yanıtın aktivasyonu, kardiyopulmoner bypass sırasında bazı pıhtılaşma faktörlerinin ve fibrinojenin azalması sayılabilir.[10]

Çalışmamızda gruplar arasında preoperatif demografik karakterler arasında sadece platelet değeri 1. ve 3. gruplar arasında istatistiksel anlamlı fark bulundu. Diğer tüm karakterler anlamlı olmadığı için "*propensity score match yöntemi*" ile örneklem grupları oluşturulmadı. Ayrıca 1 ve 3. gruplarda ki düşük platelet değerleri klinik olarak trombositopeni olarak yorumlanmadı.

Peroperatif hasta maksimum ısısı arasındaki anlamlı fark klinik olarak önemli değildir. Çünkü bu ısılar enzim ve pıhtılaşma faktörlerinin çalışmaları için optimal ısı aralığındadır. Peroperatif maksimum hemoglobin değerinin Grup 3 için düşük olması, yoğun bakım geliş ve 24. saat hemoglobin değerlerini olumsuz etkilediği görülmektedir. Grup 3 hemoglobin değeri her iki gruptan daha düşüktür. Bu durum postoperatif total eritrosit süspansiyonu transfüzyon sayısının da anlamlı olarak yüksek olmasına neden olmuştur. Ayrıca komplikasyon sayıları oldukça düşük olmasına rağmen Grup 3 için tromboemboli sıklığı istatistiksel olarak daha fazladır. Bunun nedeni Grup 2 de insizyon öncesi uygulanan puşe TXA miktarının bir miktar etkinliğinin kardiyopulmoner

bypass sırasına azalması ve oksijenatör rezervuarındaki dilüsyonel etki olabilir. Bunun nedeni ile ilgili literatürde de bilgiye rastlanılmamıştır.

Grup 2 ve 3 arasında yoğun bakım 24. saatteki hemoglobin farkının total transfüzyona etkisi olmamıştır. Bunun nedeninin 2 ve 3. gruplarda KPB sonrası infüzyon ile verilen TXA olduğu düşünülebilir.

Aprotinin, 2008 yılındaki BART çalışmasından sonra ciddi komplikasyon oranları nedeniyle tüm dünyada piyasadan kaldırıldı.[11] Bu tarihten sonra antifibrinolitik ajanların etkinliği ve güvenliği ile ilgili çalışmalar arttı. Bu çalışmaların odağını epsilon amino kaproik asit ve TXA oluşturmaktadır. Kılavuzlarda özellikle TXA ile ilgili çalışmalar daha fazla yer almaya başladı. 2017 yılında Myles ve ark. büyük bir hasta grubunda yaptığı çalışma sonuçu 100 mg/kg doz yerine 50 mg/kg doz TXA kullanılmaya başlanmıştır.[12] Bu çalışmada 50 mg/kg dozun 30 günlük mortalite, tromboembolik komplikasyonları artırmadan transfüzyon miktarını azalttığı bulunmuştur. Ayrıca ilk defa bu çalışma ile TXA ile epileptik nöbetler arasında bir ilişki olduğu fark edilmiştir. TXA dozu için literatürde ve cerrahlar arasında fikir birliği bulunmamaktadır. Genellikle 30-150 mg/kg doz aralığı düşünüldüğünde kliniğimizde kullanılan dozaj düşüktür.[13]

TXA dozu ve uygulanma hızı, kaç kere uygulanması gerektiğine dair çok merkezli çalışmada ilginç sonuçlar bulundu. TXA yan etkilerinin düşük (50 mg/kg) dozlarda da olabileceği, fakat transfüzyon miktarının da yüksek doz (100 mg/kg) TXA ile azaldığı saptandı. Fakat yüksek doz TXA ile plazma, platelet veya kriyopresipitat transfüzyonunda istatistiksel anlamlı bir azalma olmamıştır.[14] Kliniğimizde de anestezistler arasında farklı uygulama şekilleri vardı. Dual antiplatelet tedavi özellikle Asetilsalisilik Asit ve Klopidogrelin beraber kullanıldığı hastalarda kanama riskinde artış olduğu bilinmektedir. TXAnın bu grup hastalarda kanamayı azaltmadığı saptandı.[14] Çalışmamızda "antiadezyon" ilaç alan hastalar çalışma dışı bırakılmıştır. Bunun nedeni; bu hasta grubundaki verilerimizde, antiadezyon ilacın ne zaman en son kullanıldığına dair bilgi eksikliğidir.

2022 yılında yayımlanan bir metaanalizde pediatrik vakalarda da antifibrinolitik uygun ajanların kullanımının güvenli olduğu görülmüştür.[15]

Çalışmamızın örneklem tarih aralığı oldukça eskidir. Fakat, TXA kullanımı günümüzde de önemini korumaktadır. Klasik koroner arter bypass cerrahisi tekniğinde major değişiklikler yaşanmadığı gibi yeni antifibrinolitiklerde kullanıma giremedi. Bu nedenle lizin analogları olan epsilon amino kaproik asit ve traneksamik asit ile ilgili çalışmalar literatürde her zaman önemli bir konu oldu. Yapılan çalışmalar artarak devam etti. Çünkü TXA kullanım dozu ve uygulama yöntemleri arasında kardiyak ve non-kardiyak cerrahilerde bir uzlaşma günümüzde de sağlanamadı.

Cerrahlar arasında TXAnın myokard iskemisine neden olabileceğine dair bir endişe vardır. Pediatrik hasta grubunda koroner arter hastalığı olmadığı için bu grupta yapılan çalışmalarda TXAnın myokard hasarı ile bir ilişkisi saptanmamıştır.[14,16,17] Bizim çalışmamızda nöbet geçiren hasta olmadı. TXAnın epileptik nöbete neden olma mekanizması tam olarak açıklanamamıştır. TXAnın mikro tromboemboli ya da hava embolisini artırarak nöbete neden olduğu düşünülmektedir.[18] Ancak bu hipotez kanıtlanamamıştır.

Kardiyak cerrahi sonrası kanama, sadece klinik sonuçları olumsuz etkilemez. Transfüzyon artışı, hastanede kalış süresini ve maliyeti ciddi oranda artırmaktadır.[19] Bu nedenle HKY, kan bileşenlerinin akıllı kullanımı uygulamaları ile ciddi bir tasarruf sağlanacaktır.[20] Kan bileşenleri pahalıdır ve giderek sürdürülebilir kamu sağlık maliyetlerini de olumsuz etkilemektedir.[21]

Sonuç olarak bu çalışmada, TXA uygulama farklılıkları arasında postoperatif kanama miktarları arasında anlamlı farklılık saptanmadı. Yoğun bakım kalış süresini etkilemedi. Grup 3 de, KPB sonrası TXA uygulamasının eritrosit süspansiyonu transfüzyonunda olumlu anlamlı etkisi olduğu görülmüştür. Ancak aynı grupta ciddi bir komplikasyon olan inme daha sık görüldü.

TXA kullanımı kılavuzlara ve çalışmalara rağmen kardiyak cerrahide standart protokoller ile yapılmamaktadır. Kılavuzlara göre TXA, kan kaybını, transfüzyon hızını ve toplam kan ürünü miktarını azaltmak için uygun ajandır. Yine de 30 günlük mortalite, greft oklüzyonu ve tromboembolik olaylar üzerindeki etkisi için yeni çalışmalara gereksinim vardır. TXA ile epileptik nöbetler ve inme arasındaki ilişki tedirgin edicidir.

Sınırlamalar

Bu çalışma eski bir tarih aralığındaki hasta verilerinden retrospektif olarak yapıldı. Bir çalışma protokolü oluşturulmadığı için daha fazla veri değerlendirilemedi. Örneğin, günümüzdeki en sık uygulamalardan "Bispektral İndeks İzleme" yapılamamıştır ve nörolojik izleme verileri analizlere dahil edilmemiştir. Geç dönem renal ve nörolojik sonuçları bilinmemektedir.

Açıklamalar

Makale verileri, ticari olarak sahip ve isim değiştiren bir hastanenin hasta klinik takip arşiv kayıtlarından Başhekimlik izniyle elde edilmiştir.

Çıkar Çatışması

Bu çalışmada herhangi bir kişi/kurum ile çıkar çatışması bulunmamaktadır.

Yazar Katkısı

CI: Çalışmanın planlanması, verilerin toplanması, analiz, kaynak taraması, yazım, yorum

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Research Article | Araştırma Makalesi

A SURGICAL PERSPECTIVE ON TWO ANESTHESIA TECHNIQUES USED IN CHILD AGE GROUP FOREIGN BODY ASPIRATION TREATMENT DURING RIGID BRONCHOSCOPY

ÇOCUK YAŞ GRUBU YABANCI CİSİM ASPİRASYONU TEDAVİSİNDE KULLANILAN RİJİT BRONKOSKOPİ ESNASINDA UYGULANAN İKİ ANESTEZİ TEKNİĞİNE CERRAHİ BİR BAKIŞ

Hüseyin Fatih Sezer[1*], Tülay Çardaközü[2], Aykut Eliçora[1]

[1]Kocaeli University, Faculty of Medicine, Department of Thoracic Surgery, Kocaeli, Türkiye. [2]Kocaeli University, Faculty of Medicine, Department of Anesthesiology and Reanimation, Kocaeli, Türkiye.

ABSTRACT

Objective: In our study, we aimed to reveal the advantages and disadvantages of inhalation and intravenous anesthesia techniques in the maintenance of anesthesia in pediatric patients whose tracheobronchial foreign body was removed by rigid bronchoscopy under general anesthesia.

Methods: The patients were divided into two groups with 34 individuals in each group, whose anaesthesia was maintained with sevoflurane inhalation or propofol infusion. Demographic characteristics of the patients, symptoms at presentation, radiological examinations, anaesthesia and surgical procedures, complications were analyzed. In addition, surgical difficulty and surgical comfort scales created from the experiences of our institution were used. The results obtained and the effects of inhalation and intravenous anaesthetics used in maintenance on the duration of the procedure, clinical, hemodynamic and surgical comfort were compared.

Results: Vital signs during the procedure were similar in both groups. The duration of bronchoscopy was shorter in the intravenous anaesthesia group (p=0.014), and secondarily, the duration of anaesthesia was longer in the inhalation anaesthesia group (p=0.027). While the surgical difficulty scale was similar in both groups, the surgical comfort scale was higher in the intravenous anesthesia group (p=0.017).

Conclusion: Anaesthesia maintenance with sevoflurane prolongs the duration of bronchoscopy and accordingly the duration of anaesthesia, and also reduces surgical satisfaction independent of processing time. For these reasons, we think that while rigid bronchoscopy is performed for the removal of childhood foreign body aspirations, propofol can be preferred for anaesthesia maintenance, resulting in a shorter procedure time and higher surgical satisfaction.

Keywords: Surgical comfort scale, surgical difficulty scale, rigid bronchoscopy

ÖZ

Amaç: Çalışmamızda genel anestezi altında rijit bronkoskopi ile trakeabronşiyal yabancı cisim çıkarılan çocuk hastalarda anestezi idamesinde inhalasyon ve intravenöz anestezi tekniğinin birbirlerine olan avantaj ve dezavantajlarını ortaya koymayı amaçladık.

Yöntem: Hastaların anestezi idamesi sevofluran inhalasyonu ile veya propofol infüzyonuyla yapılanlar olmak üzere 34'er kişiden oluşan iki grup oluşturuldu. Hastalara ait demografik özellikler, başvuru semptomları, radyolojik incelemeler, anestezi ve cerrahi işlemler bağlı özellikler, komplikasyonlar analiz edildi. Ayrıca kurumumuz tecrübelerinden oluşturulan cerrahi zorluk ve cerrahi konfor skalaları kullanıldı. Elde edilen sonuçlarla idamede kullanılan inhalasyon ve intravenöz anesteziklerinin işlem süresi, klinik, hemodinami, cerrahi konfor üzerine etkileri karşılaştırıldı.

Bulgular: İşlem esnasında vital bulgular her iki grupta benzerdi. Bronkoskopi süresi intravenöz anestezi grubunda daha kısaydı (p=0,014) buna sekonder olarak anestezi süresi inhalasyon anestezisi grubunda daha uzundu (p=0,027). Cerrahi zorluk skalası her iki grupta benzer iken, cerrahi konfor skalası intravenöz anestezi grubunda daha yüksekti (p=0,017).

Sonuç: Sevofluranla idame anestezisi bronkoskopi süresini uzatmakta ve buna sekonder olarak anestezi süresi uzamaktadır ayrıca işlem süresinden bağımsız olarak cerrahi memnuniyet azaltmaktadır. Bu nedenlerle çocukluk çağı yabancı cisim aspirasyonlarının çıkartılmasında rijit bronkoskopi yapılırken anestezi idamesinde propofolün tercih edilebilerek daha kısa işlem süresi ve daha yüksek cerrahi memnuniyet elde edileceğini düşünüyoruz.

Anahtar Kelimeler: Rijit Bronkoskopi, cerrahi konfor skalası, cerrahi zorluk skalası

***Corresponding author/İletişim kurulacak yazar:** Hüseyin Fatih Sezer; Kocaeli University, Faculty of Medicine, Department of Thoracic Surgery, Kocaeli, Türkiye.
Phone/Telefon: +90 (262) 303 75 75 **e-mail/e-posta:** hfs.hfs@gmail.com
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Introduction

Tracheobronchial foreign body aspiration (TBFBA) is a life-threatening condition that is frequently observed in childhood.[1,2] In the diagnosis and treatment of children TBFBAs due to the need for sedation and difficulty in cooperation in this process, rigid bronchoscopy (RB) is generally preferred under general anaesthesia instead of Flexible bronchoscopy (FB).[3-5] General anaesthesia induction and maintenance is accomplished with intravenous (iv.) or inhalation anaesthetics with spontaneous breathing or controlled breathing.[6] The choice of anaesthesia method or anaesthetic agent depends on the preference of the anesthesiologist.[3] In our study, we aimed to reveal the advantages and disadvantages of inhalation and intravenous anaesthesia techniques in the maintenance of anaesthesia in pediatric patients whose tracheobronchial foreign body was removed by rigid bronchoscopy under general anaesthesia, from both anaesthesia and surgical perspectives, different from the literature.

Methods

This study was planned retrospectively with the approval of Kocaeli University Faculty of Medicine Non-Invasive Ethics Committee, numbered 2021/69. Patients aged 0-18 years, who underwent RB under general anaesthesia (GAA) with the suspicion of TBFBA by the Department of Thoracic Surgery between 11.10.2020-10.10.2019 were included in the study. Before the procedure, the necessary laboratory and imaging tests were performed following the anamnesis and physical examination of the patients. Patients were kept under close observation until the procedure. Patients who are hemodynamically and respiratory unstable and who are too urgent to wait for a fasting period, who cannot be sure of adequate ventilation with repetitive bronchoscopy attempts and positive pressure ventilation, and therefore who do not use muscle relaxants, were excluded from the study.

It is recommended that the RB procedure be performed in the daytime than night conditions in clinically stable patients who are indicated for RB.[7] On the contrary, in our study, the procedures were performed by the same thoracic surgeon and anaesthesia team who knew each other and had experience in the procedure, at all hours of the day, taking into account the fasting period without acute conditions.

Patients were divided into two groups as anaesthesia maintenance was performed with sevoflurane inhalation (Group S) or propofol infusion (Group P). When the power analysis was performed with the G*Power program by taking effect size=0.8, α=0.05 and Power (1-β)=0.90, it was determined that a minimum of 34 people should be worked for each group. Two groups, each consisting of 34 individuals with sufficient data and suitable for inclusion and exclusion criteria, were formed. Whether premedication would be performed was determined according to the clinical condition of the patient. After standard anaesthesia monitoring (ECG, SpO2, blood pressure) was applied to the patients who were taken to the operating room, anaesthesia induction was achieved by inhalation of 100% oxygen and 8% sevoflurane with a gas flow of 5 L/min in both groups, and the concentration of sevoflurane was gradually reduced. Anaesthesia was maintained with the 100% O2 with 2.5-3.5% sevoflurane in Group S, and 100-150 µg/kg/min propofol infusion in Group P. 1 mg/kg methylprednisolone was administered intraoperatively in prolonged bronchoscopy (>30 min) and in cases where intubation was repeated more than 3 times with bronchoscopy. In patients with intense secretions in the intraoperative period or in case of bradycardia development iv. atropine (0.01 mg/kg) was administered. A rigid bronchoscope (Storz brand) of suitable size was inserted into the trachea by the thoracic surgeon 3 minutes after the muscle relaxant application. The foreign body was tried to be visualized by using 0 and 30-degree optics connected with the eye and the monitor through the working channel of the bronchoscope. Intraoperative ventilation was provided by controlled manual ventilation with 8-10 Lt/min 100% oxygen through a T-piece, one opening of which was connected to the ventilation port of the rigid bronchoscope and the other opening to the breathing circuit. Anesthesia was maintained with 2-5% sevoflurane-100% O2 inhalation in Group S, and propofol infusion of 100-150 µg/kg/min in Group P. The anaesthetic agent dose was titrated according to the patient's hemodynamic response. For analgesia, 0.1 µg/kg/min remifentanil infusion was administered to both groups. In cases where TBFB could not be seen clearly, manual ventilation was interrupted in coordination with the surgeon and only oxygen insufflation was applied with apnea periods. If the patient was desaturated during the bronchoscopy procedure, the procedure was interrupted and the RB was regressed to the trachea and manual ventilation was applied until the saturation increased. After the FB was seen, it was grasped with alligator forceps and removed from the RB lumen with forceps. After the TBFB was removed, for possible complications and residue FB, the trachea and bronchial systems were observed -checked by optics. When the procedure was over, anaesthetic drugs were discontinued, muscle relaxants were reversed in necessary patients, and the patient's respiration was manually supported with 100% oxygen until the patient's spontaneous respiration reached a sufficient level. At the end of the procedure, the patients were followed in the recovery unit until the Aldrete score reached 9 points, and then they were sent to their wards. In the meantime, the presence of pneumothorax and other possible pathologies was investigated by first taking a chest X-ray in patients who developed respiratory distress. Firstly, medical treatment (corticosteroid, humidified oxygen, nebulized adrenaline) was applied to patients who were thought to have oedema in the respiratory tract, and patients whose respiratory distress continued despite medical treatment were admitted to the intensive care unit (ICU) by endotracheal intubation.

Demographic data of patients, the passing time between TBFBA history and time of admission to the hospital (application time), symptoms at admission, features of anaesthesia applications (anaesthesia, awakening and recovery time), features of surgical practice (bronchoscopy time, surgical comfort scale and surgical difficulty scale), hemodynamic and respiratory parameters of the patient, characteristics of TBFB (structure, localization) intraoperative [patient movement, desaturation (desaturation of oxygen saturation below 90%), bradycardia (heart rate decrease below 20% of control values) and bronchospasm] and awakening and recovery complications (laryngospasm, desaturation, recurrent cough) were recorded. If symptoms and complications occurred more than once in the same patient, all were recorded.

In our study, 2 new parameters were used in terms of surgery. Surgical comfort scale and surgical difficulty scale were created by our clinic's long-term TBFBA experience and therefore they were named KOU Surgical Comfort Scale and KOU Surgical Difficulty Scale. KOU Surgical comfort scale is a partially subjective criterion that the main goal is to show the effect of anaesthesia application on the surgeon. 0 points were accepted as the lowest and 9 points highest comfort level in the scale, which is based on the principle of scoring between 0 and 9 points. Less than 5 points were considered low, 5-7 points were considered normal, and 7 points and above were considered high. With this scale, the surgeon is asked to evaluate those situations one day after the procedure; During the patient's manual ventilation, a) restriction of bronchoscope manoeuvres b) affecting the quality of observation, c) adverse effects of the airflow into the eyes d) Whether there is a decrease in performance at the time of the operation or the next day due to the pollution of the room air, or whether there are health problems such as headaches and inclination to sleep (Figure 1).

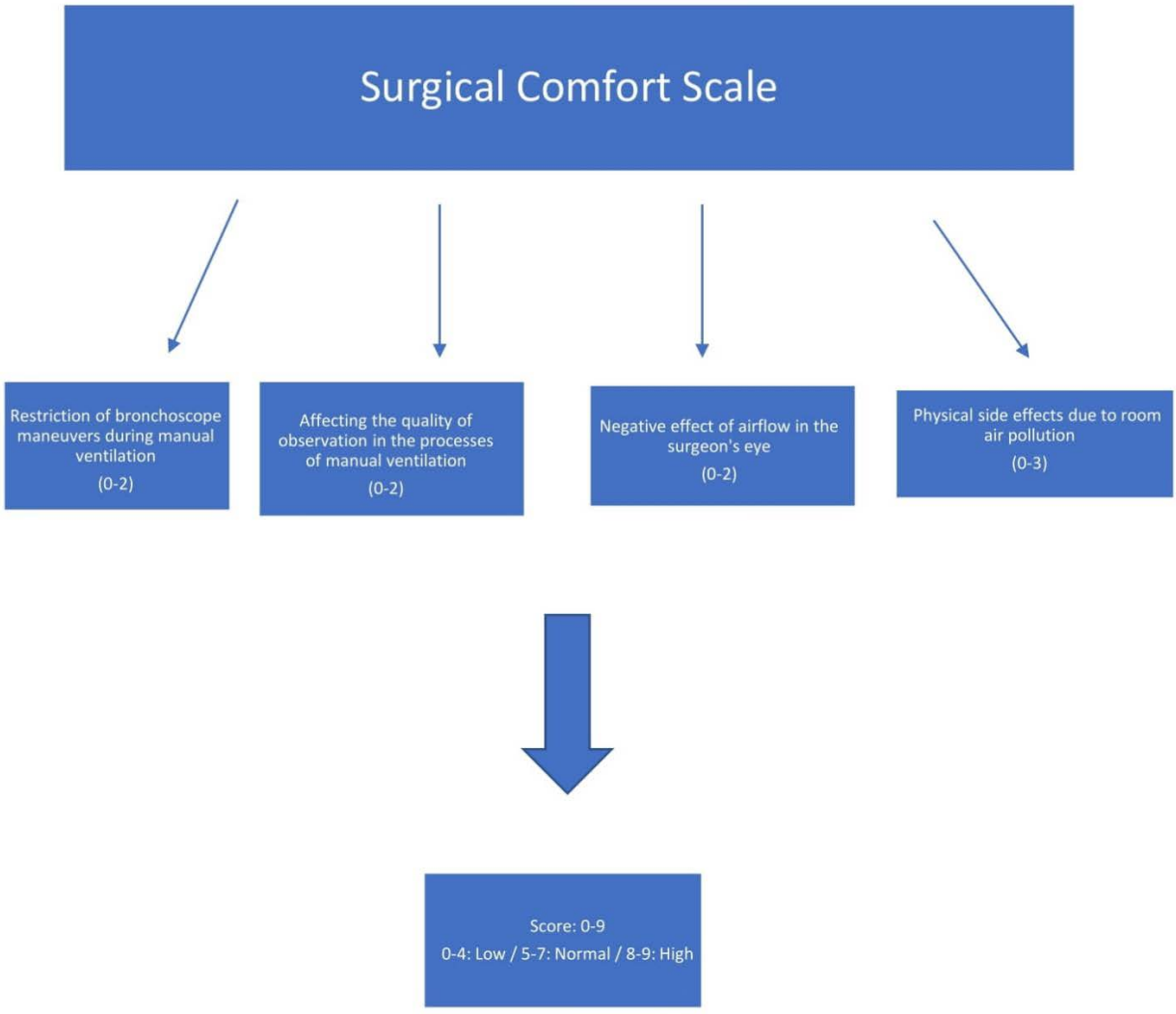

Figure 1. Surgical comfort scale

In the calculation of the Surgical Difficulty Scale; the nature of the foreign body, number of pieces, size, localization, granulation tissue formation due to foreign body in the bronchial tree, rigid bronchoscope size and subjective score reported by the surgeon (temperature-gas of the room, difficulties experienced due to the equipment used as a result of covid measures, etc.) were used. 8 points and below were accepted as easy, 9-12 points as medium, 13 points and above as difficult. (Figure 2).

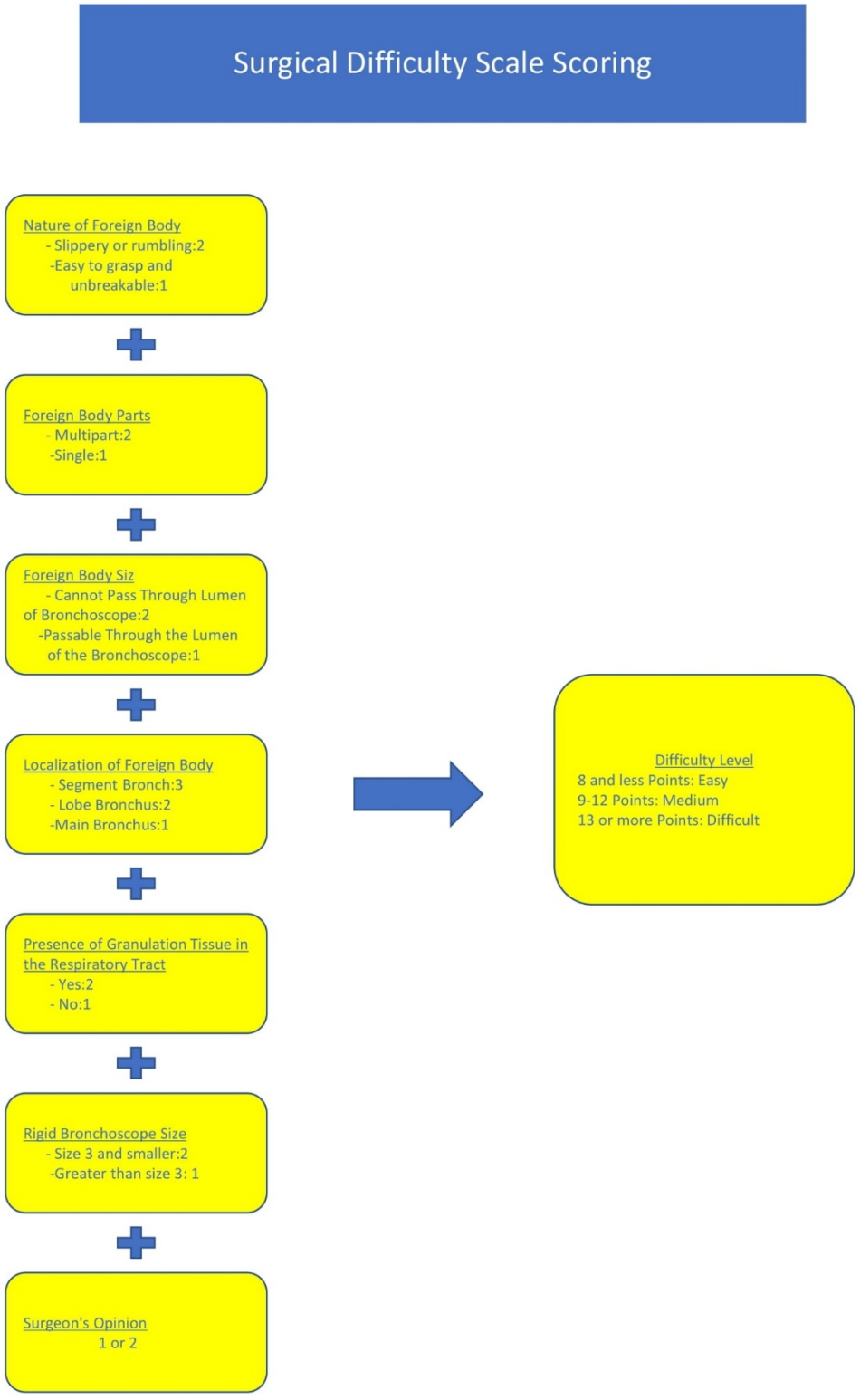

Figure 2. Surgical difficulty scale

Statistical Analysis

All statistical analyses were performed using IBM SPSS for Windows version 20.0 (SPSS, Chicago, IL, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess the assumption of normality. Numeric variables were presented with mean±standard deviation and median (25th-75th percentile). Categorical variables were summarized as counts (percentages). Since normality assumption did not hold, comparisons of numeric variables between groups were carried out using Mann-Whitney U test. Binary logistic regression analysis was used to determine the factors affecting the outcome variable. Association between two categorical variables was examined by Chi-square test. A *p*-value<0.05 was considered statistically significant.

Results

The data of 104 patients were analyzed retrospectively, 14 patients were excluded from the study, 34 patients whose anaesthesia was maintained with sevoflurane and 34 patients whose anaesthesia was maintained with propofol were randomly selected and analyzed.

Demographic characteristics, medical history and application time of the patients were similar in both groups (Table 1).

Table 1. General Features

	Total (n=68)	Group S (n=34)	Group P (n=34)	p
Age (month), mean±SD	25.19±23.11	24.26±24.65	26.12±21.80	0.372[a]
Sex, n (%)				0.460[b]
Male	40(58.8)	22 (64.7)	18 (52.9)	
Female	28(41.2)	12 (35.3)	16 (47.1)	
Weight (kg), mean±SD	13.93±6.10	13.68±6.64	14.19±5.59	0.639[a]
Disease history, n (%)				0.617[b]
Absent	54 (79.4)	27 (79.4)	27 (79.4)	
Pulmonary	6 (8.8)	3 (8.8)	3 (8.8)	
Cardiovasculer	2 (2.9)	2 (5.9)		
Other	6 (8.8)	2 (5.9)	4 (11.8)	
Application time (day), mean±SD	3.88±11.32	2.10±2.34	5.66±15.76	0.735[a]

[a]Mann-Whitney U Test, [b]Chi-Square Test, n: Number, SD: Standart Deviation

Preoperative symptoms, examination and imaging findings of the patients were similar in both groups (Table 2).

Table 2. Before Operation Symptoms and Radiological Imaging

	Total (n=68)	Group S (n=34)	Group P (n=34)	p
Preoperative Symptoms, n (%)				
Dyspnea	3(4.4)	1 (2.9)	2 (5.9)	NA
Cough	51(75)	27 (79.4)	24 (70.6)	0.575[a]
Wheeze	32 (47.1)	13 (38.2)	19 (55.9)	0.224[a]
Difference between breath sounds on auscultation	19 (27.9)	10 (29.4)	9 (26.5)	1.00[a]
Wheezing	7 (10.3)	3 (8.8)	4 (11.8)	1.00[a]
Chest X-ray Findings, n (%)				0.438[a]
No pathological image	30 (44.1)	14 (41.2)	16 (47.1)	
Aeration difference	32 (47.1)	16 (47.1)	16 (47.1)	
Atelectasis	3 (4.4)	3 (8.8)		
Appearance of foreign body	3 (4.4)	1 (2.9)	2 (5.9)	
Thorax CT, n (%)	4 (5.9)	1 (2.9)	3 (8.8)	NA

[a]Chi-square test, n: Number, NA: Not applicable

Awakening and recovery times and the ratio of drugs used were similar in both groups. Anaesthesia duration was significantly longer in Group S compared to the other (p=0.027) (Table 3).

During the procedure, heart rate and blood pressure were similar in both groups, and peripheral oxygen saturation was significantly lower in Group P at 15 and 25 minutes (p=0.023; p=0.027, respectively) (Figure 3).

The duration of bronchoscopy was statistically significantly shorter in Group P compared to the other group (p=0.014). While the surgical difficulty scale score was similar in both groups, the surgical comfort scale score was significantly higher in Group P (p=0.017). The sizes of RB used during bronchoscopy, localization and nature of TBFB, and residual TBFB were similar in both groups (Table 4).

Intraoperative complications (movement, bradycardia and desaturation), and postoperative complications were similar in both groups. The number of patients requiring manual ventilation by interrupting the procedure during the bronchoscopy procedure, and the number of patients requiring intubation was similar in both groups (Table 5).

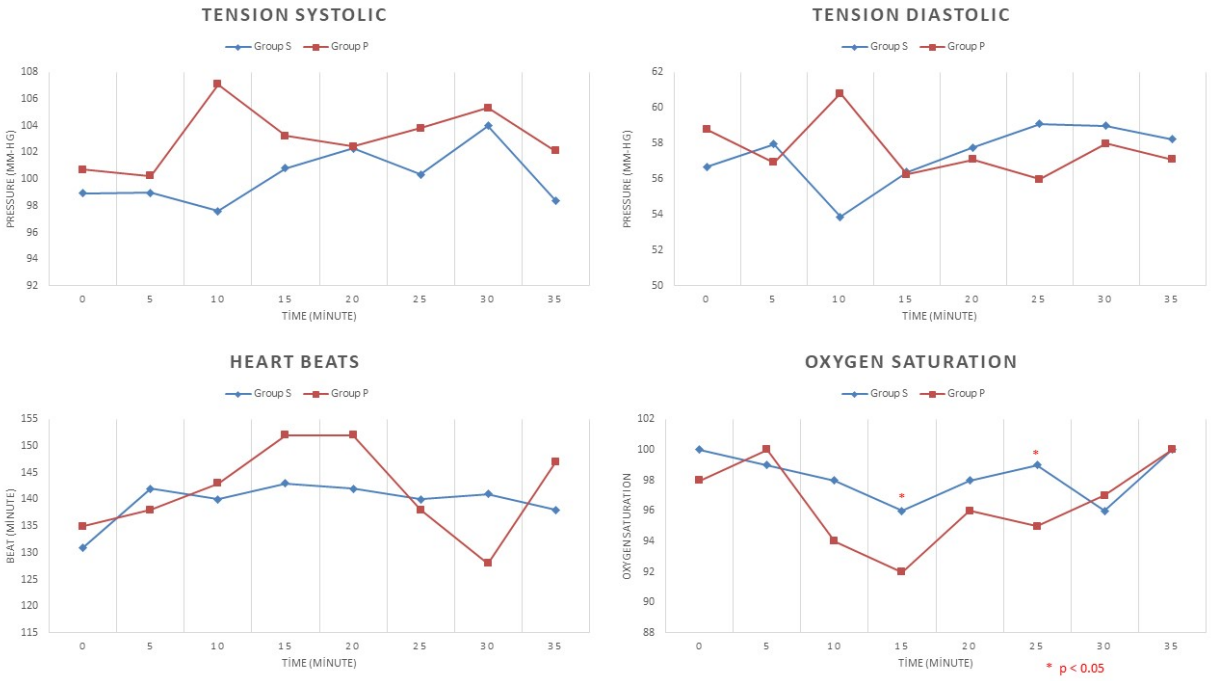

Figure 3. Vital values

Discussion

In our study, we compared two methods (inhalation-intravenous) used in the maintenance of anesthesia in rigid bronchoscopy procedures performed for foreign body aspiration, both in terms of anesthesia and surgery. While doing this, unlike the literature, we used our own scoring systems, except for the epidemiological standardization and routine surgical features.

The results of our study show that similar hemodynamic and respiratory results are obtained with inhalation or intravenous anesthesia maintenance, bronchoscopy time and accordingly anesthesia duration are longer with sevoflurane and this is not reflected in recovery and recovery times, awakening and recovery times are similar, intraoperative and postoperative complications are similar, intravenous anesthesia showed that higher surgical satisfaction scores were obtained with its maintenance.

The goals of anesthesia management during TBFB removal are adequate oxygenation and ventilation, adequate depth of anesthesia with minimal airway secretions, stable hemodynamics, controlled cardiorespiratory reflexes during bronchoscopy, rapid return of upper airway reflexes, and prevention of pulmonary aspiration.[8] Anesthesia management is difficult for the anesthesiologist because the airway is shared with the surgeon and general anesthesia is administered without tracheal intubation and the depth of anesthesia is tried to be maintained.[9]

Anesthesia technique and ventilation method (spontaneous or controlled) should provide the least risk of mortality and complications for the patient.[10] There is no clear consensus on which of these methods is ideal.[7,11]

Factors such as the characteristics of the working institution, the experience of the anesthesiologist, the localization of the foreign body, the nature of the foreign body, the level of obstruction in the airway, and the medical condition of the patient are effective in anesthesia management.[6,12]

General anaesthesia maintained is performed by intravenöz or inhalation anaesthetics with spontaneous breathing or controlled breathing. Sevoflurane is preferred because of its rapid induction and less respiratory tract irritation in inhalation anaesthesia.[6,11] In some studies, it has been reported that the induction time with propofol and sevoflurane is similar[13,14] and in some studies, the induction time with sevoflurane is shorter.[15] In the induction phase, we prefer spontaneous breathing continues until ventilation is assured, and if there is no contraindication we prefer sevoflurane inhalation with 100% oxygen in because there is no irritant effect on the respiratory tract.

There is no definite consensus in the literature regarding the administration of premedication to patients who undergo RB due to TBFBA. Because of the sedative and respiratory depressant effects of midazolam, its routine use in TBFBA removing operations is not recommended, as it may worsen existing respiratory distress.[6] In our clinic, premedication with midazolam is not routinely applied to patients who apply with the suspicion of TBFBA before RB, and the decision is made according to the clinical condition of the patient and the preference of the anesthesiologist. It has been reported that atropine may be beneficial in reducing airway secretions, preventing vagal-induced bradycardia, and alleviating cholinergic-mediated bronchoconstriction during airway manipulation.[6] Corticosteroid use is recommended before and during bronchoscopy due to its reducing

effect on airway inflammation and subglottic oedema.[16,17] While some authors have recommended the prophylactic use of dexamethasone, others have suggested its intraoperative administration.[6,18] Li et al.[19] administered methylprednisolone prophylactically before induction. Apa et al.[20] was started the steroid treatment before bronchoscopy and continued for 24 hours postoperatively. Zhang et al.[18] suggested intraoperative methylprednisolone or dexamethasone use. As can be seen, there is no consensus in the literature about which corticosteroid should be administered and for how long. In our clinic, we use dexamethasone (maximum 8 mg) in prolonged bronchoscopy (>30 min.), in case of recurrence of more than 3 intubations with bronchoscope in the intraoperative period, and methylprednisolone prophylactically in cases of chronic TBFBA. We prefer atropine in cases with intraoperative bradycardia or hypersecretion.

Although inhalation anaesthetics are widely used in the maintenance of anaesthesia in pediatric patients, the infusion of intravenous anaesthetics has also started to gain popularity recently. For this purpose, propofol is used alone or in combination with other drugs in the short-term procedures of pediatric patients.[21]

When the effects of sevoflurane and propofol are compared on the cardiovascular system and hemodynamics during anaesthesia there are some studies reporting more stability is achieved with sevoflurane[14,] on the contrary with propofol better hemodynamics was obtained,[22] or similar hemodynamic effects.[23,24] Hemodynamic side effects may also be related to the drugs used together. For example, providing more stable hemodynamics of sevoflurane was attributed to the synergistic effect of remifentanil given with propofol.[14, 25]

Different results were also obtained when propofol and sevoflurane were compared in terms of their relationship to respiratory parameters. While respiratory parameters were stable with sevoflurane in the study of Liao et al.[14], lower oxygen saturation values were found with Sevoflurane in the study of Maleki et al.[23], but respiratory complications were found to be similar.[14,22] In our study, similar hemodynamic and respiratory changes were detected with sevoflurane and propofol in addition to remifentanil infusion. The number of desaturated patients during the procedures was similar. Although the oxygen saturation at the 15th and 25th minutes were statistically significantly lower in the propofol group, we did not consider it clinically significant since it was within the reference range.

Possible gas leaks from the bronchoscope during the procedure require high gas flows to maintain the depth of anaesthesia, and this condition can pollute the atmosphere of the operating room.[14,26] Important advantages of propofol are that it is not cumulative when used for a relatively short period of time, and during the removal of the FB period that is the ventilation system is on, surgeon don't be exposed to anaesthetic vapours.[27] Propofol-based total intravenous maintenance with remifentanil, a super-short-acting opioid, is a suitable method for pediatric patients and has been shown to provide a stable level of anaesthesia.[24,25]

Complications such as laryngospasm, apnea and SpO2 reduction are frequently seen during TBFB removal.[28-30] Regardless of the ventilation method, the most frequently reported complications during the procedure are hypoxia and arrhythmia.[11] In our study, the most common intraoperative complication was desaturation with a rate of 33.3%. In our clinical observations, it was seen that patients were mostly desaturated during the removal of distal foreign bodies, desaturation occurred especially during the advancement of the RB from the main bronchus to the distal. When the relationship between intraoperative complications and anaesthetic methods is investigated, the results also differ. In one study, less cough was detected in the use of sevoflurane during bronchoscopy than in the use of propofol, and this was explained by the myorelaxant effect of sevoflurane.[24] In another study, it was reported that remifentanil together with propofol may be preferred to sevoflurane because it causes less cough and recovery agitation.[22] Farrell et al.[12] reported that the chosen anaesthetic method may be inhalation or IV-based, as there is no evidence of the superiority of one approach over the other. In our study, the rate of movement, bradycardia and desaturation, which we defined as intraoperative complications, were similar with both inhalation and intravenous methods.

Since the surgeon is directly involved in ventilation during RB, it also affects the anaesthesia management of the patient.[24] In this process, the surgeon has to be manoeuvred the foreign body without disturbing the ventilation, and the anaesthetist has to provide sufficient depth of anaesthesia.[7] Apneic oxygenation applied during the procedure makes facilitates manoeuvres, especially to distal foreign bodies.[13] For these reasons, communication between the surgeon and anesthetist is important during ventilation management.[29] The selection of the ventilation method to be applied by the anesthesiologist during rigid bronchoscopy is important because it can become a difficult situation to provide ventilation and necessary oxygenation in the pediatric age group.[31]

There are studies advocating different views on the ventilation method to be applied. The use of muscle relaxants may vary according to the localization of TBFB, and spontaneous respiration is generally preferred in proximal FBs.[32,33] The reason for this is that it allows ventilation to continue while trying to remove the FB and prevents the foreign body from causing obstruction by moving in the bronchial tree with positive pressure ventilation.[34,35] The disadvantages of the spontaneous breathing technique are the difficult prevention of cough, and patient movement.[6,27] The advantage of controlled ventilation is that it provides better oxygenation and ventilation and prevents movements that may cause complications such as coughing and moving of the patient during airway manipulation. The disadvantage is that despite preoxygenation, rapid desaturation

develops and the foreign body may move distally and cause obstruction due to positive pressure.[6] On the other hand, there are also studies reporting that there is no adverse effect with positive pressure-controlled ventilation.[36,37] The use of muscle relaxants can provide an even and adequate depth of anaesthesia for rigid bronchoscopy and reduce the anaesthetic effects on cardiac output.[36,37] Except for patients with proximal TBFB which is located proximally or has almost completely closed the trachea where we cannot be sure that we will be able to adequately ventilate with positive pressure ventilation according to TBFB localization, the thoracic anesthesiologists we work with prefer rocuronium bromide because which is more hemodynamically stable as controlled ventilation and muscle relaxant.[38]

After TBFB is removed, If the general condition of the patient is not bad before bronchoscopy airway edema has not developed and pulmonary gas exchange is not impaired, the patient is awakened by applying mask ventilation until adequate spontaneous ventilation is achieved, in some cases positive pressure ventilation may be required by intubating.[39] One of the common complications after the procedure is laryngospasm, its incidence has been reported to be 8% to 21.5% in the literatüre,[3,11,36] and it was seen in 19.1% of patients in our study. There is no significant difference between the two maintenance methods in terms of the distribution of complications. One study reported that complications were not directly related to the anesthetic agent but to the duration of bronchoscopy.[40] In our study, although the duration of bronchoscopy was longer in maintenance with sevoflurane, complications were similar to maintenance with propofol. Recovery time has been defined as one of the risk factors associated with intraoperative or postoperative hypoxemia in the RB procedure.[41] Maleki et al.[23] found a shorter recovery time in maintenance with propofol and Liao et al.[14] found a shorter recovery time in maintenance with sevoflurane, and no statistically significant difference was found in either study in terms of complications. There are also studies reporting that recovery times are similar.[42,43] Recovery times and complications were similar both methods in our study.

The most important effect of the anesthesia method used on the surgery is the movement of the foreign body, affecting the surgeon's angle of view, comfort, and difficulty of the procedure, and consequently affecting the performance of the procedure. Leaks around the bronchoscope in inhalation anesthesia can cause the desired depth of anesthesia to not be achieved and pollute the room air.[6,7,13] This may cause a decrease in the comfort of the surgeon.[44] The decrease in the comfort of the surgeon may lead to prolongation of the procedure time and an increase in the risk of complications. In his article on which he shared his personal experiences, Bould stated that in the sharing airline procedures they preferred to use intravenous agents in order to partially avoid the airline partnership and not be affected by the gas pollution of the operation team.[45] Although the surgical difficulty scale which includes bronchoscope size, localization of TBFB and features of TBFB in our study was similar in both groups, the surgeon comfort scale score was found to be lower with sevoflurane. The reason for this is the disturbing effect of sevoflurane gas coming directly from RB during bronchoscopy and the effects of spreading this gas into the room air. The long duration of anesthesia with sevoflurane maintenance can be explained by the fact that the surgeon interrupts the procedure for short periods due to the effect of the gas. Prolonging the duration of anesthesia and bronchoscopy with the maintenance of sevoflurane is important in terms of increasing the cost, although it does not affect the recovery and recovery times of complications. No study has been found in the literature reporting results with surgical comfort scales, which include a large number of parameters, as in our study. This scale consisted of parameters that could negatively affect the process of the procedure by disturbing the comfort of the surgeon during the procedure, affecting the quality of life after the procedure, and causing dissatisfaction with the procedure. In the study of Zhang et al.,[24] it was observed that they used a limited surgical satisfaction score, and no significant difference was found in the comparison made in terms of inhalation-intravenous anesthesia.

For stable patients, some authors recommend that the procedure be performed in daytime conditions, by experienced teams, and under optimum conditions.[29,45] However, it is reported that in this case, the complications of the procedure are also no different from the complications in the acute intervention period.[29] In our study, the procedure was performed at any time of the day, taking into account the fasting period, and our complication rates were consistent with the literature. For this reason, it was thought that the procedures to be done with an experienced team could be done at any time of the day.

The limitations of our study are its retrospective nature, not controlling the depth of anesthesia with BIS and the number of patients was partially limited. The fact that the surgical comfort scale and difficulty scale used in standardization between groups are partially subjective can be considered as another shortcoming.

In general anaesthesia maintained with sevoflurane inhalation or propofol infusion for removal of TBFB by RB in pediatric patients, it was detected that similar effects on complications, hemodynamics, respiratory parameters, awakening and recovery times. Anaesthesia maintenance with sevoflurane prolongs the duration of bronchoscopy and accordingly the duration of anaesthesia, and also reduces surgical satisfaction independent of processing time. For these reasons, we think that while RB is performed for the removal of childhood FBA, propofol can be preferred for anaesthesia maintenance, resulting in a shorter procedure time and higher surgical satisfaction.

Table 3. Features Based on Anesthesia Applications

	Total (n=68)	Group S (n=34)	Group P (n=34)	p
Anesthesia Time (minute), mean±SD	19.59±16.92	22.94±18.83	16.24±14.27	**0.027**[a]
Awakening Time (minute), mean±SD	12.67±13.54	9.07±5.82	16.27±17.65	0.417[a]
Recovery Time (minute), mean±SD	18.21±7.74	19.32±10.03	17.09±4.29	0.265[a]
Medicines, n (%)				
Methylprednisolone	48 (70.6)	24 (70.6)	24 (70.6)	1.00[b]
Midazolam	30 (44.1)	21 (61.8)	17 (50)	0.464[b]
Sugammadex	61 (89.7)	28 (82.4)	33 (97.1)	0.105[b]
Atropine	20 (29.4)	12 (35.3)	8 (23.5)	0.864[b]

[a]Mann-Whitney U Test, [b]Chi-Square Test, n: Number, SD: Standard Deviation

Table 4. Features Based on Surgical Applications

	Total (n=68)	Group S (n=34)	Group P (n=34)	p
Bronchoscopy Time (minute), mean±SD	15.34±16.02	18.00±17.23	12.68±14.48	**0.014**[a]
Bronchoscope Size, n (%)				0.314[b]
3.5	33 (48.5)	17 (50)	16 (47.1)	
3.7	17 (25)	6 (17.6)	11 (32.4)	
4	13 (19.1)	9 (26.5)	4 (11.8)	
5	5 (7.4)	2 (5.9)	3 (8.8)	
Surgical Difficulty Scale, n (%)				0.679[b]
Easy	29 (42.6)	13 (38.2)	16 (47.1)	
Medium	20 (29.4)	10 (29.4)	10 (29.4)	
Difficult	19 (27.9)	11 (32.4)	8 (23.5)	
Surgical Comfort Scale Score, n (%)				NA
1	7 (10.3)	4 (11.8)	3 (8.8)	
2	2 (2.9)			
4			2 (5.9)	
6	2 (2.9)	1 (2.9)	1 (2.9)	
7	7 (10.3)	7 (20.6)		
8	19 (27.9)	11 (32.4)	8 (23.5)	
9	31 (45.6)	11 (32.4)	20 (64.5)	
Foreign Body Localizations, n (%)				0.096[b]
Absent	19 (27.9)	7 (20.6)	12 (35.3)	
Trachea	2 (2.9)		2 (5.9)	
Right Main Bronchus	19 (27.9)	10 (29.4)	9 (26.5)	
Right Intermediate Bronch	4 (5.9)	2 (5.9)	2 (5.9)	
Right Lower Lobe Bronchus	7 (10.3)	4 (11.8)	3 (8.8)	
Left Main Bronchus	11 (16.2)	9 (26.5)	2 (5.9)	
Left Lower Bronchus	6 (8.8)	2 (5.9)	4 (11.8)	
Nature of Foreign Body, n (%)				0.461[b]
Hazelnut	14 (28.7)	6 (17.6)	8 (23.5)	
Peanut	11 (22.45)	6 (17.6)	5 (14.7)	
Walnut	13 (26.53)	9 (26.5)	4 (11.8)	
Other	11 (22.45)	6 (17.6)	5 (14.7)	
Total Inability to Remove (Residue), n (%)	5 (7.4)	3 (8.8)	2 (5.9)	1.00[b]

[a]Mann-Whitney U Test, [b]Chi-Square Test, n: Number, SD: Standard Deviation, NA: Not applicable

Table 5. Complications and Airway Management

	Total (n=68)	Group S (n=34)	Group P (n=34)	p
Intraoperative Complications, n (%)				
Movement	5 (7.4)	3 (8.8)	2 (5.9)	NA
Bradycardia	2 (2.9)	-	2 (5.9)	NA
Desaturation	23 (33.8)	13 (38.2)	10 (29.4)	0.192[a]
Manual ventilation by stopping the bronchoscopic manipulation, n (%)	31 (45.6)	18 (52.9)	13 (38.2)	0.657[a]
Postoperative Complications, n (%)				0.402[a]
No symptoms	52 (76.5)	28 (82.4)	24 (70.6)	
Broncho or Laryngospasm	13 (19.1)	4 (11.8)	9 (26.5)	
Hypoxemia- Desaturation	3 (4.4)	2 (5.9)	1 (2.9)	
Cough	10 (14.7)	5 (14.7)	5 (14.7)	
Postoperative Ventilation, n (%)				1.00[a]
Face Mask	63 (92.6)	31 (91.2)	32 (94.1)	
Intubation	5 (7.4)	3 (8.8)	2 (5.9)	

[a]Chi-square test, n: Number, NA: Not Applicable

Compliance with Ethical Standards

The study protocol was approved by the Kocaeli University Ethics Committee (Date: 18.3.2021/Number: 2021-69).

Conflict of Interest

The author declares no conflicts of interest.

Author Contribution

All the authors equally contributed to this work.

Financial Disclosure

None

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Research Article | Araştırma Makalesi

EVALUATION OF COLONOSCOPY AND PATHOLOGY RESULTS OF PATIENTS WHO UNDERWENT COLONOSCOPY DUE TO FECAL OCCULT BLOOD TEST POSITIVITY: A SINGLE CENTER EXPERIENCE

GAİTADA GİZLİ KAN TEST POZİTİFLİĞİ NEDENİ İLE KOLONOSKOPİ YAPILAN HASTALARIN KOLONOSKOPİ VE PATOLOJİ SONUÇLARININ DEĞERLENDİRİLMESİ: TEK MERKEZ DENEYİM

Ozkan Subasi[1*], Hamdi Taner Turgut[2], Abdullah Gunes[2], Gokhan Dindar[3]

[1]Health Sciences University, Derince Training and Research Hospital, Gastroenterology Surgery Clinic, Kocaeli, Türkiye. [2]Health Sciences University, Derince Training and Research Hospital, General Surgery Clinic, Kocaeli, Türkiye. [3]Health Sciences University, Derince Training and Research Hospital, Gastroenterology Clinic, Kocaeli, Türkiye.

ABSTRACT

Objective: The most important part of improving colorectal cancer prognosis is to detect and treat in the early stage. The most effective way to do this is screening programs. Fecal occult blood test is one of the most frequently used methods in the screening program. In this study, we aimed to evaluate the colonoscopy and histopathological findings of patients who underwent colonoscopy due to positive fecal occult blood test.

Methods: Patients who had a positive fecal occult blood test between January 2016 and December 2018 and underwent colonoscopy to investigate the etiology were included in the study. Demographic characteristics, colonoscopy findings and histopathological results of the patients were reviewed retrospectively from hospital records.

Results: A total of 325 patients were included in the study. Of the patients, 146 (44.9%) were male, 179 (55.1%) were female, and their mean age was 58.4±9.3 years. Colonoscopic findings of 140 patients (43.1%) were normal. Polyps in 89 (27.4%) patients, perianal disease in 46 (15.1%), diverticulum in 20 (6.2%), inflammatory bowel disease in 8 (2.4%), solitary rectal ulcers in 4 (1.2%) patients and colorectal cancer in 18 (5.5%) of them was detected.

Conclusion: Fecal occult blood test is a cost-effective and easy-to-apply method used in colorectal cancer screening programs together with colonoscopy, helping to detect both cancer and precancerous lesions and increase the survival rate.

Keywords: Fecal occult blood test, colonoscopy, colorectal cancer

ÖZ

Amaç: Kolorektal kanser prognozunu iyileştirmenin en önemli kısmı; erken evrede tespit edilerek tedavi edilmesidir. Bunun en etkili yolu tarama programlarından geçmektedir. Gaitada gizli kan testi, tarama programında en sık kullanılan yöntemlerin başında gelmektedir. Bu çalışmada gaitada gizli kan testi pozitif olması nedeniyle kolonoskopi yapılan hastaların kolonoskopi ve histopatolojik bulgularını değerlendirmeyi amaçladık.

Yöntem: Ocak 2016 ile Aralık 2018 tarihleri arasında gaitada gizli kan testi pozitif olan ve etiyoloji araştırılması için kolonoskopi yapılan hastalar çalışmaya alındı. Hastaların demografik özellikleri, kolonoskopi bulguları ve histopatolojik sonuçlar hastane kayıtlarından retrospektif olarak incelendi.

Bulgular: Çalışmaya toplam 325 hasta dahil edildi. Hastaların 146'sı (%44,9) erkek, 179'u (%55,1) kadın ve ortalama yaşları 58,4±9,3 idi. 140 hastanın (%43,1) kolonoskopik bulguları normal idi. Hastaların 89'unda (%27,4) polip, 46'sında (%15,1) perianal hastalık, 20'sinde (%6,2) divertikül, 8'inde (%2,4) inflamatuar bağırsak hastalığı, 4'ünde (%1,2) soliter rektal ülser ve 18'inde ise (%5,5) kolorektal kanser tespit edildi.

Sonuç: Gaitada gizli kan testi, kolonoskopi ile birlikte kolorektal kanser tarama programlarında kullanılan, hem kanserin hem de prekanseröz lezyonların tespit edilerek sağ kalım oranını artırmaya yardımcı olan maliyeti ucuz ve kolay uygulanabilen bir yöntemdir.

Anahtar Kelimeler: Gaitada gizli kan testi, kolonoskopi, kolorektal kanser

***Corresponding author/İletişim kurulacak yazar:** Ozkan Subasi; Health Sciences University, Derince Training and Research Hospital, Gastroenterology Surgery Clinic, Kocaeli, Türkiye..
Phone/Telefon: +90 (505) 384 21 09 **e-mail/e-posta:** osubasi25@hotmail.com
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Introduction

Colorectal cancer (CRC), which is common in the world, is the leading cause of cancer-related deaths.[1] Detection of CRC at an early stage allows for curative endoscopic or surgical treatment. Therefore, early diagnosis is one of the most important factors affecting prognosis and mortality.

Thanks to screening programs, it may be possible to detect precancerous lesions, treat or even prevent CRC.[2] Scan programs; It should be non-invasive, highly sensitive and specific, reliable and cost-effective. For this reason, in CRC screening programs; Various methods with different characteristics such as fecal occult blood (FOB) test, fecal immunochemical test, sigmoidoscopy and colonoscopy are applied in many countries.[3] In our country, the screening program has started to be applied to healthy individuals between the ages of 50-70 as of 2014, by performing a FOB test every 2 years and a colonoscopy every 10 years. Besides that, the age limit at which screening will be terminated in individuals with a negative FOB test is accepted as 70 years.[4]

FOB test is included in screening protocols because it is a practical and cost-effective procedure for CRC screening. Studies reports the sensitivity of the FOB test between 12.9% - 79.4% and the specificity between 86.7% - 97.7%.[5] However, pathology cannot be detected in approximately 50% of colonoscopies performed on patients with positive FOB test.[6]

Disadvantages of FOB test were disability of determination of the bleeding from upper or lower gastrointestinal parts and influence from many factors in the diet. Despite this, it is used in the first place in CRC screening programs in our country.

The aim of this study was to evaluate the colonoscopy and histopathological findings of the patients who were referred to the endoscopy unit due to FOB test positivity in a certain time period.

Methods

Between January 2016 and December 2018, 325 patients who underwent colonoscopy to investigate the etiology in the endoscopy unit of our hospital due to positive FOB test were retrospectively screened. Upper gastrointestinal endoscopy was not performed in any of the patients. This study was approved by SBU Kocaeli Derince Training and Research Hospital Health Sciences Scientific Research and Publication Ethics Committee (Decision No: 2021/40, Date: 25.02.2021).

Patients with inadequate colon cleansing, active gastrointestinal bleeding, history of colon surgery or inflammatory bowel disease were excluded from the study. Within the scope of the study, demographic data such as age and gender of the patients, findings detected in colonoscopy, histopathological results of patients who underwent biopsy and surgical procedures applied to patients requiring surgery were evaluated. Colonoscopy was performed in all patients in such a way that the entire colorectal region from the anus to the floor of the cecum was examined. Biopsy was taken from the masses detected during the procedure or if polyps were detected, polypectomy was performed and sent for pathological examination.

All statistical analyses were performed using IBM SPSS for Windows version 20.0 (SPSS, Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation. Categorical variables were summarized as counts (percentages).

Results

A total of 325 patients who underwent colonoscopy due to positive FOB test were included in the study. Of the patients, 146 (44.9%) were male, 179 (55.1%) were female, and their mean age was 58.4±9.3 years. Endoscopic diagnoses including; normal colonoscopic findings in 140 patients (43.1%), polyps in 89 patients (27.4%), perianal disease in 46 patients (15.1%), diverticula in 20 patients (6.2%), inflammatory bowel disease in 8 patients (2.4%), solitary rectal ulcer in 4 patients (1.2%) and CRC in 18 patients (5.5%) (Table 1).

Thirty-two (35.9%) of polyps in the sigmoid colon, 17 (19.1%) in the descending colon, 8 (8.9%) in the ascending colon, 11 (12.3%) in the transverse colon and 21 (23.5%) in the rectum were detected.

Table 1. Demographic data and colonoscopic findings of patient group (n=325)

	n	%
Gender		
Male	146	44.9
Female	179	55.1
Age, mean±SD	58.4±9.3	
Colonoscopy findings		
Polyp	89	27.4
Presence of malignancy	18	5.5
Diverticulum	20	6.2
Inflammatory bowel disease	8	2.4
Perianal disease	46	15.1
Solitary rectal ulcer	4	1.2
Normal findings	140	43.1

n: Number of individuals, SD: Standard deviation

When the histopathological types of polyps were examined, 52 (58.4%) of them were tubular adenomas, 16 (17.9%) of them were tubulovillous adenomas, 18 (20.2%) of them were hyperplastic polyps, and 3 (3.3%) of them were serrated adenomas. 14 (15.7%) of the polyps, had mild grade dysplasia and 6 (6.7%) of them had high-grade dysplasia (Table 2). 15 of the 18 malignancy patients underwent surgical treatment and 3 patients were out of follow-up. Nine (50%) cancers were in the rectum, 4 (22.2%) in the sigmoid colon, 2 (11.1%) in the transverse colon, 2 (11.1%) in the ascending, and 1

(5.5%) in the descending colon. Low anterior resection was performed in 7 patients with malignancy in the rectum, anterior resection was performed in 4 patients in the sigmoid colon, right hemicolectomy was performed in 3 patients in the ascending colon, and left hemicolectomy was performed in 1 patient in the descending colon (Table 3).

Table 2. Characteristics of detected colon polyps (n=89)

	n	%
Localization		
Sigmoid colon	32	35.9
Descending colon	17	19.1
Ascending colon	8	8.9
Transverse colon	11	12.3
Rectum	21	23.5
Pathology of polyp		
Tubular	52	58.4
Tubulovillous	16	17.9
Serrated	3	3.3
Hyperplastic	18	20.2
Presence of dysplasia		
None	69	77.5
Low grade dysplasia	14	15.7
High grade dysplasia	6	6.7

n: Number of individuals

Table 3. Characteristics of patients with colon cancer (n=18)

	n	%
Gender		
Male	12	66.6
Female	6	33.3
Localization		
Rectum	9	50
Sigmoid colon	4	22.2
Descending colon	1	5.5
Ascending colon	2	11.1
Transverse colon	2	11.1
Operation		
Low anterior resection	7	38.8
Anterior resection	4	22.2
Right hemicolectomy	3	16.6
Left hemicolectomy	1	5.5
None	3	16.6

n: Number of individuals

Discussion

The FOB test is an easy-to-administer and low-cost test that shows bleeding from any part of the gastrointestinal tract. It is widely used when investigating the etiology of anemia or in colon cancer screening programs. By screening the asymptomatic population; prevention of CRC, reduction of mortality, detection of precancerous lesions and successful intervention in early stage cancers can be provided.[7] Randomized studies recommended CRC screening and forming the basis of international guidelines, and observational studies conducted in different parts of the world have shown that FOB test reduces CRC mortality by 9-32%.[8-11] Although no pathology can be detected on colonoscopy in approximately 50% of patients with positive FOB test, current guidelines recommends that individuals between the ages of 50-75 should have a FOB test every two years and a colonoscopy every 10 years.[12] However, there is no clear study on the best age range in FOB test screening. Some studies suggest that FOB test screening has similar reductions in CRC-related mortality in different age ranges between 45 and 80 years of age.[13]

Brenner et al.[14] reported that when the colonoscopic findings of individuals with or without positive FOB test, CRC was found more frequently in individuals with positive FOB test. Paimela et al.[15] reported that the rate of invasive CRC among patients with positive FOB test was 8.2%, and the rate of adenoma and cancer showing only mucosal invasion was 39.7%. Andreas et al.[16] performed colonoscopy on 26,123 patients with positive FOB in their study and detected colorectal cancer in approximately 5% of them, adenoma in 15%, other colorectal pathologies in 15%, and negative colonoscopic findings in 65%. Utku et al.[17] found 7.5% colorectal cancer and 26.2% polyps in the colonoscopy of patients with positive FOB test. In our study, CRC was diagnosed in 18 (5.53%) of the patients who underwent colonoscopy due to positive FOB test. Our polyp detection rate was 27.4%. When the localization of tumors and polyps was examined, more than 50% of the lesions were detected in the rectum and sigmoid colon. This finding of our study is compatible with the literature.[8,18] Non-malignant pathology findings were detected in 24.9% of the patients. Normal colonoscopy findings were present in 43.1% of the patients. Consistent with the literature, in our study, FOB test was a beneficial test for detecting the diagnosis of pathologies such as inflammatory bowel disease, diverticulum, rectal ulcer and perianal disease as soon as detecting CRC and premalignant lesions.

The first limitation of this study is its retrospective design and single-center design. Second, upper gastrointestinal endoscopy is not used for screening purposes. Finally, prospective, multicenter large cohort studies with new developed CRC screening programs are needed.

In conclusion, FOB test is used in our country as in many countries of the world, especially in screening programs of asymptomatic individuals because of its low cost and easy accessibility. Although colon pathology is detected in approximately half of the patients with positive FOB test, even if the FOB test is negative, it should not interfere with colonoscopic screening.

Compliance with Ethical Standards

This study was approved by SBU Kocaeli Derince Training and Research Hospital Health Sciences Scientific

Research and Publication Ethics Committee (Decision No: 2021/40, Date: 25.02.2021).

Conflict of Interest

The authors declare no conflicts of interest.

Author Contribution

OS: Conceived and designed the experiment, materials, performed the experiments, wrote the paper; HTT, AG: Contributed reagents, analysis tools or data; OS, GD: Review and editing, supervision, project administration

Financial Disclosure

None.

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Research Article | Araştırma Makalesi

THE EFFECTS OF VITAMIN D LEVELS ON PREGNANCY OUTCOMES IN PATIENTS RECEIVING FROZEN EMBRYO TRANSFER

DONMUŞ EMBRİYO TRANSFERİ YAPILAN HASTALARDA D VİTAMİNİ DÜZEYLERİNİN GEBELİK SONUÇLARINA ETKİSİ

Merve Cakır Kole[1], Emre Kole[2], Goksen Gorgulu[3], Barıs Candan[4], Ahmet Gulluoglu[2], Emek Doger[5], Lale Aksoy[6]

[1] Antalya Education and Research Hospital, Department of Obstetrics and Gynecology, Antalya, Türkiye.[2] Alanya Alaaddin Keykubat University School of Medicine, Department of Obstetrics and Gynecology, Antalya, Türkiye. [3] Tepecik Education and Research Hospital, Department of Obstetrics and Gynecology, Izmir, Türkiye. [4] Süreyyapaşa Education and Research Hospital, Department of Public Health, Istanbul, Türkiye. [5] Kocaeli University School of Medicine, Department of Obstetrics and Gynecology, Kocaeli, Türkiye. [6] Geyve State Hospital, Department of Obstetrics and Gynecology, Sakarya, Türkiye.

ABSTRACT

Objective: The aim of this study is to evaluate the effects of 25-OH vitamin D on pregnancy outcomes in infertile patients undergoing a frozen embryo transfer.

Methods: In this prospective, single-blind study conducted at Kocaeli University Medical Faculty Hospital, Center for Assisted Reproductive Techniques, baseline serum levels of 25-OH vitamin D were measured at the start of treatment in 276 infertile patients who were scheduled to undergo frozen embryo transfer (FET). Cases with 25-OH-D vitamin levels lower than the level of deficiency (<20 ng/ml, group A, n=48) and higher than the level of deficiency (≥20 ng/ml, group B, n=44) were compared in terms of the rates of pregnancy as an outcome of the FET cycle, clinical pregnancy, ongoing pregnancy, live birth, implantation, pregnancy loss, and multiple pregnancy.

Results: Cases in groups 1 and 2 had similar demographic characteristics, and the serum AMH levels, one of the cycle follow-up parameters, were statistically significantly higher in group 1 compared to group 2 (p=0.014). Pregnancy (41.6% vs. 31.8%), clinical pregnancy (35.4% vs. 25%), ongoing pregnancy (25% vs. 18.2%), live birth (20.8% vs. 18.2%), pregnancy loss (18.8% vs. 13.6%) and twin pregnancy (4.2% vs. 9.1%) were similar between the groups (p=0.328, p=0.278, p=0.428, p=0.749, p=0.507, p=0.421, respectively).

Conclusion: There was no correlation between pregnancy outcomes from frozen embryo transfer and baseline serum 25-OH vitamin D levels obtained at the start of treatment. There is no indirect evidence showing that vitamin D level exerts its effects on fertility through endometrial receptivity and the implantation process.

Keywords: 25-OH Vitamin D, Frozen embryo transfer, pregnancy outcome

ÖZ

Amaç: Donmuş embriyo transferi yapılan infertil hastalarda 25-OH vitamin D'nin gebelik sonuçlarına etkisini değerlendirmek.

Yöntem: Kocaeli Üniversitesi Tıp Fakültesi Hastanesi, Üremeye Yardımcı Teknikler Merkezinde prospektif -tek kör olarak yürütülen bu çalışmada donmuş embriyo transferi (DET) yapılması planlanan 276 infertil olgunun serum 25-OH vitamin D düzeyleri tedavi başlangıcında elde edildi. 25-OH-D vitamini seviyelerinin yetmezlik seviyesinde düşük olduğu olgular (<20 ng/ml, grup A, n=48) ve yetmezlik seviyesinin üstünde olduğu (≥20 ng/ml, grup B, n=44) olgular DET siklusu sonucundaki gebelik, klinik gebelik, devam eden gebelik, canlı doğum, implantasyon, gebelik kaybı ve çoğul gebelik oranları karşılaştırıldı.

Bulgular: Grup 1 ve grup 2 olguların demografik özellikleri benzerdi ve siklus takip parametrelerinden serum AMH düzeyleri grup 1 de grup 2 'ye göre istatiksel olarak anlamlı yüksek izlenmiştir (p=0,014). Gruplar arasında gebelik (% 41,6 vs %31,8), klinik gebelik (%35,4 vs %25), devam eden gebelik (%25 vs %18,2), canlı doğum (%20,8 vs %18,2), gebelik kaybı (%18,8 vs %13,6) ve ikiz gebelik (% 4,2 vs % 9,1) benzerdi (sırasıyla; p= 0,328 p= 0.278, p= 0,428, p= 0,749, p= 0,507, p= 0,421).

Sonuç: Donmuş embriyo transferinden elde edilen gebelik sonuçları ile tedavi başlangıcında elde edilen serum 25-OH vitamin D seviyeleri arasında ilişki tespit edilmemiştir. Vitamin D seviyesinin fertilite üzerindeki etkilerini endometriyal reseptivite ve implantasyon süreci üzerinden gösterdiğine dair dolaylı kanıt elde edilmemiştir.

Anahtar Kelimeler: 25-OH Vitamin D, Donmuş Embriyo Transferi, Gebelik Sonucu.

*** Corresponding author/İletişim kurulacak yazar:** Lale Aksoy; Geyve State Hospital, Department of Obstetrics and Gynecology, Sakarya, Turkiye.
Phone/Telefon: +90532 422 70 02 **e-mail/e-posta:** laleaksoy@gmail.com
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Introduction

The current data of the World Health Organization show that 10-15% of married couples are affected by infertility. Assisted reproductive techniques are the only way to achieve pregnancy for most infertile couples.

In-vitro fertilization (IVF) is the ideal treatment method for couples who are unable to conceive naturally or by in-utero insemination, or for infertile patients who are not suitable for these methods. Pregnancy success in freeze-thaw cycles is still low in cases when embryos cannot be transferred in a fresh state, and novel strategies are being tested to improve success. One of these evaluations is whether the serum 25-OH-D vitamin levels of a female patient have an effect on pregnancy outcome in frozen embryo transfer (FET) cases.[1]

Vitamin D, produced mainly in the skin in the body, is a steroid hormone that is fat-soluble. Ergocalciferol, which is contained in plants, and cholecalciferol, which is prevalent in animal foods, are the main sources of exogenous vitamin D intake.[2] The effects of vitamin D on many systems in the body have been shown in numerous studies.[3,4] Studies both with humans and animals are conducted to clarify the potential role of vitamin D in female fertility.[5]

The purpose of this study is to compare the success of pregnancy outcomes between the cases where the baseline serum 25-OH-vitamin D level obtained from female patients at the beginning of the frozen embryo cycle is above (≥20 ng/mL) and below (<20 ng/mL) the insufficiency. Accordingly, it is aimed to reveal whether the serum vitamin D level affects endometrial receptivity and implantation success.

Methods

This study was carried out with 276 infertile women who were scheduled to undergo frozen embryo transfer (FET) at Kocaeli University Medical Faculty Hospital, Center for Assisted Reproductive Techniques. All participants were included in the study after obtaining informed consent.

Patients aged between 24-42 years who were scheduled to undergo FET and had at least one good quality frozen embryo with the diagnosis of single or combined unexplained infertility, male factor infertility, anovulation, low ovarian reserve, bilateral tubal factor and endometriosis were included in the study. Cases who did not want to participate in the study, who had endometrial polyps, submucous myomas, uncorrected uterine anomalies, hydrosalpinx, or uncontrolled systemic diseases, were excluded from the study.

Following the confirmation of ovulation in the luteal phase of the previous cycle, a suppressed FET cycle was performed in all cases by starting leuprolide acetate (Lucrin 5mg/ml/2.8ml vial, 14 Syringe Kit SC /Abbot) with a daily dose of subcutaneous 10 IU for pituitary suppression. On the third day of the menstrual cycle, the patients were then called for an ultrasound and blood tests. The patients were called for the measurement of serum 25-OH vitamin D levels, TSH, AMH, estradiol, and progesterone by drawing 3-5 cc blood daily on the third day of the menstrual cycle. The study continued using the blinded method by preventing the researcher and patient from knowing the 25-OH vitamin D levels. Cases whose TSH levels were not in the 0.5-4.5 mIU/L range were excluded from the study. Patients with an estradiol level of > 50 and a progesterone level of > 1 ng/mL continued to take Lucrin at a dose of 10 IU/day until complete suppression was achieved. Estrogen therapy was not started in these cases until it was determined by drawing blood every three days that suppression had been achieved. Estrogen treatment was not initiated in cases who had endometrial thickness of >5 mm or had a follicle cyst larger than >14mm in the adnexal area in the ultrasound examination which was performed concurrently. Estrogen therapy was started in these cases when the endometrial thickness was ≤ 5 mm and no cyst was detected in the adnexal area in the ultrasonographic follow-up performed every three days.

In cases who met the criteria (estradiol <50 ng/mL, progesterone < 1 ng/mL, endometrial thickness ≤ 5mm, no cysts in the adnexal area) in the blood tests and ultrasound examinations performed, 6 mg oestradiol ng/mL (Estrofem tablet 2 mg 28 tablets /Novo Nordisk) was started orally divided into three equal doses daily while Leuprolid acetate was continued with a daily dose of 5 IU. The patients were called for the first ultrasonographic evaluation at the earliest on the 10th day of the menstrual cycle and on day 7 of the estrogen therapy. In this evaluation, while the estrogen therapy was continued with the same dose in cases with an endometrial thickness of ≥ 8 mm and a blood progesterone level of <1 ng/mL, leuprolide acetate therapy was discontinued, and twice-a-day vaginal progesterone therapy was initiated (Crinone 90 mg gel 8% /Merck). Cases with an endometrial thickness of less than 8 mm were examined every other day to monitor for an increase in the endometrial thickness. Estrogen therapy was administered at a dose of 8 mg/day to the cases whose endometrial thickness did not increase sufficiently in 2 consecutive follow-ups. If available, two thawed embryos were transferred, and if not, one thawed embryo was transferred on the 4th day of vaginal progesterone in cases with a third-day embryo, on the 6th day of vaginal progesterone in cases with a 5th-day embryo, and on the 7th day of vaginal progesterone in cases with a 6th-day embryo.

The embryo transfer was performed under the supervision of transabdominal ultrasonography with a full bladder. After the visualization of the cervix with a speculum, the cervix was purified from drug residues with saline and cleared of mucus by aspiration with a mucus-attracting catheter. First, a mock transfer was performed to determine the cervical canal and the uterine position. Then, with a full echo soft catheter (Prodimed) the cervix was passed by using a stylet only in patients that necessitated it, and the embryo transfer was completed by applying the lowest pressure possible on the Hamilton syringe without approaching the middle

portion of the uterine cavity by more than 15 mm and without a fundal contact. No teneculum was used or no cervical dilatation was performed in any patient. The position of the air balloon was clearly observed in all cases. The embryo transfer catheter was slowly removed, and no bed rest was recommended for the patients after the transfer. Daily doses of 6 mg oral estrogen and 180 mg vaginal progesterone were continued after the embryo transfer. No vitamin treatments were recommended. On the 12th day after the embryo transfer, blood hCG levels were measured to confirm pregnancy.

The main outcomes that were aimed to achieve in this study were the pregnancy rate revealed by hCG positivity, the clinical pregnancy rate obtained by ultrasonographic confirmation of the embryonic heartbeat, and the ongoing pregnancy rate confirmed by exceeding the 10th week of pregnancy. Additionally, the secondary aim of the study was to obtain the rates of multiple pregnancy and rates of abortion. After achieving the primary aims of the study, the study was unblinded, and the pregnancy success as a result of the FET cycle was compared between the cases with blood 25-OH-D vitamin levels above the deficiency level (≥20 ng/ml) and the cases at the insufficiency level (20 ng/ml).

The data analysis was performed with SPSS for Windows 20.0 package program. A Kolmogorov-Smirnov test was completed to check if the continuous variables were normally distributed. Descriptive statistics were presented as mean ± standard deviation or median (minimum-maximum) for continuous variables, while categorical variables were presented as number and percentage (%) of cases. The student's t-test was used to determine the significance of the difference between the groups in terms of means. The nonparametric Mann-Whitney U Test was used for the data whose means could be calculated as the groups did not fit the normal distribution. The Pearson's Chi-Square Test was used for the data whose means could not be calculated. The results were considered statistically significant for a p value of <0.05.

Results

A comparison of the demographics of study groups are presented in Table 1. Both groups showed similar demographics and infertility diagnoses (Table 1).

While the comparison of the characteristics of the FET cycle between the groups is presented in Table 2, the comparison of the rates of pregnancy, clinical pregnancy, ongoing pregnancy, live birth, abortion, and twin pregnancies is presented in Table 3.

The serum AMH value of the group with a serum 25-(OH) Vitamin D level higher than 20 was statistically significantly lower than the group with a low serum 25-OH vitamin D level. No significant difference was found between the groups in terms of both the characteristics of the FET cycle and the rates of pregnancy, clinical pregnancy, ongoing pregnancy, live birth, pregnancy loss, and twin pregnancy.

The comparison of the rates of pregnancy, clinical pregnancy, ongoing pregnancy, live birth, abortion, and twin pregnancy between cases with 25-OH vitamin D levels of <20 ng/mL and cases with 25-OH vitamin D levels of ≥20 ng/mL is presented in Table 3. There was no significant difference in pregnancy achievement and pregnancy outcomes between the two groups (p>0.05).

The retrospective analysis of the means of 25-OH vitamin D levels of cases who got pregnant, achieved clinical pregnancy, had an ongoing pregnancy, and gave live birth revealed no significant differences in the means of vitamin D levels between the groups, and the results are presented in Table 4.

In the study, we did not find any relationship between the levels of serum vitamin D and achieving pregnancy in the FET cycle.

Discussion

There is no agreement on the ideal vitamin D levels for female reproductive health and fertility at the moment. Although the possible effect of vitamin D on the outcomes of assisted reproductive therapy (clinical pregnancy and live birth) has been evaluated in a limited number of studies, the data are inconsistent.[6-10]

Studies examining the relationship between serum vitamin D levels and the effectiveness of IVF cycles reported that the clinical pregnancy rate is associated with vitamin D deficiency. According to a study measuring the 25-OH vitamin D levels in follicular fluid instead of serum, high vitamin D levels are associated with significantly higher clinical pregnancy and implantation rates, and follicular fluid vitamin D levels are an independent predictor of IVF cycle success.[11] Similarly, research done over various IVF cycles has suggested a relationship between vitamin D levels and pregnancy outcome.[12-13]

In contrast to these studies, which discovered a significant correlation between vitamin D levels and the success of IVF cycles using fresh embryos, Anifandis et al. reported a negative correlation between follicular fluid 25-OH vitamin D level, embryo quality, and clinical pregnancy rate.[14]

We aimed to evaluate the data obtained from FET cycles of patients with good and very good quality embryos frozen in the previous cycle to rule out ovarian factors and reveal whether vitamin D has an effect on endometrial receptivity and the implantation process. As good quality embryos are already frozen, standardizing FET is easier. As a result, many concomitant variables associated with the patient and her partner in new cycles are eliminated, and the true effect on receptivity can be assessed. In this prospectively designed, single-blind study, no statistically significant relationship was found between the serum 25-OH vitamin D levels measured at the start of the FET cycle and pregnancy success. In this study, we evaluated case groups with vitamin D levels both below and above 20 ng/mL. Furthermore, unlike many other studies, all samples were examined

Table 1. Comparison of important characteristics of groups.

	Group 1 Vitamin D level <20 ng / mL. (n= 48)	**Group 2 Vitamin D level ≥ 20ng / mL (n= 44)**	**p value**
Age (year)*	30.48 ± 4.36	32.00 ± 4.30	0.144
Partner age (year)*	34.27 ± 4.16	35.10 ± 6.47	0.939
Marriage duration (year)*	7.62 ± 4.55 (n=24)	5.95 ± 4.39 (n=20)	0.210
Gravida (n)*	0.52 ± 1.24	0.55 ± 0.82	0.307
Parity (n)*	0.10 ± 0.31	0.14 ± 0.38	0.636
Abortion (n)*	0.35 ± 1.06	0.41 ± 0.76	0.243
BMI (kg/size^2) *	25.94 ± 4,94 (n=24)	24,99 ± 3,99 (n=33)	0.518
Smoking (n, %)	3 (6.3%)	5 (11.4%)	0.473 *
Chronic medical disease (n, %)	4 (8.3%)	9 (20.5%)	0.095
Previous uterine surgery (n, %)	9 (18.75%)	11 (25.0%)	0.468
Number of previous fresh IVF	1.08 ± 1.13	1.14 ± 0.98	0.443
Number of previous FET	0.75 ± 0.81	0.91 ± 0.74	0.728
Genetic (n, %)	1 (2.1%)	0 (0.0%)	0.522 *
Advanced age (n, %)	0 (0%)	2 (4.5%)	0.226 *
Endometriosis (n, %)	1 (2.1%)	2 (4.5%)	0.467 *
Bilateral Tubal factor (n, %)	2 (4.2%)	4 (9.1%)	0.298 *
Low ovarian reserve (n, %)	4 (8.3%)	7 (15.9%)	0.263
Azosperm (n, %)	5 (10.4%)	2 (4.5%)	0.255 *
Anovulation (n, %)	11 (22.9%)	6 (13.6%)	0.252
Unexplained infertility (n, %)	4 (8.3%)	5 (11.4%)	0.444 *

Values are given as mean ± standard deviation. *p value was calculated by Fischer Chi Square Test.**Abbreviations; BMI: Body mass index, ICSI: Intracytoplasmic sperm injection, FET: Frozen embryo transfer, PCOS: Polycystic ovary syndrome

Table 2. Comparison of FET cycle characteristics between groups

	Group 1 Vitamin D Level <20 Ng / Ml (n= 48)	**Group 2 Vitamin D Level ≥20 Ng / Ml (n= 44)**	**P Value**
AMH Level (Ng/Ml) *	9.73 ± 7.79 (n=26)	4.67 ± 4.21 (n=23)	**0.014**
TSH Level (Miu/L) *	1.96 ± 1.24 (n=41)	1.84 ± 0.77 (n=38)	0.702
AFC*	23.46 ± 16.09 (n=12)	14.38 ± 9.36 (n=16)	0.113
Estrogen Used Time (Day)*	9.79 ± 2.93	9.98 ± 2.42	0.379
Endometrial Thickness (mm)*	9.94 ± 1.86 (n=47)	10.06 ± 1.69 (n=43)	0.487
Progesterone Level at the end of Proliferation Phase (ng/ml) *	0.53 ± 0.3 (n=20)	0.43 ± 0.27 (n=24)	0.094
Number Of Embryos Transferred (n)*			
Day 3 Embryos (n, %)	18 (37.5%)	13 (29.5%)	0.420
Day 5 Embryos (n, %)	21 (43.8%)	22 (50.0%)	0.548
Day 6 Embryos (n, %)	9 (18.8%)	9 (20.5%)	0.837

*Values are given as mean ± standard deviation. ** Abbreviations; AMH: Anti-mullerian Hormone, AFC: Antral Follicle Count, TSH: Thyroid Stimulant Hormone

Table 3. Comparison of pregnancy, clinical pregnancy, ongoing pregnancy, live birth, abortion, and twin pregnancy rates between groups

	Group 1 Vitamin D level <20 ng/ml (n= 48)	Group 2 Vitamin D level ≥20 ng / ml (n= 44)	p value
Pregnancy Rate	20 (41.6%)	14 (31.8%)	0.328
Clinical Pregnancy Rate	17 (35.4%)	11 (25.0%)	0.278
Ongoing Pregnancy Rate	12 (25.0%)	8 (18.2%)	0.428
Live Birth Rate	10 (20.8%)	8 (18.2%)	0.749
Abortion Rate	9 (18.8%)	6 (13.6%)	0.507
Twin Pregnancy Rate	2 (4.2%)	4 (9.1%)	0.421 *

*p value was calculated by Fischer Chi-Square Test.

immediately without being frozen, but neither the researchers nor the patients were aware of their vitamin D levels. This reflects the strength of our work in minimizing bias. Similar to our study, Van de Vijyer et al. in their prospective cohort studies, evaluated 280 infertile cases whose FET cycle was planned, in two separate groups as cases with 25-OH vitamin D levels below and above 20 ng/mL on the day of embryo transfer, the pregnancy rate in the vitamin D deficient group was found to be similar when compared to the vitamin D sufficient group (respectively; 40.9% vs. 48.3%, p=0.2).[15] Similarly, no difference was found between the clinical pregnancy rates (32.2% vs 37.9%, respectively, *p*=0.3). Clinical pregnancy rates in this study were similar in cases of insufficiency, deficiency, and normal levels of vitamin D, and the multivariate logistic regression analysis revealed that vitamin D status was not associated with pregnancy outcomes. Moreover, in their study, which involved randomizing 114 infertile cases with 25-OH vitamin D levels of <30 ng/L into two groups with and without vitamin D replacement, Aflatoonian et al. reported that the results of the two groups had similar results in terms of ongoing FET cycle pregnancy and clinical pregnancy.[1] When these findings are considered together with the findings of our study, we are of the opinion that there is no data showing that vitamin D exerts its effects on reproductive functions through endometrial receptivity and implantation.

The fact that the study was not designed to reveal the effects of vitamin D levels at the tissue level is a limitation. However, the study focused on the clinical outcomes needed in current practices and provided explanatory information on this subject. Nevertheless, when the limitations of our study are considered, it is clear that randomized controlled trials with high quality large samples are needed to determine the optimal 25(OH) vitamin D levels and the effects of vitamin D supplementation on fertility.

Compliance with Ethical Standards

This study was approved by Kocaeli University Non-interventional Clinical Research Ethics Committee (Decision number: 2017/176, Date: 07/06/2022)

Conflict of Interest

The authors have no conflicts of interest relevant to this article.

Author Contribution

Authors have contributed equally to this work.

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Araştırma Makalesi | Research Article

KONDROİTİN SÜLFAT TAYİNİ İÇİN İMMÜNFLORESAN BOYAMALARDA OPTİMUM FİKSASYON YÖNTEMİNİN BELİRLENMESİ

DETERMINATION OF OPTIMAL FIXATION METHOD FOR CHONDROITIN SULFATE DETECTION VIA IMMUNOFLUORESCENCE STAINING

Duygu Yücel[1*]

[1]Erciyes Üniversitesi, Genom ve Kök Hücre Merkezi (GenKök), Kayseri, Türkiye.

ÖZ

Amaç: Kondroitin sülfat (KS) zincirleri embriyonik hücre bölünmesi, sitokinez ve gelişimde rol oynayan, *C. elegans*'tan fareye benzer rollerde görev alan moleküllerdir. Doku ve hücrelere mekanik destek veren, nöronal plastisite ve hücre migrasyonunda görev alan KS moleküllerinin biyosentezi, *C. elegans*'ta insandakine benzer bir mekanizma ile gerçekleştirilmektedir. Bu çalışmada, *C. elegans* model organizmasında kondroitin sülfat moleküllerinin immünfloresan boyama tekniği ile gözlemlenmesi için en etkin fiksasyon yönteminin belirlenmesi hedeflenmiştir.

Yöntem: İmmünfloresan boyamaların gerçekleştirilmesi için embriyolar, erişkin *C. elegans* 'lardan izole edilmiş ve embriyo pelletleri %4 paraformaldehid (PFA) ve metanol ile fikse edilmek üzere iki farklı tüpe ayrıştırılmıştır. İnkübasyon ve yıkama aşamalarını takiben, örnekler, chondroitinase ABC (CSase ABC) enzimi ile muamele edilmiştir. Enzimin inaktive edilmesinin ardından %10 donkey serum ile bloke edilen embriyo pelletleri, primer antikor anti-chondroitin antibody ile gece boyu inkübe edilmiştir. Sekonder antikor olarak donkey anti-mouse Alexa 488 1:250 oranında kullanılmıştır. Görüntüler inverted mikroskop kullanılarak elde edilmiştir.

Bulgular: PFA ile yapılan boyamalarda, embriyonik katman yapılarının daha iyi korunduğu görülmüştür. Metanol ile fiksasyonun, embriyonik tabakaların yapısını bozduğu ve kondroitin boyamalarının başarısını düşürdüğü gözlemlenmiştir. Yapılan analiz ve karşılaştırmalarda, PFA ile yapılan fiksasyon aşamasının otofloresan sinyalini artırsa da KS sinyalinin ve morfolojik yapısının tespiti için metanol ile yapılan fiksasyon yöntemine göre daha iyi sonuç verdiği gözlemlenmiştir.

Sonuç: Bu çalışmada, KS tayini için en etkin yöntemin PFA fiksasyon yöntemi olduğu tespit edilmiştir. Böylece, PFA ile fiksasyon yöntemi kullanılarak, KS moleküllerine dair *C. elegans* model organizmasında fenotipik değişiklikler daha nitelikli ve yüksek doğrulukta analiz edilebilecektir.

Anahtar Kelimeler: Kondroitin sülfat, fiksasyon yöntemi, *C. elegans*, immünfloresan boyama, ekstraselüler matriks

ABSTRACT

Objective: Chondroitin sulfate (CS) chains are involved in embryonic cell division, cytokinesis and development from nematode *C. elegans* to mice. CS chains provide mechanical support to tissues and cells and play roles in neuronal plasticity and cell migration. The biosynthesis of CS in *C. elegans* is similar to the mechanism in humans. In this study, we aimed to determine the optimal fixation method for chondroitin sulfate detection via immunofluorescence staining using *C. elegans* as a model organism.

Methods: In order conduct immunofluorescence staining, embryos were isolated from adult worms and embryonic pellets were allocated into two separate tubes for fixation with 4% paraformaldehyde (PFA) and methanol. After incubation and washing steps, samples were treated with chondroitinase ABC (CSase ABC) enzyme upon which the enzyme was inactivated. %10 donkey serum was used for blocking and samples were incubated with anti-chondroitin antibody overnight. As for secondary antibody, donkey anti-mouse Alexa 488 was used in a 1:250 ratio. Image analysis was performed using an inverted fluorescent microscope.

Results: The embryonic layers were conserved better in the PFA fixation method compared with the methanol fixation. In methanol fixated samples, the structure of the embryonic layers was disrupted decreasing the success of the CS staining. Although PFA fixation increased the autofluorescence, it provided a better staining profile for detection of CS signal and the morphological structure as compared with the methanol fixation.

Conclusion: In this study, PFA fixation was determined as the optimal fixation method for CS detection. With the use of PFA fixation method, phenotypic changes related to CS molecules can be analysed with a better quality and higher accuracy in *C. elegans*.

Keywords: Chondroitin sulfate, fixation method, *C. elegans*, immunofluorescence staining, extracellular matrix

***İletişim kurulacak yazar/Corresponding author:** Duygu Yücel; Erciyes Üniversitesi, Genom ve Kök Hücre Merkezi (GenKök), Kayseri, Türkiye.
Telefon/Phone: +90 (352) 207 66 66 (13638) **e-posta/e-mail:** duyguyucel@erciyes.edu.tr
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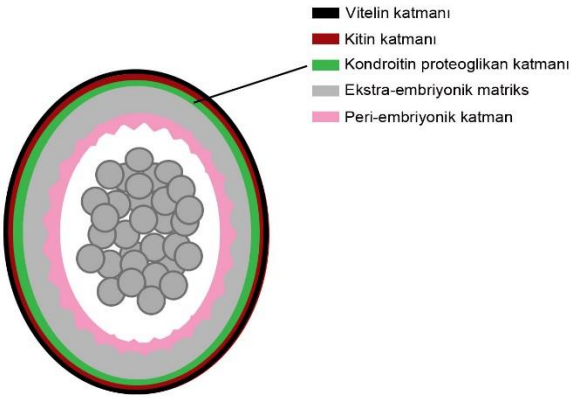
Giriş

Kondroitin sülfat (KS) zincirleri embriyonik hücre bölünmesi, sitokinez ve gelişimde rol oynayan, *C. elegans*'tan fareye benzer rollerde görev alan moleküllerdir.[1] KS moleküllerinin fonksiyonları kurtçuktan memeli sistemlere kadar korunmuştur. KS'ler lineer ve tekrar eden disakkarid birimlerden oluşan polisakkarid yapılardır. Bu polisaakraid zincirler spesifik proteinlerle kovalent olarak bağlanarak, hücre yüzeyinde ve ekstraselüler matrikste yaygın olarak bulunan kondroitin proteoglikan (KPG) yapılarını oluştururlar. Doku ve hücrelere mekanik destek veren, nöronal plastisite ve hücre migrasyonunda görev alan KS moleküllerinin biyosentezi, *C. elegans*'ta insandakine benzer bir mekanizma ile gerçekleştirilmektedir.[1,2]

C. elegans basit anatomisi, her birinin akıbeti belli olan karakterize edilmiş 959 adet somatik hücresi, genetik çalışmalara yatkınlığı ve genlerinin yarısından fazlasının insan genleriyle homoloji göstermesinden dolayı oldukça rağbet gören bir model organizmadır.[3,4] *C. elegans* ve insan arasında birçok biyolojik işlev korunmuştur. İnsan hastalık genlerinin % 65'ine *C. elegans* genomunda bir karşılık bulunabilmektedir.[3] *C. elegans*'ın kolay idame ettirilen ve genetik olarak manipülasyona açık olan sistemi insanlarda görülen birçok hastalığın moleküler mekanizmasının açığa çıkarılmasına katkı sağlamıştır. 302 adet nöronu ve bütün nöronal bağlantı haritasının bilinmesi ile nörodejeneratif hastalıklara, ortalama 20 günlük ömrü ile yaşlanma ve metabolik hastalıklar üzerine, vulval farklılaşma mekanizması ile kanser biyolojisine ışık tutmuştur.

KS moleküllerinin akıbetinin hastalık modelleri, ilaç ve kimyasal ajanlar sonucu gösterdiği fenotiplerin araştırılması açısından nematod *C. elegans*, evrimsel olarak korunmuş olan KS biyogenezi ve fonksiyonlarıyla avantajlı bir model organizma platformudur. *C. elegans*'ta embriyonik zar yapısı beş farklı katmandan oluşmaktadır (Şekil 1).



Şekil 1. Kondroitin molekülleri embriyonik katmanlardan birini oluşturmaktadır. *C. elegans* embriyonik katmanları gösterilmektedir.

Kondroitin proteoglikan (KPG) katmanı, en dıştaki vitelin ve bir altındaki kitin katmanından sonra embriyoyu sarmalayan üçüncü katmandır. Vitelin, kitin ve KPG katmanları dış zarı oluştururken, ekstra-embriyonik matriks KPG katmanının hemen altında asimetrik yapıdadır. Beşinci katman olan peri-embriyonik katman ise lipid moleküllerinden oluşan, embriyonun hidrasyonunu sağlayan permeabilite bariyeri olarak görev yapar. Kitin tabakası embriyoya mekanik destek sağlar ve polispermiyi engeller. KPG tabakası, plazma mebranının kitin tabakasına adhezyonunu engelleyerek embriyonun gelişimi için alan açılmasını sağlar.

KS moleküllerinin immünofloresan tayini için uygulanacak fiksasyon yöntemi önem arz etmektedir. Fiksatifler cross-linking yapanlar ve denatürasyon yapanlar olmak üzere iki ana kategoride sınıflandırılmaktadır.[5] Metanol dehidrasyon/ denatürasyon yoluyla fiksasyon sağlarken, paraformaldehid (PFA) cross-linking yaparak fiksasyon yapılmasına neden olmaktadır.[6] Aldehitler ve alkol bazlı fiksatifler hücre biyolojisi alanında etkinliği en çok karşılaştırılan fiksatif ajanlar arasında yer almaktadır.[7] Fiksasyon antijeniteyi bozabilecği için, farklı fiksasyon yöntemlerinin test edilmesi en iyi boyama sonucu alınması açısından önemlidir. . Bu çalışmada, PFA ve metanol ile fiksasyon yöntemleri kullanılarak KS tayini için en iyi hangi yöntemin kullanılması gerektiği incelenmiştir. Yapılan analiz ve karşılaştırmalarda, PFA ile yapılan fiksasyon aşamasının otofloresan sinyalini artırsa da, KS sinyalinin ve morfolojik yapısının tespiti için metanol ile yapılan fiksasyon yöntemine göre daha iyi sonuç verdiği tespit edilmiştir. Böylece, PFA ile fiksasyon yöntemi ile, KS moleküllerine dair *C. elegans* model organizmasında fenotipik değişiklikler daha nitelikli ve yüksek doğrulukta analiz edilebilecektir.

Yöntem

Kurtçukların İdame Ettirilmesi

Kurtçukların kültüre edilmesi için Nematode Growth Medium (3 g/L Sodium Chloride, 2.5 g/L Bacto peptone, 17 g/L Bacteriological agar) ve OP50 bakteri suşu kullanılmıştır. Deneyler 20 – 25 C'de soğutmalı inkübatör kullanılarak gerçekleştirilmiştir. Çalışmada kullanılan yabanıl tip N2 (Bristol) *C. elegans* suşu, University of Minnesota Caenorhabditis Genetics Center'dan temin edilmiştir.

Embriyo Eldesi

C. elegans kültürü, 60 mm'lik Nematode Growth Medium/ OP50 (NGM/OP50) petri kaplarında erişkin evreye gelene kadar büyütüldükten sonra, kurtçuklar dH2O kullanılarak petri kaplarından falkon tüplerine aktarılmıştır. Kurtçukların tüplerin dibine çökmesi sağlandıktan sonra supernatant uzaklaştırılmıştır. Erişkin *C. elegans* popülasyonu BS2X hipoklorür solüsyonu ile (0.5 M NaOH, 20% (v/v)) muamele edilerek erişkin hayvanların kütikülleri eritilmiş ve embriyolar elde edilmiştir. Hipoklorür solüsyonu M9 tampon çözeltisi kullanılarak (1 mM MgSO4; 22 mM KH2PO4; 42 mM Na2HPO4; 86 mM NaCl) ortamdan uzaklaştırılmıştır. M9 tampon çözeltisi içindeki embriyolar immünfloresan boyamalar için kullanılmıştır.

İmmünfloresan Boyamalar

Embriyo pelletleri %4 paraformaldehid (PFA) ve metanol ile fikse edilmek üzere iki farklı tüpe ayrıştırılmıştır. Fiksatif eklendikten sonra, embriyoların kütiküllerinde çatlak oluşturarak fiksatiflerin penetre olmasını sağlamak amacıyla tüpler sıvı azot içerisinde dört kez dondurulup çözdürülmüştür.[1,8] Ardından, %4 PFA ve metanol olmak üzere iki farklı tüp içerisindeki embriyolar gece boyu 4 C'de inkübe edilmiştir. Ertesi gün, PFA ve methanol solüsyonları % 0,25 Triton-X içeren PBS çözeltisi (PBST) ile embriyolardan uzaklaştırılmıştır. Örnekler, 0,04M Tris Acetate, pH 8 içerisinde çözülmüş ve 50mU/ µl chondroitinase ABC (CSase ABC) enzimi ile muamele edilmiştir. Negatif kontrol olarak yalnızca 0,04M Tris Acetate, pH 8 solüsyonu ile muamele edilen örnekler kullanılmıştır. CSase ABC enzimi içeren ve içermeyen iki ayrı tüp 3 saat boyunca 37 C'de inkübe edilmiştir. Enzimin inaktive edilmesi için örnekler 95 C'de 5 dakika bekletilmiştir. Örnekler oda sıcaklığında 1 saat boyunca %10 donkey serum ile bloke edildikten sonra primer antikor anti-chondroitin antibody (anti-proteoglycan ΔDi-0S antibody 1B5, 1:20; Seikagaku) ile 4 C'de gece boyu inkübe edilmiştir. Sekonder antikor olarak donkey anti-mouse Alexa 488 (Life Technologies) 1:250 oranında kullanılmıştır ve 49,6-diamidino-2-phenylindole (DAPI) içeren VectaShield mounting medium ile mikroskop preparatları hazırlanmıştır.

Mikroskop analizi

Görüntüler Leica DMI1 inverted mikroskop (DMI4000) kullanılarak elde edilmiştir. *C. elegans*'ların idame ettirilmesi ve embriyoların izole edildiğinin konfirmasyonu için stereomikroskop (Leica M165 FC) kullanılmıştır.

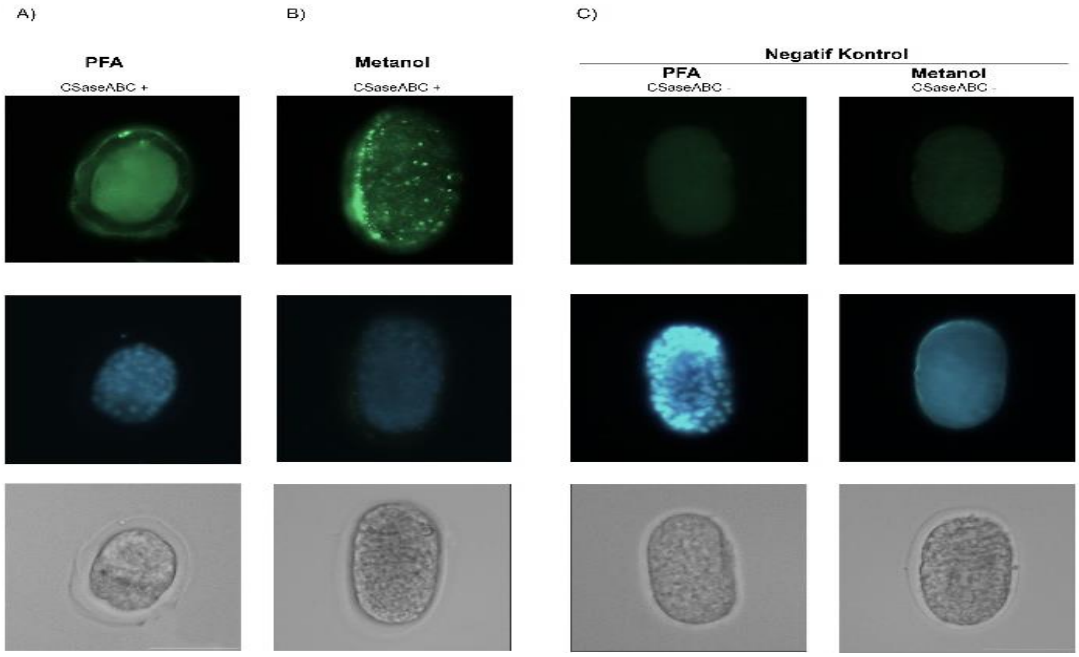
Bulgular

C. elegans embriyolarında PFA ve metanol fiksasyon yöntemlerinin etkinliğinin incelendiği ve optimum fiksasyon yönteminin belirlenmesinin hedeflendiği bu çalışmada, PFA ile yapılan boyamalarda, embriyonik katman yapılarının daha iyi korunduğu görülmüştür (Şekil 2A). Metanol ile fiksasyon işlemi gerçekleştirildiğinde, embriyonik tabakaların yapısının bozulduğu ve kondroitin boyamalarının başarısının düştüğü gözlemlenmiştir (Şekil2B).

Şekil 2A'da PFA fiksasyonu ile yapılan boyamada, birinci panelnde KS sinyali görülmektedir. Şekil 1'de embriyonik katmanların şematik gösteriminde yeşil olarak işaretlenen KPG katmanı net bir şekilde tespit edilebilmektedir. İkinci panelde, DAPI ile işaretlenmiş embriyonik hücrelerin çekirdekleri gösterilmektedir. Üçüncü panel, embriyonun ışık mikroskobu ile elde edilen görüntüsüdür. Şekil 2B'deki birinci panel ile karşılaştırıldığında, KPG katmanının metanol fiksasyonu sonucu morfolojik yapısının bozulduğu görülmektedir. KS moleküllerine ait sinyal, noktasal bir şekilde dağılmış ve ikinci paneldeki DAPI ile yapılan boyamada embriyonik çekirdek hücreleri bulanık bir şekilde görülmekte ve kaç adet çekirdek olduğu tespit edilememektedir.

Şekil 2C panelinde negatif kontrol olarak temsili görüntüleri sunulan chondroitinase ABC (CSase ABC) enzimi ile muamele edilmemiş görüntüler, KS sinyalinin spesifik olarak kondroitin moleküllerini gösterdiğini doğrulamaktadır. CSase ABC enzimi ile muamele edilerek elde edilen Şekil 2A ve 2B, enzimin KS zincirlerini degrede ederek, monoklonal antikor 1B5'in tanıyacağı kondroitin parçalarının oluşmasını sağladığı temsili görüntülerdir. Şekil 2C'de metanol fiksasyonu ile gerçekleştirilen DAPI boyamasının başarısı, Şekil 2B'dekine benzer şekilde düşüktür.

KS moleküllerinin tespit ve analizi için PFA'nın, metanol ile karşılaştırıldığında daha etkin bir fiksasyon yöntemi olduğu gözlemlenmekle birlikte PFA fiksasyonu sonucu otofloresan sinyalinin oluştuğu görülmektedir. Metanol ile yapılan fiksasyonda, otofloresan sinyali oldukça düşüktür. PFA ile fiksasyon sonucu otofloresan sinyalindeki artış, KS moleküllerine dair yapılan analizleri etkilemeyecek düzeydedir.



Şekil 2. PFA ve metanol ile gerçekleştirilen fiksasyon işlemlerinin karşılaştırılması. A) PFA B) Metanol fiksasyonu ile yapılan immünfloresan boyamalarda, yukarıdan aşağıya doğru birinci panelde KS sinyali görülmektedir. İkinci panelde embriyonik çekirdek hücreleri ve üçüncü panelde ise embriyonun ışık mikroskobundaki görüntüsü verilmiştir. C) Chondroitinase ABC (CSase ABC) enzimi ile muamele edilmemiş görüntüler, KS sinyalinin spesifik olarak kondroitin moleküllerini gösterdiğini doğrulamaktadır. Ölçü belirteci 25 mikronu göstermektedir.

Tartışma

Proteinlerin selüler ve subselüler yerleşiminin tespit edilmesi için antikorların kullanımı ve immünhistokimyasal ve immünfloresan boyamalar yaygın olarak uygulanan yöntemlerdir. Ancak *C. elegans* model organizmasında moleküler genetik teknikler antikor boyama yöntemlerinin önüne geçmektedir. Bu teknikler arasında ilgili genin Green Fluorescent Protein ile işaretlenmesi (2009 Nobel Kimya Ödülü), promotör bölgesinin veya cDNA ürünlerinin GFP ile gen füzyonları oluşturarak gözlemlenmesi en temel yaklaşımken, son gelişmeler arasında MOSSCI, CRISPR/Cas9 yöntemleri bulunmaktadır. Fonksiyon kaybına uğramadan genleri işaretleme yöntemleri geliştirilmiş olsa dahi, gen ürünlerinin direkt olarak antikorlar ile gözlenebilir olması protein fonksiyonlarının çalışılması açısından önem arz etmektedir. Bunun en temel sebebi genellikle gen fonksiyonlarının anlaşılması için kullanılan genetik ve moleküler analizlere ek olarak, immün işaretleme yoluyla gen ürünlerinin ifade paterni ve hücresel yerleşiminin tespitine ihtiyaç duyulmasıdır. *C. elegans* model organizmasında immün boyamalara dair en büyük sorunlardan biri kurtçukların dış yüzeyini kaplayan kütikül sebebiyle antikorların dokulara ulaşmasının zor olmasıdır.[9] *C. elegans*'ın dış iskeletini oluşturan ve onu dış etkenlerden koruyan, morfolojik yapısını korumasını sağlayan kütikül yapısı embriyolarda beş adet katmandan oluşmaktadır. Kütikül yapısının boyamalarda yarattığı engelin önüne geçebilmek için kullanılan yöntemlerden birisi "freeze-crack" yöntemidir. Fiksasyon solüsyonunun ve antikorun dokulara ulaşması, embriyoların sıvı azot içerisinde birkaç kez dondurulup çözülmesi vasıtasıyla sağlanabilmektedir.

Çalışmamızda, *C. elegans* embriyolarında kondroitin sülfat tayini için kullanılacak en iyi fiksasyon yöntemini araştırdığımızda, PFA ile fiksasyonun metanolle karşılaştırıldığında daha iyi sonuç verdiğini gözlemlenmiştir. Metanol membrandan lipidleri uzaklaştırıp, proteinleri çöktürerek fiksasyonu sağlamaktadır. PFA, proteinler ve lipidler arasında çapraz bağlar oluşturarak fiksasyon sağladığı için, membran yapısı korunmaktadır. KS molekülleri, hücre yüzeyinde ve ekstraselüler matrikste bulundukları için, metanolün membranı modifiye edici etkisinden dolayı, KS molekülleri ve KPG embriyonik tabakasının morfolojisi metanolle ile muamaele sonucu hasar görmüş olabilir.[10] PFA fiksasyonu otofloresan etkisinden dolayı dezavantajlı olabilmektedir. Metanol ile fiksasyon yapıldığında otofloresan etkisi az olmakla birlikte, boyamanın başarısı düşüktür. PFA ile yapılan fiksasyonda otofloresan sinyali, KS sinyalinin analiz edilmesinin önüne geçmemektedir.

KS moleküllerinin morfogenez, nöronal plastisite, iskelet-kas hastalıkları ve enfeksiyonlarda rol aldığına dair bulgular ve *C. elegans*'tan memeli sitemlerine kadar evrimsel olarak korunmuş rollere sahip olmaları, bu moleküllerin analizi için *C. elegans* model organizmasının kullanılabileceğine işaret etmektedir.[2,11] Yakın zamanda fare modelinde yapılan çalışmalar, KS moleküllerinin perinöral net (PNN) yapılarında nöroprotektif rol oynadığını ve KS degredasyonunun psikiyatrik ve nörolojik hastalıklarda etkisi olduğunu göstermiştir.[12,13] Kısa yaşam döngüsü, korunmuş hücresel işlevleri ve genetik manipülasyonlara açık sistemiyle *C. elegans*, bu çalışmada optimize edilmiş KS immünfloresan boyama yöntemi ile KS fonksiyonlarını etkileyen faktörlerin araştırılması için avantajlı bir platformdur.

Etik Standartlara Uygunluk
Çalışma etik kurul iznine tabi değildir.

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Research Article | Araştırma Makalesi

PEDIATRIC NON-HODGKIN LYMPHOMA: TEN-YEAR EXPERIENCE WITH BERLIN-FRANKFURT-MUNSTER (BFM) PROTOCOLS FROM A TERTIARY CARE HOSPITAL IN TURKIYE

PEDİATRİK NON-HODGKİN LENFOMA: TÜRKİYE'DE ÜÇÜNCÜ BASAMAK MERKEZİ'NDEN BERLİN-FRANKFURT-MUNSTER (BFM) PROTOKOLLERİ İLE ON YILLIK DENEYİM

Meriban Karadogan[1], Ugur Demirsoy[2], Yonca Anık[3], Maksut Gorkem Aksu[4], Funda Corapcioglu[5]

[1]Sakarya Training and Research Hospital, Pediatric Hematology and Oncology, Sakarya, Türkiye. [2]Kocaeli University Faculty of Medicine, Pediatric Hematology and Oncology, Kocaeli, Türkiye. [3]Kocaeli University Faculty of Medicine, Radiology, Kocaeli, Türkiye. [4] Kocaeli University Faculty of Medicine, Radiation Oncology, Kocaeli, Türkiye. [5] Acıbadem Maslak Hospital, Pediatric Hematology and Oncology, Istanbul, Türkiye.

Abstract

Objective: Progress in therapy of childhood non-Hodgkin lymphoma (NHL) is one of the stunning success stories of the past two decades. In developed countries, more than 80% of children with NHL can now be cured with modern therapy, even patients with widely disseminated disease. The aim of this study is to analyze all NHL patients who were treated in a single tertiary center in Turkiye.

Methods: An analysis of data of children with NHL, diagnosed and treated between 2003 and 2012 according to the original Berlin-Frankfurt-Munster (BFM) protocol in Kocaeli University Pediatric Oncology Department was carried out.

Results: Forty-seven children were eligible for analysis. Mean age at diagnosis was 9.6 years with a male: female ratio of 1.9. Thirty-one patients (66%) were mature B-cell NHL with 23 patients (48.9%) Burkitt lymphoma, 7 patients (14.8%) diffuse large B-cell lymphoma, one patient (%2) primary mediastinal large B-cell lymphoma; 13 patients (27.6%) were lymphoblastic lymphoma with 11 patients (23.3%) T-lymphoblastic lymphoma and 2 patients (4.2%) B-lymphoblastic lymphoma, and also 3 patients (6.3%) were mature-T cell lymphoma-anaplastic large cell lymphoma. Four-year event-free survival was 78.7% and overall survival was 80.8%.

Conclusion: These results with BFM protocol management reflect good treatment outcomes in our patients.

Keywords: Children, non-hodgkin lymphoma, Turkiye.

Öz

Amaç: Çocukluk çağı Non-Hodgkin lenfoma (NHL) tedavisindeki ilerleme, son yirmi yılın çarpıcı başarı öykülerinden biridir. Gelişmiş ülkelerde, NHL'li çocukların %80'inden fazlası, yaygın hastalık durumunda bile modern terapi ile tedavi edilebilmektedir. Bu çalışmanın amacı, Türkiye'de tek bir üçüncü basamak merkezde tedavi edilen tüm NHL hastalarını analiz etmektir.

Yöntem: Kocaeli Üniversitesi Pediatrik Onkoloji Anabilim Dalı'nda 2003-2012 yılları arasında orijinal Berlin-Frankfurt-Munster (BFM) protokolüne göre teşhis ve tedavi edilen NHL'li çocukların verilerinin analizi yapıldı.

Bulgular: Kırk yedi çocuk analiz için uygun bulundu. Ortalama tanı yaşı 9,6, erkek/kız oranı 1,9 idi. 31 hasta (%66) matür B hücreli NHL, bunların 23'ü (%48.9) Burkitt lenfoma, 7'si (%14.8) diffüz büyük B hücreli lenfoma, biri (%2) primer mediastinal büyük B hücreli lenfoma idi; 13 hasta (%27,6) lenfoblastik lenfoma, bunların 11'i (%23,3) T-lenfoblastik lenfoma ve 2'si (%4,2) B-lenfoblastik lenfoma ve ayrıca 3 hasta (%6,3) matür T hücreli lenfoma-anaplastik büyük hücreli lenfoma idi. Dört yıllık olaysız sağkalım %78,7 ve genel sağkalım %80,8 idi.

Sonuç: BFM protokolü uygulamasıyla elde edilen bu sonuçlar, hastalarımızda iyi tedavi sonuçlarını yansıtmaktadır.

Anahtar Kelimeler: Çocuk, non-hodgkin lenfoma, Türkiye.

*** Corresponding author/İletişim kurulacak yazar:** Meriban Karadogan; Sakarya Training and Research Hospital, Pediatric Hematology and Oncology, Sakarya, Turkiye
Phone/Telefon: +90 (264) 888 40 00 **e-mail/e-posta:** meribank79@gmail.com
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Introduction

Non-Hodgkin lymphoma (NHL) is caused by malignant transformation of lymphoid cells, the constituent cell of the immune system. It usually originates from the lymph nodes and tends to spread to organs such as spleen, bone marrow or central nervous system (CNS); primary bone or CNS presentations are rarely seen.[1] NHLs account for 60% of childhood lymphomas. It constitutes 8-10% of all childhood malignant diseases in developed countries. In recent years, an increase in adolescence has been reported.[2] The World Health Organization (WHO) classification from the International Lymphoma Study Group incorporates histology, immunohistochemistry, gene expression profiling, cytogenetic, molecular and clinical features for lymphoid neoplasms classification.

Childhood NHL is a widely high-grade and disseminated disease in contrast to adults where more than two-thirds of the tumors are indolent, low-grade malignancies and includes the four major subtypes; B- and T-lymphoblastic lymphoma (LL), Burkitt lymphoma (BL), Diffuse large B-cell lymphoma (DLBCL) and Anaplastic large cell lymphoma (ALCL) Commonly, enlarged lymphadenopathy due to the compression of surrounding structures cause clinical symptoms such as new onset wheezing, facial swelling, respiratory distress or acute abdominal pain, depending on the type of lymphoma and the areas of involvement. As the symptoms usually emerge rapidly (one to three weeks), efficient and appropriate handling of pathologic materials (tissue, bone marrow, cerebrospinal fluid (CSF) or pleural/ paracentesis fluid) is essential to ensure. Superior vena cava syndrome secondary to a large mediastinal mass obstruction, respiratory airway compression, tumor lysis syndrome (TLS) secondary to severe metabolic abnormalities from massive lysis of tumor cells requires immediate attention and emergency treatment.[3-5]

Combination chemotherapy is the primary modality used for the treatment of pediatric NHL. Some patients receive radiation therapy. Most children and adolescents with NHL have a good prognosis with current therapy. Long-term overall survival is achieved in >80 percent of pediatric NHL cases overall and in >90 percent of stage I or II pediatric NHL.

The aim of this study is to analyze all NHL patients who were treated in a single tertiary center in Turkey and present the results of Berlin-Frankfurt-Munster (BFM)-95 treatment protocol.

Methods

An analysis of data of children with NHL, diagnosed and treated between 2003 and 2012 according to the original BFM-95 protocol in Kocaeli University Pediatric Oncology Department was carried out. A total of 47 children, aged between 1 and 18 years old and who completed chemotherapy at least 2 years prior to the analysis, were included in the study.

The patients were evaluated regarding their ages, gender, symptoms, anatomical location of the tumor and histopathological characteristics retrospectively. Complete blood count and assessment of liver and renal function tests and serum LDH were performed. The diagnosis was made by histopathological evaluation of biopsies, bone marrow aspiration samples and for some patients, after clinical, radiological and cytological tests.

We classified our patients according to St. Jude's non-Hodgkin's lymphoma classification in children.[11] All patients were treated with BFM-95 treatment protocol. We calculated overall survival (OS) and event-free survival (EFS) for the patients. Overall survival was defined as the total follow up time of patients from the time of diagnosis; EFS was defined as relapse, tumor progression, secondary malignancy or death from any cause from the time of diagnosis.

SPSS 13.01 pack program was used for statistical analyses. Log-rank and Kaplan Meier tests were used for the evaluation of survival analysis. Statistically significant considered as $p<0.05$.

Results

A total of 47 patients were included in the study whose characteristics, primary localization and stage of the disease, diagnosis and the treatment results are summarized in Table **1**. The median age at diagnosis was 9.6 years with a male to female ratio of 1.9. According to subtypes, 31 patients (66%) were mature B-NHL with 23 patients (48.9%) BL; 7 patients (%14.8) DLBCL and one patient (2%) primary mediastinal large B-cell lymphoma (PMLBL); 13 patients (27.6%) were LBL with 11 patients (23.3%) T-lymphoblastic lymphoma (T-LBL) and 2 patients (4.2%) B-lymphoblastic lymphoma (B-LBL) and 3 patients (6.3%) were mature-T cell lymphoma-ALCL. Most patients, 36 (76.6%), were diagnosed in advanced stages, 24 (51%) stage III and 12 (25.5%) stage IV. Eleven patients (23.4%) had local disease; 2 (4.2%) stage I and, 9 (19.1%) stage II. Five patients (10.2%) had CNS involvement while 8 patients (%17) had bone marrow involvement.

Primary location of the disease was head and neck region in 17 (36%) patients while it was abdomen, mediastinum, peripheral lymph nodes and other (nasopharynx, bone, tonsil, CNS, sacral), 14 (30%), 6 (13%); 2 (4%) and 8 (17%) patients respectively. Thirty-seven patients (78.7%) were diagnosed via biopsy performed under general anesthesia. Six patients (12.7%) were diagnosed by examination of pleural fluid or ascites and 4 (8.5%) after bone marrow aspiration.

Two patients died of infections (4.2%); 6 patients (12.7%) died of disease progression and 1 patient (2.1%) died of secondary malignancy (glioblastoma multiforme). Eight patients (17%) relapsed; 7 of them were stage III and 1 of them stage IV during initial diagnosis. Median relapse time after diagnosis was 9 months (4-19 months). The mean follow-up time was 56 months (range 63±31, median 62 months). Four-year EFS was 78.7% and OS 80.8% Figure 1, 2.

Table 1. Patients Characteristics and Outcome in all NHL Patients

	LBL	B-NHL	ALCL	Total
	(n = 13) n	(n = 31) n	(n = 3) n	(N = 47) n (%)
Stage				
I	0	2	0	2 (4.2)
II	2	6	1	9 (19.1)
III	7	15	2	24 (51)
IV	4	8	0	12 (25.5)
Localization				
Abdomen	2	11	1	14 (30)
Mediastinum	5	1	0	6 (13)
Head and neck	6	10	1	17 (36)
Peripheral lymph nodes	0	2	0	2 (4)
Other	0	7	1	8 (17)
CNS	0	5	0	5 (10.6)
Bone marrow	4	4	0	8 (17)
Diagnosis				
Biopsy	8	26	3	37 (78.7)
Pleural fluid/ascites	3	3	0	6 (12.7)
Bone marrow	2	2	0	4 (8.5)
Treatment results				
Death due to disease progression	2	4	0	6 (12.7)
Death due to sepsis	1	1	0	2 (4.2)
Toxic death	0	0	0	0 (0)
Secondary malignancy	1	0	0	1 (2.1)
Survival				
OS	69.2%	81.3%	100%	80.8%
4-Year pEFS	69.2%	80.6%	100%	78.7%

Note. NHL = non-Hodgkin lymphoma; LBL = lymphoblastic lymphoma; ALCL = anaplastic large-cell lymphoma; B-NHL =mature B-cell lymphoma; CNS = central nervous system; OS = overall survival; EFS = event-free survival.

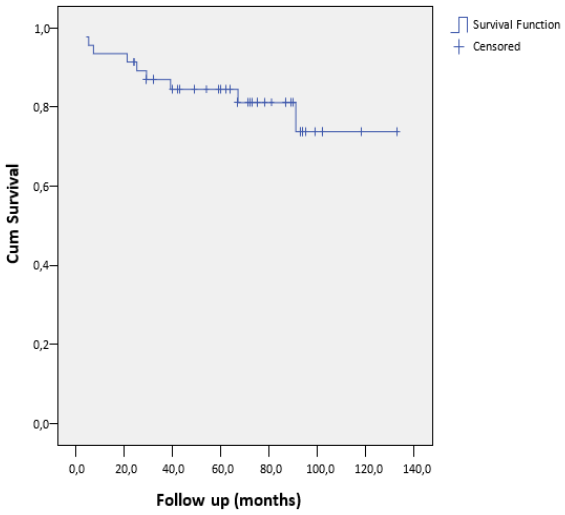

Figure 1. Kaplan-Meier estimate of overall survival. (n=47; 4-year OS = 80.8%).

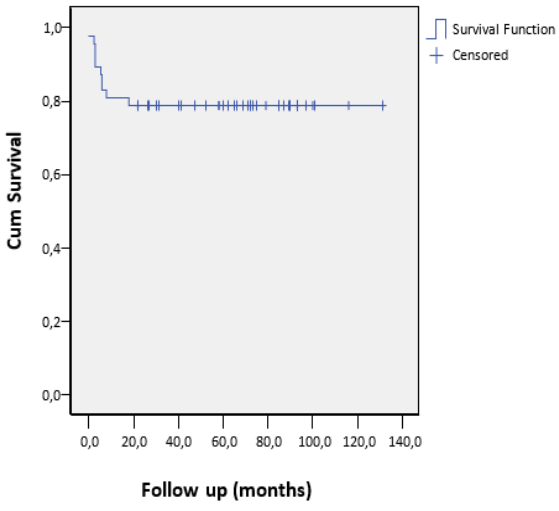

Figure 2. Kaplan-Meier estimate of event-free survival (EFS) (n=47; 4-year EFS = 78.7%)

Four-year EFS of male patients was 77.4% versus 81.3% for female patients Figure **3**, with no statistical significance (p >0.05).

All patients were stratified into 2 age groups: 0-10 years (23 patients) and 11-18 years (24 patients). The four-year EFS for these age categories was 78.3% and 79.2%, respectively Figure **4**, without reaching statistical significance (p=0.809).

Four-year EFS for different stages are shown in Figure **5**. Stage I and II survival was 100%: stage III 66.7% and stage IV 83.3%. Patients with stage I and II disease had significantly higher EFS compared to stages III and IV (p<0.05).

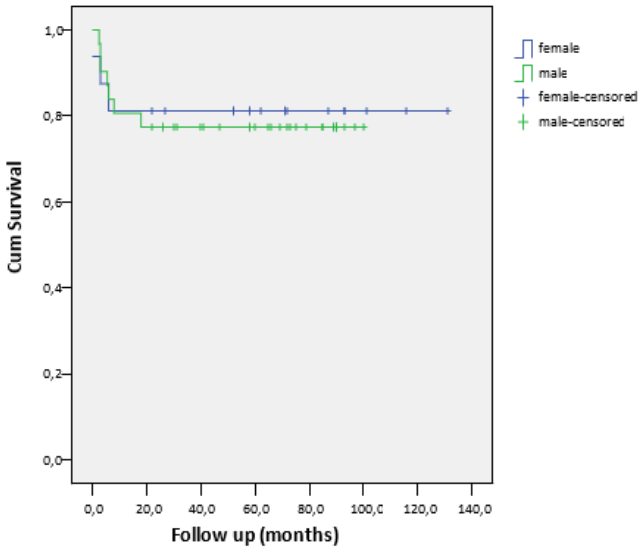

Figure 3. Kaplan-Meier estimate of event-free survival by sex; EFS = 77.4% versus 81.3% (P=0.809).

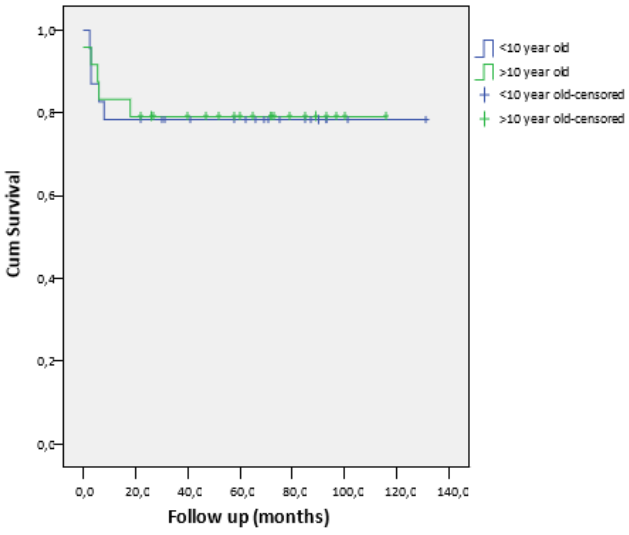

Figure 4. Kaplan-Meier estimate of event-free survival by age; EFS = 78.3% versus 79.2% (P=0.933).

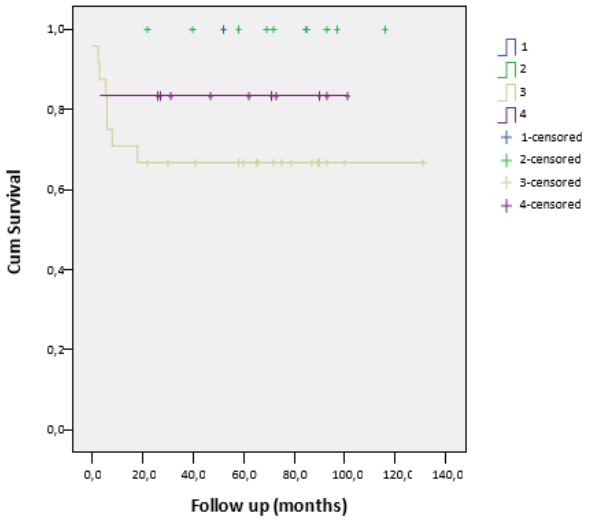

Figure 5. Kaplan-Meier estimate of event-free survival according to stage of disease (p=0.185).

The patient group with B-NHL included 23 children with BL, 7 children with DLBCL and 1 child with PMLBL. The age of the patients with BL ranged from 2.8 to 17, with a median of 11 years. Three patients had primary nasopharyngeal involvement, 2 patients had primary bone lymphoma, 1 patient had tonsillar lymphoma and 1 patient had primary CNS lymphoma. One of the patients died of sepsis and one of them relapsed 7 months after the end of treatment and had an autologous bone marrow transplant (BMT) but died of progressive disease. A patient who had an allogeneic BMT after relapse is still alive.

DLBCL patients aged between 2-15 years; median 11 years. One patient relapsed 9 months after the end of treatment and had an autologous BMT but relapsed again after BMT and died of progressive disease. A patient with DLBCL had no remission and died of progression. 17 years old boy diagnosed with PMLBL relapsed after 13 months and died of progressive disease. 4-year EFS was found 87% with BL and 71.4% with DLBCL.

Thirteen children were diagnosed with T-LBL and 2 with precursor B-LBL. The patients' ages ranged from 4.5 to 14.5; median 7.9 years; two of the patients died from progressive disease; one patient died from infections and one patient died from a secondary malignancy.

Three patients made up the ALCL patient cohort, one of whom was a 5-year-old girl who had isolated bone lymphoma when she was first diagnosed. Patients ranging in age from 4.5 to 14.5; the average age was 13 years. After treatment, there was no relapse or death. Figure 6 displays the NHL subtypes' four-year EFS.

Fourteen patients had liver toxicity, but no renal and cardiac toxicity was encountered. None of the patients died due to toxicity. Three patients had renal involvement while 1 patient had in pancreas, and another patient in adrenal gland. Ten patients had prophylactic cranial radiotherapy (pCRT). Two patients with primary bone lymphoma, 1 patient with primary mediastinal tumor and another patient with primary CNS lymphoma

received therapeutic radiotherapy (RT). RT was given to bone, mediastinum and bronchial region due to relapse of disease. Five BL and 4 DLBL patients were given rituximab.

Discussion

Because of the possible emergency complications of NHL, prompt recognition and therapy initiation at the time of diagnosis are critical. Children with NHL should be treated in a comprehensive pediatric oncology center with a multidisciplinary approach. In many series, there is a pronounced male predominance in all age groups[3,6] and the median age at diagnosis is around 10 years.[7,8,10] Demographics of our patients were as follows: male to female ratio 1.9/1 and mean age 9.6 years, median 10.4 years. The distribution of subtypes of NHL in our patients was concordant with WHO 2008 NHL Classification.[11] Burkitt lymphoma was the most diagnosed subtype, 48.9%; and the rates of DLBCL, PMLBL, LBL and ALCL were 14.8%, 2%, 27.6% and 6.3%, respectively.

No statistical difference was reached considering EFS in 2 different age groups and gender. Burkhard et al. showed the impact of gender and age on the outcome as there was a significant difference between NHL subgroups.[6] The reduced sample size in our study can be used to explain this outcome.

Diagnostic workup was made without histopathological confirmation in 10 patients (21%). Biopsy under general anesthesia should be avoided, if possible, especially in patients with significant airway narrowing or symptoms of respiratory distress.[12,13] Examination of bone marrow or pleural fluid/ ascites may be diagnostic.

The majority of patients, 36 (76.6%), had advanced stage disease, stage III in 24 (51%) and stage IV in 12. (25.5 percent). Almost 40% of children with NHL presented with stage I and II and the remainder with stage III and IV disease.[9] The frequency of patients with stage III/IV NHL was 49.4%. in China[14]; however, this frequency reaches 90% in Pakistan.[15]

Using stratification based on the biological aspects of the disease, patients were handled in accordance with BFM procedures. Four-year EFS was 78.7% and overall survival was 80.8%. Overall survival was 100% in stage I and II; while it was 66.7% in stage III and 91.7% in stage IV. Therefore, our results are similar to other study investigations using the same stratification approaches. EFS of 84.1% corresponds to the results of the largest multicenter BFM study.[6]

Death due to sepsis was encountered in 4.2 percent of patients and it is greater than the rate (2 percent) that has been recorded in developed countries but also lower than other countries rates with insufficient resources.[16,17] As a developing country, our outcomes do not linger behind the results that are reported in developed countries.

The survival outcome of children with B-NHL has noticeably improved through consecutive clinical trials in large study groups, and the cure rate of childhood B-NHL has reached 90% during the last two decades.[18-20] We showed a good survival outcome with 4-year EFS 80.6% in children with B-NHL, 87% with BL and 71.4% with DLBCL in the present study.

For LBL; the use of intensive protocols designed for children with ALL, such as the BFM regimens, have been shown to be more effective in advanced-stage disease.[21, 22] Our study showed 69.2% EFS with LBL.

Anaplastic large cell lymphoma (ALCL), within the classification of mature T-cell lymphoma, accounts for approximately 10-15 % of NHL in childhood. Event free survival achieves 70% of current treatment approaches using multiagent chemotherapy.[4,23] In our study, ALCL patients made up 6.3% of total with 100% overall survival.

CNS involvement was defined in 10.6% of patients and all had mature B-cell lymphoma. Pediatric studies have shown that CNS illness, particularly in more advanced stages of the disease, affects up to 6% of children with NHL.CNS involvement at diagnosis is not common but is most commonly seen in children with advanced BL and LL.[24] Our result was similar. Our research found that BM infiltration was 17%, which was a bit lower than rates in bigger NHL patient series.[6]

Conclusion

NHL was successfully treated in the majority of children in our clinic in which success depends on the type, stage and grade of the lymphoma. Better outcomes are associated with early diagnosis and localization of the disease in one region of the body. The clinical response to the treatment protocols was similar to the literature.

Ethical Approval

No ethics committee decision is required for the study.

Conflicts of Interests

The authors declare there are no conflict of interest.

Author Contribution

All authors contributed equally to this work.

Financial Disclosure

None.

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Research Article | Araştırma Makalesi

KEEP IN MIND OTHER CAUSES THAN INTRINSIC SPHINCTER DYSFUNCTION IN POST-PROSTATECTOMY INCONTINENCE

POST-PROSTATEKTOMİ İNKONTİNANSTA İNTRİNSİK SFİNKTER DİSFONKSİYONU DIŞINDAKİ NEDENLER DE AKILDA BULUNDURULMALIDIR

Huseyin Alperen Yildiz*, Emrullah Sogutdelen

Bolu Abant Izzet Baysal University, Department of Urology, Bolu, Türkiye.

Abstract

Objective: This study aims to determine the causes of urinary incontinence and accompanying bladder pathologies in patients with persistent incontinence 12 months after radical prostatectomy.

Methods: Thirty-four patients who underwent open radical retropubic prostatectomy (RRP) due to localized prostate cancer and incontinent after surgery between January 2015 and December 2020 and underwent urodynamic study (UDS) were retrospectively reviewed. All UDSs were performed according to the ICS protocol of good urodynamic practice and assessed by the same doctor. Patients were assessed by demographic and clinical parameters including age, body mass index (BMI), comorbidity, preoperative PSA level, prostate volume (PV), transrectal prostate biopsy Gleason score, clinical stage, pathological ISUP grade, pathological stage, lymph node positivity, international consultation on incontinence questionnaire-short form and subjective questionnaire for daily pad use.

Results: Urodynamic studies showed that 29 patients (85.2%) had intrinsic sphincter deficiency (ISD). Nine (26.4%) of 29 patients with ISD had a secondary diagnosis of overactive bladder (OAB), impaired detrusor contractility (IDC) and OAB+IDC 9 (11.6%), 4 (11.6%), 1 (2.9%), respectively. Nineteen patients (55.8%) had a sole diagnosis of ISD, whereas 15 patients (44.1%) had another primary diagnosis or a secondary diagnosis other than ISD.

Conclusion: Although ISD is the most common cause of persistent urinary incontinence in patients after radical prostatectomy, it should be taken into account that overactive bladder and impaired detrusor contractility are also accompanied by a significant proportion of patients.

Keywords: prostate cancer, post-prostatectomy incontinence, urodynamics.

Öz

Amaç: Bu çalışmada radikal prostatektomiden 12 ay sonra persistan inkontinansı olan hastalarda üriner inkontinans nedenleri ve eşlik eden mesane patolojilerinin belirlenmesi amaçlanmıştır.

Yöntem: Ocak 2015 ile Aralık 2020 tarihleri arasında lokalize prostat kanseri nedeniyle açık radikal retropubik prostatektomi (RRP) uygulanan ve cerrahiden sonra inkontinansı olan ve urodinami yapılmış olan 34 hasta retrospektif olarak incelendi. Tüm ürodinamik çalışmalar, iyi ürodinamik uygulama ICS protokolüne göre yapıldı ve değerlendirmeleri aynı doktor tarafından yapıldı. Hastalar vücut kitle indeksi, komorbidite, ameliyat öncesi PSA düzeyi, prostat hacmi, prostat, prostat biyopsi Gleason skoru, klinik evre, patolojik ISUP grade, patolojik evre, kaçırma anket lenf nodu pozitifliği, uluslararası inkontinans sorgulama formu–kısa form ve ped kullanımı için öznel anket dahil olmak üzere demografik ve klinik parametreleri ile değerlendirildi.

Bulgular: Ürodinamik çalışmalar 29 hastada (%85.2) intrinsik sfinkter yetersizliği (ISY) olduğunu gösterdi. Bu 29 hastanın 9'unda (%26,4); sekonder aşırı aktif mesane (AAM), bozulmuş detrusör kontraktilitesi (BDK) ve AAM+BDK, sırasıyla 9 (%11,6), 4 (%11,6), 1 (%2,9) hastada saptandı. On dokuz hastada (%55,8) tek ISY tanısı varken, 15 hastada (%44.1) ISY dışında başka bir birincil tanı veya ikincil tanı vardı.

Sonuç: Radikal prostatektomi sonrası hastalarda persistan üriner inkontinansın en sık nedeni intrinsik sfinter yetmezliği olmasına rağmen, hastaların önemli bir kısmında aşırı aktif mesane ve bozulmuş detrüsör kontraktilitesinin de eşlik ettiği göz önünde bulundurulmalıdır.

Anahtar Kelimeler: prostat kanseri, prostatektomi sonrası inkontinans, ürodinami.

* **Corresponding author/İletişim kurulacak yazar:** Huseyin Alperen Yildiz; BAİBÜ Gölköy Yerleşkesi, 14030 Merkez/Bolu, Türkiye.
Phone/Telefon: +90 (374) 253 46 26 (3547) **e-mail/e-posta:** h.alperenyildiz@gmail.com
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Introduction

Prostate cancer is the second most common cancer among men.[1] Since prostate-specific antigen screening was first introduced in 1987, the diagnosis and incidence of prostate cancer have gradually increased. With the prolongation of life expectancy and the development of surgical techniques, more men are undergoing radical prostatectomy (RP) surgery today. Men subjected to RP have a significantly worse quality of life, and urinary incontinence is one of the most distressing complaints.[2] Although there are several studies investigating surgical techniques to reduce the morbidity of RP[3,4], incontinence remains a serious nuisance for patients. Detection of underlying pathologies is essential to provide appropriate treatment and symptomatic improvement in this large patient group. The most objective method to evaluate the bladder function after RP is urodynamic study (UDS).

This study aims to determine the causes of urinary incontinence and accompanying bladder pathologies in patients with persistent incontinence 12 months after RP.

Methods

This study was approved by the local Ethical Review Committee of our institution (2022/69). All patients consented to the use of their medical and surgical data in the context of this study. Data of the patients who underwent open radical retropubic prostatectomy (RRP) due to localized and locally-advanced prostate cancer and incontinent after surgery between January 2015 and December 2020 were retrospectively reviewed.

Patients who had a history of the urethra, prostate or bladder surgery, preoperative urinary incontinence, neurogenic bladder, overactive bladder, and postoperative urethra or bladder neck stricture, dry or who did not use security pads in postoperative month 12 were excluded. All RRP procedures were performed by two surgeons who are experts in their field.

All patients were followed in our outpatient clinic for the functional and oncological outcomes every three months in the postoperative first year and then 6-month intervals until completion of 5-year. Patients were assessed by demographic and clinical parameters including age, body mass index (BMI), comorbidity, preoperative PSA level, prostate volume (PV), transrectal prostate biopsy Gleason score, clinical stage, pathological ISUP grade, pathological stage, lymph node positivity, international consultation on incontinence questionnaire-short form (ICIQ-SF) and subjective questionnaire for daily pad use. ICIQ-SF forms were filled and evaluated by the urologists during the interview with the patient. Also, urodynamic studies (UDS) were performed by a specialist nurse with and under the supervision of a specialized physician. Before UDS, patients were undertaken for uroflowmetric study for maximum flow rate and ultrasound for residual volume after micturition were performed. All UDSs were performed using the multichannel urodynamic device (MMS/Laborie, Netherlands) according to the ICS protocol of good urodynamic practice.[5] During the procedure, the bladder was emptied with a catheter. A 7 F urethral catheter (T-DOC® Air Charged Catheters) was placed in the urethra, and a 7 F rectal catheter (T-DOC® Air Charged catheters) was placed in the rectum in the lithotomy position. EMG probes were placed. After placing the catheters and probes, cystometry was started by the filling of bladder with saline at rate of 30 mL/min at room temperature at lithotomy position. All UDS examinations and evaluations were assessed by the same doctor.

Statistical Analysis

Categorical data were presented as numbers and percentages. Levene's test was used to determine whether the distributions of continuous variables were distributed normally. Data for variables normally distributed were presented as mean and standard deviations. The frequencies of categorical variables were compared using the Pearson Chi-Square test. All data were statistically analyzed by using SPSS software version 21 (IBM Corp., Armonk, NY) and a p-value was determined as statistically significant for <.05 in 95% confidence interval.

Results

Thirty-four patients fulfilling inclusion criteria were analyzed retrospectively. The mean age of the patients was 63.2 ± 7.1 years. The mean of ICIQ-SF score and the daily pad use were 11.3 ± 3.4 and 3.2 ± 1.1, respectively. Demographics and clinical data of the patients are shown in Table 1. Urodynamic studies showed that 29 patients (85.2%) had intrinsic sphincter deficiency (ISD) (Table 2). Nine (26.4%) of 29 patients with ISD had a secondary diagnosis of OAB (overactive bladder), IDC (intrinsic sphincter deficiency) and OAB+IDC (9 (11.6%), 4 (11.6%), 1(2.9%), respectively (Table 2). Nineteen patients (55.8%) had a sole diagnosis of ISD, whereas 15 patients (44.1%) had another primary diagnosis or a secondary diagnosis other than ISD.

Discussion

Our study shows that the most common cause of post-radical prostatectomy incontinence is intrinsic sphincter deficiency, but it is accompanied by overactive bladder and impaired detrusor contractility in a significant number of patients.

Continence is maintained primarily by the external sphincter after removal of the internal sphincter during radical prostatectomy.[6] The external urethral sphincter complex is primarily located distal to the prostate apex, but an extension of striated muscle is also located inside the apex.[4,7] Full-length preservation of the urethral sphincter has been shown to provide better continence.[4]

Damage to the somatic nerve fibers, which can occur during surgery, is one of the causes of sphincteric dysfunction. The pudendal nerve branches that innervate the external sphincter divide at the level of the urogenital diaphragm and are very close to the prostatic apex dissection and urethral anastomosis area.[8] Another mechanism associated with incontinence is the development of fibrosis at the urethral anastomosis which leads to decreased elasticity of the urethra.[9]

In a urodynamic study performed by Groutz et al. in 83 postprostatectomy incontinence patients, sphincteric insufficiency (88%) was found to be the main cause 10. Likewise, several reports have shown that intrinsic sphincter deficiency was the main cause of post-prostatectomy incontinence.[11–13]

In a prospective urodynamic study, 90% of the patients with postoperative incontinence had ISD and 40% had DO 13. While ISD was not found in any of the patients without incontinence, detrusor overactivity was found in 25.6%.[13]

Previous studies in the literature have reported different rates of de novo detrusor overactivity after RRP, ranging from 2.3% to 54.5% .[13–26] Furthermore, resolution of preoperative DO was reported with the rates of 19.6%-87.5%.[13,17–19,21–24,27,28] However, these studies vary significantly in terms of follow-up length and timings of pre/post-operative urodynamic studies. Differences seen in these studies may be due to differences in study designs, as well as the improvement in detrusor function over time and differences in urodynamic study timings. It should be considered that in our study we only evaluated patients who were incontinent after 12 months of surgery without previously known bladder or urethral dysfunction.

DO and DU are thought to be linked to autonomic nerve injury during surgery.[14,24,25] This is particularly true with dissections at the bladder neck and the excision of the seminal vesicles.[29] It has been suggested that nerve-sparing surgery can also lead to the protection of some autonomic nerves, and pelvic lymphadenectomy in some patients may increase pelvic plexus damage.[30] Many alternative nerve-sparing approaches have been developed to enhance functional results in the case of presumed pathology.[31] As our study was an observational study, more randomized controlled trials are needed to investigate the results of nerve-sparing techniques.

Our study has several limitations. Although we excluded patients with known bladder or urethral pathology, it is not possible to certainly determine the relationship between our findings and surgery, since preoperative urodynamic studies were not performed. Due to retrospective cross-sectional design of our study, a cause and effect relationship could not be evaluated.

Table 1. Demographics and clinical data of the patients

Number of patients	34
Age, mean ± SD, years	63.2 ± 7.1
BMI, mean ± SD, kg/m^2	26.6 ± 3.2
Comorbidity, n (%)	
Diabetes	10 (29.4)
Hypertension	12 (35.2)
PV, mean ± SD, mL	45.0 ± 17.1
PSA, mean ± SD, ng/mL	8.6 ± 6.2
Biopsy Gleason score, n (%)	
≤6	21 (61.7)
7	7 (20.5)
8-10	6 (17.6)
Clinical stage, n (%)	
T1c	13 (38.2)
T2a	9 (26.4)
T2b	7 (20.5)
T2c	5 (14.7)
ISUP classification, n (%)	
1	20 (58.8)
2	9 (26.4)
≥ 3	5 (14.7)
Pathological stage, n (%)	
T2	25 (73.5)
T3a	5 (14.7)
T3b	4 (11.7)
Surgical margin positivity, n (%)	6 (17.6)
Lymph node positivity, n (%)	2 (5.8)
Post-operative RT, n (%)	4 (11.7)
ICIQ-SF score, mean ± SD	11.3 ± 3.4
Number of daily pad use, mean ± SD	3.2 ± 1.1

Abbreviations: SD, standard deviation; BMI, Body mass index; PV, prostate volume; PSA, Prostate-specific antigen; ISUP, International Society of Urologic Pathologists; RT, Radiotherapy; ICIQ-SF, international consultation on incontinence questionnaire-short form.

Conclusion

In conclusion; although ISD is the most common cause of persistent urinary incontinence in patients after radical prostatectomy, it should be taken into account that overactive bladder and impaired detrusor contractility are also accompanied by a significant proportion of patients.

Ethical Approval

Permission for this study was obtained from the Ethics Committee of Bolu Abant Izzet Baysal University Faculty of Medicine, numbered 2022/69.

Conflicts of Interests

No competing interests have been declared by the authors.

Author Contribution

All authors contributed equally to this work.

Financial Disclosure

None.

Table 2. Urodynamic results of patients with post-prostatectomy incontinence

	Urodynamic results			
Variables	**ISD**	**OAB**	**IDC**	***P* value**
Number, n (%)	28 (82.3)	5 (14.7)	1 (2.9)	**<0.001+**
Bladder volume, mean ± SD, mL	270 ± 35	250 ± 40	260 ± 25	0.42
Qmax, mean ± SD, mL/min	15.3 ± 8.5	16.2 ± 8.0	14.0 ± 7.8	0.14
PVR, mean ± SD	25 ± 15	30 ± 10	20± 10	0.23
Sole diagnosis, n (%)	19 (55.8)	3 (8.8)	1 (2.9)	**<0.001+**
Secondary diagnosis, n (%)	9 (26.4)	2 (5.8)		**<0.01+**
OAB	4 (11.6)			
IDC	4 (11.6)	1 (2.9)		
OAB + IDC	1 (2.9)	1 (2.9)		

Abbreviations: SD, standart deviation; ISD, intrinsic sphincter deficiency; OAB, overactive bladder; IDC, impaired detrusor contractility; Qmax, maximum flow rate; PVR, post-voiding residue; mL, milliliter; min; minute. + represents the result of Pearson chi-square test and showed as bold for the statistically significant.

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Research Article | Araştırma Makalesi

ANTIBIOTIC RESISTANCE OF *ACINETOBACTER* STRAINS IN OUR INTENSIVE CARE UNIT: A RETROSPECTIVE STUDY

YOĞUN BAKIM ÜNİTEMİZDEKİ ACİNETOBACTER SUŞLARININ ANTİBİYOTİK DİRENCİ: RETROSPEKTİF BİR ÇALIŞMA

Ebru Karakoç[1], Ayşe Ayyıldız[2], Birgül Yelken[1*]

[1]Eskişehir Osmangazi University Faculty of Medicine, Department of Anesthesiology and Reanimation, Eskişehir, Türkiye. [2]Eskişehir City Hospital, Intensive Care Clinics, Eskişehir, Türkiye.

ABSTRACT

Objective: Antibiotic resistance development in the treatment of *Acinetobacter* infection is a serious health care problem and responsible for high mortality in intensive care units (ICU). In our study, it was aimed to determine rates of antibiotics resistance of *Acinetobacter* strains isolated from various samples in our ICU.

Methods: We examined the records of *Acinetobacter* isolates and antibiotics resistance for one year followed up in our ICU. The samples from different patients and different type of samples of the same patients were evaluated. The data was analyzed with SPSS for Windows version 23.0. Categorical variables were expressed in terms of numbers and percentage.

Results: 50% of the samples were isolated from tracheostomy. 96.4% of the 138 isolates were *Acinetobacter baumannii* and 3.6% were the other strains. We found high resistance to all of antibiotics except colistin (3.6%) and tigecycline (13.1%).

Conclusion: *Acinetobacter* is the most important opportunistic human pathogen causing fatal nosocomial infections because its ability of developing resistance to new antibiotics is overly fast. Compared to the results reported from Dicle University Hospital in south east of our country it was determined that antibiotics resistance, especially colistin resistance ratio in our ICU was different. It is important to remember that antibiotic susceptibility may vary in regions, hospitals and even clinics, and resistance development should be constantly detected to make the appropriate initial therapy until deescalation.

Keywords: *Acinetobacter,* antibiotic resistance, multidrug resistant infections

ÖZ

Amaç: *Acinetobacter* enfeksiyonunun tedavisinde antibiyotik direnci gelişimi ciddi bir sağlık sorunudur ve yoğun bakım ünitelerinde (YBÜ) yüksek mortaliteden sorumludur. Çalışmamızda yoğun bakım ünitemizde çeşitli örneklerden izole edilen *Acinetobacter* suşlarının antibiyotik direnç oranlarının belirlenmesi amaçlanmıştır.

Yöntem: Yoğun bakım ünitemizde takip edilen bir yıllık *Acinetobacter* izolatları ve antibiyotik direnç kayıtları incelendi. Farklı hastalardan alınan numuneler ve aynı hastaya ait farklı tipteki numuneler değerlendirildi. Veriler SPSS for Windows 23.0 versiyon ile analiz edildi. Kategorik değişkenler sayı ve yüzde olarak ifade edildi.

Bulgular: Örneklerin %50 'si trakeostomiden izole edildi. 138 izolatın %96,4'ü *Acinetobacter baumannii* ve %3,6'sı diğer suşlardı. Kolistin (%3,6) ve tigesiklin (%13,1) dışındaki tüm antibiyotiklere yüksek direnç bulduk.

Sonuç: Acinetobacter, yeni antibiyotiklere aşırı hızlı direnç geliştirme yeteneği nedeniyle ölümcül hastane enfeksiyonlarına neden olan en önemli fırsatçı insan patojenidir. Ülkemizin güneydoğusundaki Dicle Üniversitesi Hastanesi'nden bildirilen sonuçlarla karşılaştırıldığında, yoğun bakım ünitemizde antibiyotiklerin dirençlerinin, özellikle kolistin direnç oranının farklı olduğu görülmektedir. Antibiyotik duyarlılığının bölgelere, hastanelere hatta kliniklere göre değişebileceğini ve direnç gelişiminin deeskalasyona kadar uygun başlangıç tedavisini yapmak için sürekli olarak saptanması gerektiğini hatırlamak önemlidir.

Anahtar Kelimeler: *Acinetobacter*, antibiyotik direnci, çoklu ilaca dirençli enfeksiyonlar

***Corresponding author/İletişim kurulacak yazar:** Ayşe Ayyıldız; Eskişehir City Hospital, Intensive Care Clinics, Eskişehir, Türkiye.
Phone/Telefon: +90 (506)4608533 **e-mail/e-posta:** drayseayyildiz@gmail.com
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Introduction

Acinetobacter strains are gram negative nonfermentative coco bacillus and commonly found in nature, especially in food and water.[1] It can be found in the oral, gastrointestinal, and upper respiratory tract flora of healthy people and can stay on inanimate surfaces for days.[1,2] Normally it is rare to form a disease in a healthy person because of their low virulence but it can cause serious genitourinary, respiratory and soft tissue infections in immunocompromised patients.[3,4]

Acinetobacter is the most important opportunistic human pathogen causing fatal nosocomial infections because its ability to develop resistance to new antibiotics is overly fast.[3] Uncontrolled use of antibiotics especially in hospital, increased ratio of immunocompromised patients and use of antibiotics in food industry can be counted as some of the reasons of antibiotic resistance development mechanisms of *Acinetobacter*.[5] In the genomic analyses examining resistant and susceptible strains, 52 genes responsible for resistance were detected. There are a total of 45 resistance genes localized in the same DNA region, called "resistance island". This is the largest island of resistance ever identified in a bacterium.[6]

Antibiotic resistance development in the treatment of *Acinetobacter* infection is a serious health problem and responsible for high mortality in intensive care units (ICU).[7-9] It brings along many problems, such as prolonged stay in ICU, increased treatment costs, and mortality.[10] While carbapenems were the first treatment option previously, now Acinetobacter strains have developed resistance to almost all conventional antibiotics. If precautions are not taken and new generation antibiotics can not be developed; we will have to deal with a deadly pathogen that is incurable.[6]

It is important to determine the endemic antibiotic resistance spectrum in order to defeat this pathogen, which develops resistance to antibiotics so quickly. The World Health Organization also emphasized the importance of endemic surveillance analyses in health institutions in reducing antibiotic resistance.[11] In our study, we aimed to determine the rates of resistance of *Acinetobacter* strains isolated from various samples in our ICU.

Methods

The study was carried out with the permission of the Eskişehir Osmangazi University, Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (Date: 15.05.2018 Decision No: 15). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Microbiological clinical samples of patients hospitalized in our ICU for one year between May 2017 and May 2018 were analyzed retrospectively. Specimens with *Acinetobacter* growth in sample types such as tracheostomy, blood, urine, wound, and catheter tip were included in the study. All samples from different patients and different types of samples from the same patients from which *Acinetobacter* strains were isolated, were evaluated. Subtypes of the strains, resistance patterns and sample types were examined.

Clinical samples sent from the ICU to the clinical microbiology laboratory were inoculated on 5% blood agar and Eosin Methylene Blue agar and incubated at 35°C for 24 hours.

Bacterial identification and antibiotic susceptibility were determined by the automated VITEK2 (bioMérieux, France) system and the results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) standards. The Kirby-Bauer disc diffusion method was used for tigecycline, which is not included in the CLSI interpretation criteria, and the zone diameter was accepted as ≤ 12 mm resistant and ≥ 16 mm sensitive. The minimum inhibitory concentration (MIC) values for colistin resistance of *Acinetobacter* species, which were determined to be multi-drug resistant based on routine antibiotic susceptibility profiles, were determined by liquid microdilution method.

The antibiotics evaluated in the study were imipenem, meropenem, amikacin, gentamicin, colistin, ciprofloxacin, levofloxacin, tigecycline, tobramycin, and trimethoprim/sulfamethoxazole (TMP/SMX), respectively. Statistical analysis SPSS 23.0 (IBM, Package program) was used. Categorical variables were expressed in terms of numbers and percentages.

Results

In a one-year period, 138 *Acinetobacter* strains were isolated and evaluated. Ninety six point four percent of the 138 isolates were *A. baumannii* and 3.6% were the other *Acinetobacter* strains. Seventy point two percent of the samples were isolated from endotracheal aspirate. The other sites for Acinetobacter isolation were followed by blood (13.8%), urine (5.8%) and wound (5.1%) cultures, respectively. The places where the samples were isolated are given in detail in Table 1.

Table 1. General characteristics of *Acinetobacter* strains

	n (%)
Isolated bacteria	
A. baumannii	133 (96.4%)
Other subspecies	5 (3.6%)
Material	
Endotracheal aspirate	97 (70.2%)
Blood	19 (13.8%)
Urine	8 (5.8%)
Wound	7 (5.1%)
Sputum	1 (0.7%)
Other	6 (4.3%)

While the highest antibiotic resistance was seen to be meropenem (99.3%), the lowest resistance rate was found to be against colistin (3.6%). Antibiotic resistance rates are detailed in Table 2.

When we evaluated the antibiotic resistance patterns according to the places where *Acinetobacter* strains were isolated, the highest resistance was found against ciprofloxacin and meropenem in endotracheal aspirate samples, while levofloxacin resistance took the first place in blood samples. It was seen that all colistin resistant *Acinetobacter* samples were endotracheal aspirate samples (Table 3).

Table 2. Antibiotic resistance rates of *Acinetobacter* strains.

	Antibiogram		
	Susceptible	**Intermediate susceptibility**	**Resistant**
Amikacin	43 (31.2%)	14 (10.1%)	81 (58.7%)
Ciprofloxacin	3 (2.2%)	-	135 (97.8%)
Colistin	133 (96.4%)	-	5 (3.6%)
Gentamicin	67 (48.9%)	-	70 (51.1%)
Imipenem	4 (3.4%)	13 (11.1%)	100 (85.5%)
Levofloxacin	1 (0.9%)	-	114 (99.1%)
Meropenem	1 (0.7%)	-	137 (99.3%)
Tigecycline	68 (68.7%)	18 (18.2%)	13 (13.1%)
Trimethoprim/Sulfamethoxazole	39 (28.3%)	-	99 (71.7%)
Tobramycin	53 (43.1%)	-	70 (56.9%)

Table 3. Antibiotic resistance patterns according to where *Acinetobacter* strains were isolated.

	A	**CI**	**CO**	**G**	**I**	**L**	**M**	**T**	**TMX-SMX**	**TO**
Material										
Endotracheal aspirate	61(62.8%)	95 (97.9%)	5(5.1%)	58 (59.7%)	69 (71.1%)	79 (81.4%)	97 (100%)	11 (11.3%)	73 (75.2%)	53 (54.6%)
Blood	11 (57.9%)	18 (94.7%)	-	4 (21.1%)	13 (86.7%)	15 (100%)	18 (94.7%)	1 (7.7%)	11 (57.9%)	8 (44.4%)
Urine	4 (50%)	8 (100%)	-	4 (50%)	7 (87.5%)	8 (100%)	8 (100%)	-	7 (87.5%)	4 (50%)
Sputum	1 (100%)	1 (100%)	-	-	-	-	1 (100%)	-	1 (100%)	1 (100%)
Wound	1 (14.3%)	7 (100%)	-	4 (57.1%)	6 (100%)	6 (100%)	7 (100%)	-	5 (71.4%)	3 (50%)
Other	3 (50%)	6 (100%)	-	-	5 (83.3%)	6 (100%)	6 (100%)	1 (25%)	2 (33.3%)	1 (16.7%)

A: Amicasin, CI: Ciprofloxacin, CO: Colistin, G: Gentamicin, I: Imipenem, L: Levofloxacin, M: Meropenem, T: Tigecycline, TMX-SMX: Trimethoprim/sulfamethoxazole, TO: Tobramycin

Discussion

In our study, we evaluated the *Acinetobacter* strains of our own clinics and we found that the carbapenem resistance rate was high, and the tigecycline and colistin resistance rates were lower than other studies in the literature. We have shown that tigecycline and colistin are the most effective agents for *Acinetobacter* strains in our own endemic region.[4,7-9]

In studies comparing the isolated places of *Acinetobacter* strains, the highest rate was observed in deep tracheal aspirate and tracheostomy cultures.[7,12] Parallel to this, in our study, the most common place isolated was endotracheal aspirate culture. This was followed by blood and urine cultures, respectively.

Although carbapenems are a broad-spectrum antibiotic group used in *Acinetobacter* infection, it has been shown that resistance has increased over the years in studies conducted throughout the world and our country.[5,13,14] Doruk et al. examined the four year *Acinetobacter* antibiotics resistance profile in their study and showed that while carbapenem resistance was 28.6% in 2009, this resistance increased to 100% in 2011-2013.[13] In another study of Şafak et al. the six year resistance profile was analyzed and it was shown that the resistance of meropenem increased from 78.5% in 2010 to 96.2% in 2016.[15] In our study, it was observed that carbapenem resistance was high and this rate was 85.5% for imipenem and 99.3% for meropenem, respectively. Studies have shown that patients infected with *Acinetobacter* strains with high carbapenem resistance are associated with high mortality.[16,17] We can attribute the high meropenem resistance in our clinic to the fact that we use meropenem too much in empirical antibiotic selection. We need to plan interventions to prevent colonization of this resistant strain.

Intravenous or inhaler colistin is popularly preferred especially in our country in the treatment of carbapenem-resistant A*cinetobacter* infections. Colistin is a polymyxin group antibiotic, and its systemic use was limited in the 1960s due to its nephrotoxic and neurotoxic side effects, but today it has been re-used due to hospital-acquired infections of multi-drug-resistant nonfermentative gram-negative bacteria.[18,19] However,

colistin resistance reported in recent studies has confronted clinicians with the same problem. In the study by Talan et al, in which they examined the colistin-resistant *Acinetobacter* infections, they found colistin resistance in 9 of 33 *Acinetobacter* infections (33%). They found that the length of stay in ICU was numerically higher in patients infected with resistant strains .[20] In the study conducted in the Dicle University hospital located in the southeast of Turkey, the resistance rate was found to be 6%.[21] In our study, colistin resistance rate was quite low and found to be 3.6%.

The study conducted by Celik et al.[22], reported that the antibiotic to which *Acinetobacter* species was most sensitive, apart from colistin, was trimethoprim/sulfamethoxazole and recommended it for empirical treatment. This study was conducted in 2014 and 2016 (in a three-year period) and it showed how antibiotic susceptibility changed over the years. Surveillance is a dynamic process and its importance was emphasized in this study. The most important limitation of our study was that we only evaluated the one-year resistance profile. Again, in a study by Duran et al., all antibiotic resistances except colistin resistance were examined and it was seen that the antibiotic to which it was most sensitive was TMP-SMX.[23] In our study, the TMP-SMX resistance was high and was 71.7%. This situation once again showed us how important it is to know our own endemic flora when starting empirical antibiotics.

It is known that *Acinetobacter* strains rapidly develop resistance to fluoroquinolone group antibiotics.[24] Yıldız et al.[25], in a study they conducted, looked at the pattern of resistance to fluoroquinolones and showed that this rate was 98.8% to ciprofloxacin and 98.2% to levofloxacin. They interpreted this situation as the development of resistance due to its frequent use as an empirical antibiotic in combination therapy over the years.[25] In our study, the rates were found to be similar, and it was observed that they were 97.8% for ciprofloxacin and 99.1% for levofloxacin, respectively.

In the studies, the resistance rate of tigecycline varies considerably according to the geographical regions, and it has been reported that the resistance rate is between 7-78%.[26,27] While Zer et al. found tigecycline resistance as 19%, Kuşçu et al. found this rate as 5%.[26,28] In our study, tigecycline was found to be the most sensitive antibiotic after colistin.(13.1%). Due to its lack of antipseudomonal and bactericidal activity, it is still not recommended for empirical treatment in sepsis.[29] Its use in combination therapy in multidrug-resistant *Acinetobacter* infections is still controversial.[30,31]

Compliance with Ethical Standards

This study was approved by Eskişehir Osmangazi University, Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (Date: 15.05.2018 Decision No: 15).

Conflict of Interest

The authors declare no conflicts of interest.

Author Contribution

AA, EK and BY: Study idea, hypothesis, study design; AA,EK and BY: Material preparation, data collection and analysis; AA,EK,BY: Writing the first draft of the article; AA, EK and BY: Critical review of the article finalization and publication process.

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Research Article | Araştırma Makalesi

BENZIMIDAZOLE-THIAZOLE HYBRIDS; SYNTHESIS, STRUCTURE ELUCIDATION AND CYTOTOXIC PROPERTIES

BENZİMİDAZOLE-TİYAZOL HİBRİTİ: SENTEZ, YAPI KARAKTERİZASYONU VE SİTOTOKSİK ÖZELLİKLER

Göknil Pelin Coşkun[1], Ömer Erdoğan [2], Özge Çevik[2], Zafer Şahin[3], Şeref Demirayak[4]

[1]Acibadem Mehmet Ali Aydinlar University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Istanbul, Turkiye [2]Aydin Adnan Menderes University, Faculty of Medicine, Biochemistry, Aydin, Turkiye [3]University of Health Sciences, Hamidiye Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Istanbul, Turkiye [4]Kocaeli Health and Technology University, School of Pharmacy, Department of Pharmaceutical Chemistry, Istanbul, Turkiye

Abstract

Objective: Neuroblastoma is one of the leading tumors among the childhood cancers and the treatment of the disease is currently being investigated. As the metastatic ability of the cells is high, current anticancer therapy needs improvement.

Methods: Therefore, here five novel benzimidazole-thiazole compounds were synthesized. Their structures were elucidated using spectroscopic methods like FTIR, NMR and Mass analysis. The synthesis monitoring and purity of the compounds were performed with chromatographic methods. The compounds biological activity on SH-SY5Y neuroblastoma cell line was investigated using MTT assay.

Results: All of the tested compounds showed moderate cytotoxic activity. The best IC50 was obtained from compound 6b with the IC50 value of 175,02±4,17 µM.

Conclusion: These results indicated that benzimidazole-thiazole core is important for anti neuroblastoma activity, however, the ability of intramolecular hydrogen bonding could block the anticancer activity.

Keywords: Benzimidazole, thiazole, neuroblastoma, cytotoxicity, NMR.

Öz

Amaç: Nöroblastoma, çocukluk çağı kanserleri arasında önde gelen tümörlerden biridir ve hastalığın tedavisi halen araştırılmaktadır. Hücrelerin metastatik yeteneği yüksek olduğundan, mevcut antikanser tedavisinin iyileştirilmesi gerekmektedir.

Yöntem: Bu nedenle, burada beş yeni benzimidazol-tiyazol bileşiği sentezlenmiştir. Yapıları FTIR, NMR ve Kütle analizi gibi spektroskopik yöntemlerle aydınlatılmıştır. Bileşiklerin sentezleri ve saflığı kromatografik yöntemlerle gerçekleştirilmiştir. Bileşiklerin SH-SY5Y nöroblastoma hücre hattı üzerindeki biyolojik aktivitesi, MTT deneyi kullanılarak araştırılmıştır.

Bulgular: Test edilen bileşiklerin tümü, orta derecede sitotoksik aktivite gösterdi. En iyi IC50, 175,02±4,17 uM IC50 değeri ile bileşik 6b'den elde edildi.

Sonuç: Test edilen bileşiklerin tümü, orta derecede sitotoksik aktivite göstermiştir. En iyi IC50, 175,02±4,17 uM IC50 değeri ile bileşik 6b'den elde edilmiştir.

Anahtar Kelimeler: Benzimidazole, thiazole, neuroblastoma, cytotoxicity, NMR.

*** Corresponding author/İletişim kurulacak yazar:** Göknil Pelin Coşkun; Acibadem Mehmet Ali Aydinlar University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Istanbul, Turkiye
Phone/Telefon: +90 (216) 500 44 44 **e-mail/e-posta:** goknilpelincoskun@gmail.com
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Introduction

Neuroblastoma (NB) is defined as a tumor that develops almost anywhere in sympathetic nervous system and occurrence is mainly observed in abdomen. In some NB patients, tumor growth was also recorded in adrenal gland, abdominal regions, neck, thoracia and pelvis.[1] Almost 10% of the childhood malignancies classified as NB tumors.[2] Unfortunately, metastasis is the first clinical profile in NB patients and cortical bone and bone marrow are at high risk.[3] The treatment goal is to target some important cellular pathways in NB. Some promising treatment regiments were introduced to the literature like retinoic acid, angiogenesis inhibition, histone deacetylate inhibition.[4] However, the requirement of a universal treatment is still needed. The tumor heterogeneity, the drug resistance, the drug toxicities lead treatment failure. Therefore, understanding the underlying mechanism and biological profiles of NB is highyl important.

As the drug repurposing is gaining importance in future drug development strategies, newly developed studies on neuroblastoma was investigated. Flubendazole, which has benzimidazole core ring in its structure has shown to have potential activity on neuroblastoma cells.[5] Apart from that, benzimidazole moiety kept the attraction because of their various biological activities.[6-20] The motivation behind this study is the mimic drug-repurposing strategy and keep the active pharmacophore rigid. Therefore, five new benzimidazole derivatives were designed and synthesized. Apart and also originating from the existing literature, SH-SY5Y neuroblastoma cells were used in this study. They were first obtained from metastatic bone tumor.[21]

Methods

Chemistry

All the chemicals were purchased from Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, MO). Reactions were monitored by TLC on silica gel plates purchased from Merck (Merck Co., Darmstadt, Germany). Melting points of the synthesized compounds were determined in a Stuart SMP50 Automatic Melting Point apparatus. The purity of the compounds was first checked by TLC and confirmed on LC-MS. NMR spectra were recorded on Bruker 400 MHz (Billerica, MA) for 1H-NMR. Data are reported as follows: chemical shift, multiplicity (b.s.: broad singlet, d: doublet; m: multiples, s: singlet, and t: triplet), coupling constants (Hz), integration. An Agilent 1260 Infinity II HPLC-MS spectra equipped with G7114A 1260DAD detector, G7311B 1260 Quad Pump system, G1328C 1260 manual injection unit and G6125B LC/MSD detector was used for both HPLC and mass analysis. Retention times were recorded with ACE C18 column (particle size: 3 µm, pore size: 100A). The column temperature was adjusted to 25ºC in the column compartment. The mobile phase consisted of acetonitrile- water (90:10, v/v) mixture and delivered at a flow rate of 0.8 mL/min. The injection volume was 20 µL. The high-resolution mass spectra of the compounds were determined on a Shimadzu 8040 LC/MS/MS ITTOF system (Shimadzu, Tokyo, Japan) using a mass spectrometer with the electron spray method (ESI).

Biological Activity

Cytotoxicity Experiment

SH-SY5Y (Neuroblast from neural tissue) cultured and maintained in DMEM/F12 containing 10% FBS and supplemented with penicillin/streptomycin. The cultures were incubated at 37°C in a humidified atmosphere with 5% CO2. Compounds were about 0.1-1000 µM concentration using MTT assay[22] and it is previously reported.[23] IC_{50} values were calculated and cellular analyzes were continued with the compounds 6a, 6b, 6c, 6d, and 6e. Cellular morphological changes were characterized under the microscope. The compounds were dissolved in DMSO, and MTT was performed at a concentration of 0, 0.1, 1,10,100,1000 µM. Cells were incubated for 24 hours, and IC50 values were calculated using Graphpad Prism 7.

Statistical Analysis

All experiments were performed at least in triplicates. Data are presented as mean ± standard deviation. Statistical comparisons were performed through the one-way ANOVA followed by the Tukey test. Statistical significance was set at $p < 0.05$. Statistical analysis and artwork were performed using Graph Pad Prism 7.0d (Graph Pad Software, La Jolla, USA).

Results

Chemistry

Compound 1 was supplied from Sigma-Aldrich. 2-(1-hydroxy) ethylbenzimidazole (1) is oxidized by chromium trioxide to obtain 2-acetylbenzimidazole. 2-acetylbenzimidazole was methylated by dimethyl sulfate to get 1-methyl-2-acetylbenzimidazole (2) and then brominated in acetic acid to obtain 3. Aniline derivatives (4a-e) were reacted with ammonium thiocyanate (NH4SCN) in ethanol and HCl mixture. Resulted thioureas (5a-e) were checked for their melting points and then used for the final step. At the final step, 3 and 5a-e reacted to get thiazole derivatives (6a-e) according to Hantzsch method (Figure 1).

Synthesis of 1-methyl-2-acetylbenzimidazole (2)

1-Methyl-2-(1-hydroxy) ethylbenzimidazole (0,001 mol) is dissolved in acetic acid and stirred at 90 °C until the color of chrom trioxide turns out green by adding chromium trioxide (dissolved in water). After the reaction was complete, equal volume of water was added. Extracted with chloroform. Organic layer was dried with magnesium sulfate and recrystallized from toluene. After

it was dried, dissolved in NaOH solution (including equal mol of NaOH, 1N), dimethyl sulfate was added and shaked for 1 hour. The product is precipitated and collected.

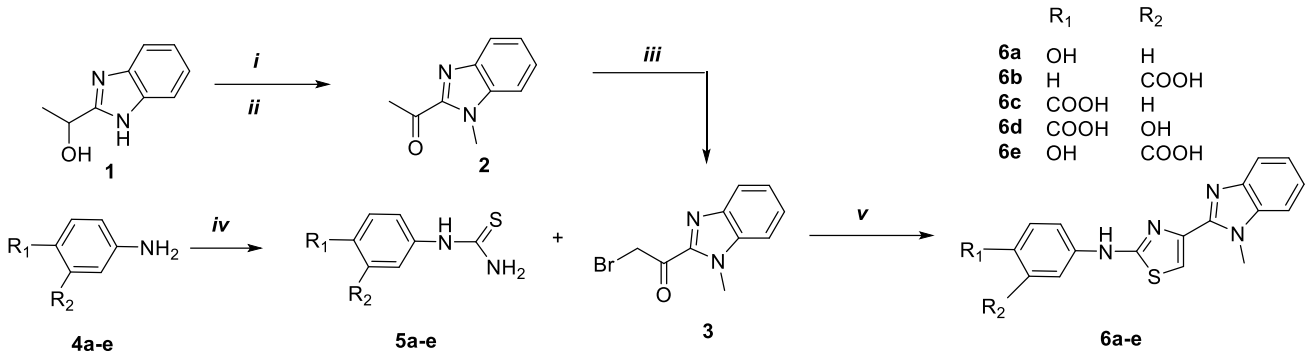

Figure 1: Synthesis scheme of benzimidazole-thiazole compounds; i: chromium trioxide, ii: dimethylsulfate, iii: HBr/acetic acid, iv: ammonium thiocyanate, HCl; v: ethanole

Synthesis of 1-methyl-2-(2-bromoacetyl)benzimidazole (3)

Compound 2 (0.001 mol) is dissolved in acetic acid. Equal mol of bromine is diluted in acetic acid and then added to the reaction dropwise. Catalytic HBr is added and stirred at room temperature for overnight. After the reaction was complete, solids are filtered and the solids dissolved in water and quenched carefully with sodium bicarbonate. Filtered off and dried.

Synthesis of N-(3,4-disubstitutedphenyl)thiourea (5a-e)

Aniline derivatives (4a-e) (300 mmol) is dissolved in ethanol. Equal mole of HCl (330 mmol) and ammonium thiocyanate (330 mmol) is added and refluxed for 4 hours. Resulted precipitation is collected and washed with ethanol for 4-5 times. Solid was recrystallized from ethanol. Melting points was checked and used for the next step.

Synthesis of N-(3,4-disubstitutedphenyl)-4-(1-methyl-1H-benzimidazol-2-yl)-1,3-thiazol-2-amine (6a-e)

Compounds 5a-e (0.001 mol) and compound 3 (0.001 mol) were dissolved in ethanol for 15 min at room temperature and refluxed for 1.5 hours. Quenched with sodium bicarbonate solution to get rid of the salt. Precipitation was collected and recrystallized.

6a: 4-((4-(1-Methyl-1*H*-benzo[d]imidazol-2-yl)thiazol-2-yl)amino)phenol

Mp.: 202 °C. **FT-IR υ_{max}** (cm^{-1}): 3402 (OH sb), 3211 (NH sb), 2982 (CH aliphatic sb), 1602 (C=N sb), 1452 (CH aliphatic bb), 913 (Ar-H bb). **^{1}H NMR (400 MHz, DMSO-d_6) δ** 10.13 (s, 1H, NH), 9.50 (s, 1H, OH), 7.69 – 7.61 (m, 1H, Ar), 7.59 (d, *J* = 7.7 Hz, 1H, Ar), 7.55 (s, 1H, thiazole H), 7.46 (d, *J* = 8.5 Hz, 2H, Ar), 7.33 – 7.18 (m, 2H, Ar), 6.77 (d, *J* = 8.5 Hz, 2H, Ar), 4.19 (s, 3H, CH3).

HRMS (M+H): For $C_{17}H_{14}N_4OS$ calcd: 323.0961, found: 323.0960.

6b: 3-((4-(1-Methyl-1*H*-benzo[d]imidazol-2-yl)thiazol-2-yl)amino)benzoic acid

Mp.: 247 °C. FT-IR **υ_{max}** (cm^{-1}): 3312 (OH sb), 3152 (NH sb), 2978 (CH aliphatic sb), 1732 (C=O sb), 1589 (C=N sb), 1456 (CH aliphatic bb), 987 (Ar-H bb) **^{1}H NMR (300 MHz) DMSO-d_6 δ (ppm):** ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (s, 1H, COOH), 10.68 (s, 1H, NH), 8.63 (s, 1H, Ar), 7.80 – 7.71 (m, 2H, Ar), 7.68 – 7.55 (m, 3H, Ar), 7.48 (t, *J* = 7.7 Hz, 1H, Ar), 7.27 (m, 2H, Ar), 4.25 (d, *J* = 1.5 Hz, 3H, CH3).

6c: 4-((4-(1-Methyl-1*H*-benzo[d]imidazol-2-yl)thiazol-2-yl)amino)benzoic acid

Mp.: 302 °C. **FT-IR υ_{max}** (cm^{-1}): 3401 (OH sb), 3234 (NH sb), 2976 (CH aliphatic sb), 1745 (C=O sb), 1616 (C=N sb), 1451 (CH aliphatic bb), 899 (Ar-H bb). **^{1}H NMR (400 MHz, DMSO-d_6) δ** 12.60 (s, 1H, COOH), 10.86 (s, 1H, NH), 7.96 (d, 2H, Ar), 7.87 – 7.74 (m, 3H, Ar), 7.71 – 7.56 (m, 2H, Ar), 7.34 – 7.17 (m, 2H, Ar), 4.22 (s, 3H, CH3).

HRMS (M+H): For $C_{17}H_{14}N_4OS$ calcd: 351.0910, found: 351.0909.

6d: 2-Hydroxy-4-((4-(1-methyl-1*H*-benzo[d]imidazol-2-yl)thiazol-2-yl)amino)benzoic acid

Mp.: 211 °C. **FT-IR υ_{max}** (cm^{-1}): 3314 (OH sb), 3298 (NH sb), 2998 (CH aliphatic sb), 1701 (C=O sb), 1579 (C=N sb), 1443 (CH aliphatic bb), 956 (Ar-H bb). **^{1}H NMR (400 MHz, DMSO-d_6) δ** 10.60 (s, 1H, NH), 9.49 (s, 1H, OH), 8.16 (s, 1H, COOH exchanged), 8.04 (dd, *J* = 6.3, 3.3 Hz, 1H, Ar), 7.85 (dd, *J* = 7.8, 2.7, 2.2 Hz, 1H, Ar), 7.69 – 7.59 (m, 2H, Ar), 7.16 (dd, *J* = 4.9, 2.3 Hz, 3H, Ar), 6.47 (s, 1H, Ar), 4.34 (s, 3H, CH3).

6e: 2-Hydroxy-5-((4-(1-methyl-1*H*-benzo[d]imidazol-2-yl)thiazol-2-yl)amino)benzoic acid

Mp.: 287 °C. **FT-IR υ_{max}** (cm^{-1}): 3398 (OH sb), 3212 (NH sb), 2956 (CH aliphatic sb), 1722 (C=O sb), 1609 (C=N sb), 1467 (CH aliphatic bb), 902 (Ar-H bb). **^{1}H NMR (400 MHz, DMSO-d_6) δ** 15.93 (s, 1H, COOH), 10.03 (s, 1H, NH), 8.13 (d, J = 2.9 Hz, 1H, Ar), 7.63 (d, *J* = 7.7 Hz, 1H, Ar), 7.58 (d, *J* = 8.1 Hz, 1H, Ar), 7.52 (s, 1H, thiazole), 7.43 – 7.32 (m, 1H, Ar), 7.32 – 7.16 (m, 2H, Ar), 6.64 (d, *J* = 8.6, 1.3 Hz, 1H, Ar), 4.23 (s, 3H, CH3), 1.70 (s, 2H, OH exchanged).

Discussion

The purity of the synthesized compounds were proven by chromatographic methods. All the compounds thin layer chromatography results ended with single stain in TLC analysis. The elucidation of the structures were proven by spectroscopic methods. The IR results proved the formation of proposed compounds. The disappearance of C=S stretching bands correlated with the overall structure. The NH stretching bands were recorded between 3298-3152 cm^{-1}. The salicylic acid derivatives among the compounds resulted with additional OH stretching band ranging from 3402-3312 cm^{-1} which also explains the hydrogen bonding. The bending peaks resulting from aromatic rings were also detected in the expected regions. In all our compounds (**6a-6e**), the band belonging to the NH group located between the thiazole and the aromatic ring was observed as a sharp peak around 10 ppm. While the aromatic OH group in **6a** and **6d** compounds is close to 9.5 ppm, in compound 6e this hydrogen has been replaced by water at 1.7 ppm. In the compounds bearing the carboxyl group (6b-e), the COOH peak belonging to the carboxyl group was observed between **12-16** ppm, it was only observed in the aromatic area in the **6d** compound due to intermolecular hydrogen bonds. The methyl group attached to benzimidazole was observed as a singlet around 4.2 ppm in all compounds. The single aromatic hydrogen in the thiazole ring was distinguished in compounds **6a** and **6e** and observed as a singlet at 7.5 ppm, mixed with aromatic hydrogens in other compounds. The total number of hydrogens for all compounds holds the total number in the molecules.

The cytotoxicity assay analysis were performed for all the compounds on SH-SY5Y neuroblastoma cell line. All the compounds were insoluble in water and the test studies were performed in dimethlysulfoxide. Inhibitory concentration, cell viability and CV stain analysis were performed for five compounds. Among them, compound **6b** was found to have the best inhibitory activity. Comparing the IC_{50} values of the compounds, hydroxyl group did not favor for inhibitory activity. The carboxylic acid structure in the meta position of the aniline moiety increased the antitumor activity. The dramatic decrease in the inhibitory activity of hydroxyl group carrying compounds could be explained as a result of addition hydrogen bonding. The ability of intramolecular hydrogen bond could block the interaction with the cancer cell and the µshould be performed in order to understand the underlying mechanism.

The MTT assay studies revealed the IC50 values of the synthesized compounds and results were presented in Table 1.

In SH-SY5Y cells, all compounds were determined the cell viability levels of the cells at a concentration of IC_{50} values in 24 hours. The cell viability results were also performed for the synthesized compounds. After 24 hours in 10µM concentration, compound **6b** inhibited the cell viability 80% (Figure 2).

Table 1. IC_{50} values of the synthesized compounds on SH-SY5Y cell lines

Compound	IC_{50} (µM)
6a	669.18±2.05
6b	175.02±4.17
6c	735.02±6.68
6d	ND
6e	481.59±7.22

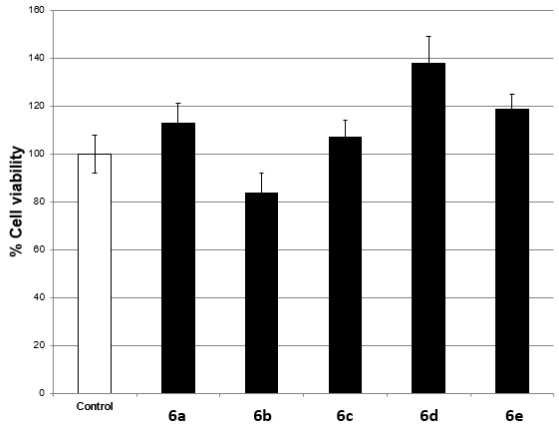

Figure 2. Cell viability results for compounds **6a-6e**.

When the results obtained in the measurement of SH-SY5Y, cell colony forming capacity was evaluated. The CV staining images were made in all tested compounds (Figure **3**). Compound **6b** is the compound that significantly reduced the colony formation levels compared to the control group.

Conclusion

Fully neuroblastoma treatment is still on the research level. The occurrence cell resistance and metastasis is the biggest threat. Within this study, we aimed to develop and synthesize novel benzimidazole-thiazole compounds which may possess anticancer activity. The tested novel five compounds exhibited moderate activity, however, one can considered to have a good potential for future studies. Compound 6b, which have meta carboxylic acid moiety showed the best inhibitory activity on neuroblastoma cells. Even though it is hard to estimate the structure activity relation with cancerous cells, it could maybe lead the idea of intramolecular hydrogen bonding may not be in favor for anticancer activity. Furthermore, benzimidazole and thiazole moiety keeps the importance of strong biological activity profile.

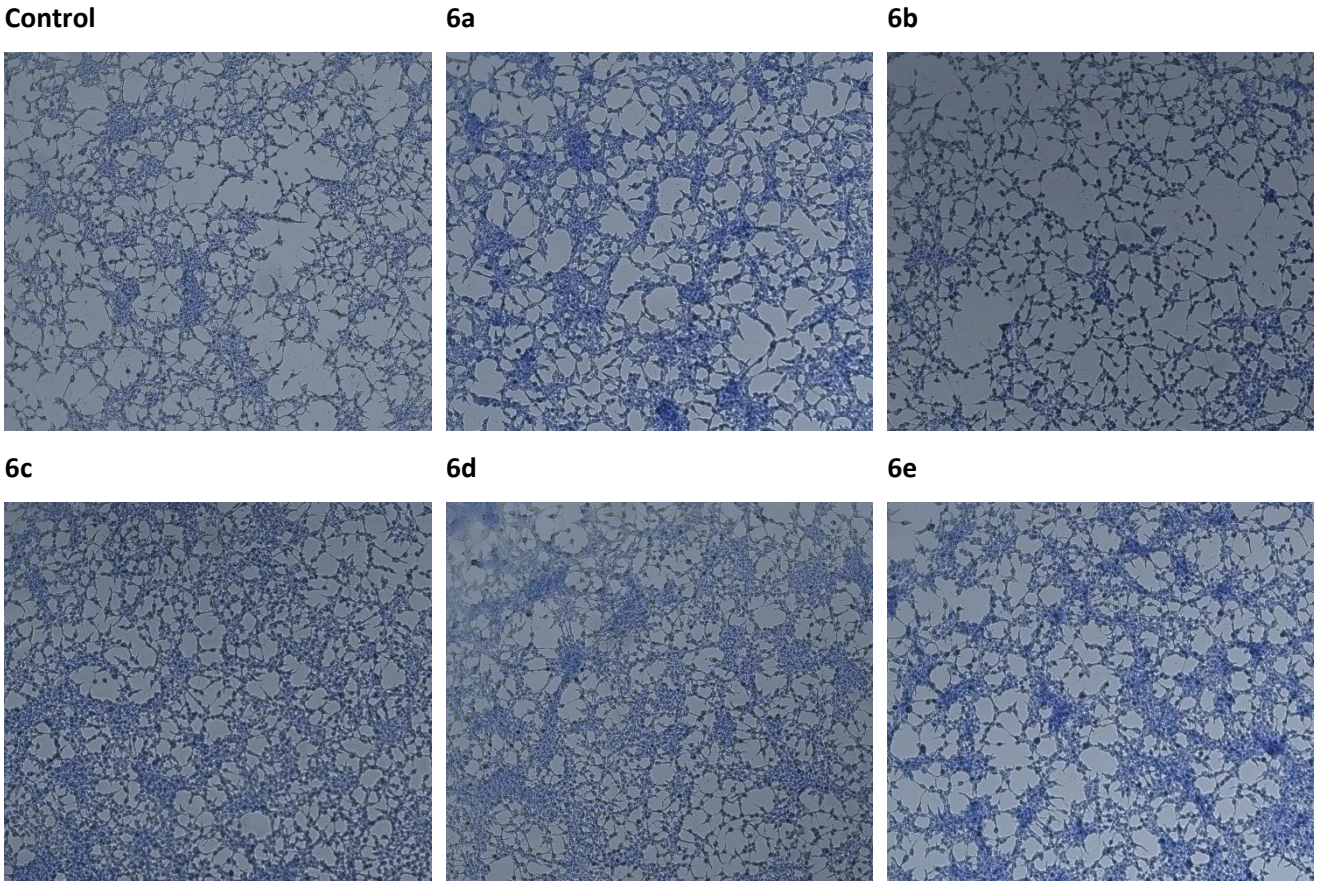

Figure 3. CV stain images of the tested compounds

Ethical Approval
No ethics committee decision is required for the study.

Conflicts of Interests
The authors declare there are no conflict of interest.

Author Contribution
All authors contributed equally to this work.

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None.

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Araştırma Makalesi | Research Article

BÖBREK DOKUSUNUN FARKLI DOKU TAKİP YÖNTEMLERİ KULLANILARAK HİSTOLOJİK OLARAK İNCELENMESİ

HISTOLOGICAL INVESTIGATION OF THE KIDNEY USING DIFFERENT TISSUE PREPERATION METHODS

Bahar Kartal*, Ebru Alimoğulları

Ankara Yıldırım Beyazıt Üniversitesi, Histoloji ve Embriyoloji Ana Bilim Dalı, Ankara, Türkiye.

ÖZ

Amaç: Doku takibi genel olarak; fiksasyon, dehidrasyon, şeffaflandırma, sertleştirme ve doku gömmeyi içermektedir. Doku takibi süreci doku histomorfolojisinin incelenmesi adına avantaj ve dezavantajlara sahiptir. Çalışmamızın amacı, diğer faktörler optimum seviyede tutularak iki farklı dehidrasyon ajanı ile hazırlanan dört doku takibi protokolünün rat böbrek dokusunun histolojik yapısına etkisini belirlemek ve en ideal yöntemi ortaya çıkarmaktır.

Yöntem: 12 adet Wistar Albino ratlardan alınan 24 adet böbrek dokularına dört farklı doku takibi yöntemi farklı dehidratanlar (alkol ve aseton) ve süreler (1 gün ve iki gün) uygulanmıştır. Elde edilen bloklardan alınan kesitler Hematoksilen&Eozin ve Masson trikrom ile boyanmış ve histomorfolojik olarak ışık mikroskobu altında değerlendirilmiştir. Bunlara ek olarak, dört farklı grupta böbrek glomerül çapları ölçülmüştür.

Bulgular: Böbrek preparatlarında glomerulus, tubulus distalis, tubulus proksimalis, inen henle, çıkan henle ve bowman kapsüllerindeki histolojik yapılar ele alınmıştır. Bu doğrultuda, böbrek dokusunda belirtilen bütün bölgeler için alkol ve aseton kullanılan gruplarda anlamlı bir fark olmadığı fakat iki gün süre ile aseton kullanılan grupta böbrek doku bütünlüğünün bozulduğu saptanmıştır. Tüm gruplarda ölçülen böbrek glomerül çapları normal değerlere yakın bulunmuştur.

Sonuç: En iyi düzeyde histomorfolojik incelemeler için, doku takibi izlenmesi gereken önemli bir süreçtir. Bizde çalışmamızın sonuçları doğrultusunda dehidratif ajan olarak uygun süreler doğrultusunda aseton ve alkol kullanımını önermekteyiz.

Anahtar Kelimeler: Doku takibi, histoloji, böbrek, dehidrasyon, aseton, rat.

ABSTRACT

Objective: The main steps of tissue processing are fixation, dehydration, clearing, and embedding. The aim of our study is to determine the effects of four different tissue protocols prepared with two different dehydration agents on the histological structure of rat kidney tissue and to reveal the most ideal method, while keeping other factors at an optimum level.

Methods: Four different tissue processing were designed and performed on 24 rat kidney samples by using two different dehydration solutions (alcohol and acetone) and two different processing times (one and two days). The samples were stained with Hematoxylin&Eosin and Masson's trichrome compared by using qualitative histomorphological criteria under the light microscope. Additionally, the diameters of renal glomerular structures were measured in all groups.

Results: The histological structure of rat kidney samples investigated in terms of renal glomerulus, Bowmans capsule, proximal and distal tubules were. There was not any significant difference among the groups. However, the histological architecture was disrupted by using the acetone for two days protocol. The diameters of the renal glomerulus were in normal values.

Conclusion: In conclusion, for optimal histomorphological examinations, it is important to follow the tissue preparation process. According to the results of our study, we can suggest that acetone and alcohol can be used as a dehydrating agents for appropriate times.

Keywords: Tissue preparation process, histology, kidney, dehydration, acetone, rat.

***İletişim kurulacak yazar/Corresponding author:** Bahar Kartal; Ankara Yıldırım Beyazıt Üniversitesi, Histoloji ve Embriyoloji Ana Bilim Dalı, Ankara, Türkiye.
Telefon/Phone: +90 (532) 1539887 **e-posta/e-mail:** bahar.kartal@outlook.com
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Giriş

Dokuların ışık mikroskobu ile incelenebilmesi için yapılan işlemler dizisine doku takibi adı verilir. Doku takibi süreci fiksasyon (tespit), dehidrasyon, şeffaflandırma, sertleştirme ve gömme basamaklarını içermektedir.[1]

Doku takibinde ilk aşama fiksasyondur.[2] Fiksasyonun amacı, dokuyu canlı organizmaya en yakın şekilde muhafaza etmek, sabitlemek ve dış etkenlerden korumaktır[3]. Fiksasyonu etkileyen faktörler arasında sıcaklık, pH, osmolarite, tespitin süresi, konsantrasyon ve dokunun boyutu yer almaktadır.[4] Tespit için kullanılan solüsyonlara fiksatif adı verilir. En yaygın kullanılan fiksatif, formalindir. Diğer fiksatifler arasında Carnoy ve Bouin sayılabilir. %10'luk formalin, formaldehitin sudaki %4'lük çözeltisidir. Formalinde fikse edilen dokular, çeşitli özel boyalar ile boyanabilir ve immünohistokimya yöntemi uygulanabilir.[5]

Doku takibinin ikinci aşaması dehidrasyon basamağı dokudan suyun uzaklaştırılması olarak tanımlanır. Dehidrasyon ajanları içerisinde alkoller (etil alkol, denatüre alkol, metanol, isopropanol, bütanol), glikol-eterler (polietilen glikol, etoksi etonol, dioksan) ve diğer dehidrasyon ajanları (tetrahidrofuran, 2,2 dimetoksipropan, aseton,) yer almaktadır. En fazla kullanılan dehidrasyon maddesi etil alkoldür. Renksiz, kolay alev alabilen, berrak, orta derece organik ve toksik çözeltiler ile karışabilen bir sıvıdır. Dehidrasyon için yükselen konsantrasyonlarda kullanılır. Diğer bir dehidrasyon solüsyonu asetondur. Aseton berrak, renksiz, karekteristik kokulu ve alev alabilen bir dehidrandır. Hızla dehidrasyon yapar. Aseton hızlıca buharlaşarak dokuların sertleşmesini sağlar.[6]

Dehidrasyondan sonraki basamak, doku içinde bulunan alkolün, şeffaflandırıcı ajan ile yer değiştirmesi olan şeffaflandırmadır.[7] Şeffaflandırıcı ajanların, dokuya nazik davranabilme, dehidrasyon maddesini temizleyebilen, sertleştirici madde ile geçimli olabilen özelliklerine sahip olması gerekmektedir. Şeffaflandırma ajanları içinde hidrokarbonlar (ksilen, tolüen, benzen, kloroform, trikloraeten) esterler ve (metil salisilat, n-bütil asetat ve metil benzoat) terpenler yer almaktadır. Genellikle sık kullanılan şeffaflandırıcı ajan ksilendir. Alev alabilen tehlikeli bir maddedir. Dokunun uzun süre ksilende kalması sonucunda dokuda sertleşme çok fazla olmaktadır.

Sürecin bir sonraki aşaması dokuların serleştirilerek mikrotom cihazı ile kesilebilecek seviyeye gelmesidir. Bu basamakta amaç dokudaki mevcut solüsyonların tutucu bir madde ile yer değiştirmesini sağlamaktır. Bu işleme "sertleştirme" denilmektedir. Bunun için kullanılan maddeler arasında parafin, alternatif maddeler (reçine yapısında mumlar, mumlar), diğer maddeler (jelatin, agar, selloidin) sayılabilir.[8] En yaygın ve sık kullanılan sertleştirici madde parafindir. İyi bir infiltrasyon için dokunun parafinde optimum sürede kalması gerekmektedir. Bunun için doku takibinin bu basamağında üç farklı parafin kabı olmalı ve son kullanılan parafin kabında şeffaflandırıcı ajan kokusu olmamalıdır.[9]

Doku takibinin son aşaması bloklama ya da gömme basamağında dokular infiltrasyon ortamı ile kaplanır. Bloklamada dokunun doğru yönde gömülmesi oldukça önemlidir. Dokular gömüldükten hemen sonra parafin soğutulmalı ve kristal yapısına dönmesi sağlanmalıdır.[10]

Daha önce yapılan bir çalışmada farklı tespit solüsyonları (Alkol, Formaldehit, Glasiyal Asetik Asit), ve formalin ile kısa ve uzun süreli tespitin farklı dokulardaki (karaciğer, böbrek, deri) histolojik etkilerini karşılaştırılmıştır.[11] Literatürde yer alan diğer bir çalışmada araştırmacılar, 3 farklı fiksatif ile hazırlanan 4 çözeltinin, doku tespit işlemi sürecinde nasıl etkilere sahip olduklarını belirlemek amacıyla, dalak ve böbrek dokuları üzerinde çalışılmışlardır.[12] Diğer bir çalışmada ise farklı fiksatif (Formalin, Glyo-Fixx, FineFIX, Cell block ve Greenfix) ve 4 farklı şeffaflandırıcı (Ksilen, Sub-X, Bio Clear, Shandon Xylene Substitute) kullanılarak 13 farklı doku takibi kurgulanıp, 13 farklı dokuya uygulanmış ve hematoksilen-eozin boyalı kesitler histomorfolojik düzeyde niteliksel olarak karşılaştırılmıştır.[13]

Yukarıda verilen bilgiler doğrultusunda literatürde genellikle farklı tespit solüsyonları kullanılarak hazırlanan doku takibi protokolleri yer almaktadır, bizde çalışmamızda farklı dehidrasyon ajanlarından olan alkol ve aseton kullanılarak, bir gün ve iki gün süreli dört farklı doku takip protokolünün böbrek dokusu üzerinde nasıl etkilere sahip olduklarını belirlemeyi amaçladık. Bu doğrultuda solüsyonların en ideal kullanımlarını ortaya çıkararak literatüre katkı sağlayacağımızı düşünmekteyiz.

Yöntem

Çalışma için etik kurul onamı Saki Yenilli Deney Hayvanları Üretim ve Uygulama Laboratuvarı hayvan deneyleri yerel etik kurulu tarafından alınmıştır. Çalışmamızda deney Hayvanları Üretim ve Uygulama Laboratuvarından temin edilen 12 adet Wistar albino türü dişi sıçanların 24 adet böbrekleri materyal olarak kullanıldı. Alınan doku örnekleri %10 formalin ile 48 saat süreyle tespit edildi. Tespit edilmesinin ardından 24 saat akan suda yıkanan örnekler, dört farklı doku takibi yöntemi ile dereceli alkol serilerinden geçirilip dehidre edildi ve parafinde bloklandı.[14,15] Hazırlanan bloklardan 4 µm kalınlığında kesitler alındı. Alınan bu kesitlere genel histolojik yapıyı belirlemek amacıyla Hematoksilen-Eosin boyama yöntemi uygulandı. Farklı doku takip yöntemleri uygulanan böbreklere ait preparatlarda glomerulus, tubulus distalis, tubulus proksimalis, inen henle, çıkan henle ve bowman kapsüllerindeki histolojik yapılar ele alınmıştır. Bu yapıların incelenmesi sağlanarak grupları arası farkı ortaya çıkarmak amaçlanmıştır. Ayrıca bu gruplarda böbrek glomerül çapları daha önce yapılan çalışmalara uygun olarak ölçülmüştür.[8] Böbrek doku hasarı (40X) ışık mikroskobu altında dokuda etkilelenilen yüzde derecelendirilerek skorlandı. 0, 0%; 1, <30%; 2, 31% - 60%; 3, 61% - 100%. Elde dilen tüm skorlar toplandı, ortalama değerler alındı ve grafik üzerinde sergilendi[16].

Hazırlanan preparatlar Olympus BX43F (Japonya) ışık mikroskobun altında incelendi ve ilgili kısımlarından

Tablo 1. Histolojik doku takibi protokolleri

Grup I: 1 günlük asetonsuz doku takip protokolü	**Grup II: 1 günlük asetonlu doku takip protokolü**	**Grup III: 2 günlük asetonsuz doku takip protokolü**	**Grup IV: 2 günlük asetonlu doku takip protokolü**
Formalin, %10 24 saat	Formalin, %10 24 saat	Formalin, %10 24 saat	Formalin, %10 24 saat
Akan su altında 2 saat	Akan su altında 2 saat	Akan su altında 2 saat	Akan su altında 2 saat
Alkol %75 30 dk	Alkol %75 30 dk	Alkol %75 1 saat	Alkol %75 1 saat
Alkol %96 30 dk	Alkol %96 30 dk	Alkol %75 1 gece	Alkol %75 1 gece
Alkol %96 30 dk	Aseton 1 30 dk	Alkol %96 1 saat	Alkol %96 1 saat
Alkol %100 30 dk	Aseton 2 30 dk	Alkol %96 1 saat	Alkol %96 1 saat
Alkol %100 30 dk	Aseton 3 30 dk	Alkol %96 1 saat	Aseton 1 30 dk
Ksilen 15dk	Ksilen 15dk	Alkol %100 1 saat	Aseton 2 30 dk
Parafin I'de 1 saat	Parafin I'de 1 saat	Alkol %100 1 saat	Aseton 3 30 dk
Parafin II'de 1 saat	Parafin II'de 1 saat	Ksilen 15dk	Ksilen 15dk
Parafin III'de 1 saat	Parafin III'de 1 saat	Parafin I'de 1 saat	Parafin I'de 1 saat
Bloklara gömme	Bloklara gömme	Parafin II'de 1 saat	Parafin II'de 1 saat
		Parafin III'de 1 saat	Parafin III'de 1 saat
		Bloklara gömme	Bloklara gömme

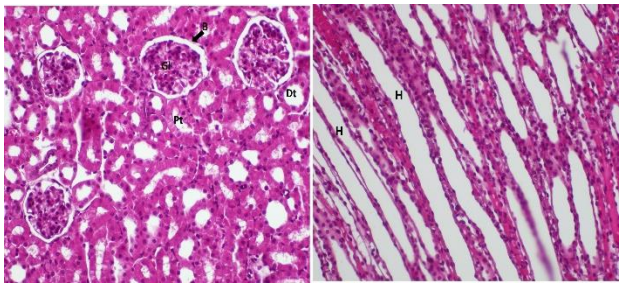
fotoğraf çekimleri yapıldı. 4 farklı doku takibi işlemi manuel olarak yapılmış olup Tablo 1 de sunulmuştur.

İstatiksel Analiz

Verilerin istatistiksel değerlendirmesi SPSS 20.0 (SPSS Inc., Chicago, USA) programında tek yönlü varyans analizi ve çoklu karşılaştırmalar için One -way Anova ve post-hoc Tukey's testleri kullanılarak yapılmıştır. İstatistiksel anlamlılık $p < 0.05$ olarak kabul edilmiştir.

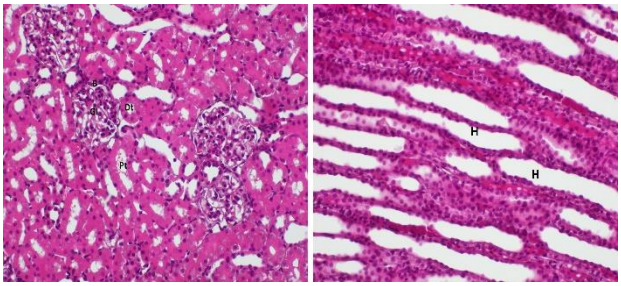
Bulgular

Bu çalışmada dört farklı doku takip yöntemi uygulanan böbreklere ait preparatlarda glomerulus, tubulus distalis, tubulus proksimalis, inen henle, çıkan henle ve bowman kapsüllerindeki histolojik yapılar ele alınmıştır. Buna göre belirtilen bütün bölgeler için Grup I bir günlük asetonsuz (Şekil 1), Grup II bir günlük asetonlu doku takibi (Şekil 2), ve Grup III iki günlük asetonsuz doku takip protokolleri (Şekil 3) ile hazırlanan böbrek preperatları incelendiğinde böbrek dokularının histolojik yapıları arasında anlamlı fark gözlenmedi.

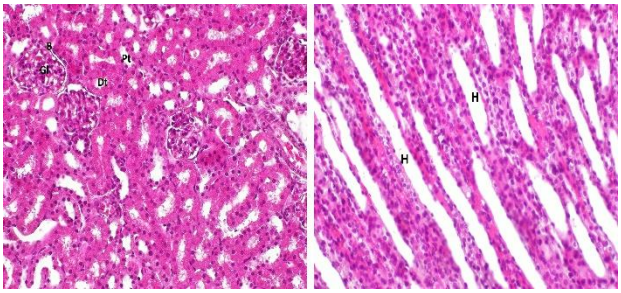


Şekil 1. Grup I: Bir günlük asetonsuz doku takip protokolu örneği ile elde edilen bir ratın böbrek kesiti. *Gl: Glomerül yumağı; Pt: Tubulus Proksimalis, Dt: Tubulus Distalis,bir ratın böbrek kesiti (HE, 40X).*

Bu gruplarda glomerül yumağı ayrılmaların olmadığı ve proksimal ve distal tübüller, henle kulpu yapılarının da bir bütün halinde olduğu gözlendi.

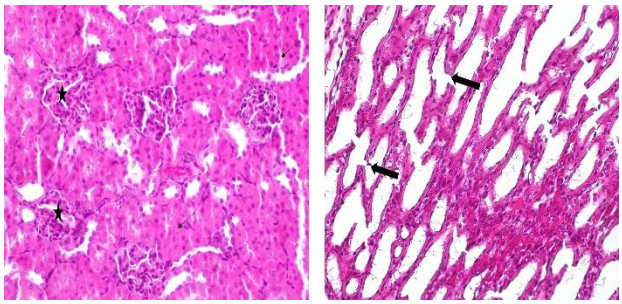


Şekil 2. Grup II: Bir günlük asetonlu doku takip protokolü örneği ile elde edilen bir ratın böbrek kesiti. *Gl: Glomerül yumağı; Pt: Tubulus Proksimalis, Dt: Tubulus Distalis,bir ratın böbrek kesiti (HE, 40X).*



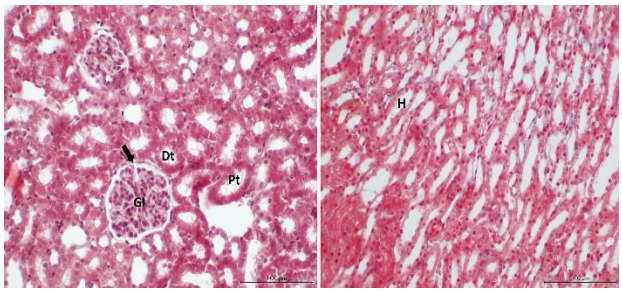
Şekil 3. Grup III: İki günlük asetonsuz doku takip protokolü örneği ile elde edilen bir ratın böbrek kesiti. *Gl: Glomerül yumağı; Pt: Tubulus Proksimalis, Dt: Tubulus Distalis,bir ratın böbrek kesiti (HE, 40X).*

Grup IV iki günlük asetonlu doku takip protokolu izlenerek elde edilen böbrek preperatları incelendiğinde glomerül yumağında parçalanmalar tespit edildi. Bu grupta tubulus proksimalis, tubulus distalis, çıkan henle ve inen henlelerin hücre sınırlarında parçalanmaların meydana geldiği gözlendi (Şekil 4).



Şekil 4. Grup IV: İki günlük asetonlu doku takip protokolü örneği ile elde edilen bir ratın böbrek kesiti. *Glomerül yumakta parçalanmalar (yıldız) proksimal ve distal tübüllerde ayrılmalar (ok) gözlenmiştir. Medullada Henle kulpu yapısında parçalanmalar (ok) tespit edilmiştir. (HE,40X)*

Grup I,II ve III doku takip yöntemleri uygulanarak hazırlanan böbrek dokularında tüm oluşumların bütünlüklerini korudukları belirlendi. Tüm gruplara ait böbrek dokularında yer alan glomerülus, Bowmann kapsülü, tubulus distalis, tubulus proksimalis, inen ve çıkan Henle yapılarındaki hasar dereceleri ve ölçülen glomerül çaplarının ortalama değerleri tablo 2 de ve semikantitatif analizi Şekil 9 da sunulmuştur.



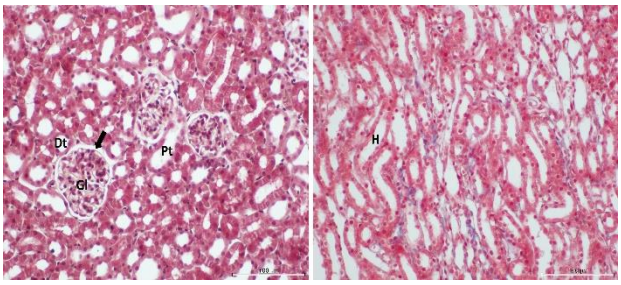
Şekil 5. Grup I: Bir günlük asetonsuz doku takip protokolü örneği ile elde edilen bir ratın böbrek kesiti Masson Trikrom Boyaması. *Gl: Glomerül yumağı; Pt: Tubulus Proksimalis, Dt: Tubulus Distalis, bir ratın böbrek kesiti (40X).*

Bunlara ek olarak Masson trikrom ile boyanan böbrek doku kesitleri grup I (Şekil 5), grup II (Şekil 6) ve grup III (Şekil 7) de resimlenmiştir. Grup I,II ve III doku takip yöntemleri uygulanarak hazırlanan böbrek dokularında histomorfolojik olarak tüm yapıların bütünlüğünü koruduğu tespit edilmiştir. Fakat Grup IV te (Şekil 8) böbrek dokusunda yer yer parçalanmalar gözlenmiştir.

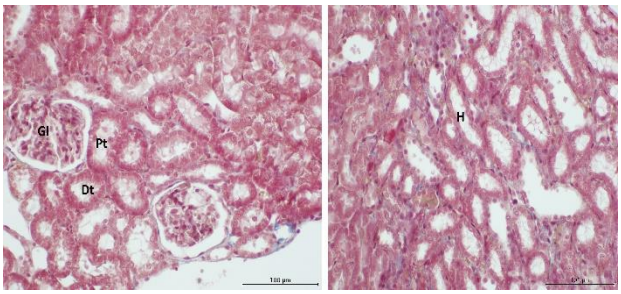
Tablo 2. Gruplardaki glomerülus, Bowmann kapsülü, tubulus distalis, tubulus proksimalis, inen ve çıkan Henle hasar oranlarının gösterimi

	Glomerülus	Bowmann Kapsülü	Tubulus distalis	Tubulus proksimalis	İnen Henle	Çıkan Henle	Glomerül çap
G-I	0,16	0,16	0,16	0,33	0,5	0,5	88,96
G-II	0,6	0,3	0,33	0,33	0,33	0,33	108,64
G-III	0,5	0,5	0,6	0,33	0,33	0,33	114,64
G-IV	2,3	1,33	1,33	1,33	1,33	1,5	109,74

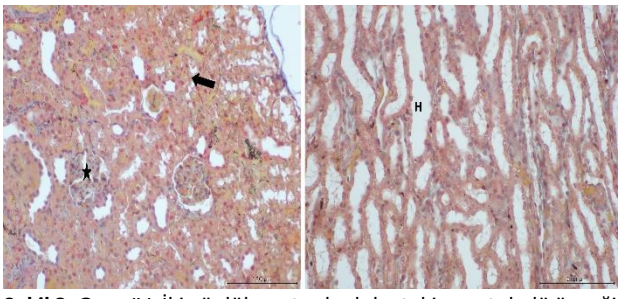
**G: Grup*



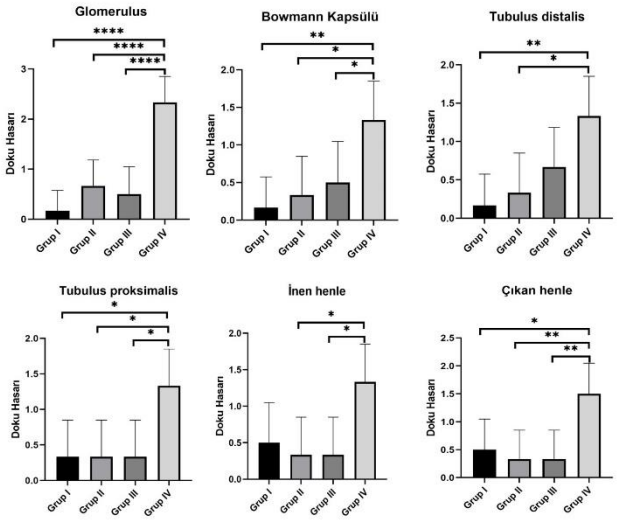
Şekil 6. Grup II: Bir günlük asetonlu doku takip protokolü örneği ile elde edilen bir ratın böbrek kesiti Masson Trikrom Boyaması. *Gl: Glomerül yumağı; Pt: Tubulus Proksimalis, Dt: Tubulus Distalis,bir ratın böbrek kesiti (40X).*



Şekil 7. Grup III: İki günlük asetonsuz doku takip protokolü örneği ile elde edilen bir ratın böbrek kesiti. Masson Trikrom Boyaması. *Gl: Glomerül yumağı; Pt: Tubulus Proksimalis, Dt: Tubulus Distalis,bir ratın böbrek kesiti (40X).*



Şekil 8. Grup IV: İki günlük asetonlu doku takip protokolü örneği ile elde edilen bir ratın böbrek kesiti Masson Trikrom Boyaması. *Glomerül yumakta parçalanmalar (yıldız) tübüllerde ayrılmalar (ok) gözlenmiştir. (40X)*



Şekil 9. Böbrek doku hasarı semikantitatif analizi. *Glomerülus, Bowmann kapsülü, tubulus distalis, tubulus proksimalis, inen ve çıkan Henle hasarı, Grup IV te diğer gruplara oranla anlamlı derecede artmıştır.*

Tartışma

Dokunun canlı organizmadan uzaklaştırılmasının hemen ardından ilk aşama fiksasyon ve doku takibi sırasında, kimyasal ve yapısal bütünlük göz önüne alınarak verilen ödünlerin minimum düzeyde tutulması gerekir. Doku takip basamaklarına etki eden faktörler arasında takip şekline bağlı olanlar, takip ajanlarına ait olanlar ve dokuya ait olanlar şeklinde sınıflandırılabilir. Takip ajanlarına ait faktörler solüsyonların konsantrasyonu, viskoziteleri, polaritesi, birbirleri ile uyumu ve buharlaşma hızıdır. Doku takibinin aşamaları fiksasyonu takiben dehidrasyon, şeffaflandırma, sertleştirme ve gömmedir.[10]

Farklı fiksasyon yöntemleri ve bu amaç için kullanılan çeşitli fiksasyon ajanları vardır. Şu zamana kadar tüm dokular için uygun tek bir fiksatifin bulunmaması araştırmacıların faklı fiksatifler üzerine yoğunlaşmasına neden olmuştur. Literatürde yer alan çalışmalar incelendiğinde, formalinin genel olarak moleküler açıdan bir ya da birkaç farklı fiksatifle karşılaştırıldığı tespit edilmiştir. Çalışmaların birçoğunda, formaline alternatif olan fiksatiflerin moleküler metodların kullanımı bakımından formalinden daha üstün oldukları gösterilmiştir. Hindi[17] ve koyun[18] böbrek dokularının ve bazı memeli türlerinde[19] dalağın histolojik yapısını değerlendirmeye yönelik çalışmalar vardır. Ratların böbrek dokuları üzerine yapılan bir çalışmada 0.1 M fosfat tamponlu gluteraldehit, 0.1 M fosfat tamponlu %10 formaldehit, 0.1 M kakodilat tamponlu paraformaldehit tespit solüsyonlarıyla immersiyon tespit yöntemi kullanılmıştır. Çalışmanın sonucunda böbrek tübüllerinde ve Bowman kapsülünde parçalanmaların meydana geldiği bildirmiştir[20]. Literatürde yer alan diğer çalışmalarda ise, farklı fiksasyon tipleri akciğer[21] ve kolon kanserlerinde[22], ince iğne aspirasyonlarında[23], tiroid dokusunda[24], karaciğer dokusunda[25], normal kolon mukoza örneklerinde[26] değerlendirilmiştir. Bu ideal fiksatif arayışları, dehidratif alternatiflerini geri planda bırakmıştır.

Dehidrasyon ajanlarını seçerken dikkat edilecek özellikler arasında ekonomik olması, doku takibinde yer alan diğer solüsyonlar ile uyumlu olması ve dokuyu ne kadar küçülttüğü sayılabilir. Dehidrasyon yeterli olmadığı taktirde şeffaflandırma ve infiltrasyon basamakları da kötü olacağı için çamur gibi, ortası yumuşak dokular elde edilebilir. Tam tersi olarak fazla dehidrasyon ise zor kesit alınabilen, çok sert ve kırılgan dokular elde edilmesine sebep olur.

Biz de çalışmamızda, diğer çalışmalardan farklı olarak böbrek dokusuna dehidratifleri ve süreleri farklı olan dört adet doku takibi yöntemi uyguladık. İki doku takibi protokolünde dehidratif olarak alkol diğer ikisinde aseton, sırasıyla bir ve iki gün süre ile uygulandı. Ancak dokular formalin dışında alternatif bir fiksatif ile fikse edilmedi.

Çalışmamızda kullandığımız farklı dehidratifler ve farklı doku takipleri ile elde ettiğimiz kesitler, mikroskobik olarak alışılagelmiş morfolojiye benzer ya da daha iyi özellikte sonuç vermiştir. Değerlendirilen tüm parametreler Grup I, Grup II ve Grup III te doku bütünlüğü ve tüm yapılar göz önüne alındığında genel olarak, morfolojik açıdan benzer sonuç verdiği gözlendi. Ancak iki günlük doku takipleri karşılaştırıldığında aseton kullanılarak uygulanan protokolde kesitlerde parçalanmalar olduğu ve böbrek doku bütünlüğünün kaybedildiği gözlendi. Bunlara ek olarak Gruplarda ölçülen glomerül çaplarının ortalama değerleri literatüre yakın bulunmuştur.[27]

Sonuç olarak, rutin histolojik incelemelerde, doku morfolojisinin korunmasında en önemli etken doku takibidir. Bu süreç ne kadar başarılı ise mikroskoptaki ayrıntı düzeyi de o derecede iyi olacaktır bu durumda temel histomorfolojik incelemeler için uygun bir zemin oluşturacaktır. Bizde çalışmamızda elde ettiğimiz verilere dayanarak doku takibi sürecinde dehidratif ajan olarak aseton kullanımını önermekteyiz.

Etik Standartlara Uygunluk

Bu çalışmanın etik kurulu Saki Yenilli Deney Hayvanları Üretim ve Uygulama Laboratuvarı hayvan deneyleri yerel etik kurulu tarafından 01.10.2021 tarihinde, karar numarası: 33 ile onaylanmıştır.

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Yazarlar arasında çıkar çatışması bulunmamaktadır.

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Research Article | Araştırma Makalesi

EVALUATION OF THE FREQUENCY OF MALNUTRITION IN CHILDREN AND ADOLESCENTS REFERRED TO PEDIATRIC ENDOCRINOLOGY OUTPATIENT CLINIC FOR SHORT STATURE

BOY KISALIĞI NEDENİYLE PEDİATRİK ENDOKRİNOLOJİ POLİKLİNİĞİNE BAŞVURAN ÇOCUK VE ERGENLERDE MALNÜTRİSYON SIKLIĞININ DEĞERLENDİRİLMESİ

Elif Söbü[1], Mehmet Tolga Köle[1]

[1]Kartal Dr. Lutfi Kırdar City Hospital, Department of Pediatrics, Istanbul, Turkey.

ABSTRACT

Objective: The aim of this study was to describe the characteristics and the etiological factors of children and adolescents and the prevalence of malnutrition with the complaint of short stature.

Methods: This retrospective study was conducted in the pediatric endocrinology outpatient clinic of a tertiary care city hospital in İstanbul, Turkey, between May 2019 and May 2021. Patients aged 1-18 years who were referred to the pediatric endocrinology outpatient clinic because of short stature were included in the study. Short stature was defined as a height below the third percentile for the corresponding age and gender in the Turkish children's growth chart. Mild malnutrition was defined as weight for height SDS (standard deviation score) between -1 and -2, moderate malnutrition between -2 and -3 and severe malnutrition less than -3.

Results: The study included 980 patients aged between 1 and 18 years. Forty-five percent of the patients were female (n=444) and 55% (n=536) were male. Height SDS was less than -2 SDS in 408 patients, while 572 (58%) patients had a height SDS value greater than -2 SDS. When the whole group was evaluated, 38% of the cases (n:371) had a weight for height SDS value less than -1 and had varying degrees of malnutrition. There was no statistically significant difference between girls and boys in the distribution of malnutrition according to gender (p:0.46).

Conclusion: Results of our study indicate that stunting due to protein-energy deficiency is still a serious health problem in our country and mild malnutrition can be easily overlooked.

Keywords: Short stature, malnutrition, stunting

ÖZ

Amaç: Bu çalışmanın amacı, boy kısalığı şikayetiyle başvuran çocuk ve ergenlerde klinik bulguları, etiyolojik faktörleri ve malnütrisyon sıklığını tanımlamaktır.

Yöntem: Bu retrospektif çalışmada, Mayıs 2019 ile Mayıs 2021 tarihleri arasında Türkiye'de üçüncü basamak bir şehir hastanesinin çocuk endokrinoloji polikliniğine boy kısalığı nedeniyle yönlendirilen 1-18 yaş arası olgular değerlendirildi. Boy kısalığı Türk çocuklarının büyüme eğrilerine göre boyun -2 standart deviasyon skoru (SDS) altında olması olarak tanımlandı. Hafif malnütrisyon boya göre ağırlık SDS değerinin -1 ile -2 arasında, orta derecede malnütrisyon -2 ile -3 arasında ve ağır malnütrisyon -3'ün altında olması şeklinde tanımlandı.

Bulgular: Çalışmaya yaşları 1 ile 18 arasında değişen 980 hasta dahil edildi. Hastaların %45'i kız (n=444) ve %55'i (n=536) erkekti. Boy SDS değeri 408 hastada -2 SDS'den düşükken, 572 (%58) hastada -2 SDS'den yüksekti. Tüm grup değerlendirildiğinde, vakaların %38'inin (n:371) boya göre tartı SDS değeri -1'den düşüktü ve çeşitli derecelerde malnütrisyonu vardı. Malnütrisyonun cinsiyete göre dağılımında kız ve erkek çocuklar arasında istatistiksel olarak anlamlı bir fark bulunmadı (p:0,46).

Sonuç: Çalışmamızın sonuçları, protein-enerji eksikliğine bağlı bodurluğun ülkemizde hala ciddi bir sağlık sorunu olduğunu ve hafif malnütrisyonun kolayca gözden kaçabileceğini göstermektedir.

Anahtar Kelimeler: Boy kısalığı, malnitrisyon, bodurluk

***Corresponding author/İletişim kurulacak yazar:** Elif Söbü; Kartal Dr. Lutfi Kırdar City Hospital, Department of Pediatrics, Istanbul, Turkey.
Phone/Telefon: +90 (216) 458 00 00 **e-mail/e-posta:** elifsobu@yahoo.com
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Introduction

Short stature is one of the most common reasons for referral to the pediatric endocrinology outpatient clinic and defined as a height that is 2 standard deviations (SD) or more below the mean height for individuals of the same sex and chronologic age in a given population.[1] However, this definition is not appropriate for children of unusually taller or shorter than normal parents; therefore, a more appropriate definition would be 2 SD below the mean parental SD score (SDS). Severe short stature is defined as being more than -3 SD shorter than children of the same age, gender and race. The probability of finding an organic cause of short stature in children between -2 and -3 SDS is about 10%; when height is below -3 SDS, organic causes may constitute 58% of the etiology.[2-5]

Most children with short stature are described as variants of normal, such as familial short stature, puberty delay or idiopathic short stature. Pathological causes include growth hormone deficiency (GHD), hypothyroidism, celiac disease and Turner syndrome, chronic diseases (renal, hepatic and gastrointestinal) and genetic syndromes.[6]

Nutritional deficiency may manifest as short stature. The World Health Organization defines 'wasting' as weight-for-height <-2 SD, 'stunting' as height-for-age < -2 SD, and 'underweight' as weight-for-age < -2 SD.[7]

The aim of this study was to describe the characteristics and the etiological factors of children and adolescents and the prevalance of malnutrition with the complaint of short stature evaluated in a pediatric endocrinology clinic of a tertiary center. Auxological data and laboratory results were presented.

Methods

The study has been reviewed by the local ethical committee of Kartal Dr. Lütfi Kırdar City Hospital and has therefore been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (ethics approval number:2021/514/204/10). This retrospective study was conducted in the pediatric endocrinology outpatient clinic of Kartal Dr. Lütfi Kırdar City Hospital in İstanbul, Turkey, between May 2019 and May 2021. A total of 980 patients aged 1-18 years who were referred to the pediatric endocrinology outpatient clinic because of short stature were included in the study.

Age at presentation, gender, weight, weight SD, height, height SD, body mass index (BMI), body mass index SD, pubertal status, bone age, target height, target height SD, thyroid function tests, insulin like growth factor (IGF), IGF SD, FSH, karyotype, tissue transglutaminase IgA values and comorbidities were recorded.

During the height measurements, the patients were wearing a thin layer of clothing and the measurements were performed without shoes. Height measurements were made by the same physician using a stadiometer sensitive to millimeters (Seca, Germany).

Short stature was defined as a height below the third percentile for the corresponding age and gender in the Turkish growth chart that was revised in 2008 by Neyzi et al[5]. The BMI is defined as a child's weight in kilograms divided by the square of his or every height in the mistress (kg/m^2). Weight-for-height SD score was calculated and recorded for subjects aged 1-5 years.

Waterlow and World Health Organization data were used to define malnutrition. According to the Waterlow classification, weight-for-height above 90% is defined as normal, 81-90% as mild, 70-80% as moderate and below 70% as severe acute malnutrition. In addition to this definition, the range of 81-90% corresponds to the range where the Z score is -1/-2 in the WHO classification. Although it is defined as mild malnutrition in Waterlow, according to the WHO, it is now excluded from the definition of malnutrition.[8] WHO defines moderate acute malnutrition as BMI-for-age ≤−2 SD and ≥−3 SD of the median, or mid-upper arm circumference ≥115 mm and <125 mm. And severe acute malnutrition is defined as weight-for-length/height or BMI-for-age <− 3 SD of the median or mid-upper arm circumference <115 mm, or bilateral pitting oedema. Weight for height was assessed to evaluate malnutrition in patients aged 1-5 years.[8]

Statistical analysis

The SPSS version 21.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis. The variables were investigated using visual (histogram, probability plots) and analytic methods (Kolmogorov–Smirnov/Shapiro–Wilk's test) to determine whether or not they are normally distributed. Descriptive analyses were presented using proportions, medians, minimum (min), and maximum (max) values where appropriate. Differences in proportions between groups were evaluated by the Chi-square test or Fisher's exact test where appropriate. Mann–Whitney U test was used to compare the non-normally distributed continuous data between two groups. The homogeneity test was used to compare dependent and multi-category variables. Categorial variables are defined as number (n) and percentage (%) values. *p* value < 0.05 was considered as significant.

Results

The study included 980 patients aged between 1 and 18 years. Forty-five percent of the patients were female (n=444) and 55% (n=536) were male. The median (minimum-maximum) age of all patients was 9 (1-18) years. The median age of girls was 9 (1-18) and of boys 10 (1-18 years). Boys' age at presentation was statistically higher than that of girls (p=0.002)

The median weight was 24 (min-max: 7-73) kg, median weight SDS -1 (min-max: -4.93 -2.99), median height 125 (min-max: 74.7-178) cm, median height SDS -2 (min-max: -5-1), median BMI 16 (min-max:10.7-32.8) kg/m2, median BMI SDS -1 (min-max: -5.04-3.08).

The median insulin-like growth factor 1 (IGF-1) level was 122 (min-max: 20.8-498.3) ng/ml and the median IGF-1 SDS was -1 (min-max: -3-2.8). Seventy-two percent of the cases (n=706) were prepubertal and 28% (n=274) were pubertal at presentation. Height SDS was less than -2 SDS in 408 patients, while 572 (58%) patients had a height SDS value greater than -2 SDS.

When 408 patients with short stature were evaluated, 63 of these patients had a height SDS value of -3 SDS and below. In the group with severe short stature, 17 cases had malnutrition (27%), 9 cases had growth hormone deficiency (9.19%), 3 cases had constitutional delayed puberty, 2 cases had hypothyroidism, 2 cases had celiac disease, and 2 cases had the diagnosis of bioinactive growth hormone, 1 case was diagnosed as nutritional rickets, 1 case as Turner syndrome, 1 case as DEND syndrome, 1 case as Peliazeus Merzbacher syndrome, 1 case as Klippel Feil syndrome and 1 case as dilated cardiomyopathy and malnutrition. Fourteen of the patients with severe short stature were referred to the genetics outpatient clinic because pathologic short stature was found in the mother and/or father and endocrine tests were normal. Eight patients did not continue their follow-up after the first control.

In our study, 344 patients had height SDS values between -2 SD and -3 SD. In this group, 116 (33.6%) were diagnosed as familial short stature, 24 (0.07%) as growth hormone deficiency, 21 as constitutional delayed puberty, 7 as skeletal dysplasia, 6 as hypothyroidism, 4 as syndromic short stature, 1 as Noonan syndrome and 1 as celiac disease. Thirty-one of the patients did not continue their follow-up after the first control. In 133 cases, no reason other than malnutrition was found to explain short stature. Malnutrition was also the most common cause in this group.

Among 572 patients with a height between -2 SDS and 1.45 SDS who were referred to the endocrine outpatient clinic because of short stature, 26% (n=152) had mild malnutrition, 8% (n=45) had moderate malnutrition and 0.008% (n=5) had severe malnutrition. Thyroid function tests and tissue transglutaminase IgA levels were evaluated in all cases. Only one of these patients had celiac antibody positivity and one had hyperthyroidism.

When the whole group was evaluated, 38% of the cases (n:371) had a BMI SDS value less than -1. According to WHO classification, 10.1% (n=99) of the patients were moderately or severely malnourished. In 40% (n:162) of the patients with height SDS value lower than -2 SDS, weight SDS value was lower than -1 SDS. The frequency of malnutrition was similar in children with short stature when evaluated in the whole group. When the cases were divided into 3 groups as 1-5, 6-12 and >13 years of age, the distribution of the number of cases according to the severity of malnutrition between the groups is shown in Table 1. The distribution of mild, moderate and severe malnutrition was statistically similar between age groups (p=0.093). Also, there was no statistically significant difference between girls and boys in the distribution of malnutrition (p=0.46).

Table 1. Distribution of malnourished patients according to age groups

Age (years)	Mild (n)	Moderate (n)	Severe (n)	Total
1-5	47	21	2	70
6-12	144	34	4	182
>13	81	31	7	119
Total (n)	272	86	13	371

n: total count

Discussion

Short stature may be a sign of an endocrine disorder or may develop as a result of a chronic disease or malnutrition. Early diagnosis and treatment is important due to long-term permanent losses and psychosocial effects. In our study, cases referred to the pediatric endocrinology outpatient clinic for short stature in a 2-year period were evaluated. Fourty-one 41% of these patients had a height less than -2 SD. In fact, almost 1/3 of the patients referred for short stature were found to have various degrees of malnutrition.

Malnutrition can lead to stunting in children and mild forms of malnutrition can easily be overloooked.[9,10] In 2006, Gür et al. evaluated 1576 school children aged 6-18 years and reported a prevalence of 5.6% for stunting, 4.6% for underweight, 1.0% for wasting.[11] Although the rates were found to be lower in the healthy population, the data of our study support that weight-for-height SDS was low in 10.1% in our study group. This may be due to the fact that, especially cases referred with the complaint of short stature were evaluated in our study.

The American Society for Parenteral and Enteral Nutrition (ASPEN) defines malnutrition as an imbalance in nutrient requirements and intake leading to total energy, protein or micronutrient deficiencies that may adversely affect growth and development.[12] Also malnutrition is defined as a deficiency or excess of protein, energy and other nutrients, resulting in a decrease in body mass as a nutritional disorder that causes measurable negative effects on the body and its functions.[13] Inadequate or unbalanced nutrient content affects growth and development, defense system, inflammation process, autoimmune triggers. Many studies reported that the risk of mortality in childhood is significantly increased in cases with malnutrition.[14-16]

Also in a recent study El-Shafie et al. evaluated 33150 school children in Egypt, the prevalence of underweight was reported to be 8.2% and short stature 17%.[17]

Several studies show that socioeconomic and demographic factors influence the prevalence of stunting, with improvement in socioeconomic status leading to a marked improvement in nutritional status and a significant reduction in the prevalence of stunting, especially in children under 5 years of age.[18,19]

In a cross-sectional study in China, Wang et al reported the prevalence of stunting in the 10-18 age group 19%.[20]

In a study conducted in Pakistan, the prevalence of stunting in school children aged 6-12 years was reported to be 16.5%.[21]

A detailed nutritional history, physical examination, interpretation of anthropometric measurements using appropriate reference standards (including weight, length and head circumference in young children) and, where possible, basic laboratory tests are the main considerations in the assessment of nutrition in children.[22] In the case of nutrient deficiencies, there may not always be visible symptoms. However, in this case a chronic condition that can cause lifelong growth and developmental retardation and reduced productivity which is a form of malnutrition. Chronic malnutrition, especially occurred in the first two years of life, which is characterized with rapid growth and development period of life, the lifelong effects are more pronounced.

The limitations of our study should be mentioned. Since the study was retrospective, mid-upper arm circumference measurements, which are other markers of malnutrition, were not available. Also, unfortunately, we did not have data on the socioeconomic status of the patients.

In conclusion, the results of our study indicate that stunting due to protein-energy deficiency is still a serious health problem in Turkey and mild malnutrition can be easily overlooked. When children remain under the influence of malnutrition for more than three months, it is defined as chronic malnutrition and it should be kept in mind that this condition is associated with permanent growth and developmental retardation.

Compliance with Ethical Standards

Since the study was planned retrospectively, informed consent form was not taken. This study was approved by Ethical Committee of Kartal Dr. Lütfi Kırdar City Hospital (2021/514/204/10).

Conflict of Interest

The authors declare no conflicts of interest.

Author Contribution

Authors contributed equally to this work.

Financial Disclosure

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Research Article | Araştırma Makalesi

ELECTRONIC AND TOPOLOGICAL INVESTIGATION OF ANTITUBERCULAR TRYPTANTHRIN ANALOGUES AND THEIR INTERACTION WITH THE ENOYL-ACP REDUCTASE USING IN SILICO METHODS

ANTİTÜBERKÜLOZ TRİPTANTRİN ANALOGLARININ ELEKTRONİK VE TOPOLOJİK ÖZELLİKLERİNİN VE ENOİL-ACP REDÜKTAZ İLE ETKİLEŞİMLERİNİN İN SİLİKO YÖNTEMLERLE İNCELENMESİ

Fatma Aksakal*

*Department of Analytical Chemistry, Faculty of Pharmacy, Kocaeli Health and Technology University, Kocaeli, Türkiye.

Abstract

Objective: This study aimed to investigate the structure-antitubercular activity relationships of a series of tryptanthrin analogues and the binding mechanisms of these analogues with InhA, *Mycobacterium tuberculosis* enoyl-acyl carrier protein (enoyl-ACP) reductase.

Methods: Firstly, pharmacophores and anti-pharmacophores have been determined with the Electronic-Topological Method (ETM) and activity prediction models have been developed with the ETM-Neural Networks (NN) approach. In the second step, the binding affinities and conformations of the compounds to the InhA enzyme and noncovalent interactions between them were investigated with the molecular docking method. Finally, these interactions were discussed with the electron density distribution of the frontier molecular orbitals (FMO).

Results: The results of the ETM-NN application to the series of compounds in view are pharmacophores and anti-pharmacophores, which are characteristic of the class of compounds demonstrating activity against *Mycobacterium tuberculosis* strain H37Rv. The statistical characteristics of five pharmacophores (Phi) and five anti-pharmacophores (APhi) entering the forecasting system, are 0.90 and 0.86, respectively. Molecular docking and electron density distribution analyzes revealed that the active compounds bind more strongly to the InhA enzyme.

Conclusion: A model of prognosis for the antitubercular activity of *Mycobacterium tuberculosis* H37Rv was developed on the base of the pharmacophores found, docking results, and electronic structure calculations. This model allows for designing of new potent antitubercular drugs.

Keywords: Tryptanthrin analogues, *Mycobacterium tuberculosis* H37Rv, InhA, electronic-topological method, molecular docking, density functional theory.

Öz

Amaç: Bu çalışmanın amacı, bir dizi triptantrin analogunun yapı-antitüberküloz aktivite ilişkilerini ve bu analogların *Mycobacterium tuberculosis* enoyl-acyl taşıyıcı protein (enoyl-ACP) redüktaz (InhA) ile bağlanma mekanizmalarını incelemektir.

Yöntem: İlk olarak, elektronik-topolojik yöntem (ETM) ile bileşiklerin yapısındaki farmakofor ve anti-farmakoforlar tespit edilmiş ve ETM-Sinir Ağları (*Neural Networks*, NN) yaklaşımına dayalı olarak aktivite tahmin modelleri geliştirilmiştir. İkinci aşamada, moleküler kenetlenme yöntemi kullanılarak bileşiklerin InhA enzimine bağlanma afiniteleri ve konformasyonları elde edilmiş; bağlanmada etkili olan kovalent olmayan etkileşimler incelenmiştir. Son aşamada, bu etkileşimler sınır moleküler orbitallerin (FMO) elektron yoğunluğu dağılımı üzerinden tartışılmıştır.

Bulgular: İnceleme altındaki bileşik serileri üzerine ETM-NN yönteminin uygulaması sonucunda *Mycobacterium tuberculosis* H37Rv suşuna karşı aktivite gösteren bileşiklerin karakteristik farmakofor ve anti-farmakofor özellik gösteren moleküler fragmanları tespit edilmiştir. Aktivite tahmin sistemine giren beş farmakofor (Phi) ve beş anti-farmakoforun (APhi) istatistiksel özellikleri sırasıyla 0,90 ve 0,86 olarak elde edilmiştir. Moleküler kenetlenme ve elektron yoğunluğu dağılımı analizleri, aktif bileşiklerin InhA enzimine daha kuvveti bağlandığını ortaya koymuştur.

Sonuç: Tespit edilen farmakofor gruplar, kenetlenme sonuçları ve elektronik yapı hesaplamalarına dayalı olarak, *Mycobacterium tuberculosis* H37Rv'nin antitüberküloz aktivitesi için bir prognoz modeli geliştirilmiştir. Bu prognoz modeli, yeni potansiyel antitüberküloz ilaçların tasarımında kullanılabilecektir.

Anahtar Kelimeler: Triptantrin analogları, *Mycobacterium tuberculosis* H37Rv, InhA, elektronik-topolojik yöntem, moleküler kenetlenme, yoğunluk fonksiyoneli teorisi.

*** Corresponding author/İletişim kurulacak yazar:** Fatma Aksakal; Department of Analytical Chemistry, Faculty of Pharmacy, Kocaeli Health and Technology University, Kocaeli, Turkiye
Phone/Telefon: +90 (262) 999 80 85 **e-mail/e-posta:** fatma.dagdelen@kocaelisaglik.edu.tr
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Introduction

Tuberculosis (TB) is an infection arising from the bacillus *Mycobacterium tuberculosis* (*Mtb*). Globally in 2021, around 1.6 million people died from this infectious disease, of which 187000 were HIV co-infected.[1] Occurrence and spread of multidrug resistant tuberculosis (MDR-TB) along with extensively resistant tuberculosis (XDR-TB) make harder the problem, since few new medicines have been approved to fight the resistant forms.

Tryptanthrin (indolo[2,1-b] quinazoline-6,12-dione) is a compound from the class of tryptophan derived alkaloids produced by diverse plants[2-5] and fungi.[6] Tryptanthrin (TRYP) and its derivatives have been shown to induce a large number of biological effects in different *in vitro* and *in vivo* studies. These effects are antibacterial activity against *Bacilus subtilis*[6], *Escherichia coli*[7], MRSA[8]; antiprotozoal activity against *Leishmania donovani*[9], *Plasmodium falciparum*[10,11], *Trypanosoma brucei*[12], *Toxoplasma gondii*[13,14]; antifungal activity against *Malassezia furfur*[8], *Trichophyton mentagrophytes*[4]; cytostatic or cytotoxic activity against MCF-7[15-17], K-562[18], NCI-H460, SF-268[15], HeLa, SKOV-3[17],WEHI-3B JCS[19], U-937, HL-60[20] and A-498[17] cell lines, and antiproliferative effects against tumor cells in Lewis lung cancer tumor-bearing mice[17] and WEHI-3B JCS cells in syngeneic BALB/c mice.[21]

Interestingly, TRYP has been found to reduce resistance to some anticancer agents in breast cancer cells.[16,17] One of the important activity of TRYPs is their following immunomodulatory effects: down-regulation of interleukin-4 production by Th2 cells[19], inhibition of nitric oxide and prostaglandin E2 synthesis in macrophages[22], inhibition of interferon-γ and interleukin-2 production by mouse spleen cells and Peyer's patch lymphocytes *in vitro*[23], indole amine 2,3-dioxygenase inhibition[24], significant decrease of the levels of TSLP, IL-4, IFN-γ, IL-6, TNF-α, chemokine, and caspase-1 in atopic dermatitis (AD), repression of the histidine decarboxylase levels with consequent reduction of histamine levels in AD.[25]

TRYP and compounds derived from TRYP were proposed as immunotherapeutic agents to treat cancer, BCG, cholera, plague, typhoid, hepatitis B infection, influenza, inactivated polio, rabies, measles, mumps, rubella, oral polio, SARS, yellow fever, tetanus, diphtheria, Haemophilus influenzae type b, meningococcus and pneumococcus infections.[26,27] Another reported activity of TRYP and its derivatives is antimycobacterial activity.[28,29]

Enoyl-acyl carrier protein reductase (InhA) from *Mtb* is one of the key enzymes involved in the mycobacterium fatty acid biosynthetic pathway and is an effective antimicrobial target. InhA inhibitors are promising candidates for the development of novel antitubercular agents.[30]

Molecular modelling and structure-activity relationships (SAR) studies play an essential role in the design of potential ligands that are both sterically and chemically compatible with the binding site of a target bio-macromolecule.[31,32] It should be noted that in literature there are a few computational works devoted to the ligand-receptor interaction for the enzyme InhA from *Mtb*.

Novel antitubercular compounds identification through a hybrid virtual screening approach was investigated and molecular docking was performed to select the compounds on the base of their binding energies, binding modes, and tendencies to form reasonable interactions with InhA.[33] Effect of the explicit flexibility of the InhA enzyme from *Mtb* in molecular docking computations can be found in the literature.[34] The fully-flexible receptor models of InhA explicitly characterize the overall movements of the amino acid in helices, strands, loops and turns, allowing the ligand to properly accommodate itself in the receptor's binding site. Tripathi and coworkers[35] performed molecular docking analysis of TRYP and its analogues with InhA from *Mtb*. The study revealed that the alkaloid and its two analogues show a high affinity for the InhA binding site. Syntheses and docking of new modifications of the isoniazid-structure molecules are also presented in the literature.[36] *In silico* modeling on the InhA confirmed that longer alkyl substituents are advantageous for the molecular interactions and affinity to the enzyme. In one of the papers[37] on the inhibitors InhA from *Mtb*, docking of the compounds taken from the National Cancer Institute compound library against InhA was carried out. Analysing data on virtual screening, the authors described two promising and novel fragment hits that inhibit InhA activity.

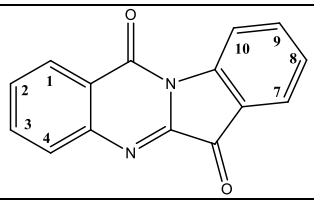
In this study, molecular design of TRYP analogues and their interactions with InhA were reported. Firstly, structural and electronic factors influencing antitubercular activity of TRYP analogues were investigated. The structure-activity relationships study was performed using the electronic-topological method combined with Neural Networks (ETM-NN). [38,39] Then, an antitubercular activity prediction model was improved to carry out computer screening of TRYP analogues. The prediction model was supported by the results of molecular docking and electronic structure computations of TRYP analogues in the active site of InhA enzyme.

Methods

Data set

Initial data for the search of the structure-activity relationship is a learning series of compounds together with data on their activities.[28,29] Molecular skeletons of the compounds are presented in Figure 1, while molecules with their substituents are given in Table 1.

Table 1. Compounds of the training and testing sets with different substituents

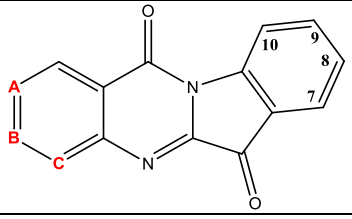


№	1	2	3	4	7	8	9	10	A/LA*
					Training set				
1	H	H	H	H	H	H	H	H	A
2	H	Cl	H	H	H	F	H	H	A
3	H	H	H	H	H	Br	H	H	A
4	H	H	H	H	H	NO_2	H	H	A
5	H	H	H	H	H	Cl	H	H	A
6	H	H	H	OCH_3	H	F	H	H	A
7	H	H	F	H	H	F	H	H	A
8	H	H	H	H	H	CO_2Et	H	H	A
9	H	H	H	H	H	H	F	H	LA
10	H	H	H	OCH_3	H	I	H	H	A
11	H	I	H	I	H	CO_2Et	H	H	LA
12	H	H	F	H	H	Cl	H	CH_3	A
13	H	F	NBP	H	H	I	H	H	LA
14	F	Br	H	Br	H	I	H	H	A
15	F	Br	H	Br	H	H	H	H	LA
16	H	F	3-MNBP	H	H	I	H	H	LA
17	H	H	H	OCH_3	H	CO_2Et	H	H	A
18	H	H	H	OCH_3	H	SO_2n-octyl	H	H	LA
19	H	H	H	OCH_3	H	SO_2NMP	H	H	LA
20	H	H	H	OCH_3	H	CO_2H	H	H	LA
21	H	NO_2	H	H	H	H	H	H	LA
22	Cl	H	Cl	H	H	H	H	H	LA
23	H	H	H	H	H	NH_2	H	H	LA
24	H	F	3-MP	H	H	CO_2H	H	H	LA
25	H	OPO_3Na_2	H	H	H	H	H	H	LA
26	H	OH	H	H	H	H	H	H	LA
27	H	octyl	H	H	H	H	H	H	LA
28	H	6-acoctenyl	H	H	H	Cl	H	H	LA
29	H	H	H	H	H	Cl	Pip	H	LA
30	H	H	H	H	H	CF_3	H	H	A
31	H	H	F	H	H	OCF_3	H	H	A
32	H	F	H	H	H	Cl	H	H	A
33	H	H	H	H	H	OCF_3	H	H	A
					Test set				
34	H	H	H	H	H	F	H	H	A
35	H	H	H	OBn	H	F	H	H	LA
36	H	H	H	H	H	I	H	H	A
37	H	F	H	H	H	F	H	H	A
38	H	Br	H	H	H	F	H	H	A
39	H	CH_3	H	CH_3	H	F	H	H	A
40	H	CH_3	H	H	H	H	H	H	LA
41	H	I	H	I	H	I	H	H	LA
42	H	2-AG	H	H	H	Cl	H	H	LA
43	H	H	octyl	H	H	octyl	H	H	LA

The compounds are the TRYP analogues possessing high and middle level of antitubercular activity. As seen from Figure **1**, the molecules differ from each other by their substituents in the rings they contain. To adjust to the series more representative, it was added a few active heterocyclic compounds (III-X) with structures that are quite different from the structure of TRYP.

The search for pharmacophores responsible for the compounds, bioactivity was carried out in two groups of compounds taken from the initial series. In one of them there were highly active compounds (43 molecule, MIC< 0.125 μg/ml), and the other included low-active compounds (29 molecules, MIC > 4 μg/ml). Besides, training set (63 molecules, including III-X) and test set (17 molecules) were formed to evaluate the effectiveness of the activity/inactivity fragments found.

Table 1. Continued



№	A	B	C	7	8	9	10	A/LA*
					Training set			
44	CH	CH	N	H	Br	H	H	A
45	CH	CH	N	H	Cl	H	H	A
46	CH	CH	N	H	F	F	H	LA
47	CH	CH	N	H	I	H	H	A
48	CH	CH	N	H	CO_2Et	H	H	A
49	N	CH	CH	H	I	H	H	A
50	N	CH	CH	H	Cl	H	H	A
51	N	CH	CH	H	Br	H	H	A
52	N	CH	CH	H	1-octenyl	H	H	A
53	N	CH	CH	H	n-octyl	H	H	A
54	CH	CH	N	CF_3	I	H	H	A
55	N	CH	CH	H	CF_3	H	H	A
56	CH	CH	N	CF_3	Cl	H	H	A
57	N	CH	CH	H	OCF_3	H	H	A
58	N	CH	CH	H	n-butyl	H	H	A
59	N	CH	CH	H	CH(i-propyl)OCH_3	H	H	A
60	CH	N	CH	H	OCF_3	H	H	A
61	N	H	H	H	H	Pip	H	LA
62	N	H	H	H	H	Mor	H	LA
63	N	H	H	H	H	Prz	H	LA
64	H	H	N	H	H	Pip	H	LA
65	H	H	N	H	H	H	H	LA
					Test set			
66	N	CH	CH	H	SO_2CH_3	H	H	LA
67	N	CH	CH	H	1-hexyl	H	H	A
68	N	CH	CH	H	DMDOx	H	H	A
69	N	CH	CH	H	MH	H	H	A
70	N	CH	CH	H	cyclohexyl	H	H	A
71	N	CH	CH	H	2-octyl	H	H	A
72	CH	CH	N	H	OCF_3	H	H	A

*A: highly active, LA: low-active; 2-AG: 2-(acetoxymethyl)-6-(allyloxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate; NBP: 3-n-butylphthalide; NMP: N-Methyl-2-pyrrolidone ; 3-MNBP:3-methyl-3-n-butylphthalide; DMDOx: 2-(5,5-dimethyl-l,3-dioxanyl); MH: 3-(2-methylheptyl); 3-MP: 3-methyl-2-pyrrolidinone; Pip: piperidine; Bn: benzyl; Mor: morpholine; Prz: piperazinyl.

Description of Electronic-Topological Method (ETM)

Since ETM is explained in detail in the literature,[39-46] the most distinctive features of ETM compared to other structure-activity relationship approaches are given in this section. ETM belongs to the so-called structural methods. So, the base for the method is a language for the compounds' structure description. This language reflects the discrete nature of molecules as groups of atoms, some of which are chemically bonded. Labeled graphs appeared to be the most appropriate mathematical counterparts for chemical structures together with relationships on their atoms and bonds.

The representative of a graph is a matrix of order *n x n*; where *n* is the number of vertices of the graph. Therefore, ETM proposes Electronic-Topological Matrices of Contiguity (ETMC) for the identification of chemical compounds. Bonds have no direction, so matrices are symmetric with respect to their left diagonal, and it is sufficient to have only the upper right triangle of any such matrix with its diagonal.

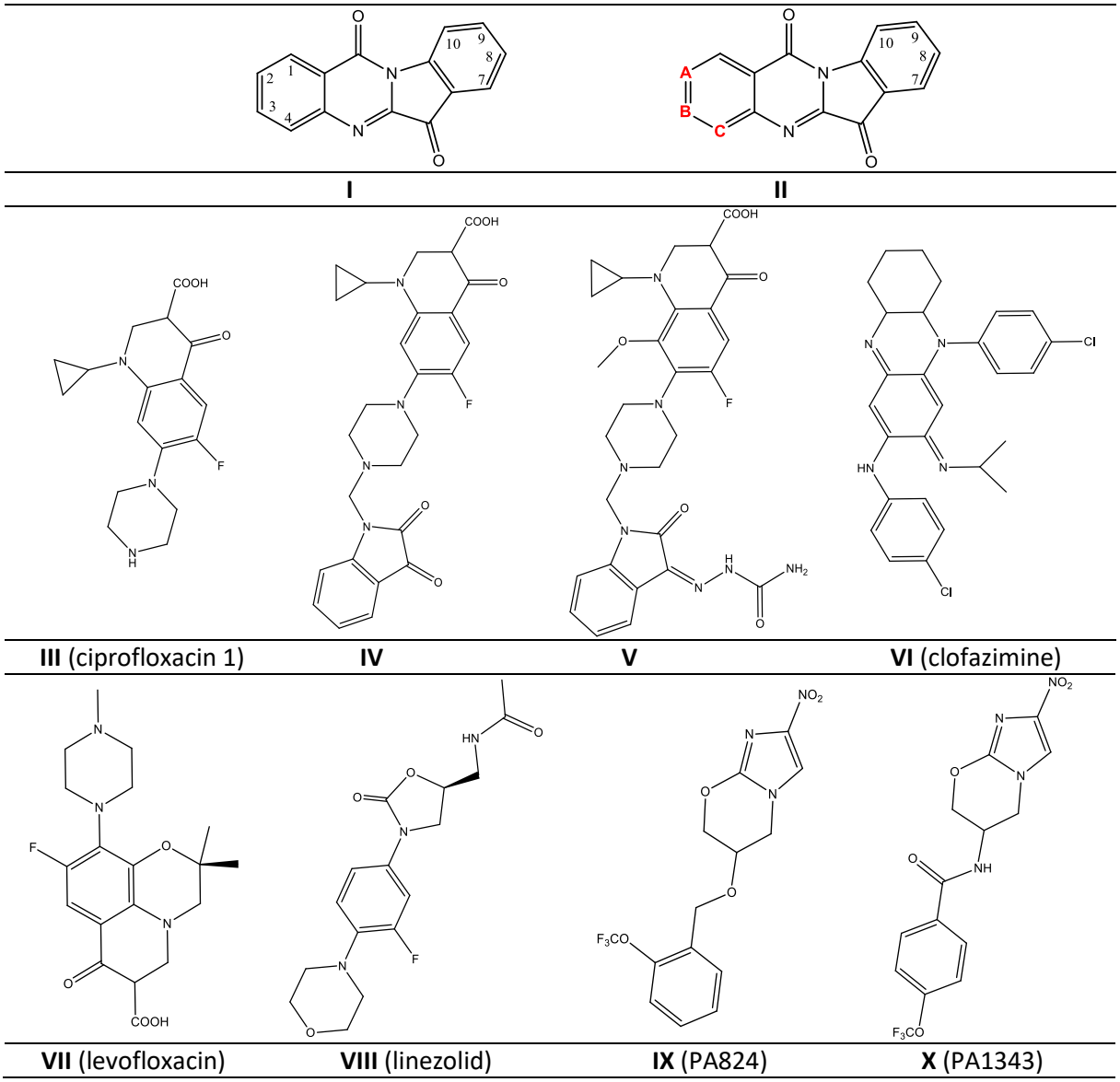


Figure 1. Structures of tryptanthrin (**I**), its analogues (**II**) and some antitubercular drugs (**III-X**)

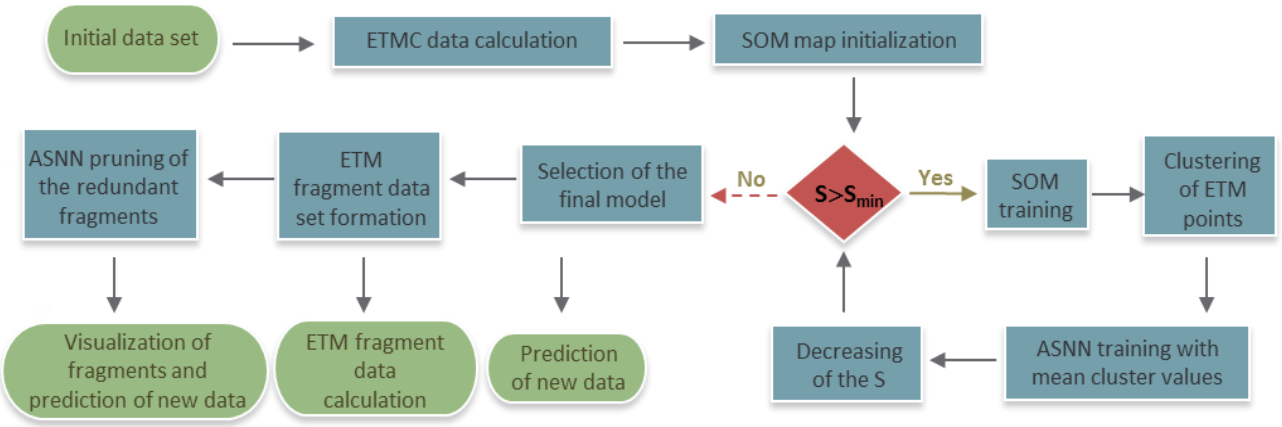


Figure 2. Block-scheme of data analysis with ETM-NN.

Vertices of such graphs (diagonal elements a_{ii} of the ETMCs) are naturally labeled by values representing atomic characteristics (charges-q_i, frontier molecular orbital coefficients *etc.*). For off-diagonal elements there are two possibilities. If they represent chemical bonds, then one of the bond characteristic is to be chosen for all matrices and fixed (here, Wiberg's indices, W_{ij}). Otherwise, the corresponding distance value is taken for the given pair of atoms (R_{ij}).

For a selected activity, the goal of the ETM approach is to identify molecular fragments common to all active compounds (pharmacophores, Ph) and not found in all inactive compounds (anti-pharmacophores, APh) with similar structures. A number of fragments characterizing inactive compounds will likewise be found. The pharmacophores and the anti-pharmacophore set are essential molecular substructures that provide a model for the prediction of the activity in view. This model can be used both for testing newly synthesized compounds, and for the computer modeling of new candidates for the purposeful syntheses.

To automate the activity estimation procedure, ETM application is followed by an associative neural networks (ASNNs) application (with unsupervised and supervised learning algorithms), and this approach is named as ETM-NN.[38] The ANNs application uses data of the ETM computations being electronic-topological submatrices of contiguity (ETSC) for Ph and APh as input for a new algorithm developed on the base of Volume Learning Algorithm. It had been used previously for the CoMFA analysis.[47,48] This algorithm is implemented as a recurrent iterative application of the Kohonen self-organizing maps (SOMs) and ASNNs.[49,50]

The later version of ETM using neural networks (ETM-NN) for data optimization and features extraction is working with the structures of compounds optimized through their interaction with receptor (in the process of the ETMCs formation), *i.e.* with the structures that are mostly profitable for the interaction with receptor conformations. This helped to solve the problem of finding the proper initial conformation for flexible molecules (flexibility problem). Conformational analysis and the electronic properties study are carried out for all compounds by using Gaussian 09 program.[51] General block-scheme of ETM-NN is given in Figure 2.

The first step is to prepare an initial data set for the SOM algorithm training (triples computation). The data sample is a triple (*d1, d2, d3*), where *d1* and *d2* are charges of two atoms and *d3* is a connection between them. The d_i values, i=1, 2, 3 are taken from the ETMCs. The total number of data samples corresponds to the amount of all two-atom connections taken from all ETMCs.

The second step includes initialization and training of the Kohonen's network of the size $S=x*y$. The initial size of Kohonen's maps was $S=2*S_{ETM}$, with S_{ETM} being the size of the largest ETM matrix. Upon subsequent compressions, the map size was decreased by two units in both x and y directions. When using the Kohonen's networks, it is possible to create a nonlinear projection of high-dimensional data set onto a low-dimensional domain. To train a Kohonen's map, two phases are needed. The first phase of 50,000 iterations is used for the weight vectors of the map neurons rough ordering. During the second phase (100,000 iterations), the values of the weight vectors are fine-tuned. The initial learning rate and neighborhood radius of the SOM under training are selected to be $\alpha_1=0.6$, $\sigma_1=2/5(x*y)^{0.5}$ and $\alpha_2=0.15$, $\sigma_2=2/5\sigma_1$ for the first and the second phase, respectively. To form a compressed sample data set, the cluster centers (C_n) were calculated by averaging over all values entering into the given cluster, $C_n = Xn_i/m$, where Xn_i was the value of the i-th element of the ETMC for the n-th molecule, and m was the number of ETMC values entering into the cluster for the molecule n.

At the third step, the compressed data are tested on the three-layer ASNNs. From the mean values of input parameters for each cluster, a new data set is formed for the ASNN learning. The number of neurons in the input layer corresponds to the number of clusters obtained as the output of the SOM. The hidden layer contains five neurons. The bias neuron is presented both on the input and hidden layer. An ensemble of M=100 neural networks was trained. The activity values were calculated for each network (ASNN) and averaged over all M networks. This value was used to calculate the statistical coefficients. The quality of each final model was assessed by the leave-one-out (LOO) method. By the method, each molecule is removed from the training set, and the remaining set is used to separate molecules into classes of activity, thereby predicting the activity of this molecule and evaluating the quality of the decision rule.

The fourth step is the computation of pharmacophores as the ETMCs fragments. At the fifth step, the weight of each fragment is estimated for each compound as its projection error, E_q, relative to the same nodes of the Kohonen's map as its comprising ETMC. The weight is taken as the inverse of its E_q: $W_{ij}=1/E_{qij}$ (i: molecule's number, j: fragment's number). A new table is formed based on the weight coefficients computed using the fragment weights as parameters.

In the final step, pruning algorithms targeting the selection of the most relevant ETMC fragments are applied. Optimized fragments, found to be important for displaying the activity analyzed by the molecules, are used to visualize these regions of the molecules under study.

Molecular docking computations

In order to gain insight into the binding mode of compounds to InhA and make interpretation of the obtained results, molecular docking was carried out. 3D-crystallographic structure of the target enzyme InhA was retrieved through RCSB Protein Data Bank (http://www.rcsb.org/pdb/), under the accession code 4U0J.[30] Before the molecular docking, the geometry of initial structures has been built and optimized by using the Gaussian 09 program. All these structures were calculated by using Density Functional Theory (DFT) at the B3LYP (Becke, three-parameter, Lee-Yang-Parr)/6-31G(d,p) level. [52,53]

For the docking studies, MOE[54] software was used to estimate free energies of the enzyme-ligand binding. The enzyme-ligand complexes were minimized up to a gradient of 0.01 kcal/(mol Å), and hydrogens were added using the force field AMBER99. Charges on the protein were assigned using the force field AMBER99, while the charges on the ligands were assigned by using force field MMF94X. The docked poses for the compounds under

study were scored using London ΔG scoring function for finding the best docking pose.

Results

Analysis of pharmacophores and anti-pharmacophores

The ETM application made it possible to reveal in the two groups both molecular fragments characterizing the presence of activity (pharmacophores) and fragments inhibiting the activity (anti-pharmacophores). A pharmacophore (Ph) is represented by a submatrix of an ETMC, which isomorphically enters the structures of all active compounds. Its realization in this class can be assessed by a P_a parameter, which characterizes effectiveness of the pharmacophore. The closer its value to the unit, the more guaranty that a compound belongs to the class of active molecules. Analogous estimates can be calculated for the class of low-active compounds when searching for the anti-pharmacophoric molecular fragments.

In the matrix comparison stage (testing whether atoms and bonds match) the optimum values of variations allowed were found as $\Delta_1 = \pm 0.07$ for diagonal elements (q_i) and $\Delta_2 = \pm 0.20$ for off-diagonal values (W_{ij} and R_{ij}). The lowest level of probabilistic estimates, P_a, was taken as 0.80 to determine the most informative activity features. To form the basis of a model for the antitubercular activity prediction, compound **57** possessing the highest activity was taken first of all as a template for the comparison. In Fig. 3a, a submatrix of this template ETMC (ETSC) is given, which corresponds to one of pharmacophores revealed (Ph1).

As seen, Ph1 consists of negatively charged atoms attached to the A and D rings. Their charges are changing in the range between -0.01 ē and -0.20 ē, and maximum distance between the atoms belonging to the fragment is 10.36 Å (N2.....O20) (Figure 3a). The other pharmacophore, Ph2, includes 7 atoms that are negatively charged as well. Maximal value of distance equals 11.15 Å (C2.....O22). Ph2 (template compound **8**) is characterized by the presence of atoms belonging to the cycles A, B and by the oxygen atom of the $-CO_2Et$ group, attached to the cycle D (Figure 3b). The probabilities of Ph1 and Ph2 realization, *i.e.* P_a values, are 0.94 and 0.92, respectively.

Analysis of the APh1 and APh2 anti-pharmacophores (corresponding to template compounds **20** and **25**) has shown that they differ from Ph1 and Ph2 in the part of charge distribution on the atoms and by their spatial topology as well (Figure 4).

While the class of active compounds is characterized by the presence of a nitrogen in the cycle A, then the class of low-active compounds is represented by the phenyl cycle with varying substituents.

In Table 2, some of the pharmacophores (Phi) and anti-pharmacophores (APhi) are given, which have been found for different template molecules. All data are given both for training set and for the test set of molecules. As seen from Table **2**, the values of P_a vary in the limits of 0.87–0.94 and 0.82–0.90 for pharmacophores and anti-pharmacophores, respectively.

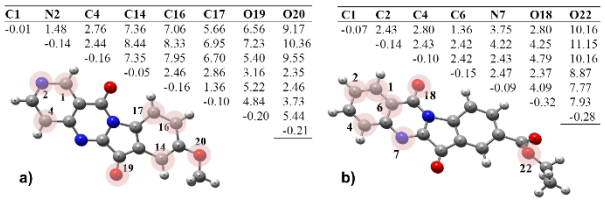


Figure 3. Two pharmacophores, Ph1 and Ph2, found relative to corresponding active templates **57** (**a**) and **8** (**b**)

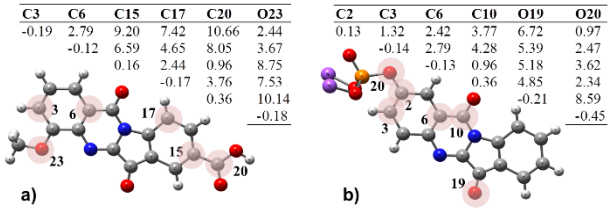


Figure 4. Two anti-pharmacophores, APh1 and APh2, found relative to corresponding inactive templates **20** (**a**) and **25** (**b**)

Table 2. Statistical characteristics for some of pharmacophores (Ph_i) and anti-pharmacophores (APh_i) calculated by ETM

Ph_i (template compound)	P_a	P_{ia}	APh_i (template compound)	P_a	P_{ia}
Ph1 (57)	0.94	0.06	APh1 (20)	0.10	0.90
Ph2 (8)	0.92	0.08	APh2 (25)	0.11	0.89
Ph3 (5)	0.90	0.10	APh3 (12)	0.14	0.86
Ph4 (44)	0.88	0.12	APh4 (23)	0.16	0.84
Ph5 (54)	0.87	0.13	APh5 (29)	0.18	0.82
Average	0.89	0.11	Average	0.15	0.85

Application of ETM-NN Approach

The first stage of the ETM-NN data analysis was to find a cluster distribution model capable of reflecting the realistic internal structure of the data; and its results are given in Table 3.

For the training set, 54 clusters were found. ASNNs recognized correctly 92% (58 from 63 compounds), while for the test set the predictive ability was a bit lower, namely, 88% (15 compounds from 17). For the summary set, the result was 90% (73 compounds from 80). These results tell in favor of high quality of the cluster distribution model and its fitness for the analysis of new data sets, *i.e.* for the search for pharmacophores.

At the second stage, only 25 fragments were selected for the training and test sets (Table 4). On the base of the weights calculated for the molecular fragments represented by ETSCs, ASNN were capable of recognizing 95% (60 compounds from 63) in the training set, and 88% (15 compounds from 17) in the test set. In total, the network classified correctly 94%, or 75 compounds from 80.

Table 3. Cross-validated q^2 coefficients calculated for Tryptanthrin analogues.

All pharmacophores			
Data set	WD_s* number	Molecule	
		Amount	Predicted (P_a)
Training set	54	63	58 (0.92)
Test set	54	17	14 (0.86)
Total	54	80	72 (0.90)

* WDs: weight descriptors

Table 4. Cross-validated q^2 coefficients calculated for Tryptanthrin analogues on the base of fragment data set.

		Param. number	Molecule	
			Amount	Predicted (%)
All Ph*	Training set	25	63	60 (95)
	Test set	25	17	15 (88)
	Total	25	80	75 (94)
Ph selected by pruning methods	Training set	20	63	59 (94)
	Test set	20	17	15 (88)
	Total	20	80	74 (93)

*Ph: Pharmacophores

The pruning methods application afforded to select only 20 of them as the most influential fragments. By this, ASNN classified correctly 94%, that is 59 compounds from 63 in the training set, and 88%, or 15 compounds from 17, in the test set. In the summary set, ASNNs classified correctly 93% (74 compounds from 80).

In our case, comparison of two models (one model based on the cluster distribution found in a straightforward manner, and another based on training the network by the ETMCs for fragments) tells in favor of close correspondence between their results. However, the first model is not stable enough and depends severely on the structures of compounds selected for the training set. However, in comparison with other commonly used approaches, the approach presented in this study has shown quite satisfactory results, which tell about workability of both models found, and both can be applied for the design of new potent antitubercular drugs.

Based on the pharmacophores and anti-pharmacophores found, the model for the antitubercular activity prediction had been successfully applied to the activity estimation in a series of Tryptanthrin analogues. However, molecular docking and frontier molecular orbital (highest occupied molecular orbital, HOMO and lowest unoccupied molecular orbital, LUMO) analyses were performed for these compounds to understand the mechanism of their actions in detail.

Molecular docking results

In the framework of the ETM-NN study, docking of template compounds into the active site of InhA has been carried out. The best docking poses of active compound **57** and inactive compound **20** in the active site of InhA were represented in Figure 5.

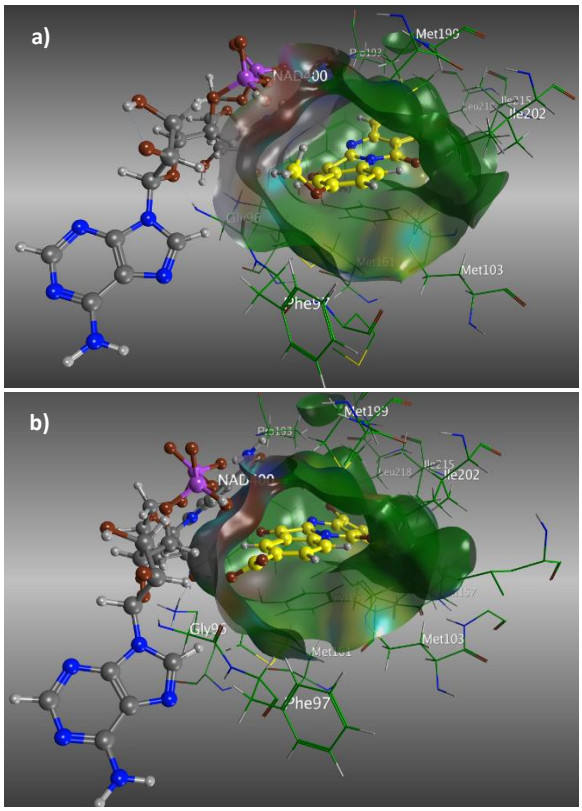


Figure 5. 3D representation of docked poses of compounds **57** (**a**) and **20** (**b**) in the active site of InhA (PDB code: 4U0J)

Close interaction with such amino acids as Tyr158, Phe149, Met161, Ile215, Gly96, and Met199 is observed in the case of template compound **57**. As to low-active compound **20**, amino acids Gly96, Tyr158, Phe149, and Met199 are involved into the interaction. It should be stressed that there exists a noticeable difference in the energy of binding with receptor in the two cases. For the active compound **57** it is -15.5 kcal mol^{-1}, for the low-active **20** it equals to -7.3 kcal mol^{-1}. Analogous situation is observed for the other pair of template compounds, **8** and **25**. The energy of binding with receptor is considerably higher for the high-active molecule **8** than for the low-active molecule **25** (-16.1 and -10.8 kcal mol^{-1}, respectively).

Frontier Molecular Orbital (FMO) analyses

The analysis of specificity of the enzyme-ligand interaction is closely related to the analysis of frontier orbitals (HOMO and LUMO) in molecular systems. The electron density distribution on the frontier orbitals of the enzyme-ligand complexes under study tells about donor-acceptor character of the interaction inside the complexes. The electronic structure computations were carried out with the Gaussian 09 using DFT at the B3LYP/6-31G(d,p) level. 3D structures of ligand and active site of enzyme were taken from the molecular docking.

When active compounds bind with InhA, atoms of amino acid residues and atoms of ligands form the frontier orbitals of the formers. For the low-active compounds, the character of the electron density distribution is a bit

different. As an example, Fig. **6** shows the electron density distribution on the frontier orbitals formed by the active sites of InhA with active compound **57** (Figure **6a** and **6b**) and low active compound **20** (Figure **6c** and **6d**). It is worth to be noted that the electron density distribution on the frontier orbitals of InhA-**57** complex is such that donor-acceptor interaction is specific for the HOMO, while low acceptor interaction is characteristic of the LUMO.

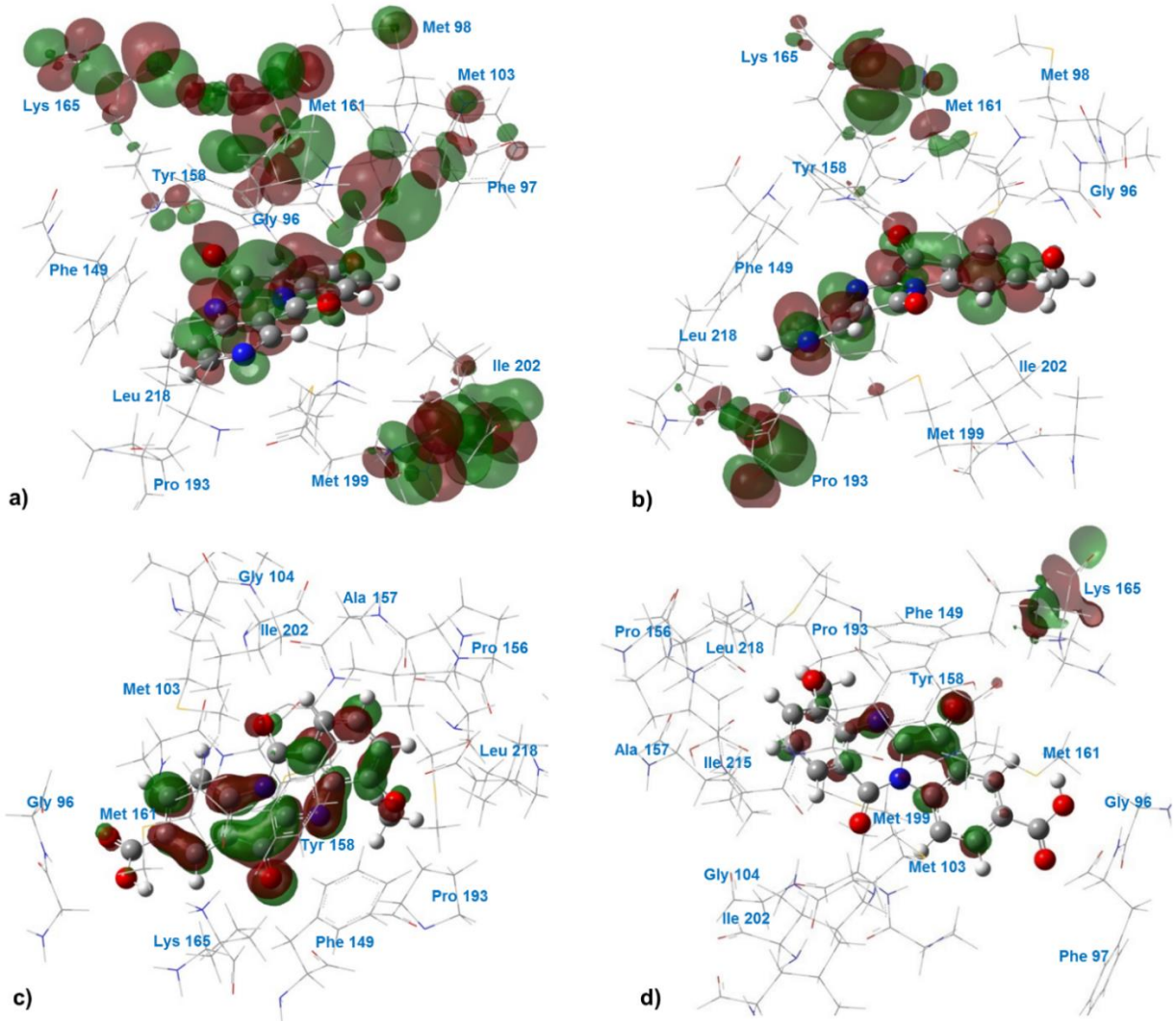


Figure 6. 3D view of HOMO (**a, c**) and LUMO (**b, d**) for the active site of InhA with active compound **57** (**a, b**) and inactive compound **20** (**c, d**)

In addition, HOMO is formed by the atoms of both **57** and amino acids of the InhA active site (Lys165, Met161, Gly96, Phe97, Ile202, Met199, Leu218) (Figure 6a). As to the LUMO, the electron density is concentrated on the atoms of ligand and those of amino acids Met161 and Pro193 (Figure 6b).

For the template low-active compound **20** and InhA complex, electron density is distributed mainly on the atoms of the ligand (Figure 6c and 6d). Moreover, the role of frontier orbitals in the ligand-receptor interaction is not significant. The analysis of the electron distribution in two groups of compounds (active and low active ones) shows that it is the frontier orbitals' nature in the ligand-receptor complexes within active site of InhA that plays very important role for the antitubercular activity prediction in the series of tryptanthrin analogues. Both groups enter the system of the activity prediction.

Conclusion

A series of Tryptanthrin analogues were studied with the aim of finding peculiarities of conformational and electronic structures of compounds. The researches were carried out for structurally diverse set of 80 molecules. The results of the ETM-NN application to the series of compounds in view are pharmacophoric and anti-pharmacophoric molecular fragments, which are characteristic of the class of compounds demonstrating activity against *Mtb* H37Rv. The statistical characteristics of five pharmacophores (Ph_i) and five anti-pharmacophores (APh_i) entering the forecasting system, are 0.90 and 0.86, respectively. The fragments selected serve as a base for the further prognosis and design of antitubercular molecules.

Analysis of the molecular docking and electron density distribution has shown that the more effective binding

with receptor was observed for active compounds. A model of prognosis for the antitubercular activity of *Mtb* H37Rv was developed on the base of the pharmacophores found, docking results, and electronic structure computations. This prognosis model allows for carrying out screening and design of new potent antitubercular drugs.

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Ethical Approval
No ethics committee decision is required for the study.

Financial Disclosure
None.

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Research Article | Araştırma Makalesi

DOES ASPARTATE AMINOTRANSFERASE PLATELET RATIO INDEX PREDICT GESTATIONAL DIABETES MELLITUS? A RETROSPECTIVE COHORT STUDY

ASPARTAT AMİNOTRANSFERAZ TROMBOSİT ORANI İNDEKSİ GESTASYONEL DIABETES MELLITUS ÖNGÖRÜR MÜ? RETROSPEKTİF BİR KOHORT ÇALIŞMASI

Mujde Can Ibanoglu[1*], Seval Yilmaz Ergani[1], Busra Sahin[1], Hande Nur Oncu[1], Cantekin Iskender[1], Yaprak Engin Ustun[1]

[1]Department of Obstetrics, Ankara Etlik Zubeyde Hanım Women's Health Training and Research Hospital, Ankara, Türkiye.

ABSTRACT

Objective: Our aim was to determine the significance of aspartate aminotransferase platelet ratio index (APRI), neutrophil to lymphocyte ratio (NLR), and platelet to lymphocyte ratio (PLR) in predicting gestational diabetes mellitus (GDM) at first trimester screening in a retrospective study of pregnant women.

Method: In this study, a retrospective analysis of pregnant women (n=216) screened at the antenatal clinic and diagnosed with gestational diabetes between 24-28 weeks of gestation was performed. APRI score, NLR and PLR were calculated in the blood of these pregnant women in the first trimester and compared with the results of patients in the control group (n=250).

Results: The ROC -analysis for APRI yielded an AUC value of 0.489 (p=0.684). Maternal age was found to be an independent risk factor for GDM. The risk increased 1.162-fold with increasing maternal age (p<0.001). The optimal cutoff value for NLR was 3.55, sensitivity was 65%, specificity was 49%, and the area under the ROC curve was 0.544.

Conclusion: In the results we compared with those of the control group, we found no significant change in APRI value and PLR. However, we found that NLR has a predictive value for GDM.

Keywords: Gestational diabetes mellitus, aspartate aminotransferase platelet ratio index, liver fibrosis, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio.

ÖZ

Amaç: Bu retrospektif çalışmada amacımız, aspartat aminotransferaz trombosit oran indeksi (APRI), nötrofil lenfosit oranı (NLR) ve trombosit lenfosit oranının (PLR) birinci trimester taramasında gestasyonel diyabeti (GDM) öngörmedeki önemini belirlemekti.

Yöntem: Bu çalışmada antenatal polikliniğinde taranan ve 24-28. gebelik haftaları arasında gestasyonel diyabet tanısı alan gebelerin (n=216) retrospektif analizi yapılmıştır. Bu gebelerin ilk trimesterdeki kanlarında APRI skoru, NLR ve PLR hesaplandı ve kontrol grubundaki hastaların (n=250) sonuçları ile karşılaştırıldı.

Bulgular: APRI için ROC analizi, 0,489'luk bir AUC değeri verdi (p=0,684). Anne yaşı GDM için bağımsız bir risk faktörü olarak bulundu. Artan anne yaşı ile risk 1,162 kat arttı (p< 0,001). NLR için optimal kesme değeri 3,55, duyarlılık %65, özgüllük %49 ve ROC eğrisinin altındaki alan 0,544 idi.

Sonuç: Kontrol grubu ile karşılaştırdığımız sonuçlar da APRI değeri ve PLR'de anlamlı bir değişiklik bulamadık. Ancak NLR'nin GDM için prediktif bir değere sahip olduğunu bulduk.

Anahtar Kelimeler: Gestasyonel diabetes mellitus, aspartat aminotransferaz trombosit oranı indeksi, karaciğer fibrozisi, nötrofil-lenfosit oranı, trombosit-lenfosit oranı.

***Corresponding author/İletişim kurulacak yazar:** Mujde Can Ibanoglu; Etlik Zubeyde Hanim Women's Health Training and Research Hospital, 06010, Ankara, Türkiye
Phone/Telefon: +90 532 308 94 88 **e-mail/e-posta:** drmujdecan@gmail.com
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Introduction

Obesity and its consequences are an important global health problem, and women of childbearing age also represent an important population.[1] Gestational diabetes mellitus (GDM) and macrosomia during pregnancy are known to be associated with subsequent obesity and adverse pregnancy outcomes.[2,3] GDM develops due to a metabolic disorder in women with normal carbohydrate metabolism before pregnancy.[3] It is known that when GDM develops during pregnancy, in addition to insulin resistance, secretion of some diabetogenic placental hormones and pancreatic dysfunction increase accordingly.[4] Some inflammatory markers known to support this process have already been studied and shown to be effective in this pathogenesis (e.g. visfatin).[4] GDM is a clinical condition that must be correctly diagnosed and monitored because it causes polyhydramnios, increased cesarean section rates, maternal complications such as preeclampsia, and other complications such as macrosomia, birth trauma, and intrauterine growth retardation.[5] The 75-g glucose test recommended by the American Diabetes Association (ADA) and the 50-g glucose test recommended by the American College of Obstetricians and Gynecologists (ACOG) are now standard.[6] The sensitivity of the 50-g glucose test, which is widely used in the diagnosis of GDM, ranges from 60% to 80%.[7] This test is used in pregnant women between 24 and 28 weeks of gestation, and in the next phase, the 100-g oral glucose tolerance test (OGTT) is used in patients whose blood glucose is 140 mg/dl or more in the first hour after sugar loading without fasting.[8,9]

The aspartate aminotransferase platelet ratio index (APRI), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) are inflammatory markers used in many studies and in various fields.[10-15] They have recently gained popularity because they can be easily calculated from routine blood values and are inexpensive.[10] NLR and PLR have been used as prognostic markers in metastatic cancer, inflammatory bowel disease, and ischemic heart disease.[16] The same markers have also been used as screening tests for complications of diabetes mellitus (DM).[17] To determine the systemic inflammatory response, hematologic parameters such as NLR and PLR from blood counts have been useful to determine the presence and severity of disease in cardiovascular, oncologic, and metabolic disorders. NLR and PLR have been shown to be predictors of mortality, particularly in patients with coronary artery disease and acute coronary syndromes. Recent studies have also shown that these markers can predict pregnancy-related complications such as preeclampsia.[11] In studies performed to date, APRI has predictive value for conditions such as PE, HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count), and cholestasis, in which liver function is impaired.[14,15] These inflammatory markers may be indirect markers for hepatic and pancreatic insufficiency, in which gluconeogenesis occurs.

In view of this information, these markers were not studied together in the GDM group. Our objective was to determine the value of APRI, NLR, and PLR values in predicting first-trimester screening GDM in a retrospective study of pregnant women diagnosed with gestational diabetes mellitus.

Methods

The study was approved by the board of the Health Sciences University Etlik Zubeyde Hanım Women's Health Education and Research Hospital on July 26, 2022, as number 09. Because it was a study with human participants, it complied with the principles of the Declaration of Helsinki.

In this study, participants admitted to obstetric department between 2015 and 2020. The study group included 216 patients with gestational age between 24-28 weeks and GDM as a result of glucose tolerance test. Venous plasma cutoff values for the 75 g OGTT were accepted as fasting ≥ 95 mg/dl, 1st hour ≥ 180 mg/dl, 2nd hour ≥155 mg/dl. Patients with a high value of 1 as a result of the test were diagnosed with GDM. According to Carpenter and Coustan (C&C) criteria, the cut-off values in venous plasma for the 100-g-OGTT fasting were ≥ 95 mg/dl; 1 hour ≥ 180 mg/dl; 2nd hour ≥ 155 mg/dl; 3rd hour was accepted as ≥ 140 mg/dl.18 GDM is diagnosed when there are two or more cut-off values for the 100-g-OGTT. One patient with a value above 200 was diagnosed with GDM. These patients were retrospectively analyzed, and the APRI score, NLR, and PLR were calculated using first-trimester screening blood values. There were no problems in pregnancy in 250 patients, and these patients formed the control group. The control group was selected from patients who presented to the outpatient clinic during the first two days of the month and met the inclusion criteria during the same period. Inclusion criteria were age between 18 and 40 years and singleton pregnancy. Exclusion criteria were multiple pregnancy, fetal anomalies, chronic diseases (diabetes, thyroid dysfunction, uncontrolled endocrine diseases or renal dysfunction, autoimmune diseases, chronic inflammatory diseases), intrauterine exitus, placental anomalies, pregnancy by assisted reproductive technology, active smokers.

Accordingly, 216 patients were included in the study group. The total number of patients was 466, and data were obtained from the patients' computerized medical records, which are continuously updated in real time during delivery and throughout the hospital stay. Data included various demographic, general medical, obstetric, and neonatal variables. In addition, patient data such as age, weeks of gestation, birth weight, diagnosis, operative time, transfusions, and results of swab specimen cultures were obtained from medical records.

Venous blood samples from patients were collected into tubes containing ethylenediaminetetraacetic acid (EDTA) after admission. Blood samples were centrifuged at 1500

g/min for 10 minutes and analyzed using the automated hematology instrument (Mindray BC-6000) available in our laboratory.

The Statistical Package (SPSS 23.0, Inc. Chicago, IL, USA) was used to analyze the data. Receiver operating characteristic curve analysis (ROC) was used to determine the optimal cut-off value for the independent markers, and sensitivity and specificity were calculated. Data were expressed as arithmetic mean, standard deviation, median, and minimum-maximum values. For normally distributed data, the independent-samples t test was used, and for nonnormally distributed variables, the Mann Whitney U test was used. For analysis of categorical variables, the chi-square test or the Fisher extract test was used.

In the study, a p value of less than 0.05 was considered statistically significant.

Results

In the study, 216 patients with gestational diabetes mellitus and 250 pregnant women in the control group were observed. The demographic characteristics of these patients are shown in Table 1. The mean age of the patients included in the study was 30.07± 5.54 years, and the body mass index was 30.93±3.44 kg/m2. The NLR value was higher in the study group (3.65±1.17) than in the control group (p=0.001). However, this difference could not be statistically demonstrated for APRI and PLR values.

Table 1. Clinical characteristics of patients and control group

	Control Group (n: 250) mean±SD	Study Population (n: 216) mean±SD	p
Age (years)	27.93±5.80	32.22±5.28	**<0.001**
BMI (kg/m²)	31.08±3.62	30.78±3.27	0.335
Birth weeks (weeks)	38.3±0.09	39.2±0.07	**<0.001**
Birth weight (gram)	3291.25±33.25	3251.17±30.15	0.117
White Blood cell (cells/mL)	8362.32±2158.40	8375.80±2149.78	0.946
Platelet (cells/µL)	258.444±56.61	266.580±64.28	0.147
APRI	0.168±0.108	0.170±0.176	0.923
PLR	161.54±61.30	160.62±63.11	0.873
NLR	3.30±1.05	3.65±1.17	**0.001**

SD: Standard deviation, BMI: Body mass index, APRI: Aspartate aminotransferase platelet ratio index, PLR: Platelet-to-lymphocyte ratio, NLR: Neutrophil to lymphocyte ratio

In the ROC analysis for APRI, the AUC value was 0.489 (p=0.684). In univariate analysis, only maternal age was found to be an independent risk factor. Increasing maternal age increased the risk of a positive diagnosis by 1.144-fold (p<0.001). APRI and BMI were not found to be independent risk factors in multivariate analysis (p values 0.484 and 0.609, respectively). The risk increases 1.162-fold with increasing maternal age (p<0.001), (Table 2). The optimal cutoff value for NLR was 3.55, sensitivity 65%, specificity 49%, and area under the receiver operating characteristic curve 0.544 (Table 3, Figure 1).

Table 2. Multiple logistic regression analysis between the control and study groups

			Univariate		Multivariate	
	Control (n=250)	Study (n=217)	OR (95% CI)	p	OR (95% CI)	p
APRI	0.169±0.108	0.17±0.177	1.064 (0.301-3.761)	0.923	1.62 (0.419-6.261)	0.484
BMI	31.08±3.629	30.78±3.275	0.975 (0.925-1.028)	0.350	0.985 (0.93-1.044)	0.609
Maternal Age	27.93±5.801	32.22±5.289	1.144 (1.104-1.186)	**<0.001**	1.162 (1.114-1.212)	**<0.001**

Table 3. ROC result between control and GDM groups

	Cut-off	AUC (95% CI)	Sensitivity	Specificity	p
NLR	3.55	0.544 (0.512-0.575)	65	49	**0.007***

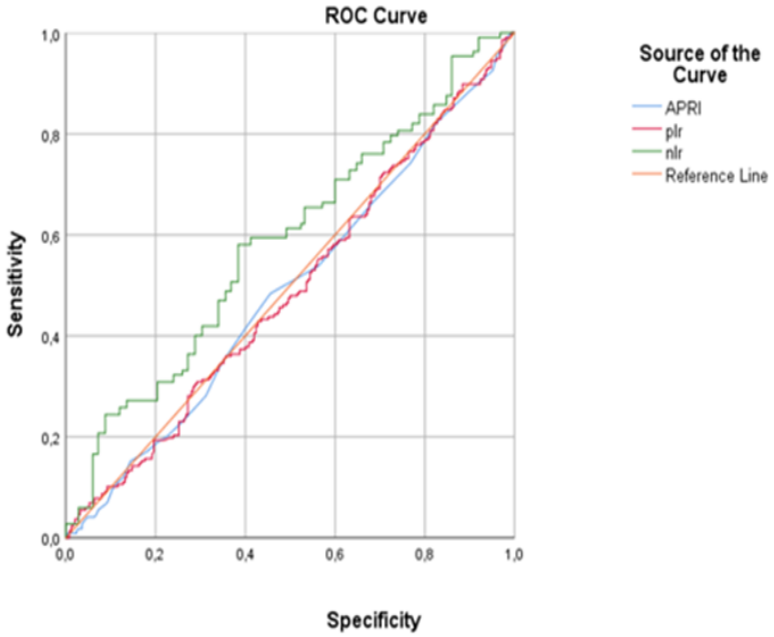

Figure 1. ROC analysis for APRI, PLR, NLR in GDM

Discussion

In this study, we retrospectively evaluated patients diagnosed with GDM in our hospital and investigated whether the APRI score, PLR score, and NLR score predict this condition in the context of first-trimester screening. In the results we compared with the control group, we found no significant change for the APRI score and the PLR score. However, we found that the NLR score measured in the first weeks was predictive of future GDM.

Complete blood count, renal and liver function tests: these are routine, readily available, automated and inexpensive tests. When used for early diagnosis, prediction, or prognostic monitoring of disease, these parameters are of great importance to clinicians because they allow easy access and use of the test. During the literature search on PLR and NLR, it was found that there are many studies and many different results. Adipose tissue plays an important role in glucose and lipid metabolism.[19] The proliferation of this tissue triggers inflammatory cytokines that lead to obesity (DM, PE).[19] In one study, it was found that the value tested in the first trimester that is important for predicting GDM is the leukocyte count.[20] In another study, a leukocyte count above 15,000 in the second trimester blood count was found to be important in predicting GDM.[21] In a study that examined PE, it was found that NLR was higher and PLR was lower in blood parameters measured in the last trimester than in the control group.[22] One study highlighted that NLR and PLR parameters were not sufficient to predict preeclampsia.[23] In the study conducted by Sargın et al, NLR and PLR were found to be inadequate for predicting GDM.[24] In our study, NLR was found to be the only independent variable suitable for predicting GDM in blood parameters measured in the first trimester.

Literature search did not find any study that investigated the association between gestational diabetes and APRI. However, the APRI score is considered a promising parameter, especially in pregnancy-related conditions such as HEELP syndrome and cholestasis, in which liver functions are impaired.[25] In a 2020 retrospective study by Şaşmaz et al, a multivariate regression analysis between study groups with HELLP syndrome (n=40) and normotensive patients (n=124) concluded that APRI score predicted HELLP syndrome better than AST alone.[15] In the ROC curve, the sensitivity of AST was 71.1% and the specificity was 91.2% to discriminate HELLP patients from the control group.[15] When the cut-off value of APRI score in the study was set at 0.339, the sensitivity was 82.6% and specificity was 87.6%. It is suggested that there is an association between diabetes mellitus and liver dysfunction. However, liver biopsy, an invasive procedure, is the method that best detects deterioration of liver function in the general population.[26] Therefore, there is great interest in introducing approved noninvasive fibrosis markers into clinical practice. A population-based study that demonstrated the association between type 2 DM and liver fibrosis showed a weak correlation with APRI.[27] However, there is a stronger association between overweight and obese diabetics and APRI.[27] On the other hand, many complications due to liver dysfunction occur in macrosomic infants who develop due to GDM. For this reason, the association between maternal APRI blood level at the beginning of pregnancy and macrosomia might be stronger. However, in our study, this difference between the study group and the control group could not be clearly demonstrated because treatment for GDM was started at the time of diagnosis.

The main limitation of this study is the small sample size and retrospective design. In addition, the sample size had to be kept smaller in this study because we had to use the pre-pandemic values COVID -19 and it was not known

whether the pandemic would affect the complete blood count and liver function parameters. Now that the effects of the pandemic are diminishing, it is appropriate to conduct prospective studies on this topic.

Conclusion

This study explores the association between APRI and GDM. Although the association has not been fully proven, more extensive studies are needed to examine the complications associated with GDM. APRI score, thanks to its ability to detect early; it is considered a promising marker because it can reduce mortality and morbidity in pregnant women. NLR score is a parameter that can be used to predict GDM, according to the literature. Our findings pave the way for randomized, controlled, prospective studies with larger numbers of cases.

Compliance with Ethical Standards

This study followed the Declaration of Helsinki on human-subject research. The present study was approved by the Ethics Committee for Ankara Etlik Zubeyde Hanim Women's Health Training and Research Hospital on July, 2022, with approval number 09.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contribution

MCI, HNO, CI: Study idea, hypothesis, study design; BS: Material preparation, data collection and analysis; MCI: Writing the first draft of the article; YEU: Critical review of the article finalization and publication process.

Financial Disclosure

None.

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Research Article / Araştırma Makalesi

THE EFFECTS OF LONG-ACTING METHYLPHENIDATE FORMULATIONS AND ATOMOXETINE ON CARDIAC FUNCTIONS IN CHILDREN WITH ATTENTION DEFICIT AND HYPERACTIVITY

DİKKAT EKSİKLİĞİ VE HİPERAKTİVİTE BOZUKLUĞU OLAN ÇOCUKLARDA UZUN ETKİLİ METİLFENİDAT BİLEŞİKLERİNİN VE ATOMOKSETİNİN KARDİYAK FONKSİYONLAR ÜZERİNDEKİ ETKİLERİ

Ajda Mutlu Mıhçıoğlu[1*], Tayfun Kara[2]

[1]University of Health Sciences, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Department of Pediatrics, Division of Pediatric Cardiology, Istanbul, Türkiye. [2]Alanya Alaaddin Keykubat University, Faculty of Medicine, Department of Child and Adolescent Psychiatry, Antalya,Türkiye.

ABSTRACT

Objective: Methylphenidate (MPH) and atomoxetine (ATX) are most commonly prescribed for the treatment of attention deficit hyperactivity disorder (ADHD). In the literature, there are studies performed to evaluate each of these drugs, but there is no study comparing these three medications together. Therefore, we aimed to evaluate the cardiac effects of these medications.

Methods: Forty-six children with ADHD using osmotic-release oral system MPH (OROS-MPH), 46 using extended-release MPH (ER-MPH), and 46 using ATX for at least six months were included in the patient groups. Forty-six children with normal cardiac findings were enrolled as the control group. All participants were evaluated using a sociodemographic information form, 12-channel electrocardiography (ECG), transthoracic 2D, Doppler and tissue Doppler echocardiography.

Results: Statistically significant differences were observed in terms of A wave velocity, E/A and E' septal values between the groups (p=0.002, p<0.001, p=0.002, respectively). Children using ATX had higher A wave velocities than the OROS-MPH users and controls (p<0.001 and p=0.007, respectively) and lower E/A values than the OROS-MPH users and controls (p<0.001 for both). Children using ER-MPH and ATX had lower E' septal values compared to the control group (p<0.001 and p=0.002, respectively).

Conclusion: Atomoxetine treatment showed impairment in some of the myocardial relaxation parameters more than long acting-MPH medications. In conclusion, although the drugs used in the treatment of ADHD are cardiac safe in general and do not cause clinical findings of heart failure, patients should be evaluated for cardiac involvement. Further studies are needed to support the findings in our study.

Anahtar Kelimeler: Methylphenidate, Atomoxetine, children, tissue Doppler imaging, attention deficit and hyperactivity disorder.

ÖZ

Amaç: Dikkat Eksikliği ve Hiperaktivite Bozukluğunun (DEHB) tedavisinde metilfenidat (MPH) ve atomoksetin (ATX) en yaygın olarak reçete edilen ilaçlardır. Literatürde bu ilaçların kalp üzerine etkilerini tek tek karşılaştıran çalışmalar olmakla bu üç ilacı birlikte karşılaştıran çalışmalar yoktur. Dolayısıyla bu çalışmada DEHB tedavisinde kullanılan bu ilaçların kalp üzerine etkilerini karşılaştırmayı amaçladık.

Yöntem: Hasta gruplarına en az 6 aydır ozmotik-salınımlı oral sistem metilfenidat (OROS-MPH) kullanan 46 hasta, geç-salınımlı metilfenidat (ER-MPH) kullanan 46 hasta ve ATX kullanan 46 DEHB tanılı çocuk dahil edildi. Normal kardiyak bulguları olan 46 çocuk kontrol grubu olarak alındı. Tüm katılımcılar sosyodemografik bilgi formu, 12 kanallı elektrokardiyografi (EKG), transtorasik 2D, Doppler ve doku Doppler ekokardiyografi kullanılarak değerlendirildi.

Bulgular: Gruplar arasında A dalga hızı, E/A ve E' septal parametrelerinde istatistiksel olarak anlamlı fark izlendi (p=0,002, p<0,001, p=0,002, sırasıyla). Atomoksetin kullanan çocuklarda, OROS-MPH kullananlara ve kontrollere göre daha yüksek A dalga hızı (p<0,001, p=0,007, sırasıyla) ve OROS-MPH kullananlara ve kontrollere göre daha düşük E/A (p<0,001, her ikisi için) saptandı. Geç-salınımlı metilfenidat ve ATX kullanan çocukların E' septal değerleri kontrol grubuna göre daha düşüktü (p<0,001, p=0,002, sırasıyla).

Sonuç: Atomoksetin tedavisi, bazı miyokardiyal relaksasyon parametrelerinde uzun etkili MPH tedavilerine göre daha fazla bozulmaya sebep olmuştur. Sonuç olarak, DEHB tedavisinde kullanılan ilaçlar genel olarak kardiyak açıdan güvenli olsalar ve klinik olarak kalp yetersizliğine sebep olacak sonuçlar doğurmasalar da hastalar kardiyak açıdan değerlendirilmelidirler. Çalışmamızdaki sonuçları desteklemek için daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Metilfenidat, Atomoksetin, çocuklar, doku doppler görüntüleme, dikkat eksikliği ve hiperaktivite bozukluğu

İletişim kurulacak yazar/Correspondingauthor: Ajda Mutlu Mıhçıoğlu;[1] University of Health Sciences, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Department of Pediatrics, Division of Pediatric Cardiology, Istanbul, Türkiye.
Telefon/Phone: +90 (212) 414 71 71 (5031) +90 (505) 648 64 68 **e-posta/e-mail:** ajdamutlu@yahoo.com
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Introduction

Attention deficit and hyperactivity disorder (ADHD) is a psychiatric condition presenting with hyperactivity, inattention and impulsivity.[1] It affects 5-7% of school-age children.[2] The prevalence is three times higher in boys than in girls.[1] Stimulant and non-stimulant drugs are used for treatment.[2] One of the best known stimulant medications is methylphenidate (MPH), while non-stimulant medication therapy is provided with atomoxetine (ATX). There are different forms of MPH, such as immediate-release (IR-MPH), extended-release (ER-MPH), and osmotic-release oral system methylphenidate (OROS-MPH).[3]

Treatment with psychostimulant drugs is usually the option of choice, with once-daily doses of OROS-MPH and ER-MPH producing higher adherence to therapy than IR-MPH, which is administered three times a day. Atomoxetine involves similar effects and dosages to those of long-acting forms of MPH.[4] Osmotic release-MPH (Concerta®) (Janssen-Cilag Ltd, High Wycombe, UK) uses the osmotic controlled-release delivery system³. It dissolves within 1 to 2 hours and releases 22% of the total dose of MPH. The remaining 78% of the dose is osmotically controlled (osmotic-release oral system technology) and is released over 10 hours. The maximum effect occurs 6 - 8 hours after dosing.[5] Extended release-MPH (Medikinet® retard, MEDICEPharma GmbH and Co., KG, Iserlohn, Germany) provides 50% of the racemic dose immediately, the remaining 50% being released over 12 hours.[6] The maximum effect occurs 8 hours after dosing.[7] Atomoxetine (Strattera®, The Netherlands) is one of the non-stimulant drugs and can be administered once or twice-daily.[8]

Methylphenidate exhibits its effects by blocking dopamine and noradrenaline transporters, thus increasing the concentrations of dopamine and noradrenaline in the presynaptic region.[1,2] Atomoxetine is a selective inhibitor of noradrenaline transporters that regulates noradrenaline transmission by inhibiting the reuptake of noradrenaline into presynaptic nerve terminals. This in turn causes the effects of sympathomimetic amines such as noradrenaline and dopamine to appear in the body.[8]

Increased sympathomimetic impacts are expected to be observed in different systems of the body.[3] In general, heart rate (HR), blood pressure (BP) and, electrocardiographic findings have previously been investigated. There are also case reports showing that these psychostimulant drugs affect cardiac functions and precipitate myocardial infarction.[6,9,10,11] Several reports have also suggested that increasing sympathetic activity or low parasympathetic activity exacerbate the risk of sudden cardiac death, independently of other risk factors.[12] A study evaluating the cardiac safety of these drugs showed that methylphenidate usage revealed no significant association with either cardiovascular events like angina, dysrhythmia or transient cerebral ischemia.[13] They stated that clinical diagnoses of cardiovascular events and symptoms were rare and not associated with stimulant use.[13] Another study was performed in children who use MPH to investigate cardiovascular functions by echocardiography. They revealed that there was no clinical differences between the children using MPH and controls declaring that MPH usage does not impair cardiovascular functions at short-term follow-up.[14] But there has been no pediatric studies evaluating and comparing the effects of the these three widely used medications together on diastolic and systolic cardiac functions. Therefore this study was performed to investigate the effects of MPH formulations and ATX on cardiac functions in pediatric patients with ADHD.

Methods

Study design

Forty-six children with ADHD using OROS-MPH (Concerta®), 46 using ER-MPH (Medikinet® retard), and 46 using ATX (Strattera®) were included in the study. Additionally, 46 healthy children without any disease were included as the control group. All the patients with ADHD had been using their medications for at least six months. The patient groups and control group were matched in terms of weight, age, and gender parameters. None of the patients enrolled in the study experienced any significant adverse events requiring drug discontinuation. All participants were evaluated using a sociodemographic information form prepared by experienced researchers. Patients with any known systemic disease or using drugs other than MPH or ATX were excluded from the study. Children were also excluded in case of a contraindication to any of the medications, or if another drug combination was required. Individuals capable of completing the questionnaires and with no sensorial deficit capable of obstructing communication with the psychiatrist were included in the patient group. They were interviewed about cardiovascular adverse events such as chest pain, palpitation, and syncope and non-cardiac adverse events such as nausea, abdominal pain, decreased appetite, and headache. All procedures were performed in accordance with the ethical standards of the relevant institutional research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent forms were obtained from all patients and their parents. All the groups in the study were subsequently evaluated by a pediatric cardiologist by means of physical examination and electrocardiographic and echocardiographic findings. Echocardiographic evaluation was performed using conventional, color Doppler and tissue Doppler echocardiography.

Cardiac evaluation

Twelve-channel electrocardiographic evaluation was performed to all of the participants. Transthoracic echocardiographic studies were conducted by using an EPIQ 7 Ultrasound System (Philips, Heide, The

Netherlands) with S8 and S5 probes. Following echocardiographic evaluation, BP was measured using an age-appropriate sphygmomanometer. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated using the averages of three measurements.

Echocardiography

Measurements were performed using conventional and Doppler echocardiography. Left ventricular systolic functions were measured by using M-mode.[13] All echocardiographic measurements were taken in three repetitious cycles, and their mean values were calculated. M-mode records were taken at a rate of 50 mm/s, and Doppler records at 100 mm/s. Mitral early diastolic flow velocity (E), E wave deceleration time (EDT), late diastolic flow velocity (A) and its duration (A duration), and E/A were measured with the sample volume of pulse wave (PW) placed on the level of the mitral annulus in apical four-chamber view.[15,16]

Tissue Doppler imaging

All participants underwent tissue Doppler imaging examinations. Acquisition by pulsed tissue Doppler imaging was recorded at a high frame rate (>180 fr/sec) in apical four-chamber imaging at the mitral annulus. An appropriate velocity scale was chosen in order to avoid data aliasing. A sample volume of PW was placed on the conjunction of both the septal and lateral walls of the left ventricle and the mitral annulus. Peak early diastolic wave velocity septal and lateral (E'septal and E'lateral), peak late diastolic wave velocity septal and lateral (A'septal and A'lateral), E/E' septal and lateral, isovolumetric relaxation time (IVRT) septal and lateral, isovolumetric contraction time (IVCT) septal and lateral, mitral annular peak velocity septal and lateral (S' septal and S' lateral) were measured by using tissue Doppler imaging to elicit information concerning left ventricular systolic and diastolic functions.[17]

Statistical Analysis

Analyses were performed with MedCalc Statistical Software version 12.7.7 (MedCalc Software Bvba, Ostend, Belgium; http://www.medcalc.org; 2013). Descriptive statistics were used to express continuous variables (mean ± standard deviation). Student's t test was applied in the comparison of two independent variables with normal distribution, while two independent variables without normal distribution were compared using the Mann Whitney U test. One-Way ANOVA was employed in the comparison of more than two independent variables exhibiting normal distribution. The Kruskal Wallis test was applied in the comparison of more than two independent variables without normal distribution. The chi-square test (or sometimes Fisher's Exact test) was used for evaluating relationships between categorical variables. Statistical significance (p) was determined as <0.05.

Results

The study was conducted with 184 participants, consisting of 138 cases (three groups) and 46 controls. Age, gender, weight, and height were similar between the groups, although SBP, HR, duration and dose of medication differed between the groups (p=0.001, p=0.014, p <0.001, and p<0.001, respectively). Systolic blood pressure was not higher in the patient groups than the controls, and DBP was similar in all four groups. Heart rate was higher in the ATX group than in the other groups (p=0.002). Rates of adverse events were higher in the OROS-MPH group (26.1%) than in the other groups (15.2%, 4.3%, p<0.001). Cardiac adverse events and family histories of cardiac events were similar in the groups (p>0.05). Patients using OROS-MPH exhibited a longer duration of medication than those using ER-MPH and ATX (31.9±16 months, 19.89±11.4 months, 16.2±14.43 months, respectively, p<0.001 for both). The lowest medication dosage was observed in the patients using ER-MPH (p<0.001) (Table 1 and Table 3).

A, E/A, and E' septal differed significantly between the groups (p=0.002, p<0.001, and p=0.002, respectively). The ATX group exhibited a higher A wave velocity and lower E/A than the OROS-MPH (60.65±13.22 cm/s vs 51.50±9.70 cm/s, 1.67±0.44 vs 2.02±0.4, p<0.001, for both) and control groups (60.65±13.22 cm/s vs 54.90±12.60 cm/s, 1.67±0.44 vs 1.94±0.43, p=0.007 and p<0.001, respectively). Additionally, the ER-MPH group exhibited lower E/A than the OROS-MPH group (1.75±0.52 vs 2.02±0.41 p =0.001). E' septal values were lower in both the ER-MPH and ATX groups than in the controls (13.77±2.69 cm/s vs 15.54±2.31 cm/s p<0.001 and 14.08±2.83 cm/s vs 15.54±2.31 cm/s p=0.002) (Table 2 and Table 3).

Discussion

Methylphenidate and ATX are used as first-line therapies in the treatment of ADHD. Studies investigating the cardiac safety of these drugs have reported inconsistent results. A study including 1,200,438 participants aged 2-24 years showed no evidence that medications used for ADHD lead to severe cardiovascular events such as sudden death, myocardial infarction, and stroke.[18] One potential mechanism proposed to account for severe cardiac events caused by ADHD medication involves acute tachycardia-induced cardiomyopathy. This result is usually associated with supraventricular or ventricular tachycardia, and after controlling the rhythm, cardiac functions return to normal.[13,19-21] According to the literature it is known that activation of sympathetic system triggers sudden death but there is also a different theory, which suggests that increased HR variability as a result of increased parasympathetic activity diagnosed by 24 hour Holter monitoring may be beneficial in terms of predicting the

risk of sudden cardiac death.[12] Clinical findings such as palpitation, chest pain and syncope becomes prevalent as a result of increased sympathetic activity.[12] There are studies in the literature showing that ATX and MPH had no difference in terms of adverse cardiac events.[22,23] Our patients were followed-up and asked about cardiac adverse events. Consistent with the previous literature, no difference was observed between the groups and controls in terms of cardiac adverse events in the present study. The OROS-MPH group exhibited higher rates of non-cardiac adverse events such as nausea, abdominal pain, decreased appetite, and headache than the other groups. This finding may be attributed to the longer duration of OROS-MPH use than the other medications.

Table 1. Demographic, hemodynamic and electrocardiographic characteristics of the patients

		OROS-MPH n=46 Mean ±SD Med (min-max)	**ER-MPH** n=46 Mean ±SD Med (min-max)	**ATOMOXETINE** n=46 Mean ±SD Med (min-max)	**Controls** n=46 Mean ±SD Med (min-max)	**p**
Age (year)		10.20±2.11 10 (7-16)	10.02±2.07 9 (7-16)	10.09±2.42 9.5 (6-16)	10.00±2.00 9 (7-17)	0.953
Gender (M/F)		34/12	35/11	33/13	34/12	0.995
Weight (kg)		38.00±7.00 38 (25-54)	37.22±10.89 35 (24-73)	37.13±12.54 33.5 (20-67)	37.90±10.80 34.5 (24-66)	0.180
Height (cm)		143.00±12.00 142 (104-168)	141.62±13.14 137.5 (126-176)	141.39±15.89 136 (123-183)	142.00±13.00 139 (124-175)	0.350
SBP (mmHg)		103.30±10.15 100 (90-124)	99.37±11.14 100 (75-130)	98.93±10.98 100 (80-120)	107.05±11.17 110 (85-128)	**0.001**
DBP (mmHg)		64.40±7.00 60 (49-80)	65.09±7.45 65 (50-85)	65.63±6.92 65 (50-80)	63.00±10.36 64 (41-82)	0.483
HR (min)		85.16±14.10 84 (47-113)	88.22±16.84 86 (52-129)	93.02±16.12 90 (61-132)	83.25±14.18 82 (43-113)	**0.014**
QTc (s)		0.40±0.02 0.39 (0.35-0.44)	0.39±0.07 0.39 (0.36-0.43)	0.40±0.04 0.39 (0.35-0.44)	0.39±0.03 0.4 (0.33-0.44)	0.954
Duration of medication (month)		31.9±16 30 (6-60)	19.89±11.4 19 (6-48)	16.2±14.43 12 (6-72)	-	**<0.001**
Dose of medication (mg/kg)		0.94±0.17 0.91 (0.56-1.42)	0.69±0.22 0.67 (0.27-1.25)	1.03±0.17 1 (0.67-1.4)	-	**<0.001**
Adverse event	No Yes	34 (73.9%) 12 (26.1%)	39 (84.8%) 7 (15.2%)	44 (95.7%) 2 (4.3%)	46 (100.0%) 0 (0.0%)	**<0.001**
Cardiac adverse event	No Yes	40 (87.0%) 6 (13.0%)	42 (91.3%) 4 (8.7%)	43 (93.5%) 3 (6.5%)	45 (97.8%) 1 (2.2%)	0.282
Cardiac event in family history	No Yes	32 (69.6%) 14 (30.4%)	35 (76.1%) 11 (23.9%)	31 (67.4%) 15 (32.6%)	30 (65.2%) 16 (34.8%)	0.700

Kruskal Wallis test, Fisher'sExact p<0.05 DBP: Diastolic blood pressure, F: female, HR: Heart rate, M: male, n: number, SBP: Systolic blood pressure, SD: Standard deviation, QTc: corrected QT interval, ATX: Atomoxetıne MPH: Methylphenidate, ER: extended-release, OROS: osmotic-release oral system, Bold values denote statistical significance at the p < 0.05 level

Dopaminergic effects associated with MPH are thought to increase HR and BP, with an expected rise in epinephrine levels.[24,25] It is described in the studies that the overall cardiac risks associated with ADHD medication is unimportant. According to the literature usage of MPH and ATX was associated with small elevations of BP and HR with no changes in electrocardiographic parameters[26,27] In contrast, increase in HR and SBP more than controls was stated with stimulant therapy and in the ATX group more than the MPH group.[1] An open study consisting of children aged 6-17 years, reported that OROS-MPH caused no significant increase in SBP, while ATX was associated with significant increases in DBP and HR.[28] In the present study, HR was higher in the ATX group than the controls, with no differences between the long–acting MPH drugs and accompanied with similar BPs.

Dopamine is thought to alter left ventricular filling pressures by raising the pressures supported by alpha receptor-mediated arterial vasoconstruction.[29] In addition to the expected increase in HR and BP described in some studies, these variables can also alter cardiovascular hemodynamics. Accordingly, we examined cardiovascular involvement in this study. Thickening of the posterior wall or interventricular septum is important in terms of predicting left ventricular hypertrophy or diastolic dysfunction, and we detected no increase in ventricular wall diameters. Dilated cardiomyopathy with an ejection fraction of 25% was described in an obese 18-year old male patient associated with the use of MPH. These findings support the idea of an increased risk of heart failure after exposure to MPH.[9]

Table 2. Conventional, Doppler, tissue Doppler echocardiographic measurements of the patients

	OROS-MPH n=46 Mean ±SD Med (min-max)	**ER-MPH** n=46 Mean ±SD Med (min-max)	**ATOMOXETINE** n=46 Mean ±SD Med (min-max)	**Controls** n=46 Mean ±SD Med (min-max)	**P**
IVSd (cm)	0.74±0.12 0.7(0.5-1)	0.70±0.10 0.68(0.5-0.9)	0.71±0.11 0.7(0.5-0.9)	0.75±0.13 0.7(0.5-1)	0.135
LVEDd (cm)	3.93±0.42 3.9(3-5)	3.92±0.32 3.9(3.3-4.6)	3.85±0.52 3.8(2.95-5.4)	4.05±0.49 3.95(3.2-5.2)	0.155
LPWDd (cm)	0.71±0.13 0.7(0.5-1.1)	0.69±0.10 0.65(0.45-0.9)	0.68±0.10 0.69(0.5-1)	0.73±0.13 0.7(0.5-1)	0.190
LA (cm)	2.36±0.29 2.3(1.9-3.2)	2.30±0.30 2.3(1.8-3.1)	2.32±0.38 2.3(1.6-3.8)	2.33±0.29 2.3(1.8-3)	0.717
EF (%)	71.10±4.22 70(65-79)	72.39±4.35 73(65-81)	71.09±4.04 71.5(65-78)	73.15±4.26 73(65-82)	0.078
FS (%)	40.34±4.70 39(35-49)	41.02±3.59 41(35-49)	40.09±3.20 40(35-46)	42.11±4.67 41(35-51)	0.128
E (cm/s)	101.30±14.8 102(59.4-134)	99.77±16.06 99(72-138)	96.93±13.4 94.85(74.2-135)	103.00±16.90 100(66-144)	0.273*
A (cm/s)	51.50±9.70 52.2(34-70.8)	60.93±18.57 57.7(31.5-114)	60.65±13.22 60.6(28-90.7)	54.90±12.60 54(33-100)	**0.002**
E/A	2.02±0.41 2 (1.29-3.02)	1.75± 0.52 1.66(0.76-3.24)	1.67± 0.44 1.51(1.01-2.98)	1.94±0.43 1.92(1.14-2.89)	**<0.001**
EDT (ms)	109.47±15.25 107.6(86-158.96)	103.65±17.81 99.41(70-155)	103.07±18.96 102(44.4-146)	104.45±18.74 99.41(68.39-151)	0.264
A duration (ms)	111.38±16.28 109.65(85.03-172)	105.06±14.35 104(81-143)	104.61±16.92 104.5(63-145)	109.93±18.15 107.1(81.33-184)	0.206
S' septal (cm/s)	7.92±1.16 7.87(5.97-11)	8.51±1.25 8.36(6.58-12.3)	8.06±1.56 7.74(5.27-13.4)	8.25±1.26 8(6.96-12)	0.116
E' septal (cm/s)	14.44±2.57 14.85(9.25-20)	13.77±2.69 13.65(6.11-22.2)	14.08±2.83 14(8.22-26.4)	15.54±2.31 15(10-20)	**0.002**
A' septal (cm/s)	6.64±1.19 6.13(4-9)	7.24±1.53 7(4.25-10.8)	7.33±1.57 7.26(4.48-10.8)	7.12±1.58 7(5-15)	0.090
IVCT septal (ms)	54.76±7.42 55(40.67-73.94)	53.25±7.48 54.3(36.97-67)	55.83±9.83 55(40-86)	54.72±9.65 55.45(31.47-90.57)	0.564*
IVRT septal (ms)	55.45±9.95 55.23(35.12-85)	56.13±7.40 58(35-69)	55.37±8.59 55(39-79)	52.94±6.45 53.6(35.12-68.39)	0.265*
E/E' septal	7.11±1.36 7.05(4.37-10.55)	7.48±1.78 7.35(4.39-14.5)	7.07±1.43 7.15(4.05-11.5)	6.73±1.28 6.65(4.12-9.64)	0.104
S' lateral (cm/s)	10.79±2.32 10.8(6-17)	10.69±2.32 10.35(7-17.3)	10.76±3.39 10.1(6.17-28)	10.67±2.16 10.15(7-15)	0.857
E' lateral (cm/s)	19.86±4.09 19.5(10.5-27)	19.57±2.87 20(11.6-25.4)	18.57±3.35 18.55(12.5-26.2)	20.42±2.99 20(15-27.9)	0.065*
A' lateral (cm/s)	7.10±1.30 7(4.58-10)	7.58±2.10 7.21(5-17)	6.88±1.73 6.47(4.35-12.5)	6.75±1.73 6.55(4-14)	0.080
IVCT lateral (ms)	54.56±12.54 51.38(36.97-110.91)	52.13±7.92 52(36-70.24)	56.26±9.46 56(40-85)	54.98±9.76 52.41(36.97-77.63)	0.264
IVRT lateral (ms)	54.69±11.39 53.8(29.57-83)	54.47±8.81 53.8(33-70)	56.65±8.82 55.5(40-77)	51.13±8.50 51.73(33.27-68.39)	0.053
E/E' lateral	5.25±1.07 5.19(3.18-7.53)	5.18±1.05 4.95(3.64-8.27)	5.34±0.99 5.15(3.52-8.2)	5.07±1.07 5(3.02-8.1)	0.575

*Kruskal Wallis test, *One Way ANOVA test*A: Late diastolic flow velocity, A': Peak late diastolic wave velocity, E: Mitral early diastolic flow velocity, E': Peak early diastolic wave velocity, EDT: E wave deceleration time, EF: Ejection fraction, FS: Fractional shortening, IVCT: Isovolumetric contraction time, IVSd: Interventricular septal diastolic diameter, IVRT: Isovolumetric relaxation time, LA: Left atrium, LPWDd: Left posterior wall diastolic diameter, LVEDd: Left ventricular end diastolic diameter, S': Mitral annular peak systolic velocity, SD: Standart deviation, ATX: Atomoxetıne, MPH: Methylphenidate, ER: extended-release, OROS: osmotic-release oral system, Bold values denote statistical significance at the $p < 0.05$ level

Heart failure is classified in terms of systolic and diastolic dysfunctions of the heart. Systolic dysfunction is the condition when heart cannot pump blood to the body. The chambers of the heart enlarges with decrease in wall thickness. Diastolic dysfunction is the condition when there is not enough filling blood in the heart to pump accompanied with increase in pulmonary venous pressure. This finding can be explained with the impairment of left ventricular relaxation, increase in myocardial wall diameter and left ventricular stiffness.

The most commonly used measurements with echocardiography are the velocities of E and A waves. Patients with a normal left ventricle, had most of the diastolic filling in the early phase, which is expressed by the prominent E wave. Late phase represents atrial contraction and it is expressed by A wave. Since atrial contraction plays a lesser role in diastolic filling, the A wave is smaller than the E wave. However, if higher pressures are required for passive filling of the left ventricle, diastolic filling becomes more dependent on atrial contraction. Therefore, the velocity of A wave becomes higher than E wave. Evaluation of filling velocities is performed by measurement of E wave and A wave velocities, IVRT and calculation of E/A ratio.[30] IVRT is described as the time interval beginning with the closing of the aortic valve and ending with the opening of the mitral valve.[31]

Table 3. Post hoc analysis of the parameters with significance

	OROS-MPH vs. Controls	**OROS-MPH** vs. **ER-MPH**	**OROS-MPH** vs. **ATX**	**ER-MPH** vs. **Controls**	**ATX** vs. **Controls**	**ER-MPH** vs. **ATX**
A (cm/s)	0.291	0.013	**<0.001**	0.146	**0.007**	0.412
E/A	0.432	**0.001**	**<0.001**	0.023	**<0.001**	0.193
E' septal (cm/s)	0.044	0.174	0.317	**<0.001**	**0.002**	0.574
HR (min)	0.304	0.475	0.021	0.096	**0.002**	0.154
SBP (mmHg)	0.063	0.060	0.057	**0.001**	**0.001**	0.978
Duration of medication (month)	-	**<0.001**	**<0.001**	-	-	0.021
Dose of medication (mg/kg) **	-	**<0.001**	0.061	-	-	**<0.001**

*Mann-Whitney U test,**Tukey test, p<0,008 Bonferroni* A: Late diastolic flow velocity, E': Peak early diastolic wave velocity, HR: Heart rate, SBP: Systolic blood pressure, ATX: Atomoxetine, MPH: Methylphenidate, ER: extended-release, OROS: osmotic-release oral system, Bold values denote statistical significance at the p < 0.008 level

Filling patterns vary depending on the degree of the disease. In grade 1 diastolic dysfunction, there are relaxation changes characterized by smaller E waves, larger A waves, and increased E wave deceleration time. In grade 2 diastolic dysfunction, there is a pseudo-normalization pattern characterized by apparently normal E and A waves. In grade 3 or restrictive filling pattern, there is a very prominent E wave with a short and sharp deceleration time and a small A wave. The patients with grade 3 diastolic pattern have a significantly worse prognosis than others.[30] Furthermore, tissue Doppler imaging can be used to measure myocardial motion, specifically the amount the mitral annulus recoils towards the base during early diastole (E') and the wave associated with the phase of atrial contraction (A') reflecting myocardial relaxation.[32] The left ventricular filling pressures can be estimated by the E/E' ratio. The clinical findings associated with diastolic dysfunction of the left ventricle are dyspnea with exertion, ortopnea, tachypnea, tachycardia, pulmonary edema and are related with respiratory system. In the presence of diastolic dysfunction, left ventricular compliance decreases and over time, firstly the left ventricular pressure, then the left atrial pressure increases, and eventually left atrial dilatation develops. In diastolic dysfunction, blood is no longer pulled in by the left ventricle but instead is pushed by increased left atrial pressure.[32]

There are patients with heart failure as a result of coronary vasospasm during the use of MPH.[11] Systolic cardiac functions are evaluated with of ejection fraction, fractional shortening, IVCT, and S' parameters. IVCT is described as the time interval beginning with the closing of the mitral valve and ending with the opening of the aortic valve.[31] S' is defined as the wave when the cusps of the valve migrate towards the apex.[32] Parameters involving systolic functions were similar between the groups in the present study, indicating that conventional and Doppler parameters of systolic functions were preserved.

Ventricular diastolic functions are detected by means of Doppler and tissue Doppler parameters. Accordingly, a decrease in E and an increase in A with decreased E/A may be expected in the presence of diastolic dysfunction. Prolongation of IVRT and EDT also support the idea of diastolic dysfunction. E' is correlated with left ventricular relaxation, preload, and filling pressures. In normal hearts, left atrial pressure exerts a powerful effect on E' velocity. In addition to decrease in E', increase in E/E' is expected with diastolic dysfunction.[16,17,33] Tissue Doppler parameters are used for a more exact identification of diastolic dysfunction, since transmitral parameters alone do not correlate well with left ventricular filling pressures in patients with normal systolic function.[16] E/E' exhibits good correlation with left ventricular filling pressures in patients with cardiac disease. An apparent relationship between E' and relaxation of the left ventricle has been observed in both human and animal studies.[17] In case of impairment in left ventricular relaxation, E' is minimally affected by left ventricular filling pressures and preload. However, when relaxation of left ventricle is normal, E' is affected by preload. Therefore, in the patients with normal hearts, E' is affected by preload, while E/E' does not always correlate with left ventricular filling pressures. Structural changes in the left ventricle and left atrium may help to differentiate normal and abnormal diastolic functions.[17] MPH showed a dose-dependant increase in

D2 expression in myocytes,[25] which causes vaso-constriction and increase in left ventricular filling pressures.[29] The reference values of systolic and diastolic parameters evaluated in the study show a wide normality range in the literature related with the ages or body surface areas of the participants. Therefore the normality of these parameters should be evaluated whether according to age distribution or the mean values of age and gender matched controls like in our study. The OROS-MPH group had similar diastolic findings with controls. A study performed in Turkey, stated that children using OROS-MPH exhibited lower E' septal values than the controls, with no differences in other parameters indicating diastolic dysfunction.[14] The ATX group had higher A, lower E/A, lower E' septal than controls with similar E/E' septal and lateral, IVRT and EDT in our study indicating detoriation in more than one parameter representing diastolic function suggesting impairment in myocardial relaxation. The ER-MPH group had lower E' septal values than controls without any differences in the other diastolic parameters like the study previously performed in our country.

The ER-MPH group had lower E/A than the OROS-MPH group and similar findings with the ATX group. The ATX group had higher A and lower E/A than OROS-MPH group, suggesting that ATX medication may affect diastolic functions more than OROS-MPH. There was not a medication among these drugs that had an effect on all of the diastolic parameters but impairment of the other parameters may emerge overtime during clinical follow-ups.

According to the recommendations of the American Academy of Pediatrics, patients who receive ADHD medications should be monitored periodically in terms of HR and BP.[28] In view of the safety concerns described, it is also recommended to evaluate the patients before psychostimulant or non-stimulant therapy is initiated, together with personal histories of syncope, dizziness, palpitations and chest pain and family histories of premature sudden death, and with careful cardiac examination.[34] They should be referred for specialist cardiac evaluation if initial findings suggest such medical history or presence of cardiovascular disease. Patients should be monitored before and during treatment with BP and HR after every dose adjustment and at least every six months to detect clinically important increases. Tachycardia and hypertension caused by medication should be evaluated with a specialist cardiac evaluation to consider the need for beta-blockers and antihypertensive treatment. Atomoxetine and MPH should not be used in children suffering from severe cardiovascular or cerebrovascular disorders. They should not be used in the patients who have the risk for clinical deterioration, accompanied with increase in HR and SBP.[1]

Conclusions

To the best of our knowledge, this is the first study to evaluate the effects of OROS-MPH, ER-MPH, and ATX on systolic and diastolic functions together with a control group, in a pediatric population with ADHD. In our study, ATX use showed impairment in some of the diastolic findings supporting myocardial relaxation more than long acting-MPH medications. There was no apparent clinical findings in the children receiving the medications, also without changes in the rest diastolic parameters. When the results were subjected to clinical evaluation, we concluded that these findings were not sufficient enough to cause apparent cardiac dysfunction but these patients should be monitored to determine the changes in other diastolic parameters during time. Further studies should be performed to support the findings detected in our study.

Compliance with Ethical Standards

The methodology and questionnaire for this study were approved by the University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Committee (decision number 2019-14-01). The authors assert that all procedures contributing to this work comply with the ethical standards of Bakırköy Dr. Sadi Konuk Training and Research Hospital and the Declaration of Helsinki of 1975, as revised in 2008.

Limitations

Heart rate is affected by numerous variables, such as noise and anxiety. It would therefore have been preferable to monitor HR using 24-hour Holter ECG, although it would not be practical to evaluate all the patients in this way.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contribution

AMM, TK: Study idea, hypothesis, study design; AMM, TK: Material preparation, data collection and analysis; AMM: Writing the first draft of the article; AMM, TK: Critical review of the article finalization and publication process.

Financial Disclosure

None

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Araştırma Makalesi | Research Article

FOLDED STENT: THE THIRD GENERATION TRICK TO IMPROVE THE MICROVASCULAR STENTING TECHNIQUE

FOLDED-STENT: MİKROVASKÜLER STENTLEME TEKNİĞİNİ GELİŞTİRMEK İÇİN ÜÇÜNCÜ NESİL YÖNTEM

Çağdaş Pamuk[1*]

[1] Silivri Anadolu Special Hospital, Orthopaedics and Traumatology Department, İstanbul, Türkiye.

ABSTRACT

Objective: Although microsurgical vessel anastomoses have become a routine procedure today, they are still among the difficult techniques to apply. The most important step in the success of the technique is the clear presentation of the vessel lumen. In this study, we aimed to describe a new technique that will provide an easier anastomosis by keeping the lumen open during microvascular anastomoses.

Methods: Four groups were formed using the chicken wing radial artery, which is an inanimate animal model. One of these was the control group who underwent standard microvascular repair. The second and third groups that followed were those in which two previously defined intravascular stenting techniques were applied. The fourth group was the Folded stent group that we just defined.

Results: Anastomosis time was found to be significantly longer when compared to other groups in our newly defined technique. This time was an average of 3 minutes. No significant difference was observed in terms of the number of sutures used in anastomoses. No posterior wall suture complication was observed in any specimen.

Conclusion: It has been seen that the "Folded-Stent" technique can be used safely like other stenting techniques in terms of keeping the lumen open during anastomosis and preventing the adhesion of the vessel walls. In clinical practice, it is an advantageous technique that can be used to prevent posterior wall suturing of small vessels, especially during venous repairs.

Keywords*:* Vascular anastomosis, microsurgery, stent, suture

ÖZ

Amaç: Mikrocerrahi damar anastomozları günümüzde rutin bir işlem haline gelmesine rağmen halen uygulanması zor teknikler arasındadır. Tekniğin başarısındaki en önemli adım damar lümeninin net olarak gösterilmesidir. Bu çalışmada mikrovasküler anastomozlarda lümeni açık tutarak daha kolay anastomoz sağlayacak yeni bir tekniği tanımlamayı amaçladık**.**

Yöntem: Cansız bir hayvan modeli olan tavuk kanadı radial arter kullanılarak dört grup oluşturuldu. Bunlardan biri standart mikrovasküler onarım yapılan kontrol grubuydu. Takip eden ikinci ve üçüncü gruplar, önceden tanımlanmış iki intravasküler stentleme tekniğinin uygulandığı gruplardı. Dördüncü grup ise yeni tanımladığımız Folded stent grubuydu.

Bulgular: Yeni tanımlanan tekniğimizde anastomoz süresi diğer gruplara göre anlamlı olarak daha uzun bulundu. Bu süre ortalama 3 dakikaydı. Anastomozlarda kullanılan sütür sayısı açısından anlamlı bir farklılık gözlenmedi. Hiçbir örnekte arka duvar sütür komplikasyonu gözlenmedi.

Sonuç: "Folded-Stent" tekniği, anastomoz sırasında lümenin açık kalması ve damar duvarlarının yapışmasını önlemesi açısından diğer stentleme teknikleri gibi güvenle kullanılabilir olduğu görülmüştür. Klinik pratikte özellikle venöz onarımlar sırasında küçük çaplı damarların arka duvar dikilmesini önlemek için kullanılabilecek avantajlı bir tekniktir.

Anahtar Kelimeler: Vasküler anastomoz, mikrocerrahi, stent, sütür

[*]**İletişim kurulacak yazar/Corresponding author:** Çağdaş Pamuk; Silivri Anadolu Special Hospital, Orthopaedics and Traumatology Department, Mimar Sinan Mah, Mimar Sinan Cd. No:72, 34570 Silivri, Istanbul, Türkiye
Telefon/Phone: +90 (505) 813 33 36 **e-posta/e-mail:**cagdaspamuk@hotmail.com
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Introduction

With the possibility of microsurgical vessel repair, many operations that once seemed impossible, such as replantation of severed limbs, and free tissue transplants, have become possible.[1] However, although microsurgical vessel repair is a routine procedure, it is still a difficult technique to apply today.[2] The most important of these difficulties and the situation that causes vascular anastomosis is the suturing of the posterior wall. At the same time, an appropriate number of sutures should be placed homogeneously at equal intervals throughout the vessel lümen.[3,4]

These problems become an even greater problem with veins because their walls stick together more easily than those of arteries. As a result, some authors have developed the technique of intravascular stenting (IVaS), which consists of placing a nylon monofilament suture in a vein to act as a temporary stent.[5] Studies comparing this technique with traditional sutures have reported difficulties with stent insertion and removal, especially thrombosis like complications due to stent related trauma to the vessel Wall.[6] Therefore, the “clip stent” technique was recommended to improve the IVaS technique.[7] In this technique, the double-needle monofilament suture is placed in the lumen of both ends of the vascular anastomosis and then the needle is passed through the vessel wall and pulled out from both sides, and the anastomosis is completed with the help of this stenting. An advantage of this technique is that stent removal is not traumatic. The disadvantage is that it causes leaks in each of the two remaining holes after needle removal.[7] It has been proposed to develop the “clip stent” technique to perform microsurgical anastomoses, by showing that it can be applied as a single needle with a technique called “pull out stent”, which can be improved by removing the needles and placing and removing the stent with one hole in two places.[8] In this study, we further developed these two techniques, which were developed to keep the vessel lumen open during microsurgical vessel anastomosis, and defined a new technique we named “folded stent”. To validate our hypothesis, we performed an experimental study by completing arterial anastomoses in the chicken wing radial artery, which is a commonly used microsurgical application model, with clip stent, pull out stent, and newly defined folded stent techniques.

Methods

This study was planned using the chicken wing radial artery microsurgery training model, which is a non-living microsurgery training model. Ethics committee approval was not obtained for the study, since there is no need for ethical committee approval in studies with non-living animal models.

All anastomoses were performed by an experienced orthopedic and traumatology specialist, who routinely performs hand and microsurgery operations. Our study was performed on 40 standard microsurgical anastomosis models. While preparing the anastomosis, the injured vessel model was created by finding the radial artery in the muscle cleavages, following the skin incision with a longitudinal incision so that the radius bone of the chicken wing remains on the surgeon's side. The mean diameter of the proximal part of the artery was 0.5 mm after the removal of the adventitia. Four groups, each containing ten anastomosis models, were formed. In group I (control group), the artery was repaired using standard end-to-end anastomosis with simple spaced sutures (10-0 nylon, Ethilon, Johnson & Johnson) . Group II was also repaired with end-to-end anastomosis using the 'clip-on stent' technique (Figure 1). In Group III, the artery was repaired by end-to-end anastomosis with the pull-out stent technique (Figure 2), and in Group IV, the artery was repaired using the newly defined “folded stent” technique. All anastomoses were performed using a stereo microscope (Soif Optical Instruments, China) with up to 40x magnification. The “Folded-stent” technique consisted of three steps (Figure 3). First, a curved 6-0 nylon monofilament was inserted, and folded into the arterial lumen. This stent was removed from the wall on one side by inserting it into the lumen and then the other end into the contralateral lumen. The second step consisted of the anastomosis with simple cut 10-0 nylon sutures (Ethilon, Johnson & Johnson). The third step consisted of removing the stent and closing the exit hole with a 10/0 nylon suture if leakage was detected. The results were recorded in terms of measuring the time to perform an anastomosis (in minutes), evaluating the homogeneous distribution of the sutures, evaluating the anastomotic transition and leaks by injecting methylene blue (Figure 4) into the vascular system, and finally, whether suturing should be applied to the stent removal site.

Differences between the four groups were statistically evaluated for each of three quantitative variables (time to completion, number of stitches per anastomosis including exit hole in groups II, III, and IV, number of leaks) and one qualitative variable (whether or not leak). Considering the sample size, non-parametric Kruskal-Wallis and Wilcoxon tests were used at a significance level of 0.05.

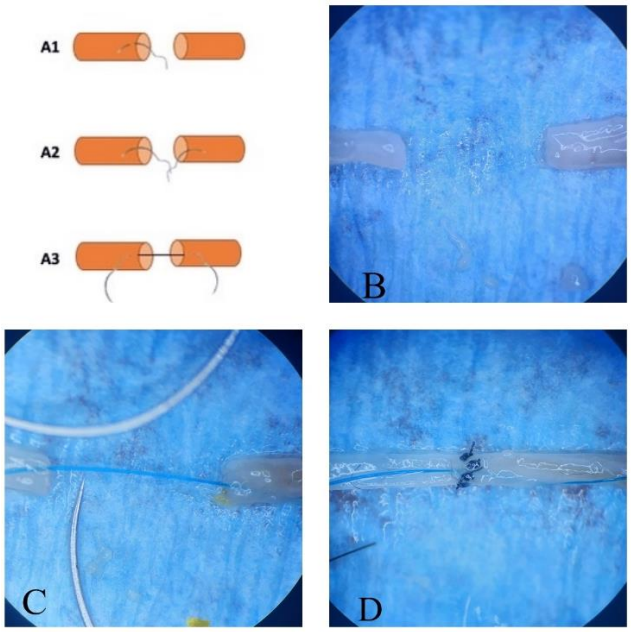

Figure 1. A1, A2 and A3 a schematic explanation of the Clip stent technique. B. The prepared state of the arteries used in the model for repair, C. The application of the Clip stent technique before anastomosis, D. The completed anastomosis with the Clip stent.

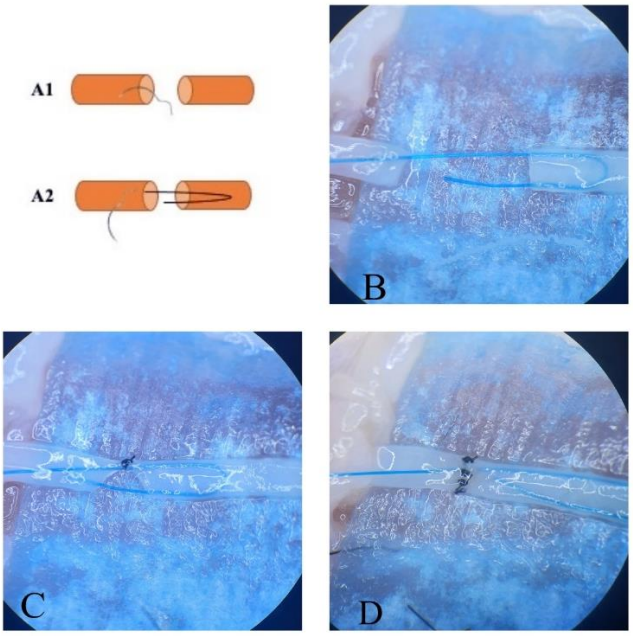

Figure 3. A1 and A2 a schematic explanation of the Folded stent technique. B. The application of the Folded stent technique before anastomosis, C. The application of the first suture of Folded stent technique, D. The completed anastomosis with the Folded stent.

Results

The results for each group are shown in Tables 1-4, respectively. The shortest anastomosis time was in group I, followed by group III, then group II and finally group IV. The difference between Groups I and II (p=0.0051), groups

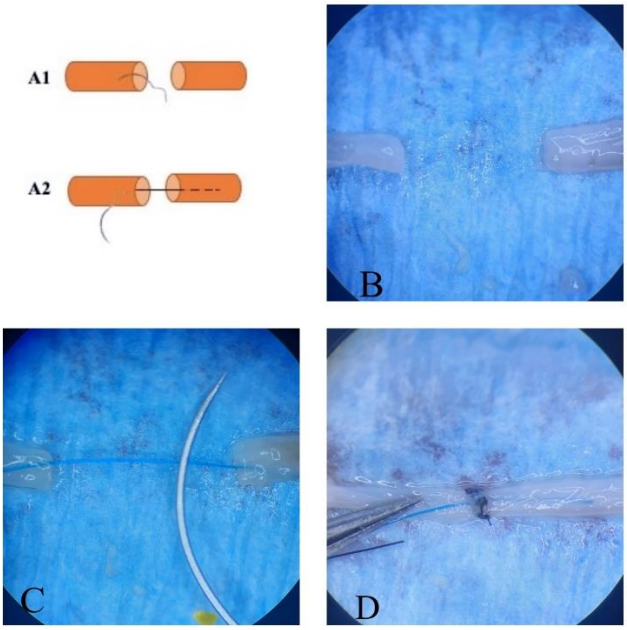

Figure 2. A1 and A2 a schematic explanation of the Pull-out stent technique. B. The prepared state of the arteries used in the model for repair, C. The application of the Pull-out stent technique before anastomosis, D. The completed anastomosis with the Pull-out stent.

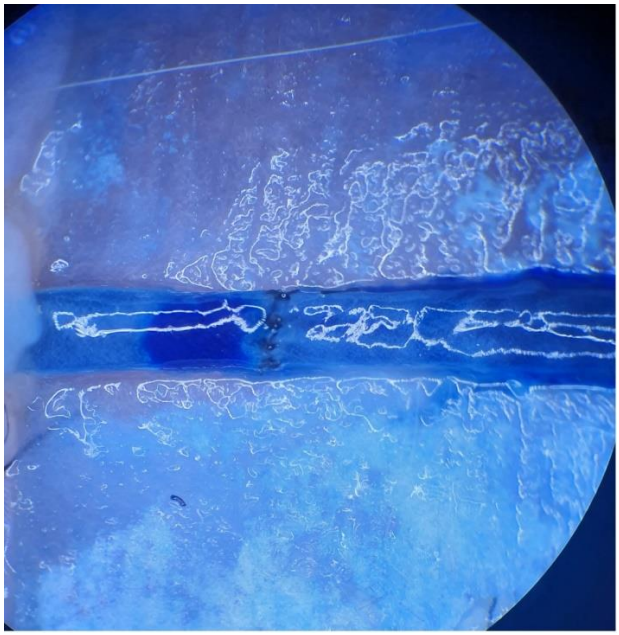

Figure 4. Appearance after injection of methylene blue for evaluation of anastomotic patency.

I and III (p=0.0069), and groups I and IV (p= 0.0051) was statistically significant when compared to the control group Group I. The mean time per anastomosis was longer. The number of sutures was 6.6±0.51 in group I, 6.5±0.52 in group II, 6.6±0.51 in group III, and 6.5±0.52 in group IV. When compared with the control group, there was no statistically significant difference between the groups (group II p=0.338, group III p=0.404, group IV p=0.404) in the terms of sutures. In Groups II and III, an additional 1 suture was required in a specimen to close the exit hole of the stent. In this respect, the difference was not statistically significant (p = 0.368). The number of

o leaks per anastomosis was 0 in group I, 2 specimens in group II, 1 specimen in group III, and 0 in group IV. Leakage rates were not significantly different in any group compared to the control group (p=0.472). It was observed that the anastomosis permeability was positive, since the methylene blue crossed the anastomosis line in all anastomoses since the anastomosis was permeable. There was no significant difference between the groups (p=1).

Table 1. End-to-end anastomoses performed without stenting

Specimen No	Time (min)	Stictches (n)	Leaks (n)	Patency (+/-)
1	14	7	0	+
2	13	6	0	+
3	15	7	0	+
4	17	7	0	+
5	19	7	0	+
6	11	6	0	+
7	16	7	0	+
8	10	6	0	+
9	12	6	0	+
10	18	7	0	+
Mean±SD	14.5±3.02	6.6±0.51		

SD: Standard Deviation, Min: Minutes

Table 2. End-to-end anastomoses performed with "Clip-Stent"

Specimen No	Time (min)	Stictches (n)	Leaks (n)	Patency (+/-)
1	18	6	0	+
2	24	7	0	+
3	17	6	0	+
4	27	7	0	+
5	29	7	1	+
6	28	7	1	+
7	19	6	0	+
8	26	7	0	+
9	20	6	0	+
10	23	6	0	+
Mean±SD	23.1±4.38	6.5±0.52		

SD: Standard Deviation, Min: Minutes

Table 3. End-to-end anastomoses performed with "Pull-out Stent"

Specimen No	Time (min)	Stictches (n)	Leaks (n)	Patency (+/-)
1	20	6	0	+
2	18	6	0	+
3	23	7	0	+
4	28	7	0	+
5	17	6	0	+
6	27	7	1	+
7	25	7	0	+
8	16	6	0	+
9	29	7	0	+
10	22	7	0	+
Mean±SD	22.5±4.69	6.6±0.51		

SD: Standard Deviation, Min: Minutes

Table 4. End-to-end anastomoses performed with "Folded-Stent"

Specimen No	Time (min)	Stictches (n)	Leaks (n)	Patency (+/-)
1	21	6	0	+
2	24	6	0	+
3	26	6	0	+
4	25	6	0	+
5	34	7	0	+
6	33	7	0	+
7	31	7	0	+
8	35	7	0	+
9	23	6	0	+
10	30	7	0	+
Mean±SD	28.2±5	6.5±0.52		

SD: Standard Deviation, Min: Minutes

Discussion

The development of microsurgical techniques made it possible to repair vessels with a diameter of 1 mm, followed by supermicrosurgical techniques with vessel repairs of 0.5 mm and less. Anastomosis of increasingly smaller blood or lymphatic vessels can be performed with these techniques.[9–11] The development of techniques has prompted the quest to develop more powerful microscopes[12], finer instruments, and more accurate suturing techniques. In this context, some authors have tried to develop methods to facilitate the removal of these delicate sutures. IVaS, "Clip-stent" followed by "Pull-out stent" techniques have been described in recently published studies.[7,8] The IVaS technique is based on a technique in which a silastic tube is inserted into the vascular lumen to facilitate suturing of vessels larger than 1 mm.[5] Although there are no studies showing the superiority of IVaS, it can be applied according to the surgeon's preference.[6] The "clip stent" technique was developed due to the complications and difficulties encountered during the removal of the stent during IVaS application.[7] This "Clip-stent and Pull-out stent" technique has many advantages.[8] The possibility of damage to the vascular intima during anastomosis is reduced. In the IVaS technique, the stent must be removed before all the sutures in the anastomosis are completed, whereas in these two stent techniques, the sutures can be made with the stent in place. In addition, vascular clamps may be released while the stent is in place to check for leakage at the anastomosis. In the event of a leak, additional sutures can be made without the risk of transfixing the vessel wall. The disadvantage of the "Clip-stent" technique is that it requires two passes through the vessel wall with two needles. Holes formed can cause leaks, therefore requiring additional stitches. For this reason, the "Pull-out stent" technique has been developed, which transforms these holes into one rather than two holes. However, the "Folded-Stent" technique, which we described in our study, is a third-generation application that improves

these techniques, and while providing the advantage of a single exit hole, it ensures that the vessel lumen is seen more clearly and remains open. At the same time, when evaluated in terms of damage to the vascular intima, it has the same advantages as the "Pull-out stent" technique. The most important step in the successful application of microsurgical vessel anastomosis is proper visualization of the lumen and no sutures to the posterior wall. Although a significant increase in the application time was observed in this newly defined technique compared to other techniques, it caused an increase of three minutes in the longest anastomosis time. Considering the advantages of making the anastomosis more ideal and seeing the lumen better, the "Folded-stent" technique is an advantageous technique in terms of anastomosis quality. In general, the benefits of stenting are better patency of vascular anastomoses, more regular sutures, and fewer posterior wall sutures.[5,7,8] The main disadvantage is leakage from the needle exit hole as its diameter constantly exceeds that of the suture, which can cause parietal tears. A “Pull out stent” is theoretically better than a “Clip-stent” because it uses a needle-free suture and enters the vessel wall through a single hole. Because the diameter of 4/0 nylon is smaller than the diameter of the needle, the hole obtained after placing an inclined suture through the vessel wall is smaller than that of the needle. Reducing the number of holes in the wall is expected to reduce the risk of leakage, as well as the advantage of better visualization of the lumen in the "Folded-stent" technique. In practice, we think that all of our results were not statistically significant because they were applied in an experimental model by an experienced surgeon. For example, the time to anastomose with the “Pull out stent” was significantly shorter than with the “Clip-stent”, but the “Folded-stent” technique was longer than the others. Although the leak rate was not significantly different from one group to the next, the "Pull out stent" and "Folded-stent" had less leakage than the "Clip-stent". Apart from these techniques, there is a theoretical risk of stent migration in the IVaS technique, which is the standard first stenting technique. Other techniques, the third generation of which were developed with our technique, do not have this risk. However, there is a theoretical risk of thrombosis due to the perforation holes in the stent, as in the "Clip-stent". Considering the clinical applications, the veins are the veins that have the most difficulty in revealing the lumen of the veins. At the same time, the risk of posterior wall suture is more common in vein repairs. We believe that the “Folded-stent” technique, which we have just defined in routine clinical applications, especially during vein repair, will benefit the surgeon in terms of anastomosis quality.

The most important limitation of our study is that it is difficult to evaluate anastomotic quality in a non-living animal model. Although anastomotic permeability can be better evaluated from animal studies, our main aim in this study is to introduce a new technique in which the vessel lumen can be seen more clearly by the surgeon, not anastomotic permeability.

Conclusion

In the non-living animal model, the “Folded-Stent” technique provides advantages like other stenting techniques in terms of keeping the lumen open during the anastomosis and preventing the adhesion of the vessel walls. It is an advantageous technique that can be used in clinical practice, especially during venous repairs, to prevent posterior wall suturing of small caliber vessels.

Compliance with Ethical Standards

Ethics committee approval is not required for the study

Conflict of Interest

The authors declare no conflicts of interest.

Author Contribution

ÇP: Study idea, hypothesis, design, material preparation, data collection and analysis, writing the first draft of the article, critical review of the article finalization and publication process

Financial Disclosure

None

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Research Article | Araştırma Makalesi

DOES LOW MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION REALLY PREDICT MORTALITY IN ACUTE EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE?

DÜŞÜK ORTALAMA KORPÜSKÜLER HEMOGLOBİN KONSANTRASYONU KRONİK OBSTRÜKTİF AKCİĞER HASTALIĞININ AKUT ALEVLENMELERİNDE MORTALİTEYİ GERÇEKTEN ÖNGÖRÜYOR MU?

Hasret Gizem Kurt[1], Deniz Celik[2*], Murat Yildiz[1], Ozlem Ertan[1]

[1]Health Sciences University, Ankara Atatürk Sanatorium Education and Research Hospital, Department of Pulmonology, Ankara, Türkiye. [2]Alanya Alaaddin Keykubat University, Faculty of Medicine, Department of Pulmonology, Alanya, Antalya, Türkiye.

ABSTRACT

Objective: Several studies have shown low mean corpuscular hemoglobin concentration (MCHC) associations with mortality and poor clinical course in conditions associated with chronic inflammation, such as cardiac failure and COPD. Thus, this study aimed to determine the link between MCHC and readmission mortality in a large patient population with a minimum of 1 year of follow-up.

Methods: We recorded clinical data at admission, laboratory data, the number of admissions to the emergency room due to acute exacerbation of chronic obstructive pulmonary disease (AECOPD) following the discharge of the last patient recruited, the number of admissions to the pulmonology unit, and the number of intensive care unit admissions between 2018 and 2019. The follow-up duration ranged between 12 and 36 months.

Results: A total of 339 patients were included. Based on a ROC analysis, the cut-off value for MCHC was 32.35 g/dl. Comparison of clinical data according to this cut-off value showed an increase in the incidence of pneumonia during admission, hypercapnic respiratory failure, need for noninvasive mechanical ventilation (NIV), and the number of intensive care unit admissions within one year, as well as reduced survival in non-anemic subjects with MCHC ≤ 32.35 g/dL. In multivariate cox-regression analysis, MCHC was not an independent predictor of mortality risk.

Conclusion: We recommend careful monitoring and assessing comorbidities in acute exacerbation of COPD patients with low MCHC but without anemia. MCHC was not found to be an independent predictor of mortality, but there was a significant correlation between MCHC and survival in patients without anemia.

Keywords: COPD, MCHC, mortality, anemia

ÖZ

Amaç: Birkaç çalışma, düşük ortalama korpüsküler hemoglobin konsantrasyonunun (MCHC), kalp yetmezliği ve KOAH gibi kronik inflamasyonla ilişkili koşullarda mortalite ve kötü klinik seyir ile ilişkisini göstermiştir. Bu nedenle, bu çalışmada, minimum 1 yıllık takip süresi olan geniş bir hasta popülasyonunda MCHC ile yeniden yatış mortalitesi arasındaki bağlantıyı belirlemeyi amaçladık.

Yöntem: 2018 ve 2019 yılları arasında hastaneye kabul sırasındaki klinik veriler, laboratuvar verileri, kronik obstrüktif akciğer hastalığının akut alevlenmesi nedeniyle acil servise başvuru sayısı, göğüs hastalıkları ünitesine yatış sayısı ve yoğun bakım yatış sayısı, çalışmaya alınan son hastanın taburcu edilmesini takiben kaydedildi. Takip süresi 12 ile 36 ay arasında değişmekteydi.

Bulgular: Toplam 339 hasta dahil edildi. MCHC için eşik değeri 32.35 g/dl olarak bulundu. Bu cut-off değerine göre klinik verilerin karşılaştırılması yapıldı. 1 yıllık takip süresi içinde yeniden başvurularda pnömoni, hiperkapnik solunum yetmezliği, non-invaziv mekanik ventilasyon (NIV) ihtiyacı ve yoğun bakıma yatış sayısında artış olduğu gösterildi. Ayrıca MCHC ≤ 32.35 g/dL olan ve anemik olmayan kişilerde sağkalımın azaldığı gösterildi. Anemisi olmayan MCHC ≤ 32.35 g/dL grubunda artmış mortalite ile ilişkili faktörler incelendiğinde, ileri yaş, demans varlığı, karaciğer yetmezliği, 1 yıl içinde KOAH akut alevlenmesine bağlı acil servis başvuru sayısı, yoğun bakım ünitesi başvuru sayısı ve başvuru sırasında NIV ihtiyacı olduğu görüldü. Fakat kabul sırasında elde edilen MCHC değeri, mortaliteyi öngörmede bağımsız bir değişken olmadığı görüldü.

Sonuç: Düşük MCHC'li ancak anemisi olmayan KOAH akut alevlenmesi hastalarında diğer komorbiditelerin dikkatli bir şekilde izlenmesini ve değerlendirilmesini öneriyoruz. MCHC değeri, mortaliteyi öngörmede bağımsız bir değişken olmadığı görüldü ancak anemisi olmayan hastalarda MCHC ile sağkalım arasında anlamlı bir ilişki saptandı.

Anahtar Kelimeler: KOAH, MCHC, mortalite, anemi

*** Corresponding author/İletişim kurulacak yazar:** Deniz Celik; Alanya Alaaddin Keykubat University, Faculty of Medicine, Department of Pulmonology, Alanya, Antalya, Türkiye.
Phone/Telefon: +90 5524036040 **e-mail/e-posta:** drdenizcelik@hotmail.com
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Introduction

COPD is the third leading global cause of death[1], with a trend toward progressively increasing mortality.[2] The most critical determinant of COPD mortality is the number of acute exacerbations during the disease.[3]
Approximately 10% to 30% of COPD patients have anemia[4], associated with reduced exercise capacity, perceived dyspnea, and need for oxygen support.[5] The purported causes of anemia in COPD include increased cytokine production due to chronic inflammation, iron deficiency, and anemia.[6,7] As a result of chronic inflammation, the incidence of cardiovascular diseases is also increasing, causing an increase in mortality. Baykal and Bulcun reported in their study that chronic hypoxemia in patients with COPD led to pulmonary vascular remodeling and increased pulmonary artery pressure.[8] In their study, Şahan and Bulut reported that as the clinical severity of COPD progresses, hypoxia increases, pulmonary hypertension appears, and some pathological changes occur in the right heart, which leads to atrial fibrillation.[9]
Only a few published studies offer insights into the relationship between COPD and iron deficiency. A cross-sectional study involving a multivariate analysis of lung capacity and serum nutrition parameters identified a direct link between the forced expiratory volume at 1 sec (FEV1) and serum iron levels.[10] MCHC is a hematological index of hemoglobin and total iron stores.[11,12] Studies have suggested that MCHC is a reliable parameter of functional iron status.[13]
In a study of 197 outpatients with chronic cardiac failure, Simbaqueba et al. showed that MCHC was a reliable prognostic indicator, particularly in those without anemia. These authors found a higher risk of mortality and increased admissions due to cardiac failure during a 5-year follow-up in patients with low MCHC. Such observations confirm the association between relatively low MCHC, chronic cardiac failure, and functional iron deficiency.[14] Again, Kento Sato et al. found higher 1-month mortality in AECOPD patients with low MCHC.[15]
The objective of this study was to evaluate the prognostic value of MCHC during the clinical course of AECOPD patients.

Methods

Study Population

All patients admitted to our tertiary chest diseases branch hospital were evaluated between January 2018 and January 2019 with AECOPD. AECOPD was defined as acute exacerbation, acute worsening of respiratory symptoms requiring antibiotic and or steroid therapy. According to the treatment they received and their clinical status, the patients were categorized into the following groups; mild acute exacerbation (no treatment), moderate acute exacerbation (steroid and or antibiotic therapy), severe acute exacerbation (steroid and or antibiotic therapy combined with noninvasive mechanical ventilation (NIV) for respiratory failure or the need for oxygen therapy). We recorded age, gender, and presence of obstructive sleep apnea syndrome (OSAS), hypertension (HT), diabetes mellitus (DM), coronary artery disease (CAD), arrhythmia, dementia), thyroid dysfunction, cerebrovascular events, epilepsy, rheumatologic diseases, smoking history in all patients over 18 years of age hospitalized due to AECOPD. Also, we recorded the number of emergency room visits, hospital admissions, and intensive care unit admissions during a minimum one-year follow-up after discharge.
Survival of the patients was calculated by the difference between AECOPD and hospitalization and date of death. We determined mortality during a minimum follow-up of 1 year for all patients after the last recruited patient.
The study did not include patients with chronic heart failure, malignancy, interstitial lung disease, and inflammatory disease along with AECOPD. Among 491 patients who presented with AECOPD within a year, 70 had chronic heart failure, 74 patients had malignancy, four had interstitial lung disease, one had organized pneumonia, and one had acute cerebrovascular ischemia, and two patients had myotonic dystrophy. In total, 339 patients were included in the study. A hemoglobin level of < 13 g/dl and < 12 g/dl was considered diagnostic for anemia in male and female patients.
This study was designed to obtain data retrospectively. The ethics committee of Ankara Keçiören Training and Research Hospital approved this retrospective study. (Number of Approval: 2012-KAEK 15/2418; Date of Approval: November 9th, 2021).

Statistical Analysis

We used SPSS 22.0 (Statistical Program for Social Sciences) software package for statistical analysis. Kolmogorov Smirnov test was used to determine variables with normal distribution. The homogeneity of the variance was tested with Levene's test. For quantitative data, descriptive statistics such as arithmetic mean and standard deviation were presented for data with normal distribution and median (min-max) for data without normal distribution. Also, we provided frequencies and percentages for qualitative data. We compared comparisons between two independent groups with Student's t-test for normal distribution and Mann-Whitney U test for data without normal distribution. We compared qualitative data between the groups with chi-square or Fisher's exact test. Univariate and multivariate Cox regression analyses were carried out to determine the effect of risk factors on mortality. Cut-off values for the relationship between mortality and MCHC were identified using ROC under the curve analysis. Survival curves according to median MCHC and exacerbation severity were prepared using the Kaplan-Meier methodology, and log-rank tests were used to compare the groups. We evaluated the association between continuous variables with Spearman's correlation analyses. All statistical analyses were

performed at a 95% confidence interval and p-level of < 0.05.

Results

The mean age of 339 patients included in this study was 70.54, and 62.8% were male. The minimum follow-up period of the patient population was one year. Demographic data and comorbidities are shown in Table 1. The smoking histories of the patients were classified as active users, quitters, and non-smokers. According to this classification, 19.2% of the patients were active users, 52.4% quit, and 28.4% never smoked. The median hemoglobin value was 13.7 g/L (0.11-113.7). The median MCHC value was 31.8 g/dL (20.9-54.9). According to the ROC analysis to predict mortality (AUC=0.589, 95% CI:0.527-0.652, p=0.006), we found the best cut-off point of the MCHC value to be 32.35, the sensitivity of MCHC at this point was 75.4%, the selectivity was 40.6%, positive and negative predictive values were respectively; 42% and 75% (Figure 1).

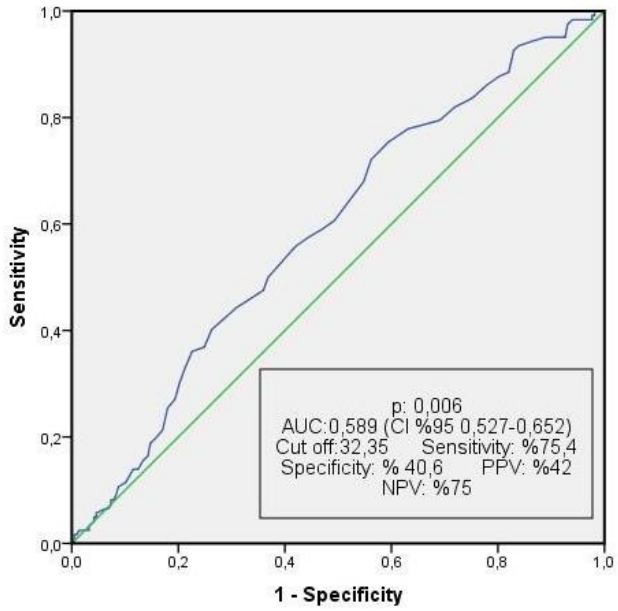

Figure 1. ROC curve of MCHC levels to predicting mortality

Table 1. Demographic characteristics of cases according to MCHC levels regarding the cut-off point obtained from ROC analysis

		TOTAL (n=339)	MCHC ≤ 32,35 (n=122)		MCHC > 32,35 (n=217)		p-value
		n (%)	n	%	n	%	
Gender	Female	126 (37.2%)	93	(42.1%)	33	(28.0%)	**0.010**
	Male	213 (62.8%)	128	(57.9%)	85	(72.0%)	
Age (year)		70.54 ± 10.86	71.20±10.64		66.93±11.22		0.122
Smoking History	None	96 (28.4%)	70	(31.7%)	26	(22.2%)	0.185
	Active	65 (19.2%)	41	(18.6%)	24	(20.5%)	
	Quit	177 (52.4%)	110		67	(57.3%)	
Diabetes Mellitus		107 (31.6%)	74	(33.5%)	33	(28.0%)	0.298
Hypertension		158 (46.6%)	103	(46.6%)	55	(46.6%)	0.999
Obstructive Sleep Apnea Syndrome		54 (15.9%)	36	(16.3%)	18	(15.3%)	0.804
Coronary Artery Disease		73 (21.5%)	46	(20.8%)	27	(22.9%)	0.659
Cardiac Arrhythmia		23 (6.8%)	15	(6.8%)	8	(6.8%)	0.998
Hypothyroid		7 (2.1%)	4	(1.8%)	3	(2.5%)	0.699
Pneumonia		268 (79.1%)	183	(82.8%)	85	(72.0%)	**0.020**
Bronchiectasis		105 (31.0%)	62	(28.1%)	43	(36.4%)	0.112
Anemia		101 (29.8%)	75	(33.9%)	26	(22.0%)	0.022
Moderate Liver Failure		27 (8%)	20	(9.0%)	7	(5.9%)	0.339
Severe Liver Failure		3 (0.9%)	3	(1.4%)	-	-	
Moderate-Severe Kidney Failure		71 (20.9%)	44	(19.9%)	27	(22.9%)	0.522
MCHC level		31.8 (20.9-54.9)	-	-	-	-	-

MCHC: Mean corpuscular hemoglobin concentration

There was a statistically significant difference in long-term mortality between the groups with MCHC higher and lower than 32.35 g/dL (p<0.001). Accordingly, we found the mortality rate higher for the group with MCHC≤ 32.35 g/dL (p<0.011).

MCHC less than 32.35 is associated with mortality in patients who had emergency admissions in the previous year. MCHC less than 32.35 is associated with mortality in patients who have had acute exacerbations in the previous year. For last year, there was no statistically significant relationship between the MCHC cut-off value

and mortality in AECOPD patients admitted to the intensive care unit. For the group with MCHC≤32.35, the incidence of hypercapnic respiratory failure ($PaCO_2$> 45 mmHg) (p<0.001), the rate of noninvasive mechanical ventilation use during hospitalization (p<0.001), the rate of admission to intensive care unit within one year (p=0.002) and pneumonia incidence (p=0.020) were found to be higher compared to the group with MCHC>32.35 (Table 2).

Table 2. Clinical characteristics of cases according to MCHC levels regarding the cut-off point obtained from ROC analysis

Clinical characteristics of the patients	TOTAL (n=339)		MCHC ≤ 32.35 (n=221)		MCHC > 32.35 (n=118)		p
	n	(%) or mean (min-max)	n	(%) or mean (min-max)	n	(%) or mean (min-max)	
Moderate COPD Acute Exacerbation	18	5.3%	10	4.5%	8	6.8%	0.378
Severe COPD Acute Exacerbation	321	94.7%	211	95.5%	110	93.2%	
Acute Hypercapnic Respiratory Failure	60	17.7%	45	20.4%	15	12.7%	0.079
Chronic Hypercapnic Respiratory Failure	279	82.3%	176	79.6%	103	87.3%	
Admissions to Emergency Service within 1 Year	304	89.7%	201	66.1%	103	33.9%	**0.001****
Admissions with Acute Exacerbation of COPD within 1 Year	334	98.5%	218	65.3%	116	34.7%	**0.002****
Admissions to the Intensive Care Unit with Acute Exacerbation of COPD within 1 Year	52	15.3%	44	84.6%	8	15.4%	0.129
Decompensated Respiratory Failure in Arterial Blood Gas at Admission (pH<7.35)	57	17.0%	43	19.6%	14	12.0%	0.074
Hypercapnic Respiratory Failure in Arterial Blood Gases at Admission ($PaCO_2$> 45 mmHg)	145	42.8%	113	51.1%	32	27.1%	**<0.001**
Hemoglobin levels (g/dL)	-	13.6 (8.30-21.1)	-	13.3 (8.30-18.3)	-	14.2 (10.2-21.1)	**0.001**
Survival duration (months)	-	30 (1-39)	-	29 (1-39)	-	31 (1-39)	**0.011**
Use of nasal oxygen therapy during hospitalization	319	94.1%	210	95%	109	92.4%	0.324
Use of NIV therapy during hospitalization	92	27.1%	75	33.9%	17	14.4%	**<0.001**

COPD: Chronic Obstructive Pulmonary Disease
NIV: Noninvasive Mechanical Ventilation

There was a significant correlation between MCHC and survival in patients without anemia (p=0.005). Table 3 shows the results of univariate Cox proportional hazards regression analysis for all possible factors thought to have an impact on overall survival. Univariate analysis showed associations between mortality and advanced age (HR=1.030, 95% CI:1.012-1.048, p=0.001), dementia (HR=4.539, 95%CI:1.845-11.167, P=0.001), pneumonia (HR=1.828, 95% CI: 1.095-3.051, P=0.021), liver failure (HR=1.999, 95% CI: 1.212-2.298, P=0.007), higher number of admissions due to AECOPD within a 1-year period (HR=1.082, 95%CI:1.009-1.160, p=0.027), more prolonged stay at intensive care unit due to acute exacerbation of COPD within a 1 year period (HR=1.354, 95% CI: 1.172-1.563, p<0.001), use of NIV at admission (HR=1.759, 95% CI:1.217-2.541, p=0.003), and MCHC ≤ 32.35 (HR=0.559, 95% CI: 0.370-0.844, p=0.006).

We fitted values associated with mortality in the univariate cox regression analysis into multivariate cox regression models. This analysis showed that advanced age, dementia, liver failure, increased emergency unit visits and ICU admissions due to COPD exacerbation within one year, and increased need for NIV during admission was associated with mortality. At the same time, MCHC was not an independent predictor of mortality (Table 4).

Discussion

MCHC is a hematologic laboratory parameter utilized for diagnosing and monitoring patients with iron deficiency anemia and measuring the oxygen-carrying capacity of red blood cells.[13,16]

Iron deficiency associated with chronic inflammation is a known indicator of poor long-term prognosis, independent of anemia.[17] Median Hg in our patient group was 13.7 g/dL, and MCHC was 31.8 g/dL. Overall, 101 patients were found to have anemia. However, we did not measure serum iron levels in our participants. According to our observations, MCHC had prognostic significance in patients without anemia.

Table 3. Univariate cox regression analysis to identify variables that predict survival in patients with chronic obstructive pulmonary disease with acute exacerbation

Univariate Cox Regression	Wald	p	HR	95% CI for HR
Age	11.079	**0.001**	1.030	(1.012-1.048)
Sex (ref: Female)	0.083	0.774	0.948	(0.658-1.365)
Smoking History	1.411	0.235	0.743	(0.455-1.213)
MCHC	5.613	**0.018**	0.907	(0.837-0.983)
Diabetes Mellitus	0.219	0.640	1.094	(0.751-1.593)
Hypertension	0.017	0.896	1.024	(0.718-1.462)
Obstructive Sleep Apnea Syndrome	3.918	**0.048**	0.559	(0.315-0.994)
Coronary Artery Disease	0.058	0.809	0.947	(0.610-1.470)
Arrhythmia	0.531	0.466	1.272	(0.666-2.429)
Dementia	10.844	**0.001**	4.539	(1.845-11.167)
Pneumonia	5.317	**0.021**	1.828	(1.095-3.051)
Bronchiectasis	0.929	0.335	0.824	(0.557-1.221)
Presence of Liver Failure	7.351	**0.007**	1.999	(1.212-2.298)
Hypercapnic Respiratory Failure (ref:acute)	1.239	**0.266**	0.782	(0.507-1.206)
Number of admissions to the emergency service within 1 year	3.282	0.070	1.011	(0.999-1.023)
Number of admissions with acute exacerbation of COPD within 1 year	4.883	**0.027**	1.082	(1.009-1.160)
Number of admissions to the intensive care unit with acute exacerbation of COPD within 1 year	17.014	**<0.001**	1.354	(1.172-1.563)
Arterial Blood Gas HCO_3 at Admission	0.016	0.416	1.013	(0.982-1.045)
Arterial Blood Gas at Admission (ref: decompensated)	1.321	0.250	0.773	(0.497-1.200)
Arterial Blood Gas at Admission (ref: $PaC0_2$>45)	2.240	0.134	1.312	(0.919-1.871)
Acute exacerbation (ref: moderate acute exacerbation)	0.017	0.896	0.950	(0.443-2.039)
Hemoglobin	1.775	0.183	1.019	(0.991-1.047)
Anemia	5.804	**0.016**	1.569	(1.088-2.262)
Nasal Oxygen Use during Hospitalization	0.041	0.839	1.082	(0.504-2.321)
Use of NIV during Hospitalization	9.031	**0.003**	1.759	(1.217-2.541)

Wald: test statistics, HR: hazard radio, Statistically significant p-values are in bold.

Table 4. Multivariate cox regression analysis applied to identify variables that predict survival in patients with chronic obstructive pulmonary disease with acute exacerbation

Multivariate Cox Regression	Wald	p	HR	95% CI for HR
Model 1				
Age	12.368	**<0.001**	1.033	(1.014-1.051)
Sex (ref: Female)	0.466	0.495	1.140	(0.783-1.659)
MCHC	6.936	**0.008**	0.894	(0.823-0.972)
Model 2				
Age	8.587	**0.003**	1.028	(1.009-1.048)
Sex (ref: Female)	2.151	0.142	1.335	(0.907-1.963)
MCHC	3.639	0.056	0.921	(0.846-1.002)
Dementia	5.199	**0.023**	3.013	(1.168-7.778)
Pneumonia	3.631	0.057	1.657	(0.986-2.785)
Presence of Liver Failure	7.777	**0.005**	2.068	(1.241-3.447)
Anemia	3.210	0.073	1.413	(0.968-2.062)
Model 3				
Age	18.738	**<0.001**	1.043	(1.023-1.063)
Sex	0.216	0.642	1.095	(0.746-1.609)
MCHC	5.770	**0.016**	.905	(0.834-0.982)
Number of admissions to the emergency service within 1 year	8.868	**0.003**	1.140	(1.046-1.243)
Number of admissions to the intensive care unit with acute exacerbation of COPD in 1 year	15.004	**<0.001**	1.367	(1.167-1.602)
Model 4				
Age	16.688	**<0.001**	1.040	(1.021-1.060)
Sex	1.732	0.188	1.295	(0.881-1.902)
MCHC	3.310	0.069	0.927	(0.854-1.006)
Use of NIV during Hospitalization	11.344	**0.001**	1.991	(1.334-2.973)

Wald: test statistics, HR: hazard radio, Statistically significant p-values are in bold.

In a study by Huang et al., low MCHC in patients admitted to the intensive care unit following acute myocardial infarction was associated with an increased risk of in-hospital mortality.[18] In another study from 2013 by Simbaqueba et al.[14] involving patients with systolic heart failure, those with lower MCHC had an elevated risk of death and transplantation and an increased likelihood of hospitalization due to heart failure.

In the study of Kento Sato et al. for patients followed up with COPD acute exacerbation, a correlation was found between low MCHC value and 30-day mortality.[15] They included 195 AECOPDs patients and a one-month follow-up period for assessing mortality. We enrolled 339 patients in our study, with a follow-up period of 1-3 years.

This study investigated the prognostic value of MCHC for patients treated in the hospital (intensive care, emergency, or chest disease ward) due to AECOPD. When the patients were examined according to the determined cut-off value (32.35 g/dL), we found the survival duration lower for the group with MCHC≤32.35 g/dL. For patients with MCHC≤32.35, the incidence of hypercapnic respiratory failure during hospitalization ($PaCO_2$>45 mmHg), the rate of noninvasive mechanical ventilation use during hospitalization, the rate of admission to intensive care unit within one year, and pneumonia incidence were found to be higher. In our Cox regression analysis, MCHC did not appear to be a strong predictor of mortality risk, contrasting with many other reports. Despite a large sample size, this might have resulted from several factors, such as the retrospective design of our study, confounding factors inherently present in AECOPD, and lack of accurate information on whether AECOPD caused each death. Further prospective studies may be required to elucidate better the association between MCHC and mortality in this setting.

MCHC ≤ 32.35 g/dL was statistically significant in determining the overall survival (OS) value in our ROC analysis of MCHC measurements in estimating the survival duration. When we compared the MCHC cut-off values with other studies, we inferred that the cut-off value was similar to our research's. Simbaqueba et al. reported that the patients whose MCHC was 32.7 g/dL and below had the worst prognosis.[14] In a 2016 paper, Huang et al. reported that patients with an MCHC < 32.8 g/dl had an increased risk of in-hospital death.[18] In the study of Kento Sato et al., the MCHC cut-off value was 31.6 g/dL.[15] In our study, the MCHC cut-off value was 32.35.

In our multivariate regression models, factors associated with a significantly increased mortality risk at one year included advanced age, dementia, liver failure, a higher number of emergency room visits and ICU admissions due to COPD exacerbations during one year, and NIV use. These findings support that low MCHC may indicate poor outcomes in AECOPD patients.

One of the most important limitations is that our study is a mono-centered, retrospective data analysis and lacks a common cut-off value that we can compare. Due to the study's retrospective nature, we could not obtain serum iron levels because it isn't done regularly.

Future studies will investigate whether the MCHC value is an effective biomarker in determining the indication for intensive care hospitalization and the use of NIV in patients followed up with AECOPD. A second study should examine the association between the predictive power of MCHC and infection and determine their ability to distinguish non-bacterial AECOPD from bacteria.

Patients with AECOPD often admit to emergency services. A Hemogram examination is among the first laboratory tests requested in the emergency admissions of these patients. It is cheap and easy to access. The study's most vital feature is its exclusion of the cases with malignancy and chronic heart failure known to affect the MCHC value and its comparatively larger sample size and longer follow-up vs. most previous studies.

Conclusion

Our results showed an increased risk of ICU admission, hypercapnic respiratory failure, need for ICU use, and pneumonia among patients with lower MCHC. Clinicians should pay adequate attention to low MCHC levels among AECOPD patients regardless of anemia.

Compliance with Ethical Standards

Health Sciences University Keçiören Education and Research Hospital, Clinical Studies Ethic Board Decision date: 09.11.2021, Decision number:2012-KAEK-15/2418.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contribution

Authors contributed equally to this work.

Financial Disclosure

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Araştırma Makalesi | Research Article

NAZAL VALV VE TİP STABİLİTESİNİ KORUMAK AMACIYLA MODİFİYE SLİDİNG ALAR KARTİLAJ TEKNİĞİ UYGULANAN HASTALARIN RETROSPEKTİF ANALİZİ

A RETROSPECTIVE ANALYSIS OF THE MODIFIED SLIDING ALAR CARTILAGE TECHNIQUE FOR PRESERVING THE NASAL VALVE AND NASAL TIP STABILITY

Ömer Faruk Ünverdi[1*], Sercan Yücel[2]

[1] Serbest Hekim, Çanakkale, Türkiye. [2]Erciyes Üniversitesi Tıp Fakültesi Plastik Rekonstrüktif ve Estetik Cerrahi ABD, Kayseri, Türkiye.

ÖZ

Amaç: Rinoplastide, internal nazal valv ve eksternal nazal valv stabilitelerinin sağlanması hem görsel hem de fonksiyonel sonuçları doğrudan etkilemektedir. Scroll ligaman kompleksinin bütünlüğü korunarak hazırlanan scroll ligaman kompleksi destekli alar kartilaj flebi internal ve eksternal valv stabilitesinin korunmasında önemli bir görev üstlenmektedir.

Yöntem: Nazal deformite nedeniyle opere edilen 25 primer rinoplasti hastası çalışmaya dahil edildi. Nazal flep, scroll ligaman kompleksinin horizontal ve vertikal lifleri tamamen korunarak kaldırıldı. Tip plasti aşamasında alt lateral kartilaj, kaudalde 5 mm lik segment korunacak şekilde kaudal ve sefalik iki parçaya ayırıldı. Sefalik parça scroll ligaman kompleksi ile bütünlüğü bozulmadan flep olarak kaudal parçanın altında hazırlanan cepe yerleştirildi.

Bulgular: Çalışmaya katılan hastaların 19'u Kadın 6'sı Erkek idi. Hastaların yaş ortalaması 29,6 (18-58) yıl idi. Takip süresi 14,5 (6-21) ay idi. Hastaların tümü nefes alıp vermede belirgin iyileşme olduğunubildirdi. Görünümün 24 hastayı memnun ettiği 1 hastayı ise memnun etmediği sözel olarak öğrenildi. Kıkırdakların dışarıdan görünürlüğünde artma veya palpe edilmesi gibi bir komplikasyon gözlenmedi. Hastaların ameliyat sonrası muayenelerinde, internal veya eksternal nazal valv yetmezliği bulguları gözlenmedi.

Sonuç: Scroll ligaman kompleksi vertikal ve longitudal liflerden oluşmaktadır. Kompleksin fonksiyonunu yerine getirebilmesi amacıyla bütünlüğünün korunması veya bütünlüğü bozulmuş olgularda tekrar onarılması gerekmektedir. Scroll ligament destekli alar kartilaj flebi, internal ve eksternal nazal valv stabilitesini sağlayan ve scroll ligaman kompleksini bir bütün olarak koruyan bir tekniktir.

Anahtar Kelimeler: Rinoplasti, scroll ligament, nazal valv, alar kartilaj

ABSTRACT

Objective: Preservation of internal nasal valve and external nasal valve stabilities directly affects both visual and functional results of the rhinoplasty. Scroll ligament complex suspended alar cartilage flap, plays an important role in maintaining the stability of internal and external valve surgery.

Methods: Twenty-five primary rhinoplasty patients with nasal deformity were included in the study. The horizontal and vertical fibers of the scroll ligament complex was completely preserved during nasal flap elevation. The lower lateral cartilage was divided into caudal and cephalic parts. The cephalic piece was placed in the pocket prepared under the caudal piece as a flap without losing its integrity with the scroll ligament complex.

Results: Of the patients participating in the study, 19 were Female and 6 were Male. The mean age of the patients was 29.6 years. Follow-up time was 14,5 months. It was verbally asked and responses recorded, that all patients were satisfied with changes in breathing with 24 patients were satisfied with the appearance of the nose and 1 patient did not. No complications such as increased visibility or palpation of cartilage were observed. No signs of internal or external nasal valve failure were observed in the postoperative examinations of the patients.

Conclusion: Scroll ligament complex consists of vertical and longitudinal fibers. In order to perform the function of the complex, its integrity must be preserved or repaired in cases integrity is impaired. Scroll ligament-supported alar cartilage flap is a technique that provides internal and external nasal valve stability and protects the scroll ligament complex as a single unit.

Keywords: Rhinoplasty, scroll ligament, nasal valv, alar cartilage

***İletişim kurulacak yazar/Corresponding author:** Ömer Faruk Ünverdi; Serbest Hekim, Çanakkale, Türkiye
Telefon/Phone: +90 541 619 65 69 **e-posta/e-mail:** unverdiomer@gmail.com
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Giriş

Alt lateral kıkırdaklar burnun alt 1/3'lük bölümünü oluşturan, medial, middle ve lateral segmentler olarak adlandırılan birbiri ile devamlılık halinde olan segmentlerden oluşan yapılardır. Yukarıda üst lateral kıkırdakların kaudal kısmı ile devamlılık komşudur. Yapısal olarak 6 farklı şekilde görülebilen alt lateral kıkırdaklar düzgün-konveks (%10), konveks- konkav (%30), konkav-konveks (%25), konkav-konveks-konkav (%25), konkav (%5), ve tamamen düzensiz (%5) yapıda sınıflandırılmaktadır.[1] Alt lateral kıkırdağın şekli, yüksekliği ve üst lateral kıkırdaklar ile olan bütünlüğü burnun hem görsel hem de fonksiyonel özelliklerini doğrudan etkilemektedir.[2,3]

Her iki tarafta yer alan alt lateral kıkırdakların birbirleri ve septum ile aralarında bulunan ligamentlerin bütünlüğünün korunması veya onarılması ameliyat sonrası süreçte tip desteğinin ve stabilitesinin korunmasında önemli rol oynamaktadır.[4] Alt lateral kıkırdakların sefalik sınırı ile üst lateral kıkırdakların kaudal sınırı arasında yerleşim gösteren ve bu kıkırdakları birbirine bağlayan yapı scroll ligament olarak adlandırılmaktadır. Scroll ligamentin sadece alt ve üst lateral kıkırdakar arasında yer alan longitudinal liflerden oluştuğu düşünülürken, Saban ve Polselli transversalis kasından longitudinal liflere uzanan vertikal liflerin olduğunu da göstermiştir. Derin SMAS ile internal nazal valv arasında uzanım gösteren vertikal liflerin korunması, internal nazal valv stabilitesinin korunmasına katkıda bulunabilmektedir.[4,5]

Nazal tip plastide burun ucuna istenilen şekilin verilmesi ameliyat sonucunu doğrudan etkileyen en önemli faktörlerden birisidir. Burun ucu projeksiyonu ve rotasyonunun kararlaştırıldığı bu aşama, burun şeklinin son halinin elde edilmesinde en önemli aşamayı oluşturmaktadır.[6-9]

Burun ucundaki bulböziteyi azaltmak amacıyla en sık uygulanan ve en etkin yöntem alt lateral kıkırdağın lateral ve middle segmentlerinden sefalik eksizyonudur.[10,11] Sefalik eksizyon esnasında lateral segmentin 5 mm den daha dar planlanması, hava akımına karşı en fazla direncin gözlendiği eksternal nazal valvte zayıflamaya ve solunum problemlerine neden olmaktadır.[12,13] Hava yollarında daralmayı engellemek ve daha güzel bir burun ucu hattı oluşturabilmek amacıyla kıkırdak greftleri yaygın olarak kullanılmakla birlikte, greftin rezorbe olması, dışarıdan hissedilmesi veya greft malpozisyonu gibi riskler tamamıyla elimine edilememektedir.[14-16]

Scroll ligaman kompleksi üst ve alt lateral kıkırdaklar arasında bulunan önemli bir anatomik yapıdır. Longitudinal ve vertikal lifleri bulunan bu kompleks, internal nazal valvin stabilitesinde önemli rol üstlenmektedir.[4] Son dönemlerde yapılan çalışmalarda, scroll ligaman kompleksinin önemi belirgin şekilde ifade edilmekte ve havayolu stabilitesini güçlendirmek amacıyla bu kompleksi koruyacak farklı teknikler uygulanmaktadır.[17,18]

Bu çalışmada alt lateral kıkırdağın sefalik kısmının, scroll ligaman kompleksi destekli sliding alar kartilaj flebin, alt lateral kıkırdağın kaudal parçasını güçlendirmeye, alt lateral kıkırdaktaki konveksiteyi düzeltmeye ve burun ucu bulbözitesini azaltmaya yönelik etkileri değerlendirilmektedir.

Yöntem

Çalışma lokal etik kurulunun 2022/193 nolu onayı alınarak yapılmıştır. Çalışmaya Ağustos 2018 - Kasım 2019 tarihleri arasında opere edilen scroll ligaman kompleksi destekli sliding alar kartilaj flebi uygulanan 25 primer rinoplasti hastası dahil edildi. Hastalar ameliyat öncesinde yazılı ve sözlü olarak bilgilendirildi ve onamları alındı. Operasyon sonrası takiplerinde hastaların sözel olarak solunum ve görünüm memnuniyetleri değerlendirildi. Tip desteği manuel olarak değerlendirildi.

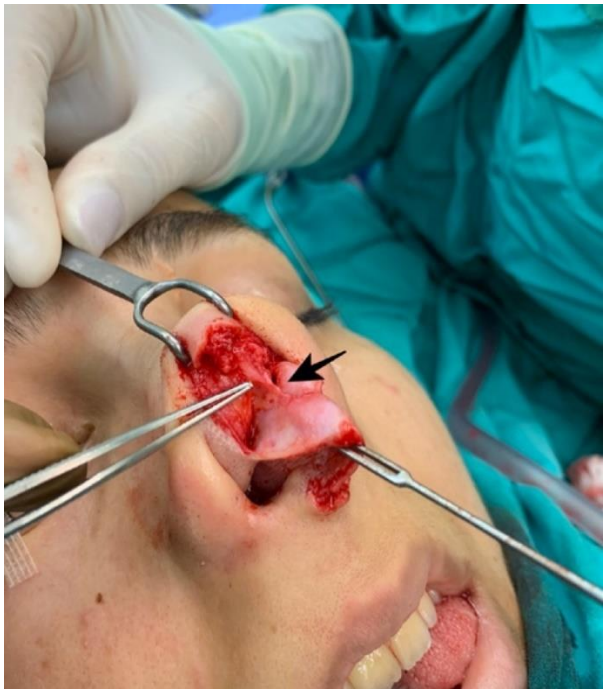
Cerrahi Teknik

Hastalar genel anestezi altında, açık rinoplasti yaklaşımı ile opere edildi. Operasyon öncesinde 1/200000lik adrenalinli lidokain enjeksiyonu yapıldı. Nazal flep alt lateral kıkırdak ve üst lateral kıkırdak seviyelerinde scroll ligaman korunarak subperikondrial planda, nazal kemik seviyesinde ise subperiostal planda disseke edilerek kaldırıldı (Şekil 1). Mukoperikondrial ve mukoperiosteal flepler kaldırılarak kıkırdak ve kemik septum eksplore edildi. Solunum güçlüğü yaşayan hastalarda kıkırdak septum skorlanarak ve 5-0 PDS dikişler kullanılarak düzeltildi. Kemik septumdan pasajı tıkayan spurlar törpülenerek pasaj açıldı. Dorsal hump eksizyonundan sonra low to low ve transvers osteotomiler yapılarak açık çatı kapatıldı. İnternal nazal valv bölgesinin daralmasını engellemek amacıyla iki taraflı spreader flepler septuma sütüre edilerek valv bölgesinde yeterli genişlik sağlandı. Planlanan tip rotasyonu ve projeksiyonunu elde etmek amacıyla yeni domlar işaretlendi. Alt lateral kıkırdağın kaudalinde 5 mm lik segment korunacak şekilde insizyon yapıldı ve kıkırdak kaudal ve sefalik parçalara ayırıldı (Şekil 2). kaudal parçanın altında, sefalik kıkırdağın genişliği ve yüksekliği boyunca cep hazırlandı. Scroll ligaman kompleksi destekli sefalik flep kaudal kıkırdağın altında hazırlanan ceplere ilerletildi. Kıkırdaklar sefalik sınırlarından atılan iki adet 6-0 PDS ile birbirine sütüre edildi (Şekil 3). Tip plasti tamamlandıktan sonra 1 adet septokolumellar sütür ile tip rotasyonu desteklenerek burun ucu şekillendirmesi tamamlandı.

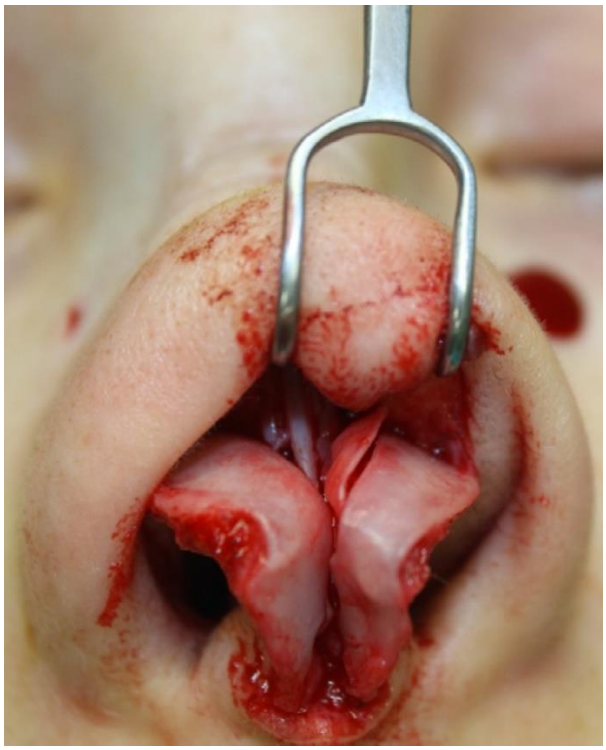
Bulgular

Çalışmaya dahil edilen hastaların 6'sı erkek 19'u kadındı. Yaş ortalaması 29,6 (18-58) yıldı. Takip süresi ortalama 14.5 (6-21) aydı. Hastaların 14' ü sadece burun şeklinden şikayetçiyken, 11 hastada hem burun şeklini beğenmeme hem de nefes almakta güçlük şikayetleri mevcuttu. Hastalarda takip dönemi boyunca nostril asimetrisi, alar retraksiyon veya pinch görüntüsü oluşmadı. Flebin kaudal kıkırdağa dikilmesinin konveks şekilli alt lateral kıkırdaklarda konveksiteyi azalttığı ve lateral segmentin daha yassı bir hal aldığı gözlendi. Hastalarda kıkırdağın

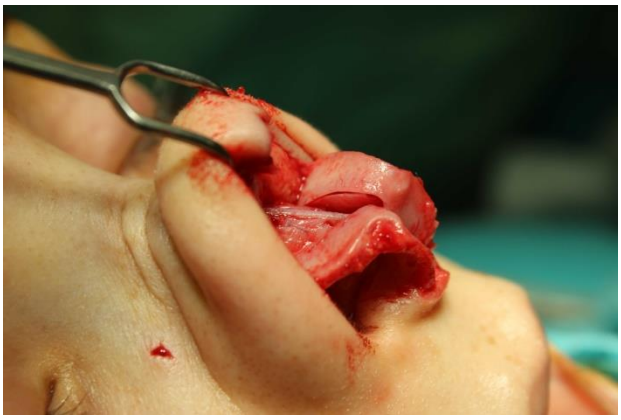
dışarıdan hissedilmesi veya kontür düzensizliği gözlenmedi (Şekil 4,5). Bir hasta burun ucu görünümünden memnun olmazken 24 hasta elde edilen sonuçtan memnun kaldı. Memnun olmayan hastaya revizyon ameliyatı planlandı. Hastaların tamamı nefes alıp vermede rahatlama olduğunu sözel olarak iletti. Derin inspiryumda hastaların hiçbirinde eksternal nazal valvde kollaps gözlenmedi.



Şekil 1. Nazal fiep kaldırılırken scroll ligamanın vertikal ve longitudinal liflerinin korunması

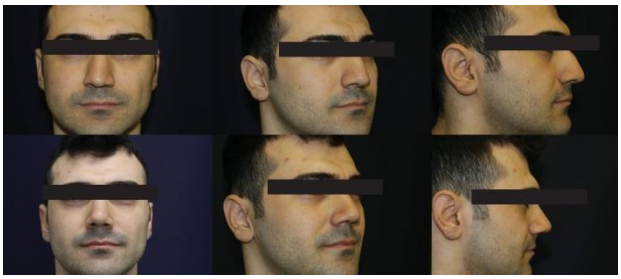


Şekil 2. Lateral crusun sefalik ve kaudal parçalara ayırılması



Şekil 3. Modifiye sliding alar kıkırdak flebinin kaudal parçanın altında hazırlanan cebe yerleştirilmesi sonrasında lateral crustaki konveksitenin işlem sonrasında düzelerek lateral crusun daha yassı bir görünüm kazanmış görüntüsü

Şekil 4. 18 yaşında kadın hastanınpreoperatif ve postoperatif 6. ay görüntüsü



Şekil 5. 27 yaşında erkek hastanın preoperatif ve postoperatif 6. ay görüntüsü

Tartışma

Rinoplastide tüm ünitelerin birbiri ile uyum içerisinde olması başarılı sonuçların olmazsa olmaz şartıdır. Farklı segmentlerin birbiri ile olan ilişkisi nedeniyle bu uyumun burun ucunda elde edilmesi için iyi bir anatomi bilgisi ve tecrübeye ihtiyaç duyulmaktadır. Temel olarak alt lateral kıkırdağın segmentlerinin şekillendirildiği burun ucunda, lateral segmentin sefalik kısmı bulböziteyi azaltabilmek amacıyla genellikle eksize edilmektedir.[9,10] Yanlış planlama nedeniyle 6 mm den daha geniş bırakılan kaudal segment bulbözitenin devam etmesine, 6 mm den daha dar bırakılan kaudal segment ise eksternal nazal valv desteğinin azalmasına neden olmaktadır. Bu durum ameliyat sonrasında burunda şekil bozukluğu ve nefes almada güçlüğe neden olmaktadır. Bu problemleri aşabilmek amacıyla revizyon ameliyatına ihtiyaç duyulmakta ve genellikle kıkırdak greftleri kullanılarak bu sorun aşılabilmektedir.[18-21] Ancak kıkırdak greftlerinin kullanılması özellikle ince derili hastalarda kıkırdağın

dışarıdan elle hissedilebilmesi ya da görünür olması gibi problemlere neden olabilmektedir.[21-24]

Scroll ligaman kompleksi üst lateral kıkırdak ve alt lateral kıkırdak arasında yerleşim gösteren, internal nazal valvin stabilizasyonunda önemli bir görev üstlenen ve longitudinal-vertikal lifleri bulunan önemli bir anatomik yapıdır.[(4)] Scroll ligaman kompleksinin korunması, ameliyat sonrasında hem tip definisyonunu sağlamakta hem de internal ve eksternal nazal valv stabilitesini korumaya yardımcı olarak hava yolu tıkanıklıklarını engellemektedir.[4,16,17]

Taş, lateral segmentin sefalik kısmını eksize etmek yerine bu dokuyu süperior bazlı transpozisyon flebi olarak hazırlamış ve scroll ligaman ile bağlantısını bozmadan lateral segmentin kaudal parçasının dış yüzüne sütüre etmiştir. Bu uygulamanın hem lateral segmentteki konveksiteyi düzelttiğini hem de internal nazal valv açısını genişlettiğini saptamıştır.[17] Özmen ve ark, lateral segmentin sefalik kısmını kaudal kısımdan ayırdıktan sonra sadece marjinal insizyon sınırındaki mukozal bağlantıların korunduğu cepler hazırlamışlardır. 'Sliding alar cartilage flep' olarak tarifledikleri flebi hazırlanan cepe yerleştirerek kaudal kıkırdağa sütüre etmiştir. Sonuçta sliding alar kartilaj flebinin tip desteğini arttırdığını gözlemlemişlerdir.[18] Sliding alar kartilaj olarak tariflenen bu teknikte scroll ligamentin yalnız longitudinal lifleri korunmuştur. Tellioğlu ve Çimen, alt lateral kıkırdağın sefalik kısmını kaudal kıkırdağın altında hazırlanan cep içerisine turn over ederek sütüre etmiş ve bu tekniğin uzun dönemde alar pinch veya collapse gelişmesine neden olmadan başarılı sonuçların elde edilebileceğini bildirmişlerdir.[25]

Çalışmamızda Özmen ve ark tekniğinden farklı olarak scroll ligaman kompleksinin longitudinal ve vertikal lifleri tamamen korunmuştur. Bu sayede alt lateral kıkırdağın sefalik kısmı ve üst lateral kıkırdağın kaudal kısmı arasındaki bağlantı bozulmamıştır. Sonuç olarak scroll ligaman kompleksinin internal nazal valvi stabilize eden etkileri sefalik eksizyondan etkilenmemiştir. Ayrıca kaudal segmentin altında hazırlanan cep, marjinal insizyon sınırına kadar değil flep genişliğince hazırlanmıştır. Bu sayede kaudal ve sefalik kıkırdakların birbiri ile temasına engel oluşturacak boşluklar elimine edilmiş ve kaudal segmentin konveksitesi giderilmiş, daha yassı bir hal alması sağlanmıştır. Taş'ın uygulamasından farklı olarak ise sefalik tarafta flep olarak hazırlanan segmentin mukoza ile bağlantıları bozulmamıştır.

Tekniğin kolay ve uygulamasının hızlı olması önemli avantajları olmasına rağmen çalışmanın bazı kısıtlılıkları da bulunmaktadır. Bu tekniğin kıkırdak yapısı çok güçlü hastalarda alt lateral kıkırdağın dışarıdan belirginliğini arttırabileceğini düşünmekteyiz. Retrospektif değerlendirme yapılması nedeniyle internal nazal valv açısındaki değişiklikleri objektif bir şekilde ortaya koyan bir değerlendirme yapılamamış olması da sonuçlar üzerinde kısıtlılık oluşturmaktadır. Burun şekline yönelik değerlendirmelerin hasta beğenisine dayanarak yapılması ise subjektif bir değerlendirmedir ve kişisel bazda farklılık gösterebilmektedir. Bu değişikliklerin solunum güçlüğü olmayan salt rinoplasti hastalarında prospektif bir çalışma ile değerlendirilmesinin tekniğin etkilerinin daha iyi anlaşılmasına yardımcı olabileceğini düşünüyoruz.

Sonuç

Scroll ligaman kompleksi destekli alar kıkırdak flebi, sefalik eksizyona bağlı oluşabilen alar kollaps ve pinch deformitesini engelleyebilen etkin bir yöntemdir. Yine de objektif veriler ile etkinliğin tespit edilmesi için prospektif bir çalışmaya ihtiyaç vardır.

Etik Standartlara Uygunluk

2022/193 karar nolu lokal Etik Kurul Onayı SY tarafından alınmıştır.

Çıkar Çatışması

Yoktur.

Yazar Katkısı

ÖFÜ: Konsept, tasarım, denetim, materyaller, veri toplama ve işlem, analiz ve yorumlama, literatür taraması, yazma, orijinal taslak, yazma inceleme ve revizyon, kritik inceleme, yazılım ve görselleştirme desteği. SY: Konsept, tasarım, denetim, materyaller, veri toplama ve işlem, analiz ve yorumlama, yazma inceleme ve revizyon, kritik inceleme, yazılım ve görselleştirme desteği.

Finansal Destek

Yoktur.

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Araştırma Makalesi | Research Article

KOCAELİ ÜNİVERSİTESİ ARAŞTIRMA VE UYGULAMA HASTANESİ 2020 VE 2021 YILLARINA AİT ÖLÜM KAYITLARININ DEĞERLENDİRİLMESİ

EVALUATION OF DEATH RECORDS OF KOCAELI UNIVERSITY HOSPITAL FOR THE YEARS 2020 AND 2021

Mehmet Ertan Güner[1*], Çiğdem Çağlayan[1]

[1]Kocaeli Üniversitesi, Tıp Fakültesi, Halk Sağlığı Anabilim Dalı, Kocaeli, Türkiye.

ÖZ

Amaç: Ölüm kayıtları epidemiyolojik değerlendirmeler için özel bir önem taşırlar. Bu araştırmanın amacı Kocaeli Üniversitesi Araştırma ve Uygulama Hastanesi'nde (KOÜAUH) 2020 ve 2021 yıllarında meydana gelen ölümlerin kayıtlar üzerinden değerlendirilmesidir.

Yöntem: Tanımlayıcı tipte bir çalışmadır. KOÜAUH, 2020 ve 2021 yıllarıı ölüm kayıtları veri kaynağı olarak kullanılmıştır. Toplam 1049 ölüm kaydı incelenmiştir. Araştırmada incelenen değişkenler, kayıtlarda bulunan; ölen hastanın yaşı, cinsiyeti, ölüm tarihi, ölüm nedeni ve ölümün gerçekleştiği bölümdür.

Bulgular: 2020 yılında, 30.178 hasta yatırılmış, 552 ölüm gerçekleşmiş; 2021 yılında 39.151 hasta yatırılmış, 497 ölüm gerçekleşmiştir. Buna göre 2020 ve 2021 yıllarında hastane mortalite hızı sırasıyla; binde 18,29 ve binde 12,69 olarak hesaplanmıştır. Ölüm sayıları, aylara göre incelendiğinde COVID-19 nedenli ölümlerin Ekim 2020 ile Şubat 2021 arası dönemde pik yaptığı görülmüştür. Ölümlerin gerçekleştiği birimlere bakıldığında 2020 ve 2021 yıllarında sırasıyla %48,4 ve %57,9 ile erişkin yoğun bakım üniteleri ilk sırada gelmektedir. Ölümlerin, 2020 yılında %25,4 ve 2021 yılında %26,8 ile en yüksek oranda COVID-19 nedeniyle gerçekleştiği görülmüştür. 2020 yılında COVID-19 nedenli ölümlerde yaş ortalaması (66,96±15,85), COVID-19 dışı nedenli ölümlerin yaş ortalamasına (55,75±26,62) göre istatistiksel olarak anlamlı düzeyde yüksek görülmüştür. (p=0,000, Mann-WhitneyU testi).

Sonuç: 2020 ve 2021 yıllarında hastanede gözlenen ölümlerde cinsiyet ve yaş dağılımının benzer olduğu görülmüştür. Ancak COVID-19 özelinde ölümlerin anlamlı derecede ileri yaş grubunda meydana geldiği görülmüştür. Her iki yılda da ölümler en sık olarak yoğun bakım ünitelerinde meydana gelmiştir. KOÜAUH'de ölüm nedenleri içinde her iki yılda da COVID-19 nedenli ölümler ilk sırada yer almıştır. Tanımlayıcı bir çalışma olarak bulgular üzerinden nedensellik kurulamasa da ölümlerin dağılımı özellikle pandemi süreciyle ilgili fikir vermektedir.

Anahtar Kelimeler: Hastane ölüm kayıtları, mortalite hızı, ölüm istatistikleri

ABSTRACT

Objective: Death records are of special importance for epidemiologic evaluations. The aim of this study was to evaluate the deaths that occurred in Kocaeli University Hospital (KOUH) in 2020 and 2021 through records.

Method: It is a descriptive study. KOUH death records for 2020 and 2021 were used as data source. A total of 1049 death records were analyzed. The variables analyzed in the study were the age, gender, date of death, cause of death and the department where the death occurred.

Results: In 2020, 30.178 patients were hospitalized and 552 deaths occurred; in 2021, 39.151 patients were hospitalized and 497 deaths occurred. Accordingly, the hospital mortality rate was calculated as 18.29 per thousand and 12.69 per thousand in 2020 and 2021, respectively. When the number of deaths were analyzed by months, it was observed that deaths due to COVID-19 peaked between October 2020 and February 2021. Looking at the units where deaths occurred, adult intensive care units ranked first with 48.4% and 57.9% in 2020 and 2021, respectively. The highest proportion of deaths were due to COVID-19 with 25.4% in 2020 and 26.8% in 2021. In 2020, the mean age of deaths due to COVID-19 (66.96±15.85) was statistically significantly higher than the mean age of deaths due to non-COVID-19 (55.75±26.62) (p=0.000, Mann-WhitneyU test).

Conclusion: In 2020 and 2021, the gender and age distribution of deaths observed in the hospital were similar. However, it was observed that COVID-19-specific deaths occurred significantly in the older age group. In both years, deaths occurred most frequently in intensive care units. In both years, COVID-19-related deaths ranked first among the causes of death in KOUH. As a descriptive study, although causality cannot be established based on the findings, the distribution of deaths gives an idea about the pandemic process.

Keywords: Hospital death records, mortality statistics, mortality rate

***İletişim kurulacak yazar/Corresponding author:** Mehmet Ertan Güner; Kocaeli Üniversitesi, Tıp Fakültesi, Halk Sağlığı Anabilim Dalı, Kocaeli, Türkiye
Telefon/Phone: +90 506 701 33 77 **e-posta/e-mail:** erguner94@gmail.com
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Giriş

Toplumun genel sağlık düzeyinin değerlendirilmesi için en temel ve en değerli kaynak olan hayati olayların kayıtlarının tutulması hemen tüm ülkelerde zorunludur. Bu kayıtların en önemlileri doğum ve ölümle ilgili kayıtlardır. Ölüm kayıtları epidemiyolojik değerlendirmeler için özel bir önem taşırlar. Bunlar sadece bir toplumda belirli sürede meydana gelen ölüm sayısını değil bunun yanı sıra ölenlerin yaş, cinsiyet, meslek gibi özelliklerini ve ölümün tıbbi nedenini de bildirir. Böylece yaş, cinsiyet, meslek ve nedene özel ölüm hızları da elde edilerek ölüm bakımından yüksek risk taşıyan gruplar ile en çok ölüme yol açan hastalıklar/nedenler bulunur. Bu sayede hangi nedenlere ve gruplara yönelik önlemler ve uygulamalar geliştirileceği belirlenir.[1]

Ölüm Belgesi, en eski ve en kapsamlı halk sağlığı sürveyans sistemlerinin temelini oluşturan ölüm istatistiklerinin kaynağıdır.[2] Ancak ölümlerle ilgili istatistiklerin kalite ve geçerlilikleri ölüm nedenlerinin tam ve doğru olarak kaydedilmesine bağlıdır. Ülkemizde ölüm bildirimleri ve kayıtları 01.01.2013 tarihinden itibaren ulusal düzeyde standart olarak Ölüm Bildirim Sistemi (ÖBS) adı verilen sistem aracılığıyla gerçekleştirilmektedir.[3]

Ölüm nedeni istatistikleri, toplumların sağlığını izlemek ve değişen epidemiyolojik koşullara etkin bir şekilde yanıt vermek için önemli bir araçtır.[4] COVID-19 pandemisi sürecinde de ölüm bildirimleri, farklı popülasyonlarda ve yerlerde güncel bir sorun olan COVID-19 pandemisinin büyüklüğünü ölçmekte kullanılmış ve vaka bildirimlerine kıyasla pandemiyi izlemek için daha güvenilir bir gösterge olarak görülmüştür. COVID-19 pandemisine bağlı ölümlerin sayısının doğru bir şekilde ölçülmesi, her ülke ve bölge için pandeminin halk sağlığı üzerindeki etkisinin büyüklüğünü anlaması açısından çok önemlidir. Nitekim yapılan bir analiz, COVID-19 salgını nedeniyle ölen insan sayısının resmi makamlarca açıklanan rakamlardan kabaca üç kat daha fazla olabileceğini ortaya koymaktadır. Ayrıca bildirilen ölümlerin güvenilirliği, konuma göre ve zaman içinde büyük farklılıklar göstermektedir.[5]

Ölümle ilgili verilerin doğru şekilde ölçülmesi, pandemiyle ilgili öngörülerde bulunabilmek ve alternatif politika seçeneklerini araştırmak için doğrudan bir veri kaynağı olduğu gibi popülasyonlar arasındaki enfeksiyon-ölüm oranındaki değişimin belirleyicilerini anlamak için gereklidir.[5]

Hastane ölüm istatistikleri de hem toplumların sağlığını izlemek, hem sağlık hizmetlerinin etkinliğinin değerlendirilmesi, hem de hastane yönetimi açısından kullanılan önemli bir epidemiyolojik kaynaktır.[6]

Bu araştırmanın amacı Kocaeli Üniversitesi Araştırma ve Uygulama Hastanesi'nde (KOÜAUH) 2020 ve 2021 yıllarında meydana gelen ölümlerin; yaşa, cinsiyete ve nedenlerine göre dağılımlarının belirlenmesi; COVID-19 pandemisinin, ölüm nedenleri ve sayıları üzerindeki etkisinin hastane boyutunda gösterilmesi ve hastane hizmetlerinin planlamasına katkı sunmaktır.

Yöntem

Araştırma Tasarımı ve Popülasyonu

Tanımlayıcı tipteki bu araştırmada, retrospektif olarak KOÜAUH'de 2020 ve 2021 yıllarında gerçekleşen hastane ölümleri incelenmiştir. Araştırmaya 01 Ocak 2020-31 Aralık 2021 arası dönemde KOÜAUH'de ölen tüm hastalar dahil edilmiştir. Araştırmanın incelenen değişkenleri kayıtlarda bulunan ölen hastanın yaşı, cinsiyeti, ölüm tarihi, ölüm nedeni ve ölümün gerçekleştiği bölümdür.

KOÜAUH'de tüm ölüm nedenleri ICD-10'a göre kodlanıp kaydedilmektedir. Analizlerde kolaylık sağlaması açısından ölüm nedenleri araştırmacılar tarafından yeniden kategorize edilerek COVID-19 Enfeksiyonuna bağlı ölümler, neoplaziler, kardiyovasküler sistem hastalıkları (KVS), solunum sistemi hastalıkları, COVID-19 dışı enfeksiyon hastalıkları, yenidoğan ölümleri ve konjenital hastalıklar, nörolojik hastalıklar ve serebrovasküler olay (SVO), genitoüriner sistem (GÜS) hastalıkları, gastrointestinal sistem (GİS) hastalıkları ve diğer nedenler olarak gruplandırılmıştır. Ölümlerin gerçekleştiği servisler erişkin yoğun bakım üniteleri, pediatrik yoğun bakım üniteleri, dahili servisler, cerrahi servisler, pediatrik servisler ve acil servis olarak gruplandırılmıştır.

İstatistiksel Analiz

Veriler SPSS 21.0 istatistik paket programıyla incelenmiştir. Tanımlayıcı istatistikler uygulanarak kategorik değişkenler sayı ve yüzde olarak, numerik değişkenler ortalama, standart sapma ve ortanca değerler olarak gösterilmiştir. Normallik varsayımı Kolmogorov-Smirnov testi ile sınanmış olup, normal dağılıma uygun olmadığı için bağımsız iki grubun ortancalarının karşılaştırılmasında Mann-Whitney U testi, kategorik değişkenlerin karşılaştırılmasında Ki-kare testi kullanılmıştır. Ayrıca hastane mortalite hızı, bir yılda ölen hasta sayısının aynı yılda yatan hasta sayısına bölünmesi ile hesaplanmış olup binde olarak ifade edilmiştir. İstatistiksel anlamlılık değeri $p<0,05$ olarak belirlenmiştir.

Etik ve Yönetsel İzinler

Araştırmanın gerçekleştirilmesi ve ölüm verilerinin temin edilmesi için KOÜAUH Başhekimliğinden yazılı izin alınmıştır. Araştırmanın etik izni için Kocaeli Üniversitesi Girişimsel Olmayan Klinik Araştırmalar Etik Kurulundan onay alınmıştır (Karar No: KOÜ GOKAEK-2021/22.19. Proje No: 2021/355).

Bulgular

KOÜ Araştırma ve Uygulama Hastanesi'nde 2020 yılında toplam 30.178 hasta yatmış ve 552 ölüm gerçekleşmişken; 2021 yılında 39.151 hasta yatmış ve 497 ölüm gerçekleşmiştir. Buna göre 2020 ve 2021 yıllarında hastane mortalite hızı sırasıyla; binde 18,29 ve binde 12,69 olarak hesaplanmıştır. Her iki yılda ölenlerin yaş, cinsiyet ve ölüm nedenlerine göre dağılımları Tablo 1'de gösterilmiştir. Buna göre cinsiyet dağılımına bakıldığında

2020 yılında ölenlerin 329'unun (%59,6) erkek, 221'inin (%40) kadın; 2021 yılında ölenlerin 289'unun (%58,1) erkek, 208'inin (%41,9) kadın olduğu görülmüş ve yıllar arasında cinsiyet açısından istatistiksel farklılık saptanmamıştır(p=0,615). Ortanca yaş her iki yılda da 65 olarak saptanırken; 2020 yılında ölenlerin yaş ortalaması 58,64±24,82, 2021 yılında ise 59,26±24,06 olarak saptanırken istatistiksel bir farklılık bulunmamıştır (p=0,686). Ölüm nedenlerine göre incelendiğinde, ölümlerin 2020 yılında %25,4 ve 2021 yılında %26,8 ile birinci sırada COVID-19 nedeniyle gerçekleştiği görülmüştür. İkinci sırada gelen ölüm nedeni 2020 yılında %16,5 ile neoplaziler olurken; 2021 yılında %18,3 ile kardiyovasküler sistem hastalıkları olduğu görülmüştür. Ölüm nedenlerinin yıllara göre karşılaştırılmasında istatistiksel bir farklılık saptanmamıştır (p=0,051). Ölenlerin ortalama yatış süresi 2020 yılında 13,1±17,8 ortanca 8 gün olurken, 2021 yılında ise ortalama yatış süresi 14,4±17,6, ortanca 8 gün olup istatistiksel farklılık saptanmamıştır (p=0,239) (Tablo 1).

Tablo 1. KOÜ Araştırma ve Uygulama Hastanesi'nde 2020 ve 2021 yıllarındaki ölümlerin temel tanımlayıcı özelliklere göre dağılımı

	n(% sütun)		p
	2020	**2021**	
YAŞ (Ortalama± Standart Sapma)	58,64±24,82	59,26±24,06	0,686
CİNSİYET			
Erkek	329(59,6)	289(58,1)	
Kadın	221(40,0)	208(41,9)	0,615
Toplam	552(100,0)	497(100,0)	
ÖLÜM NEDENLERİ			
COVİD-19 Enfeksiyonu	140(%25,4)	133(%26,8)	
Neoplaziler	91(%16,5)	79(%15,9)	
KVS Hastalıkları	82(%14,9)	91(%18,3)	
Solunum Sistemi Hastalıkları	82(%14,9)	39(%7,8)	
COVİD-19 Dışı Enfeksiyon Hastalıkları	32(%5,8)	28(%5,6)	0,051
Yenidoğan Ölümleri ve Konjenital Hastalıklar	27(%4,9)	23(%4,6)	
Nörolojik Hastalıklar ve SVO	26(%4,7)	21(%4,2)	
GÜS Hastalıkları	22(%4)	28(%5,6)	
GİS Hastalıkları	12(%2,2)	16(%3,2)	
Diğer	32(%5,8)	36(%7,2)	
YATIŞ SÜRESİ (Ortalama± Standart Sapma)	13,1±17,8	14,4 ±17,6	0,239

KOÜ Araştırma ve Uygulama Hastanesi'nde 2020-2021 yıllarında meydana gelen ölümlerin gerçekleştiği birime bakıldığında ise 2020 ve 2021 yıllarında sırasıyla %48,4 ve %57,9 ile erişkin yoğun bakım üniteleri ilk sırada gelmektedir. Bunu yıllara göre sırasıyla %27,7 ve %19,1 ile dahili servisler ardında da %12,7 ve %9,7 ile acil servis izlemektedir. 2021 yılında 2020 yılına göre erişkin yoğun bakımlardaki ölümlerin oranı artmış, dahili servislerdeki ölümlerin oranı ise azalmış olup bu değişim istatistiksel olarak anlamlı bulunmuştur. (p=0,001)

Mortalite hızlarında ise en yüksek hız yıllara göre sırasıyla ‰108,7 ve ‰98,86 ile erişkin yoğun bakım ünitelerinde iken en düşük mortalite hızı ‰0,94 ve ‰0,82 ile cerrahi servislerde görülmüştür (Tablo 2).

Tablo 2. KOÜ Araştırma ve Uygulama Hastanesi'nde 2020-2021 yıllarında meydana gelen ölümlerin servis gruplarına göre dağılımı ve mortalite hızları

SERVİS GRUPLARI	**SAYI(% sütun)**			**MORTALİTE HIZI (BİNDE)**	
	2020	**2021**	**p**	**2020**	**2021**
Erişkin Yoğun Bakım Üniteleri	267(%48,4)	288(%57,9)		108,70	98,86
Pediatrik Yoğun Bakım Üniteleri	34(%6,2)	44(%8,9)		40,18	47,26
Dahili Servisler	153(%27,7)	95(%19,1)	0,001	15,33	7,5
Cerrahi Servisler	13(%2,4)	15(%3,0)		0,94	0,82
Pediatrik Servisler	15(%2,7)	7(%1,4)		6,06	1,65
Acil Servis	70(%12,7)	48(%9,7)		-	-
Toplam	552(%100)	497(%100)		18,29	12,69

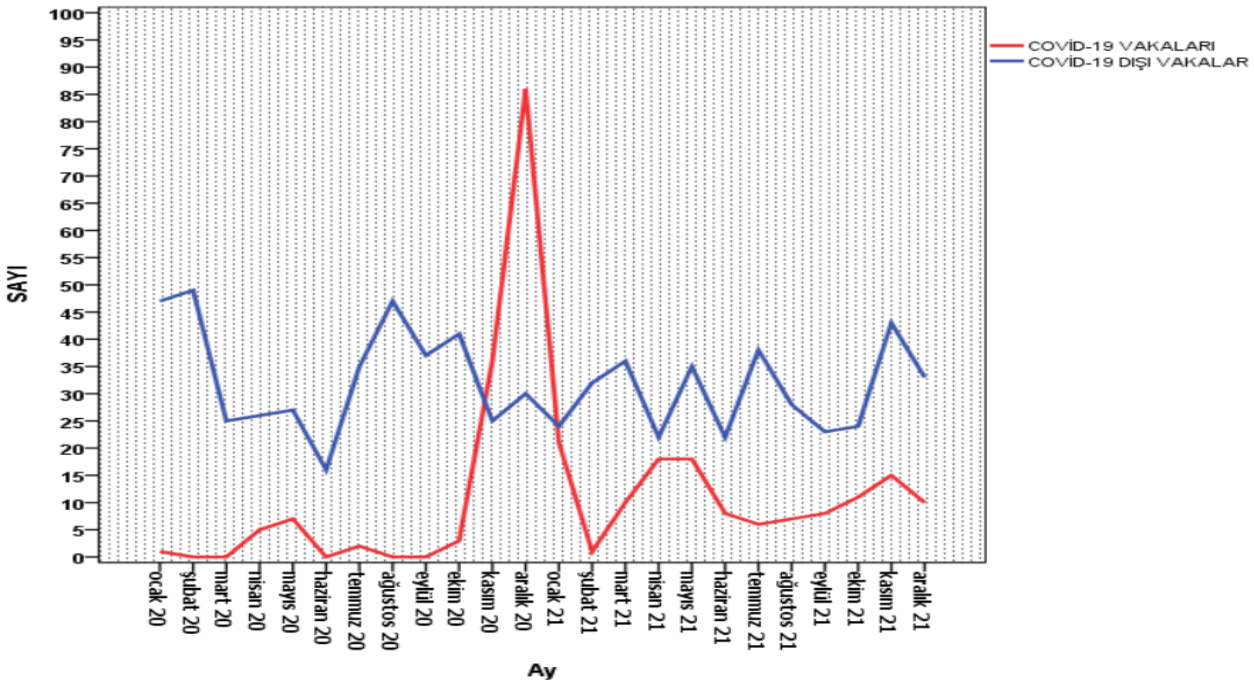
Araştırmamızda ölüm nedenlerinin cinsiyete göre dağılımı da incelenmiş olup Tablo 3'te gösterilmiştir. Buna göre her iki yılda da ölüm nedenleri ile cinsiyet açısından istatistiksel olarak anlamlı bir farklılık bulunamamıştır (sırasıyla p=0,964 ve p=0,206) (Tablo 3).

Araştırmamızda ölüm nedenleri COVID-19 nedenli ölümler ile COVID-19 dışı ölümler olarak yeniden kategorize edilerek incelenmiştir. Buna göre cinsiyet açısından bir fark bulunmazken yaş açısından incelendiğinde 2020 yılında COVID-19 nedenli ölümlerde yaş ortalaması (66,96±15,85), COVID-19 dışı nedenli ölümlerin yaş ortalamasına (55,75±26,62) göre istatistiksel olarak anlamlı düzeyde yüksek görülmüştür (p=0,000). 2021 yılında ise COVID-19 nedenli ölümlerde yaş ortalaması (63,18±16,04), COVID-19 dışı nedenli ölümlerin yaş ortalamasına (58,18±26,05) göre yüksek olduğu görülmüş ancak istatistiksel olarak anlamlı farklılık saptanmamıştır. (p=0,674). Her iki yılda gerçekleşen toplam ölümler incelendiğinde COVID-19 nedenli ölümlerin yaş ortalaması (65,09±16,02), COVID-19 dışı nedenli ölümlerin yaş ortalamasından (56,92±26,35) istatistiksel olarak anlamlı derecede yüksek görülmüştür (p=0,003, Mann-Whitney U testi) (Tablo 4).

COVID-19 nedenli ölümler ile COVID-19 dışı ölümler arasında gözlenen bir diğer farklılık ölümün gerçekleştiği aylarda saptanmıştır. Buna göre COVID-19 dışı ölümler dalgalı seyir gösterirken, COVID-19 nedenli ölümlerin Ekim 2020 ile Şubat 2021 arası dönemde pik yaptığı görülmüştür (Şekil 1). 2020 yılında ölümlerin %32,1'i Kasım-Aralık aylarında gerçekleşmiştir.

Tablo 3. Ölüm nedenlerinin 2020 ve 2021 yıllarında cinsiyete göre dağılımı

ÖLÜM NEDENLERİ	2020			2021		
	Erkek (n) (% sütun)	Kadın (n) (%sütun)	p	Erkek (n) (% sütun)	Kadın (n) (% sütun)	p
COVID-19 Enfeksiyonu	87(%26,8)	53(%24,1)		75(%26,0)	58(%28,2)	
Neoplaziler	59(%18,2)	32(%14,5)		48(%16,7)	31(%15,0)	
KVS Hastalıkları	47(%14,5)	35(%15,9)		51(%17,7)	40(%19,4)	
Solunum Sistemi Hastalıkları	47(%14,5)	35(%15,9)		21(%7,3)	18(%8,7)	
COVID-19 Dışı Enfeksiyon Hastalıkları	17(%5,2)	15(%6,8)	0,964	14(%4,9)	14(%6,8)	0,206
Yenidoğan Ölümleri ve Konjenital Hastalıklar	15(%4,6)	11(%5,0)		11(%3,8)	12(%5,8)	
Nörolojik Hastalıklar ve SVO	16(%4,9)	10(%4,5)		17(%5,9)	4(%1,9)	
GÜS Hastalıkları	13(%4,0)	9(%4,1)		19(%6,6)	9(%4,4)	
GİS Hastalıkları	6(%1,8)	6(%2,7)		13(%4,5)	3(%1,5)	
Diğer	18(%5,5)	14(%6,4)		19(%6,6)	17(%8,3)	
Toplam	325(100)	220(100)		288(100)	206(100)	



Şekil 1. Aylara göre COVID-19 ve dışı ölümlerin dağılımı

Tablo 4. COVID-19 nedenli ve COVID-19 dışı nedenli ölümlerin yıllara göre cinsiyet ve yaş ortalamaları

	COVID-19 Nedenli Ölümler		COVID-19 Dışı Ölümler		P	
	2020	**2021**	**2020**	**2021**	**2020**	**2021**
Cinsiyet(n)						
Erkek (%)	87(%62,1)	75(%56,4)	238(%58,8)	213(%59,0)	0,483	0,602
Kadın (%)	53(%37,9)	58(%43,6)	167(%41,2)	148(%41,0)		
Yaş	66,96±15,85	63,18±16,04	55,75±26,62	58,18±26,05	0,000	0,674
Toplam	65,09±16,02		56,92±26,35		0,003	

Tartışma

Bu araştırmada KOÜ Araştırma ve Uygulama Hastanesi 2020 ve 2021 yıllarına ait ölüm kayıtları değerlendirilmiş olup hastane ölüm hızlarının hesaplanması, ölümlerin tanımlayıcı özelliklerinin ortaya konması ve özellikle COVID-19 başta olmak üzere ölüm nedenlerinin belirlenmesi açısından önemli bilgiler sağlanmıştır. Bununla birlikte bazı sınırlılıklar bulunmaktadır. Öncelikle araştırmamızda incelenen veriler kayıtlarda bulunan değişkenlerle sınırlı olduğundan daha ayrıntılı inceleme yapılamamıştır. Araştırmanın diğer bir kısıtlılığı ise ölüm kayıtlarının doğru tanıyla kaydedilmemiş olma olasılığının bulunmasıdır.

Çalışmamızda 2020 yılında hastane mortalite hızı binde 18,9 olarak saptanırken, 2021 yılında mortalite hızı %43,8'lik bir azalışla binde 12,69 olmuştur. Aynı hastanede 2019 yılında meydana gelen ölümlerin incelendiği bir rapora göre 2019 yılında hastane mortalite hızı binde 14,77 olarak saptanmış olup 2020 yılında ölüm hızında %23,8'lik bir artış olduğu görülmüştür.[7] Çilingiroğlu ve ark. tarafından Hacettepe Üniversitesi Erişkin Hastanesinde 2004 yılında yapılan benzer bir çalışmada ise mortalite hızı hesaplanmamakla birlikte bir yılda yatan hastalarda ölüm oranı bizim çalışmamıza göre daha yüksek saptanmış olup %2,8 olarak bildirilmiştir.[8] Gazi Üniversitesi Tıp Fakültesi Hastanesinde 2007 yılında yapılan ölüm nedenleri çalışmasında mortalite hızı hesaplanmamakla birlikte bir yılda toplam 1065 hastanın öldüğü görülmüştür.[11] Bu farklılıkların aradan geçen zaman içinde tıbbi gelişmelere bağlı olarak ölüm oranlarının azalmasıyla ilişkili olabileceği söylenebilir.

Ölümlerin tanımlayıcı özellikleri açısından değerlendirildiğinde 2020 ve 2021 yıllarında yaş ortalamasının benzer olduğu görülmüş olup her iki yıl için de ortanca yaş 65 olarak bulunmuştur. Bu durum hastanede bir önceki yıl yapılan çalışmayla uyumlu olup, Çilingiroğlu ve ark. çalışmasında da ortanca yaş 64 olarak saptanmıştır.[7,8] Cinsiyet dağılımına baktığımızda 2020 ve 2021 yıllarında sırasıyla ölümlerin %59,6'sı ve %58,1'i erkeklerden oluşmaktadır. Önceki yıllarda yapılan diğer çalışmalarda da araştırmamızla benzer şekilde ölenler içinde erkeklerin daha fazla olduğu saptanmıştır.[7,8] Bu noktada dikkat çeken bir bulgu olarak 2020 yılında ve 2020-2021 yıllarındaki toplam COVID-19 nedenli ölümlerde istatistiksel olarak anlamlı düzeyde yaş ortalamasının yüksek olduğu görülmüştür. Bu durum COVID-19 enfeksiyonuna bağlı mortalite üzerinde, ileri yaşın önemli bir risk faktörü olduğunu gösteren diğer çalışmalarla uyumluluk göstermektedir.[6,9] Çalışmamızda COVID-19 nedenli ölümlerdeki ortanca yaş 67 ve ortalama yaş 65,0±16,02 olarak bulunurken Mena ve ark. tarafından İspanya'da bir üniversite hastanesinde yapılan çalışmada COVID-19 nedenli ölümlerin ortanca yaşının 77,5 olduğu belirlenmiştir.[6] İtalya'da yapılan başka bir çalışmada ise COVID-19 nedeniyle hastanede ölenlerin yaş ortalaması Mart-Mayıs 2020 döneminde 80.1±10.6 olarak saptanırken Haziran-Ağustos 2020 döneminde 82.8±11.1 olarak saptanmıştır.[10] Çalışmamızda COVID-19 nedenli ölümlerin yaş ortalaması diğer örneklere kıyasla daha düşüktür bu durumun olası nedenleri arasında çalışmamızın yapıldığı hastanenin bulunduğu bölgedeki tek COVID-19 hastanesi olmaması ve COVID-19 nedenli ölümlerin diğer hastanelere dağılmış olması nedeniyle temsiliyetin tam olarak sağlanamaması yer alıyor olabilir. Yaş dağılımında görülen bu durumun olası nedenlerinin aydınlatılmasına yönelik ileri araştırmalar yapılması gerekmektedir.

Ölüm nedenleri açısından değerlendirildiğinde 2020 yılında tüm dünyada yaygın bir sorun halini alan ve pandemi olarak ilan edilen COVID-19 enfeksiyonu daha önceki yıllarda görülmeyen bir ölüm nedeni olarak ölüm kayıtlarında ilk sırada yer almaktadır. 2020 yılında hastanede ölümler en sık olarak %25,4(n=140) ile COVID-19 nedeniyle gerçekleşmiştir. %22,3 ile bir önceki yılın en sık ölüm nedeni olan kardiyovasküler sistem hastalıkları ise 2020 yılında %14,9(n=82) ile en sık üçüncü ölüm nedeni olmuştur. Neoplazi nedenli ölümler ise %16,5(n=91) ile en sık görülen ikinci ölüm nedeni olmuştur.2021 yılında da 2020 ile benzer şekilde en sık görülen ölüm nedeni, ölümlerin %26,8'ini oluşturan COVID-19 olmuştur. Çalışmamızda KOÜ Hastanesi özelinde görülen bu durum, COVID-19 pandemisinin yıkıcılığı ve sağlık sistemi üzerinde yarattığı baskı hakkında genel bir fikir vermektedir. Pandemiden önceki yıllarda üniversite hastanelerinde yapılan çalışmalarda ise neoplaziler ve KVS hastalıkları nedenli ölümler ilk sıralarda yer almıştır.[7,8,11] Amerika Birleşik Devletleri'nin 2020 ve 2021 yılları ölüm istatistiklerine bakıldığında her iki yıl için de ilk sırada KVS hastalıkları nedenli ölümler, ikinci olarak neoplazi nedenli ölümler ve üçüncü sırada COVID-19 nedenli ölümlerin geldiği görülmektedir.[12]

Ülke geneli açısından değerlendirildiğinde Türkiye'de ölüm nedenleri Türkiye İstatistik Kurumu (TÜİK) tarafından açıklanmaktadır. Ancak TUİK 2020 ve 2021 yıllarına ait ulusal ölüm istatistiklerini henüz paylaşmamış olduğundan; elde edilebilen en son ulusal veri olan TUİK

2019 yılına ait ölüm nedeni istatistiklerinde incelenmiştir. Buna göre ülke genelinde en sık ölüm sebebinin %37,8 ile kardiyovasküler hastalıklar ardında da %19,3 ile neoplaziler olduğunu görülmüştür.[13] Bu noktada, COVID-19 enfeksiyonuna bağlı ölümlerin fazla olmasının yanı sıra COVID-19 dışı nedenlere bağlı hastane başvurularının pandemi nedeniyle azalmış olabileceği ve bunun da hastanede gerçekleşen diğer nedenli ölümlerde bir düşüşe yol açmış olabileceği düşünülebilir. Yapılan bir çalışmada pandemi döneminde bir yıl önceki aynı dönemle karşılaştırıldığında, hastaneye kabullerde tüm inme geçiren hasta sayılarında %50, iskemik inme sayılarında %44, intrakraniyal hemoraji sayılarında %62,5, geçici iskemik atak sayılarında %87,5 düşüş olduğu gösterilmiştir.[14] Dünya Sağlık Örgütünün yayınlamış olduğu Dünya Sağlık İstatistikleri 2022'de COVID-19' bağlı olarak ortaya çıkan mali kısıtlamalar, ulaşım zorlukları ve enfeksiyona maruz kalma korkusu nedeniyle kişilerin bakım arayışına girememesi veya isteksiz olmasının da sağlık hizmetlerini etkilediği ve bunun sonucunda milyonlarca insanın hayati önem taşıyan sağlık hizmetlerinden mahrum kaldığı bildirilmiştir.[15]

Ek olarak, doğrudan SARS-CoV-2 enfeksiyonunun neden olduğu aşırı ölüm oranı ile pandeminin dolaylı bir sonucu olarak ölüm nedenlerindeki değişiklikleri ayırt etmeye yardımcı olmak için daha fazla araştırma yapılması gerekmektedir.[5]

Sonuç olarak bu çalışmada KOÜ Araştırma ve Uygulama Hastanesi ölüm kayıtlarına göre 2020 ve 2021 yıllarında ölüm nedenleri içinde COVID-19 nedenli ölümler ilk sırada yer almıştır. Bu durumun COVID-19 pandemisi sürecinde yaşanan hizmet alma ve hizmet sunumundaki olası sorunlarla ilgisinin incelenmesi ve daha ileri araştırmalar yapılması gerekmektedir.

Etik Standartlara Uygunluk

KOÜ Tıp Fakültesi Etik Kurulundan onay alındı. Karar No: KOÜ GOKAEK-2021/22.19. Proje No: 2021/355

Çıkar Çatışması

Yazarlar arasında çıkar çatışması yoktur.

Yazar Katkısı

MEG: Çalışma fikri, veri toplama, kaynak tarama, istatistiksel analiz, makale yazımı ve yayınlanma süreci; ÇÇ: Çalışma fikri, hipotez, çalışmanın tasarımı, eleştirel inceleme, makale yazımı.

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Research Article | Araştırma Makalesi

DETERMINATION OF THE EFFECT OF RUTIN ON EPITHELIAL TO MESENCHYMAL TRANSITION IN PROSTATE CANCER CELLS

EPİTELYALDEN MEZENKİMALE GEÇİŞ ÜZERİNE RUTİNİN ETKİLERİNİN PROSTAT KANSERİ HÜCRELERİNDE BELİRLENMESİ

Esra Bal[1], Asuman Deveci Ozkan[2], Zeynep Betts[3*]

[1]Kocaeli University, Institute of Sciences, Department of Biology, Kocaeli, Türkiye. [2]Sakarya University, Faculty of Medicine, Department of Medical Biology, Sakarya, Türkiye. [3]Kocaeli University, Faculty of Arts and Sciences, Department of Biology, Kocaeli, Türkiye.

ABSTRACT

Objective: The difficulties experienced in the treatment of prostate cancer and the excess of side effects due to chemotherapy have brought the search for alternative treatment strategies. In recent studies, it is known that Rutin (RUT) has an anti-cancer effect on cancer cells. Our study aimed to determine the effects of RUT on epithelial to mesenchymal transition (EMT) in prostate cancer cells, for the first time.

Methods: The anticancer effect of RUT in prostate cancer cells (PC-3) was examined by WST-1, Annexin V ELISA, DAPI and Acridine Orange staining, and the anti-cancer and anti-metastatic properties of RUT were evaluated with the Scratch Assay test. The expression level of *Bax, Bcl-2, Snail, Twist, Vimentin* and *E-cadherin* genes was evaluated by RT-PCR.

Results: The cells were treated with RUT (500, 750, 1000 and 1500 µM) for 24 and 48 hours. The viability rates decreased with increasing RUT concentration depending on dose and time ($p<0.01$). Free Annexin V protein decreased, and apoptotic cells increased depending on the increasing RUT concentration ($p<0.01$). There was a reduction in the migrating cells, especially at 750 and 1000 µM RUT concentrations. There was a significant increase (14.22-fold) in *E-cadherin* mRNA level after 1500 µM RUT treatment ($p<0.01$). Additionally, the mRNA level of *Snail, Twist* and *Vimentin* decreased at higher RUT concentrations ($p<0.01$).

Conclusion: The existence of RUT's potential to inhibit epithelial to mesenchymal transition and promote apoptosis has been demonstrated. It is also recommended to explore the effects of RUT on EMT *in vivo.*

Keywords: Prostate cancer, rutin, epithelial to mesenchymal transition, apoptosis

ÖZ

Amaç: Prostat kanseri tedavisinde yaşanan zorluklar, kemoterapi nedeniyle görülen yan etkilerin fazlalığı alternatif tedavi stratejileri arayışını gündeme getirmiştir. Son yıllarda yapılan araştırmalarda Rutin (RUT)'in kanser hücreleri üzerinde anti-kanser etki gösterdiği bilinmektedir. Bu nedenle çalışmamızda RUT'un prostat kanseri hücrelerinde epitelyalden mezenkimale geçiş (EMT) üzerindeki etkilerinin ilk defa belirlenmesi amaçlanmıştır.

Yöntem: Prostat kanseri hücrelerinde (PC-3) RUT'un antikanser aktivitesi WST-1, Annexin V ELISA, DAPI boyama ve Akridin Oranj (AO) boyama ile belirlenerek, RUT'un anti-kanser ve anti-metastatik özellikleri Scratch (Çizik) Assay testi ile değerlendirildi. *Bax, Bcl-2, Snail, Twist*, *Vimentin* ve *E-kaderin* genlerinin mRNA ifade düzeyi RT-PCR analizi ile belirlendi.

Bulgular: PC-3 hücrelerine, farklı konstrasyonlarda (500, 750, 1000 ve 1500 µM) ve farklı zamanlarda (24 ve 48 saat) RUT uygulandı. Canlılık oranlarının doza ve zamana bağlı olarak artan RUT konsantrasyonuna bağlı olarak azaldığı gözlendi ($p<0.01$). Ayrıca artan RUT konsantrasyonuna bağlı olarak serbest Annexin V proteinin azaldığı ve apoptotik hücrelerin arttığı belirlendi ($p<0.01$). Özellikle 750 ve 1000 µM RUT konsantrasyonlarında göç eden hücre sayısında azalma olduğu ortaya kondu. RT-PCR analizi sonucu elde edilen verilerde 1500 µM RUT muamelesi sonrası *E-Kaderin* mRNA seviyesinde 14.22-kat olarak belirgin bir anlamlı artış olduğu belirlendi ($p<0.01$). Diğer yandan EMT sürecinde yer aldığı bilinen *Snail, Twist* ve *Vimentin* mRNA ifade düzeyinin artan RUT konsantrasyonuna bağlı olarak azaldığı ortaya konuldu ($p<0.01$).

Sonuç: RUT'un epitelden mezenkimal geçişi engelleme ve apoptozu teşvik edici potansiyelinin varlığı ortaya konulmuştur. Ayrıca RUT'un EMT üzerine olan etkilerinin daha ileri moleküler analizler ve *in vivo* olarak araştırılması önerilmektedir.

Anahtar Kelimeler: Prostat kanseri, rutin, epitelyalden

*** Corresponding author/İletişim kurulacak yazar:** Zeynep Betts; Department of Biology, Faculty of Faculty of Arts and Sciences, Kocaeli University, Kocaeli, Türkiye
Phone/Telefon: +90 262 303 21 67 **e-mail/e-posta:** zeynep.betts@kocaeli.edu.tr
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Introduction

Further research into the various signaling pathways involved in the pathogenesis of cancer is necessary since it is a very complex and multifaceted disease defined by the unrestricted proliferation of aberrant cells that have the capacity to assault and spread throughout the body.[1] Prostate cancer is one of the most commonly diagnosed cancers with high fatality rates, particularly among men.[2] According to the American Cancer Society, men have a 1 in 9 risks of being diagnosed with cancer during their lifetime. An estimated 1 in 41 men dies from prostate cancer.[2] Although the incidence of prostate cancer is related to age, the rate of diagnosis increases with age.[2] For the treatment of advanced prostate cancer and increased patient compliance, novel therapies also are required.

The biggest family of polyphenolic chemicals originating from plants, flavonoids have a variety of biological effects in a wide range of mammalian cell systems.[3] In recent years, the performance of flavonoids to prevent and treat prostate cancer has become the focus of attention due to the inverse relationship between a diet enriched with flavonoids and prostate cancer incidence.[4] One of the most well-known flavonoids, rutin (RUT), is best known for its anti-inflammatory and antioxidant capabilities.[5] RUT is a plant-derived flavonoid found in various vegetables, fruits and medicinal plants.[1] RUT, a phytochemical with anti-carcinogenic properties, is one of the powerful antioxidants with biochemical effects, particularly in cancer treatment.[6] In the literature, many studies have shown that RUT inhibits cancer cell proliferation through cell cycle arrest and apoptosis in some cancer types.[3,6,7] It has been shown that RUT inhibits the *in vitro* proliferation of cancer cell.[7]

The epithelial to mesenchymal transition (EMT) program is defined as a condition that causes tumor metastasis, in which epithelial cells slowly transform into mesenchymal cells, where they lose epithelial functions.[8] The earliest occurrence in EMT can be expressed as the loss of cell polarity and occurs due to the loss of epithelial proteins (E-cadherin and Vimentin).[8] By regulating the expression of the cadherin family of proteins (Snail and Twist) modulate cell adhesion.[9] Snail and Twist stand out as EMT-inducing transcription factors and have an important role in EMT.[10] The results of many studies have shown a relationship between EMT markers and poor prognosis in carcinoma types, including but not limited to prostate, breast, liver, lung, pancreatic and bladder carcinomas.[10]

The pharmacokinetic restrictions and unfavorable side effects of chemotherapeutic medicines in the treatment of metastatic prostate cancer have recently drawn attention to the development of novel anticancer agents.[11] The pleiotropic tasks of EMT related to metastasis and therapy resistance create great therapeutic opportunities to increase the effectiveness of chemotherapy, targeted therapy or immunotherapy, and to prevent EMT.[11] Therefore our work was aimed to investigate the effects of RUT on EMT in prostate cancer cells.

Methods

Cell Culture Conditions

RUT (Tokyo Chemical Industry) was purchased commercially PC-3 cell line was used as the prostate cancer cell line and is available in Department of Biology. PC-3 cells were cultured in RPMI 1640 medium containing 10% FBS (Gibco) and penicillin/streptomycin at 37°C and 5% CO_2. HUVEC cells were used only for viability analysis. HUVEC cells were cultured in DMEM medium containing 10% FBS and penicillin/streptomycin at 37°C and 5% CO_2.

Cell Viability Analysis

The WST-1 assay utilize tetrazolium salts to measure metabolic activity in active cells. According to the assay methodology, cellular mitochondrial dehydrogenases convert the tetrazolium salt to formazan. For this reason WST-1 analysis was performed to determine the cytotoxic effect of RUT in PC-3 cells. For this purpose, cells were first seeded in 96-well cell culture plates at $2x10^4$ cells/well. They were treated with different concentrations of RUT (500, 750, 1000 and 1500 µM) for 24 and 48 hours. After, WST-1 dye addition to the cell culture medium incubated for 1-4 hours at 37°C, and measurements were made in a microplate reader (Chromate) at 450 nm wavelength. Control cell viability not treated with RUT was accepted as 100%, and viability rates of experimental cells were expressed as "%".

ELISA Analysis

Annexin V ELISA analysis was made to determine the apoptotic effect of RUT on PC-3 cells. For this purpose, cells were first cultured in six-well plates at $1x10^5$ cells/well. They were treated with different concentrations of RUT (500, 750, 1000 and 1500 µM) for 24 hours. Following incubation, the Human Annexin V ELISA kit (ab119503) was used in accordance with the manufacturer's instructions.

Acridine Orange (AO) and DAPI (4',6-diamidino-2-phenylindole) Staining

AO and DAPI staining was used to evaluate the effect of RUT on cell and nuclear morphology in PC-3 cells. For this purpose, cells were first cultured in six-well plates at $2x10^5$ cells/well. They were treated with different concentrations of RUT (500, 750, 1000 and 1500 µM) for 24 hours. The cells were fixed with a 4% paraformaldehyde (PFA). Then, the cells were washed and treated with AO dye (100 mg/mL) and DAPI, and the images were taken under a fluorescent microscope (Olympus).

Scratch Assay

The scratch assay was performed to explore the anti-migrating capacity of RUT. For this purpose, the cells were first cultured in six-well plates at $2x10^5$ cells/well and the "+" shape was given to the wells with a 1000 ml pipette tip. They were treated with RUT (500, 750, 1000 and 1500 µM) for 24 hours. After incubation, cells were fixed with a 4% paraformaldehyde (PFA) solution for 30 minutes. Afterward, the images were taken under an inverted microscope (Nikon).

RT-PCR Analysis

To determine the effect of RUT on the mRNA levels of *Bax, Bcl-2, Snail, TWIST1, Vimentin* and *E-cadherin* in PC-3 cells, RT-PCR analysis was performed. For this purpose, cells were seeded in T_{25} cell culture flasks at $1x10^6$ cells/well. They were incubated with different concentrations of RUT (500, 750, 1000 and 1500 µM) for 24 hours. After incubation, RNA isolation was performed using Xtrazol (Biofroxx) according to the appropriate kit protocol. The obtained RNA levels were measured at 260 nm in a spectrophotometer (Thermo Fisher Scientific) and after controlling the quality and concentration, 5 µg of RNA was converted into cDNA using appropriate kits containing Reverse Transcriptase. The obtained cDNA is diluted with nuclease-free distilled water and after the primers suitable for *Bax, Bcl-2, Snail, TWIST1, Vimentin* and *E-cadherin* genes are obtained from the company, the appropriate reaction mixture was prepared. As a final step, the reaction was carried out by adjusting the RT-PCR conditions and the number of cycles (Bio-Rad, CFX Connect).

Statistical Analysis

"SPSS 22.0" statistical program was used to evaluation and $p<0.05$ was accepted as statistically significant. Differences between cell viability percentages were evaluated by one-way analysis of variance (Post-Hoc Tukey). The differences in mRNA expression levels, which vary depending on the dose and time, were compared statistically with the web-based analysis software (https://www.qiagen.com/tr/shop/genes-and-pathways/). All analyzes were carried out in Kocaeli University, Department of Biology, Biotechnology laboratory.

Results

The Effects of RUT on Cell Viability

WST-1 analysis was used to determine the cytotoxic effect of RUT on PC-3 and HUVEC cell lines (Figure 1). Our findings demonstrate that the proliferation rates of cells treated with different concentrations of RUT (62.5, 125, 250, 500, 1000 and 1500 µM) for 24 and 48 hours decreased significantly depending on the RUT concentration and time in PC-3 cells (Figure 1A, $p<0.05$). However, it was determined that RUT at 500 and 750 µM concentrations had a toxic effect on HUVEC cells for 48 h (Figure 1B). Based on the data obtained from the viability analysis results, RUT concentrations of 500, 750, 1000 and 1500 µM were determined to be used for 24 hours, respectively, in further analysis.

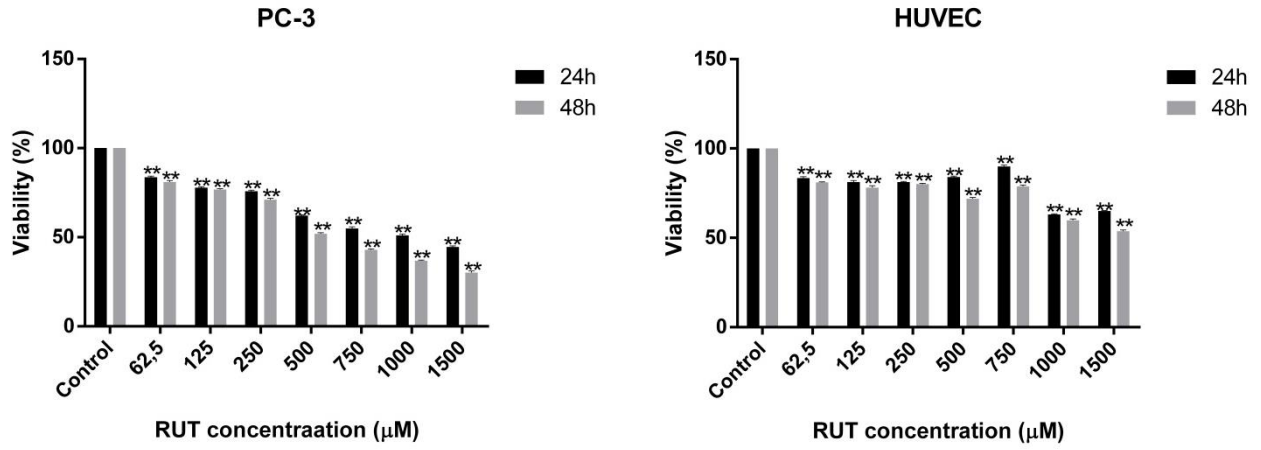

Figure 1. The cytotoxic effect of RUT in PC-3 and HUVEC cells. The viability rate was determined after treated with different concentrations of RUT (62.5, 125, 250, 500, 750, 1000 and 1500 µM) and statistically analyzed for 24 and 48 h (*$p<0.05$, **$p<0.01$).

The Effects of RUT on free Annexin V Protein Level

It was observed that there was a significant decrease in free Annexin V protein level ($p<0.01$, Figure 2). It has been determined that 500, 750, 1000 and 1500 µM RUT treatment decreased free Annexin V protein levels (7.12, 5.85, 4.82, 3.87 and 3.39 ng/mL, respectively) in PC-3 cells ($p<0.05$). Therefore, it was determined that RUT induced apoptosis depending on the increasing concentrations.

The Effects of RUT on Cell and Nucleus Morphology

Morphological changes caused by different concentrations of RUT in PC-3 cells and nuclei were visualized by AO and DAPI staining (Figure 3). In PC-3 cells, it was observed that apoptotic morphological changes increased in cells and nuclei depending on the increasing concentration of RUT. Chromatin condensation, cell and/or cytoplasmic shrinkage were observed in PC-3 cells. In addition, nuclear blebbing was determined especially in PC-3 cells that were treated with 1000 and 1500 µM RUT. Compared to the control

group, PC-3 cells treated with RUT showed disruption of cell membrane integrity, nuclear blebbing and cytoplasmic shrinkage. It was determined that apoptotic cell and nucleus changes were observed the most in PC-3 cells, especially in 1000 and 1500 µM RUT in PC-3 cells.

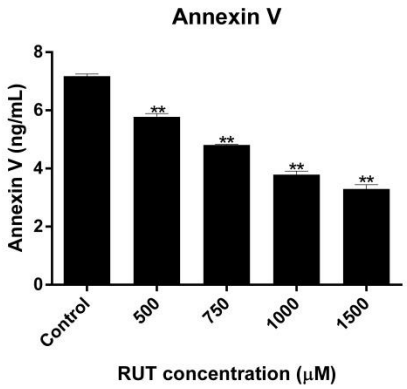

Figure 2. The effect of RUT on the level of free Annexin V protein in PC-3 cells. The level of free Annexin V protein was determined after treated with different concentrations of RUT (500, 750, 1000, and 1500 µM) and statistically analyzed for 24 h (**p<0.01).

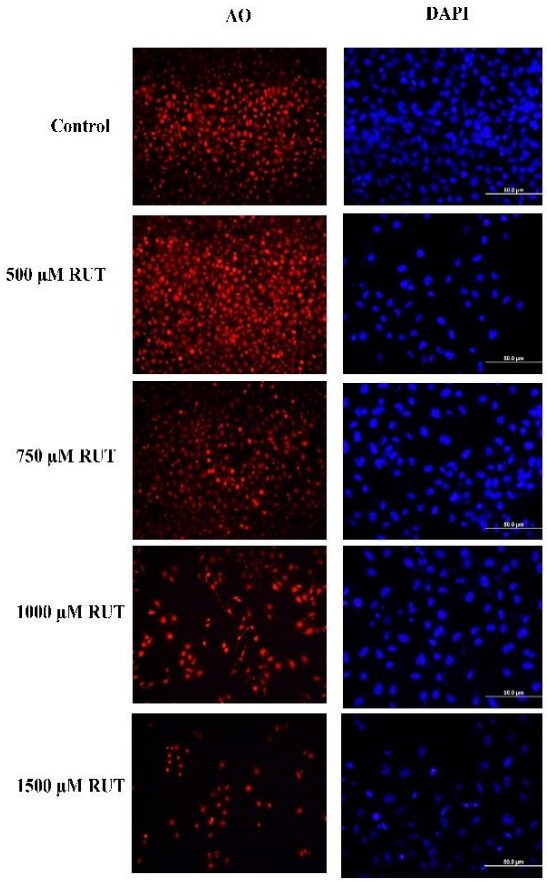

Figure 3. The effect of RUT on cell and nucleus morphology in PC-3 cells. The cells treated with RUT (500, 750, 1000 and 1500 µM), general morphology by AO staining and nuclear morphology by DAPI staining were visualized.

The Effects of RUT on Cell Migration

The effect of RUT on the migration capacity of cells was visualized by performing a scratch assay (Figure 4). PC-3 cells treated with RUT showed that the rate of migration of cells was decreased especially at 750 and 1000 µM RUT. These results reveal that RUT has anti-migratory properties as well as apoptotic properties in PC-3 cells.

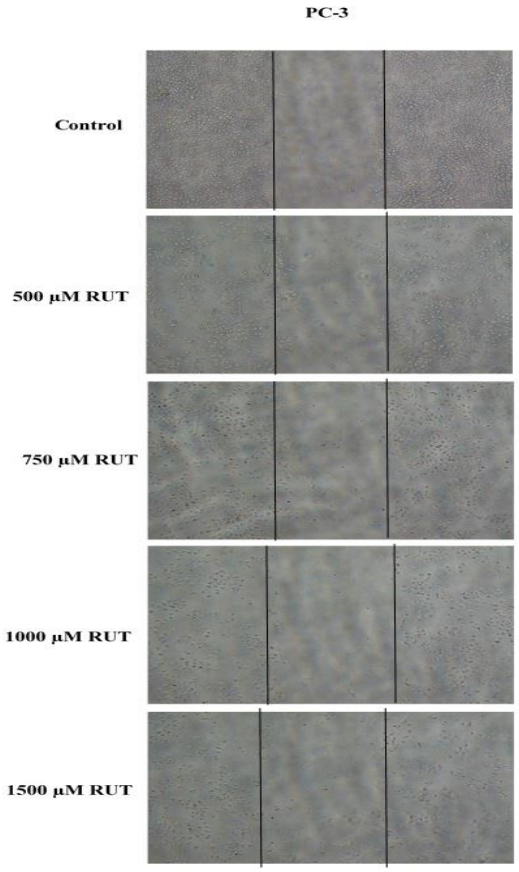

Figure 4. The effect of RUT on migration capacity in PC-3 cells. The cells treated with RUT (500, 750, 1000 and 1500 µM) and visualized.

The Effects of RUT on Gene Expression Levels

The effect of RUT on mRNA levels of *Bax, Bcl-2, E-cadherin, Snail, Twist* and *Vimentin* in PC-3 cells was determined by RT-PCR analysis (Figure 5). It was determined that EMT-inducing factors *Snail, Twist* and *Vimentin,* and *Bax* gene expression with pro-apoptotic effect decreased due to increasing RUT concentration, and there was a significant increase in mRNA levels of *Bcl-2* and *E-Cadherin* gene expression, which have anti-apoptotic activity. (p<0.01).

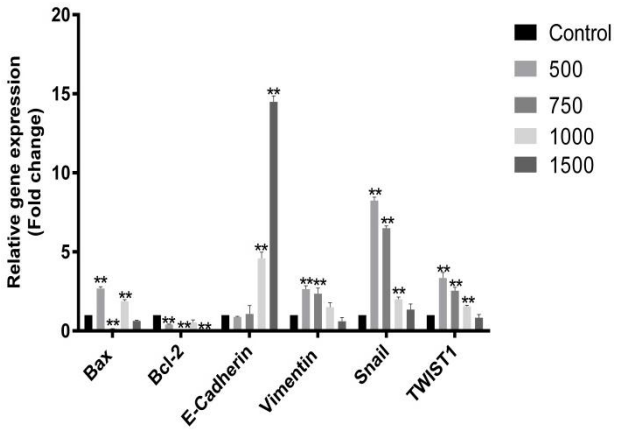

Figure 5. The effect of RUT on mRNA levels of *Bax, Bcl-2, Snail, TWIST1, Vimentin* and *E-cadherin* in PC-3 cells was determined by RT-PCR analysis and statistically analyzed (**p<0.01).

The increase in *Bax* expression and the decrease in *Bcl-2* expression, which supported the increase in apoptosis with the increase in RUT concentration, supported the Annexin V ELISA analysis and AO staining images, and the decrease in the expression of *Snail, Twist* and *Vimentin.* Together with the gene-level data we obtained, the existence of RUT's potential to inhibit mesenchymal transition from the epithelium and promote apoptosis has been demonstrated.

Discussion

RUT has been shown to decrease the cell viability of cancer cells.[6] RUT, has been shown to regulate several signaling pathways. More *in vitro* and *in vivo* researches are needed to assess the potential of RUT as an adjuvant and chemotherapeutic agent.[6] Therefore, in our study, the effect of RUT on the EMT program was evaluated for the first time. When we evaluated the obtained results, the suppressive effect of RUT on the EMT program was revealed for the first time.

It has been reported that up-regulation of Bcl-2 prevents cell death and creates disease pathogenesis with cytokine production. Bax protein initiates the process cascade via releasing cytochrome c out from mitochondria, which leads to apoptosis.[12] RUT causes growth inhibition in human glioblastoma cells by suppressing the expression of pro- and anti-apoptotic genes, induction of cell cycle arrest.[13] Similarly, Khan et al.[14] demonstrated that RUT causes apoptosis by arresting the cell cycle in cervical cancer cells. Additionally RUT induces apoptosis in colon cancer cells using mitochondria-mediated apoptotic pathways.[15] Satari et al.[6] found that after the combined treatment of anticancer drug (5-fluorouracil, 5-FU) and RUT at different concentrations to PC-3 cells, the application of 5-FU/ and RUT separately or using a combination, increased apoptosis in PC-3 cells. In addition, the combination of 5-FU/RUT has determined a high level of Bcl-2 protein suppression in PC-3 cells. In our study, *Bcl-2*, which has anti-apoptotic and pro-apoptotic activity, and *Bax* gene expression, which has the pro-apoptotic effect, decreased depending on increasing RUT concentration. The increase in *Bax* expression and decrease in *Bcl-2* expression, which supported the increase in apoptosis with the increase in RUT concentration, supported the Annexin V ELISA analysis and AO staining images.

EMT is linked to cancer growth, migration, and metastasis during prostate carcinogenesis, and mesenchymal markers and transcription factors are substantially elevated. Epithelial cell markers such as E-cadherin are downregulated. While there is a loss of cell-cell adhesion in the EMT process, mesenchymal markers such as vimentin and N-cadherin increase and allowing cells to migrate to secondary sites or organs. This process has been evaluated as a molecular target to inhibit EMT in prostate cancers.[16] RUT is also effective in colon adenocarcinoma cells by cell cycle arrest in G1 phase and regulation of microRNAs, messenger RNAs and transcription factors and exhibits anti-angiogenic properties *in vitro*.[17,18] Additionally Ben Sghaier et al.[19] demonstrated that RUT inhibits proliferation and decreases adhesion and migration of human lung and colon cancer cells. In our study, the increase in *E-cadherin* gene expression and the decrease in *Snail, Twist* and *Vimentin* expression after the highest concentration of RUT (1500 µM) treatment support the existence of its anti-migratory effect. Despite the promising anticancer effects of RUT in preclinical studies, barriers to its clinical transition have been observed. The low solubility, high metabolism, low gastrointestinal absorption, and low bioavailability of RUT limit its ability to reach appropriate concentrations in tumor tissues.[16] To increase the bioavailability and effectiveness of RUT as an anticancer agent, many strategies include the combination of RUT with other chemotherapeutic medications, RUT synthetic analogs, and RUT-nano-formulations are additional choices.

Conclusions

With the findings obtained at the end of the present study, an important development will be created in the preparation of biologically sourced, specific, biotechnologically innovative chemotherapeutics that can be used in the treatment of various cancer types. While increasing the benefit to the patient, undesirable side effects may decrease or disappear completely because the patient will be exposed to lower doses. We concluded that RUT has value as a new anti-metastatic agent and can be considered as promising multi-targeted nutraceutical agent that provides various health benefits in general.

Compliance with Ethical Standards

No ethics committee decision is required for the study.

Conflict of Interest

Authors declare that there is no conflict of interest regarding this work.

Author Contribution

Authors contributed equally to this work.

Financial Disclosure

None.

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Research Article | Araştırma Makalesi

EVALUATION OF INDETERMINATE LESIONS ON COMPUTED TOMOGRAPHY IN PATIENTS WITH COVID-19 PNEUMONIA PRESENTED TO THE EMERGENCY DEPARTMENT

ACİL SERVİSE BAŞVURAN VE COVID-19 PNÖMONİSİ OLAN HASTALARDA BİLGİSAYARLI TOMOGRAFİDEKİ BELİRSİZ LEZYONLARIN DEĞERLENDİRİLMESİ

Sevtap Dogan[1], Nurettin Ozgur Dogan[2*], Elmire Dervisoglu[1], Emel Azak[3], Aynur Karadenizli[4], Melike Kurt[4]

[1]Kocaeli University, Faculty of Medicine, Department of Radiology, Kocaeli, Türkiye. [2]Kocaeli University, Faculty of Medicine, Department of Emergency Medicine, Kocaeli, Türkiye. [3]Kocaeli University, Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Kocaeli, Türkiye. [4]Kocaeli University, Faculty of Medicine, Department of Microbiology, Kocaeli, Türkiye.

ABSTRACT

Objective: Indeterminate CT findings can complicate and delay the diagnosis of COVID-19 pneumonia in the emergency department in comorbid patients. The aim of the study is to analyze indeterminate chest CT findings and differentiate predictive features in RT-PCR positive and negative patients, which can be diagnostic for COVID-19 pneumonia.

Methods: In this cross-sectional study, patients suspected of COVID-19 pneumonia whose CT reports were indeterminate were retrospectively reviewed. All CT variables and comorbidities were recorded blindly. Lesions were compared by means of location, multiplicity, configuration, distribution, and margin characteristics between RT-PCR positive and negative patients.

Results: A total of 81 patients were enrolled in the study. Thirty-five (43.2%) had positive RT-PCR tests, and 46 (56.8%) had negative tests. Well-defined central GGO and tree-in-bud nodules were frequently seen in RT-PCR negative patients (p=0.016 and p=0.027, respectively). The lesions were located in the left lower lobe in 16 (45.7%) of the RT-PCR positive patients and 34 (73.9%) of the RT-PCR negative patients (p=0.010). Non-dependant location was recorded in 68.6 % of the RT-PCR positive patients and 54.3% of the negative patients. Regarding the binary regression analysis, only the presence of non-dependent density (OR: 4.91, 95% CI:1.12–20.21) and the absence of tree-in-bud nodules (OR: 0.15, 95% CI:0.03–0.78) were found to be independent predictors of positive RT-PCR test results.

Conclusion: Lesions in the non-dependent part of the lung may be related to positive RT-PCR test results. The presence of tree-in-bud nodules may be a predictor of negative results.

Keywords: Computed tomography, pneumonia, coronavirus, COVID-19

ÖZ

Amaç: Belirsiz BT bulguları, acil serviste komorbiditeleri olan hastalarda COVID-19 pnömonisi tanısını zorlaştırabilir ve geciktirebilir. Bu çalışmanın amacı, belirsiz akciğer BT bulgularını analiz etmek ve COVID-19 pnömonisi için tanısal olabilecek RT-PCR pozitif ve negatif hastalarda prediktif özellikleri ayırt etmektir.

Yöntem: Bu kesitsel çalışmada, BT raporlarında belirsiz olarak tanımlanan lezyonları olan ve COVID-19 pnömonisinden şüphelenilen hastalar retrospektif olarak incelendi. Tüm BT değişkenleri ve komorbiditeler kör olarak kaydedildi. Lezyonlar, RT-PCR pozitif ve negatif hastalar arasında yer, çokluk, konfigürasyon, dağılım ve sınır özellikleri açısından karşılaştırıldı.

Bulgular: Araştırmaya toplam 81 hasta dahil edildi. Hastaların 35'inin (%43,2) RT-PCR testi pozitifken, 46'sının (%56,8) negatifti. RT-PCR negatif hastalarda iyi tanımlanmış merkezi GGO ve tomurcuklu ağaç nodülleri sıklıkla görüldü (sırasıyla p=0,016 ve p=0,027). RT-PCR pozitif hastaların 16'sında (%45,7), RT-PCR negatif hastaların 34'ünde (%73,9) lezyonlar sol alt lob yerleşimliydi (p=0,010). RT-PCR pozitif hastaların %68,6'sında ve negatif hastaların %54,3'ünde non-dependan yerleşim olduğu izlendi. Regresyon analizi sonucuna göre; non-dependan yerleşim varlığı (OR: 4,91, %95 GA: 1,12–20,21) ve tomurcuklanan ağaç nodüllerinin olmaması (OR: 0,15, %95 GA: 0,03–0,78) pozitif RT-PCR test sonucunun bağımsız belirteçleri olarak bulundu.

Sonuç: Akciğerde non-dependan lezyonların görülmesi ve tomurcuklanan ağaç nodüllerinin görülmemesi; pozitif RT-PCR test sonucu ile ilişkili olabilir.

Anahtar Kelimeler: Bilgisayarlı tomografi, pnömoni, koronavirüs, COVID-19

*** Corresponding author/İletişim kurulacak yazar:** Nurettin Ozgur Dogan; Kocaeli University, Faculty of Medicine ,41380, Kocaeli, Türkiye
Phone/Telefon: +90 262 303 75 75 **e-mail/e-posta:** nurettinozgurdogan@gmail.com
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Introduction

Coronavirus disease 2019 (COVID-19) was confirmed as a pandemic by the World Health Organization. To date, over 500 million confirmed cases and over 6 million deaths were reported.[1] Although different disease variants were previously documented, typical clinical findings were reported as fever, cough, dyspnea, and myalgia, although some patients may be asymptomatic.[2] Early and accurate diagnosis of COVID-19 is quite challenging and demands a cohesive approach among clinical, radiological, and laboratory data. A definitive diagnosis of COVID-19 is based on laboratory testing, which is most often the reverse transcriptase-polymerase chain reaction (RT-PCR) test assay. The RT-PCR test detects viral RNA and the inadequate technique and timing of sampling may cause false-negative results. The sensitivity of RT-PCR testing in clinical practice was reported to be between 42% and 83%.[3,4]

Typical computed tomography (CT) findings for COVID-19 pneumonia were reported as bilateral, multifocal, peripheral ground-glass (GGO) opacities and consolidations with a rounded or confluent pattern.[5-7] The halo and atoll signs were also described.[6-8] Although the sensitivity of chest CT in detecting COVID-19 pneumonia was reported as high as 56–98%, many other conditions may resemble its appearance, including pneumonia due to other viruses and organizing pneumonia.[9] Standardized nomenclature and categorized reporting of chest CT for suspected COVID-19 cases have great importance in these circumstances. The recent Radiological Society of North America expert consensus statement on chest CT reporting proposed four categories (*typical appearance, indeterminate appearance, atypical appearance, and negative for pneumonia*).[9] Indeterminate CT findings are reported by radiologists when it is not possible to completely rule out the disease radiologically[9]. In certain patients with other clinical conditions, such as congestive heart disease, lung edema, interstitial lung disease, and chronic obstructive pulmonary disease (COPD), both clinical symptoms and chest CT findings may overlap. Due to higher COVID-19 mortality rates in the elderly and those with other comorbidities, accurate and rapid diagnosis is vital.[10, 11]

The purpose of this study was to assess the features of indeterminate CT findings that can influence the diagnosis of COVID-19 pneumonia in patients in the emergency department with appropriate clinical symptoms.

Methods

Patients

This was a cross-sectional study of retrospectively collected single-center data on patients with clinically suspected COVID-19 infection between March and April 2020. Ethical committee approval was obtained before the study started.

All patients who had presented to the emergency department with suspected symptoms of COVID-19 pneumonia were enrolled in the study. The RT-PCR test results were extracted from each patient's electronic medical record in the hospital data system. Patients were grouped according to their latest RT-PCR test results if the test had been repeated.

Adult patients with clinically suspected COVID-19 pneumonia and indeterminate chest CT findings were enrolled in the study. Patients with normal chest CT findings, patients with typical chest CT findings for COVID-19 pneumonia, patients having a different diagnosis after chest CT, and patients without RT-PCR test results were excluded from the study.

CT Technique and Interpretation

CT examinations were performed on a 16-section scanner (Toshiba Aquilion 16, Toshiba Medical Systems, Otawara, Japan). The low-dose, non-contrast chest CT scanning variables were as follows: tube voltage, 120 kVp; tube current, 60 mAs; detector thickness, 1 mm; rotation time, 0.75 seconds; pitch, 1.5; reconstruction interval, 1.0–5.0 mm. Two thoracic radiologists, one with 15 and the other with four years of experience, who were blinded to the RT-PCR test results reviewed the CT images on the same day. All images were viewed on both lung (width, 1500 HU; level, -700 HU) and mediastinal (width, 350 HU; level, 40 HU) settings. The basic CT terminology described by the Fleischner Society glossary was used.[12]

Indeterminate CT findings included; non-peripheral (central or peribronchovascular), diffuse, or amorphous GGO / consolidations, and coexisting findings such as nodules, effusions, or septal lines.

For each patient, the following CT variables were evaluated:

Lesions: Ground-glass opacity, consolidation, tree-in-bud nodules, interlobular septal thickening, subpleural band, halo sign, atoll sign, pleural effusion, and pericardial effusion.

Location of the lesion: Lesions locations were categorized as peripheral or non-peripheral (central / peribronchovascular). The lesion was recorded as peripheral if located in the outer one-third of the lobe. Otherwise, it was defined as a central lesion. Dependent or non-dependent localization of lesions was also recorded.

Configuration, multiplicity, and margins of the lesion: Lesions were categorized as round, amorphous, and diffuse in terms of their configurations. Lobar or segmental distribution was recorded if present. The number of lesions was recorded as solitary, few (1-5), or multiple (more than 5). Margin characteristics were recorded as well-defined or unidentifiable margins.

Statistical Analysis

Data analysis was performed using SPSS Statistics for Windows version 20.0 (IBM Corp., Armonk, NY, USA). The

Shapiro-Wilk test was used to test normal distribution of continuous variables. Continuous variables were tested using the Mann-Whitney U test and expressed as median and interquartile ranges. Categorical variables were tested using the Chi-square test and Fisher's exact test. A multivariable logistic regression model was performed to evaluate independent predictors of positive RT-PCR test results. Only variables that were statistically significant at a level of $p<0.25$ were included in the multivariable model. Before the final model was established, a multicollinearity analysis was performed. The Hosmer-Lemeshow test was used to assess the fitness of the model, and the effects sizes were expressed with odds ratios (ORs) and 95% confidence intervals (CIs). All the statistical analyses were two-sided, and an alpha value <0.05 was considered to be the nominal level of significance.

Results

During the study period, a total of 262 adult patients with suspected COVID-19 were assessed for eligibility. Eighty patients were excluded because of entirely normal chest CT findings, and 39 were excluded because they had typical findings for COVID-19 pneumonia. Another 62 patients were excluded because their chest CT findings were consistent with other pathologies and atypical for COVID-19. The remaining 81 patients with indeterminate CT findings were ultimately included in the study.

Of the 81 patients, 35 (43.2%) had positive and 46 (56.8%) had negative RT-PCR test results. Demographic characteristics, RT-PCR test results, CT variables, and comorbidities are shown in Table 1.

Table 1. Univariate comparisons of variables regarding to RT-PCR status

	PCR (+) n=35	PCR (-) n=46	p value
Age, yr (IQR)*	46 (32-60)	58 (39-71)	**0.048**
Lesions			
Peripheral GGO**, well-defined margins, n (%)	19 (55.9%)	14 (30.4%)	**0.022**
Peripheral GGO, unidentifiable margins, n (%)	20 (57.1%)	29 (63.0%)	0.590
Peripheral GGO, diffuse, n (%)	1 (2.9%)	3 (6.5%)	0.630
Central GGO, well-defined margins, n (%)	7 (20.0%)	21 (45.7%)	**0.016**
Central GGO, unidentifiable margins, n (%)	3 (8.6%)	2 (4.3%)	0.647
Consolidation, well-defined margins, rounded or amorphous, n (%)	8 (22.9%)	15 (32.6%)	0.335
Consolidation, lobar- segmental distribution, n (%)	3 (8.6%)	1 (2.2%)	0.311
Tree-in-bud nodules, n (%)	3 (8.6%)	13 (28.3%)	**0.027**
Halo sign, n (%)	3 (8.6%)	1 (2.2%)	0.311
Interlobular septal thickening, n (%)	1 (2.9%)	8 (17.4%)	0.070
Pleural efusion, n (%)	4 (11.4%)	14 (30.4%)	**0.042**
Pericardial efusion, n (%)	1 (2.9%)	6 (13.0%)	0.133
Non-dependent location, n (%)	24 (68.6%)	25 (54.3%)	0.195
Subpleural discoid bands, n (%)	3 (8.6%)	8 (17.4%)	0.335
Lesion number			
Multiple (>5), n (%)	19 (54.3%)	36 (78.3%)	**0.022**
Few lesions (1-5), n (%)	16 (45.7%)	10 (21.7%)	
Lesion location			
Right upper lobe, n (%)	13 (37.1%)	15 (32.6%)	0.671
Right middle lobe, n (%)	14 (40.0%)	22 (47.8%)	0.483
Right lower lobe, n (%)	29 (82.9%)	38 (82.6%)	0.977
Left upper lobe, n (%)	13 (37.1%)	14 (30.4%)	0.526
Left lower lobe, n (%)	16 (45.7%)	34 (73.9%)	**0.010**
Comorbidities			
Bronchopneumonia or aspiration pneumonia, n (%)	5 (14.3%)	4 (8.7%)	0.490
Increased cardiothoracic ratio, n (%)	4 (11.4%)	16 (34.8%)	**0.016**
Malignancy, n (%)	3 (8.6%)	2 (4.3%)	0.647
Chronic obstructive pulmonary disease or severe emphysema, n (%)	2 (5.7%)	8 (17.4%)	0.174

*IQR: interquartile range

**GGO: ground-glass opacity

According to the univariate analysis results, patients with negative RT-PCR test results were older ($p=0.048$). Peripheral GGO lesions with well-defined margins were recorded in 19 of the positive RT-PCR test group (55.9%)

and 14 of the negative RT-PCR test group (30.4%). In contrast, centrally located GGO with well-defined margins were predominantly seen in patients in the negative RT-PCR test group (n=21, 45.7%), whereas only in the 7 (20.0%) of the patients in the positive RT-PCR test group. Similarly, tree-in-bud nodules and pleural effusion were more frequently reported in the RT-PCR negative patients (28.3% vs 8.6% and 30.4% vs 11.4%, respectively). Multiple lesions were reported in 36 (78.3%) patients in the RT-PCR negative group and 19 (54.3%) patients in the positive group. Regarding comorbidities, patients with increased cardiothoracic ratio were predominantly seen in the RT-PCR negative patients (34.8% vs 11.4%).

Only the variables that had an alpha value under 0.25 in the univariate hypothesis tests were included in the final multivariate model. According to multicollinearity analysis, a high correlation between the increased cardiothoracic ratio and pleural fluid was observed (r=0.658, p<0.001), and only cardiothoracic ratio was included in the final model. Model fitness was established in the multivariate model (Hosmer-Lemeshow test, p=0.613).

Regarding the results of the binary regression analysis, the non-dependent location of the lesion and absence of tree-in-bud nodules were found to be independent predictors of positive RT-PCR test results. The absence of tree-in-bud nodules increased approximately 6.7 times (OR: 0.15, 95% CI: 0.03–0.78), and the presence of non-dependent location of the densities increased 4.9 times (OR: 4.91, 95% CI: 1.12–20.21) the positive RT-PCR test diagnosis (Table 2).

Table 2. Multivariate logistic regression model for independent predictors of positive RT-PCR result

	Wald	Odds ratio (95% CI)	p value
Age, yr	0.716	0.98 (0.95 - 1.02)	0.398
Peripheral GGO*, well-defined margins	0.538	1.59 (0.46 - 5.53)	0.463
Central GGO, well-defined margins	2.291	0.35 (0.09 - 1.37)	0.130
Multiple lesions (>5)	0.021	1.12 (0.24 - 5.30)	0.884
Left lower lobe location	1.993	0.37 (0.09 - 1.47)	0.158
Tree-in-bud nodules	5.114	**0.15 (0.03 - 0.78)**	**0.024**
Increased cardiothoracic ratio	0.278	0.59 (0.08 - 4.22)	0.598
Interlobular septal thickening	0.347	0.43 (0.03 - 7.14)	0.556
Non-dependent location	4.892	**4.91 (1.12 - 20.12)**	**0.027**
Subpleural discoid bands	0.672	0.34 (0.03 - 4.47)	0.412
Pericardial efusion	1.422	0.19 (0.01 - 2.94)	0.233
Severe emphysema	1.015	0.35 (0.05 - 2.71)	0.314

*GGO: ground-glass opacity

Discussion

Establishing a common and standard language in the radiological reporting of COVID-19 pneumonia is important in the emergency department where rapid diagnosis is vital. To achieve this, RSNA has created 4 categories in the standard COVID-19 pneumonia reporting system published in 2020.[9] In the category defined as indeterminate, the lesions are not specific to COVID-19 and have a wide differential diagnosis. There may be an increase in indeterminate chest CT lesions with increasing patient age and the coexistence of pulmonary comorbidities in COVID-19 patients. Pulmonary comorbidities complicate CT diagnosis of COVID-19 pneumonia for radiologists and necessitate scrupulous attention to detail because patients with comorbidities have poorer clinical outcomes than those without.[10]

In our study, well-defined peripheral GGO lesions were observed more frequently in RT-PCR positive patients, whereas well-defined central GGO lesions, tree-in-bud nodules, pleural effusion, multiple lesions, left lower lobe localization, and the increased cardiothoracic ratio were more prominent in RT-PCR negative patients. Among the variables in the study, it was determined that the factors most strongly predicted RT-PCR positivity were the non-dependant localization of the GGO/consolidations and the absence of the tree-in-bud nodules.

Similar to previous studies, we observed in our study that peripheral GGO lesions were more common in RT-PCR positive patients, while central GGO lesions were less common.[13-16] But it seems that it is not very accurate to speculate about RT-PCR positivity based on GGO lesions according to the multivariate analysis results of our study. It is also inappropriate to predict the COVID-19 status based solely on the presence of single or multiple GGO lesions. In addition, halo and atoll signs have been highly described for COVID-19 pneumonia in the literature, but their frequency in our study was quite low.[14,15] This may be related to the very early CT imaging of patients in the emergency department.

In our study non-dependent location of the lesions in chest CT was an independent predictor for RT-PCR positivity (Figure 2). In previous studies, COVID-19 lesions were detected more often in the posterior portions of the lung.[6,9,17] However, in these studies, patients were generally in the group who applied to the outpatient clinic and had low comorbidities, and typical COVID-19

lesions were investigated. Since our patient group consists of patients who presented to the emergency department, their comorbidities were probably high. Most of the accompanying lesions such as edema, pleural fluid, compressive atelectasis, and aspiration in these patients may involve the posterior dependant regions of the lungs, which may reinforce the importance of evaluating the non-dependent portions of the lung for COVID-19 pneumonia.

Positive RT-PCR test results also had a significant negative relationship with tree-in-bud lesions. Tree-in-bud lesions are more specific for airway diseases and non-COVID-19 infections.[9,16] Therefore these nodules are described in the atypical category for COVID-19 pneumonia in the literature.[7,9,13] However, since these nodules often accompany GGO, they are included in the indeterminate group in another study like ours.[16] In our study, of the 16 patients who had tree-in-bud nodules, only three had positive RT-PCR test results (3/16, 18.75%). All three had peripheral ground-glass lesions together with nodules, and one patient had bacterial co-infection clinically in addition to COVID-19 pneumonia (Figure 1). Even in the presence of ground-glass opacifications, tree-in-bud nodules may discourage the diagnosis of COVID-19 pneumonia.

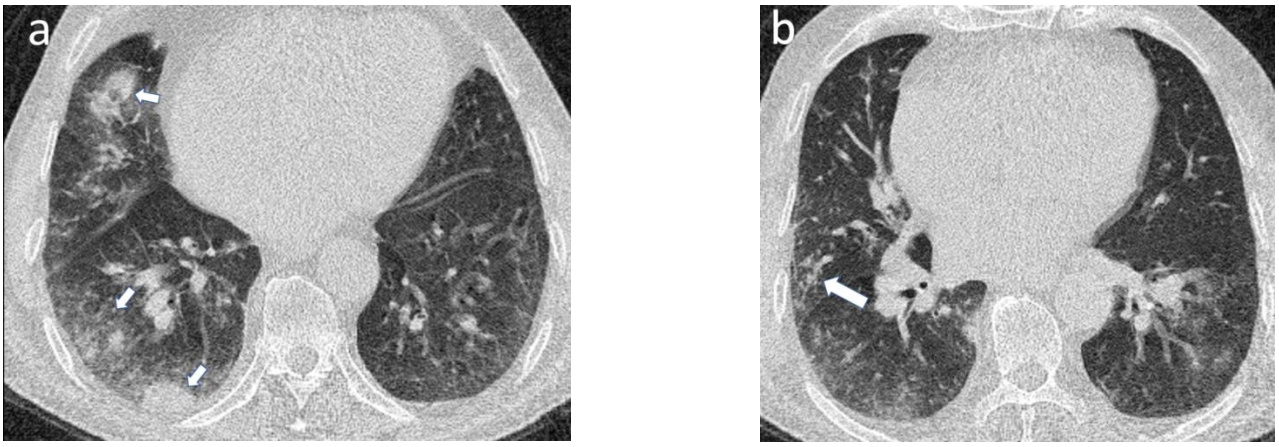

Figure 1. A 60-year-old man with a positive RT-PCR test presents with fever and dyspnea
a: Chest CT shows bilateral, peripheral located, rounded ground-glass lesions and consolidations with well-defined margins (arrows)
b: There are also centrilobular and tree-in-bud nodules (arrow) not consistent with COVID-19 infection in the same patient. The patient is diagnosed with COVID-19 pneumonia with co-infection

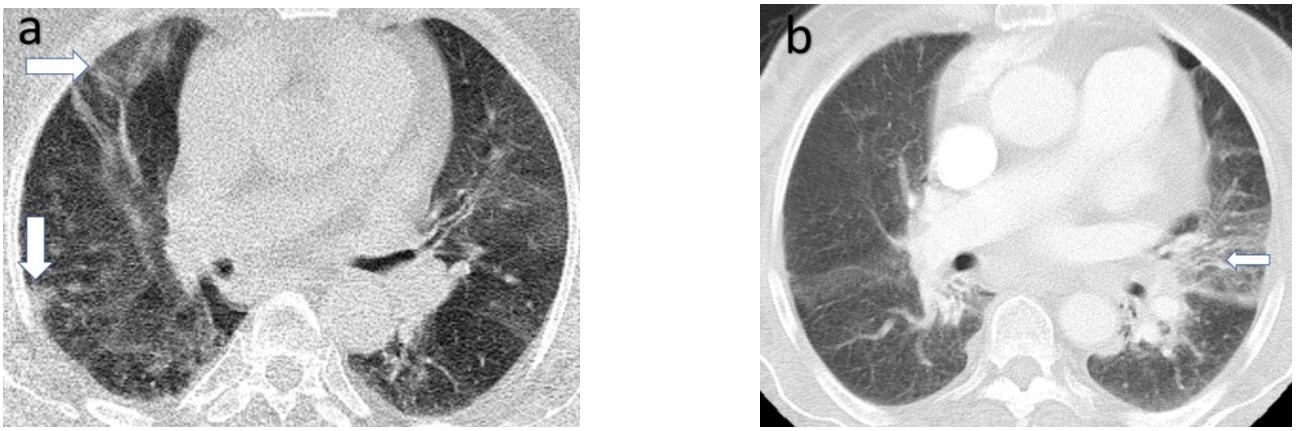

Figure 2. a: An 81-year-old man with a positive RT-PCR test. Chest CT shows bilateral, peripheral, amorphous ground-glass lesions with well-defined margins in the non-dependant region of right upper and lower lobes (arrows)
b: A 70-year-old woman presents with dyspnea and cough with a negative RT-PCR test, who has a history of cardiovascular disease and pulmonary hypertension. Chest CT shows amorphous, ground-glass lesions in the dependent portion of the left upper lobe (arrow)

Although pleural effusion was revealed as atypical for COVID-19 pneumonia in many reports of RT-PCR test-positive patients, its association with cardiac comorbidities was not mentioned before.[17-19] In our study pleural effusion had a strong relationship with increased cardiothoracic ratio but not with positive RT-PCR test.

There are several limitations to this study. First, it is a retrospective study from a single center. Second, a larger sample size of patients with a wide range of co-morbidities with indeterminate lesions in different patients would reflect more precise results. Third, some patients had difficulty breathing during their CT scans due to comorbidities, so their images were expiratory and had motion artifacts. As a result, it was difficult to evaluate the images in this population. Fourth, other atypical infections that can mimic COVID-19 pneumonia were absent from the study because they were categorized as atypical for COVID-19 pneumonia. There could be atypical appearances of COVID-19 pneumonia in that group, and including these patients would affect the results.

Conclusion

Our study evaluated the indeterminate chest CT findings in patients with suspected COVID-19 pneumonia who applied to the emergency department. The differential diagnosis of GGO/consolidations for COVID-19 pneumonia is difficult in this patient group due to comorbidities and advanced age. The localization of GGO lesions in non-dependent areas strongly supports the diagnosis of COVID-19 pneumonia. Tree-in-bud nodules and radiological clues of congestive heart failure, such as increased cardiothoracic ratio and pleural fluid, may distract us from the diagnosis of COVID-19 pneumonia.

Compliance with Ethical Standards

Ethical committee approval was obtained from Kocaeli University (KÜ-GOKAEK-2020/09.25).

Conflict of Interest

None declared.

Author Contribution

NOD, SD: Design, ED, EA, AK, MK: Patients, NOD: Methodology-Statistics, SD: Writing, NOD: Critical Revision.

Financial Disclosure

None declared.

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Research Article / Araştırma Makalesi

INVESTIGATION OF THE RELATIONSHIP BETWEEN INFLAMMATION PARAMETERS AND BLOOD GROUPS IN NEWLY DIAGNOSED TYPE 2 DIABETES PATIENTS

YENİ TANI ALAN TİP 2 DİYABET HASTALARINDA İNFLAMASYON PARAMETRELERİNİN KAN GRUPLARI İLE İLİŞKİSİNİN İNCELENMESİ

Orkun Sarıçam [1] *

[1] Ankara Pursaklar State Hospital, Department of Internal Medicine. Ankara, Türkiye.

ABSTRACT

Objective: Type 2 diabetes mellitus (T2DM) is a chronic disease with an increasing prevalence, accounting for 90-95% of all diabetics. It is considered that the disease is induced by inflammation. In this study, we aimed to investigate the inflammation parameters and the relationship of these parameters with blood groups in newly diagnosed T2DM patients.

Methods: The study included 80 newly diagnosed T2DM patients and 80 healthy volunteers. Demographic characteristics, body mass indexes (BMI), biochemistry and hemogram test results, C-reactive protein (CRP) values, and blood groups of the patient and control groups were recorded and compared between the groups.

Results: The mean age was 51.64 years and 53.8% of the patients were male in the newly diagnosed T2DM group. The counts of white blood cells (WBC), neutrophils, lymphocytes, platelets, and monocytes, the values of the monocyte/HDL ratio (MHR) and the systemic immune-inflammatory index (SII), and the CRP levels of T2DM patients were statistically significantly higher than those of the control group ($p<0.05$). Of the T2DM patients, the platelet counts were statistically significantly higher in the blood type B group compared to the blood type O group ($p=0.022$).

Conclusion: We think that the hematological parameters of inflammation and the high MHR and SII ratios the presence of chronic systematic inflammation in newly diagnosed T2DM patients before the development of complications. Therefore, these parameters may guide the diagnosis and preventive treatment in prediabetic patients.

Keywords: Type 2 diabetes mellitus, inflammation, blood group

ÖZ

Amaç: Tip 2 diyabetes mellitus (T2DM), inflamasyonun indüklediği düşünülen, tüm diyabetlilerin %90-95'ini oluşturan prevelansı giderek artan kronik bir hastalıktır. Bu çalışmada yeni tanı almış T2DM olan hastalarda inflamasyon parametrelerini ve bu parametrelerin kan grupları ile ilişkisini araştırmayı amaçladık.

Yöntem: Bu çalışmaya 80 yeni tanı almış T2DM hastası, 80 sağlıklı gönüllü dâhil edildi. Hadta ve kontrol grubunun demografik özellikleri, vücut kitle indeksi (VKİ) biyokimya, hemogram, C-reaktif protein (CRP) ve kan grupları kayıt gruplar arasında karşılaştırıldı.

Bulgular: Yeni tanı diyabet alan T2DM hatalarının yaş ortalaması 51,64'dü ve hastaların %53,8'i erkekti. T2DM hastaların beyaz kan hücresi (BKH), nötrofil, lenfosit, platelet, monosit, monosit/HDL oranı (MHO), sistemik immün-inflamatuar indeks (Sİİ) ve CRP düzeyleri kontrol grubundan istatistiksel olarak anlamlı derecede yüksek saptandı ($p<0,05$). T2DM'li hastalarda kan grubu B olanların platelet değerleri, kan grubu O olan gruptan istatistiksel olarak anlamlı derecede yüksek saptandı ($p=0,022$).

Sonuç: Yeni tanı T2DM hastalarında inflamasyonu gösteren hematolojik parametrelerin ve MHO ve Sİİ oranlarındaki yüksekliğinin, hastalarda komplikasyon gelişmeden kronik sistemik inflamasyonun varlığını gösterdiğini, prediyabetik hastalarda tanıda ve koruyucu tedaviye başlamada yol gösterici olabileceğini düşünüyoruz.

Anahtar Kelimeler: Tip 2 diyabetes mellitus, inflamasyon, kan grubu.

İletişim kurulacak yazar/Corresponding author: Orkun Sarıçam; Ankara Pursaklar State Hospital of International Medicine. Ankara, Turkey.
Telefon/Phone: +90 (505) 629 84 77 **e-posta/e-mail:** orkunsar@hotmail.com
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Introduction

Diabetes mellitus is a chronic metabolic and systemic disorder characterized by impairments in fat metabolism, protein and carbohydrate due to partial or complete insulin deficiency and/or insulin resistance leading to chronic hyperglycemia.[1] Type 2 diabetes mellitus (T2DM) accounts for 90-95% of all diabetes cases. Despite the evidence for the significant role of genetics, the prevalence of T2DM is on the rise, especially in children and young adults, due to increasing obesity rates.[2]

Significant evidence has been established in recent years showing that low-grade inflammation is an important pathogenic determinant of T2DM.[3] Although the underlying mechanisms have not been clarified yet, supporting evidence is available for the hypothesis that the increase in central fat mass due to chronic inflammation and the production of proinflammatory cytokines such as Tumor Necrosis Factor-alpha (TNFα) and Interleukin-1 (IL-1) constitute the risk factors for the development of T2DM.[4,5] Furthermore, acute phase proteins and certain cytokines are involved in several metabolic pathways such as insulin regulation and the functioning of lipoprotein lipases and adipocytes, contributing to the development of insulin resistance.[6] In recent years, easily accessible and cost-effective biomarkers have been investigated such as neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) for their association with T2DM.[7] Some studies have shown the association of the NLR ratio with glucose control in T2DM.[8]

It has been suggested in the literature that ABO blood group antigens may play a role in the pathogenesis of cancers and cardiovascular, endocrine, and metabolic disorders.[9,10] It has been documented in studies that the increased levels of plasma lipids and inflammatory markers are associated with the variants of ABO gene loci, especially of the A and B antigens and that they are well-known risk factors for DM.[11,12] The inflammatory mechanisms in the development of T2DM and the relationship of ABO blood groups with diabetes and inflammation have not been fully elucidated, yet. In this study, we aimed to investigate the inflammatory parameters and the relationship of these parameters with blood groups in newly diagnosed T2DM patients.

Methods

The study was approved by Health Sciences University Dışkapı Yıldırım Beyazıt Training and Research Hospital Ethics Committee (2021-11/123/04). All procedures were conducted in accordance with the principles of the Declaration of Helsinki. The study included 80 patients, who presented to the internal medicine outpatient clinic for routine examinations and were diagnosed with T2DM for the first time based on relevant laboratory parameters. Patients with a history of chronic disease (hypertension, hematologic disorders, or chronic diseases of the liver, kidney, heart, lung, or thyroid), immunodeficiency, acute or chronic inflammatory diseases, oncological diseases, thalassemia, infections in the past 1 month, pregnant women, or those with a history of drug use were not included in the patient group. In the control group, 80 healthy volunteers, who presented to the outpatient clinic for routine check-up visits were included.

Demographic characteristics, the results of biochemistry and hemogram tests, C-reactive protein (CRP) levels, and blood groups of the patients and healthy controls were recorded. PLR indicates the ratio of platelet to lymphocyte, NLR indicates the ratio of neutrophil to lymphocyte, and MHR indicates the ratio of monocyte to high-density lipoprotein (HDL). Systemicimmune-inflammation index (SII) = neutrophil count X platelet count/lymphocyte count.[13] SII = neutrophil count X platelet count/lymphocyte count. The measurement of HDL and fasting plasma glucose levels of the study population after 12-hour fasting was conducted by enzymatic colorimetric assay. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters. Diabetes mellitus was diagnosed in patients with a blood glucose value of ≥126 mg/dL and a Hemoglobin A1c (HbA1c) value of ≥ 6.5% mmol/mol, both of which were measured after at least 8 hours of fasting.[1]

Statistical Analysis

Study data were collected from 160 people, of whom 80 were patients and 80 were healthy controls. Descriptive statistics (median, mean, standard deviation) and frequencies (percentage, number) were used for categorical and numerical variables, respectively. IBM SPSS Statistics 26 package software were used for analyzes.

Kolmogorov Smirnov test of normality was used for the normality assumptions of the numerical variables and it was found that the variables were not normally distributed. Therefore, non-parametric statistical methods were used in the study. Spearman's Rho Correlation coefficient was used for the relationships between two independent numerical variables. Chi-square analyses were used for the relationships between two independent categorical variables. Differences among more than two independent groups were evaluated with the Kruskal-Wallis test. Differences between two independent groups were evaluated with the Mann-Whitney U Test. Statistical significance was interpreted at the 0.05 level.

Results

The mean age was 51.64±10.145 years and 48.54±11.966 years in the T2DM and control groups, respectively. Males constituted 53.8% of the T2DM patients and 56.3% of the individuals in the control group. BMI was 30.60±4.610 kg/m² in the T2DM group and 24.11±3.236 kg/m² in the control group ($p<0.001$). The distribution of blood types

was not different between the T2DM and control groups (p>0.05) (Table 1).

Table 1. Demographic characteristic of patients (N=160).

	T2DM (n=80) Mean±SD	Control (n=80) Mean±SD	Z	p
Age (years)	51.64±10.145	48.54±11.966	-1,682	0,093
BMI	30.60±4.610	24.11±3.236	-8,286	**0,000***
	N (%)	**N (%)**	**Chi-Square**	**p**
Gender				
Female	37 (46.3)	35 (43.8)	0.101	0.751
Male	43 (%53.8)	45 (56.3)		
Blood Group				
A	25 (31.3)	30 (37.5)	4.792	0.188
B	25 (31.3)	14 (17.5)		
AB	13 (16.3)	12 (15.0)		
0	17 (21.3)	24 (30.0)		

Z: Mann Whitney U Analizi *: p<0,05 BMI: Body mass index, T2DM: Type 2 diabetes mellitus

The values of glucose and HbA1c; the counts of white blood cells (WBC), neutrophils, lymphocytes, platelets, and monocytes; and the levels of MHR, SII, and CRP were statistically significantly higher in the T2DM patients compared to the control group (p<0.05). HDL levels of T2DM patients were significantly lower than those of individuals in the control group (p<0.001) (Table 2).

The comparison of the blood types with the inflammatory parameters in the T2DM group revealed that diabetes patients with blood type B had statistically significantly higher platelet counts compared to those with blood type O (p=0.022) (Table 3).

Table 2. Biochemical Test Results and Differences by Groups (N=160)

	T2DM (n=80) Mean±SD	Control (n=80) Mean±SD	Z	p
Glucose (mg/dl)	243.00±79.261	93.43±8.918	-10.923	**0.000***
HbA1c (%)	9.98±2.765	5.23±0.730	-10.925	**0.000***
WBC (×10³ µl)	8.22±1.562	7.18±1.586	-3.625	**0.000***
Neu (×10³ µl)	5.07±1.349	4.04±1.152	-4.780	**0.000***
Lym (×10³ µl)	2.50±0.820	2.22±0.696	-2.123	**0.034***
Plt (×10³ µl)	293.34±74.732	258.39±70,783	-2.386	**0.017***
Mon (×10³ µl)	0.53±0.759	0.46±0.686	-2,403	**0.016***
HDL (mg/dl)	46.09±11.049	54.55±12.975	-4.238	**0.000***
NLR	2.27±1.007	1.96±0.794	-1.853	0.064
PLR	130.81±58.81	124.81±46.332	-0.183	0.855
MHR	0.01±0.023	0.01±0.012	-4.107	**0.000***
SII	670.46±374.772	497.38±222.013	-3.100	**0.002***
CRP (mg/l)	0.64±0.740	0.26±0.387	-6.580	**0.000***

Z: Mann Whitney U Analysis, *:p<0,05, T2DM: Type 2 diabetes mellitus, HbA1c: Hemoglobin A1c, WBC: White blood cell, Neu: Neutrophils, Lym: Lymphocytes, Plt: Platelets, Mon: Monocytes, HDL: High-density lipoprotein, NLR: Neutrophil/lymphocyte ratio, , PLR: Platelet/lymphocyte ratio, MHR: Monocyte/HDL ratio, SII: Systemic immune-inflammation index, CRP: C-reactive protein

Discussion

It is predicted that T2DM will continue to be on the rise in the coming years, mostly in individuals between the ages of 45-64 years and more commonly in developing countries.[14] Although there are some differences in gender ratios across countries, T2DM is usually diagnosed in males at a lower age and with low body

Table 3. Biochemical Results According to Blood Groups in T2DM patients (N=80)

	1) A group Mean±SD	2) B group Mean±SD	3) AB group Mean±SD	4) 0 group Mean±SD	KW	p
WBC (×10³ µl)	8.37±1.723	8,36±1.256	8.27±1.794	7.75±1.585	1.522	0.677
Neu (×10³ µl)	5.14±1.405	5.13±1.158	5.33±1.643	4.7±1.333	2.268	0.519
Lym (×10³ µl)	2.75±0.96	2.44±0.759	2.55±0.76	2.16±0.647	4.971	0.174
Plt (×10³ µl)	293.68±80.15	311.36±70.488	317.38±86.58	247.94±41.198	9.661	**0.022* 0-B**
Mon (×10³ µl)	0.46±0.14	0.72±1.34	0.47±0.144	0.38±0.121	4.585	0.205
HDL	48.06±13.089	47.44±9.337	42.69±7.488	43.82±12.218	3.35	0.341
NLR	2.06±0.748	2.38±1.159	2.32±1.03	2.39±1.123	0.723	0.868
PLR	118.96±50.559	143.98±70.849	137.1±65.699	124.06±43.645	2.18	0.536
MHR	0.01±0.008	0.02±0.04	0.01±0.005	0.01±0.002	2.173	0.537
SII	603.9±271,653	755.32±477.361	736.4±407.479	593.12±297.318	2.126	0.547
CRP(mg/l)	0.68±0.537	0.77±1.158	0.52±0.411	0.48±0.256	1.612	0.657
BMI	31.12±4.746	31.52±4,265	27.62±4.835	30.74±4.164	6.848	0.077

Z: Mann Whitney U Analysis, *:p<0,05, T2DM: Type 2 diabetes mellitus, HbA1c: Hemoglobin A1c, WBC: White blood cell, Neu: Neutrophils, Lym: Lymphocytes, Plt: Platelets, Mon: Monocytes, HDL: High-density lipoprotein, NLR: Neutrophil/lymphocyte ratio, , PLR: Platelet/lymphocyte ratio, MHR: Monocyte/HDL ratio, SII: Systemic immune-inflammation index, CRP: C-reactive protein

mass indexes. Obesity, which is the most significant risk factor, is more common in women.[15] The mean age of newly diagnosed T2DM patients in our study was 51.64 years and 53.8% of our patients were men. The majority of patients, who were diagnosed with T2DM, were obese with a mean BMI of 30.60 kg/m², which was significantly higher than that of the control group.

Evidence obtained from studies shows that there is a relationship between the diabetes risk and the total peripheral WBC or leukocyte count, which is a non-specific inflammatory marker.[16] Zhang et al. reported significantly increased total counts of WBC, neutrophils, and lymphocytes in patients with newly diagnosed diabetes compared to those without diabetes.[17] Similarly, we found significantly higher counts of WBC, neutrophils, lymphocytes, and monocytes in newly diagnosed T2DM patients compared to the control group in our study. Studies have shown evidence that CRP levels are predictive of developing diabetes, indicating the relationship between inflammation and glycemic control in patients with T2DM.[18,19] It has been observed in T2DM patients that the activation, adhesion, and aggregation of platelets increase due to the dysregulation of various signaling pathways.[20] Thus, platelets have become proinflammatory entities that cause inflammation in T2DM.[21] In our study, we found that platelet counts and CRP levels in newly diagnosed T2DM patients were significantly higher than those of the control group, suggesting that these increased levels represented the indicators of inflammation.

Various studies with diabetic patients have shown that inflammatory ratios such as NLR and PLR are predictive of diabetes-related microvascular and atherosclerotic complications and that such ratios have prognostic values.[22,8] In our study, there were no significant differences in NLR and PLR values between newly diagnosed T2DM patients and the control group but MHR and SII values were found to be significantly higher in T2DM patients. There are only a few studies in the literature investigating the relationship of hematological parameters such as MHR and SII with T2DM. In a study, Cardoso et al. reported an association between MHR and an increased risk for cardiovascular mortality in patients with T2DM.[23] Wang et al. showed that a high SII value may be a risk factor for depression in diabetic patients.[24] In our study, we have thought that a high SII value and increased MHR in newly diagnosed T2DM patients compared to the control group have indicated the presence of chronic systemic inflammation before the development of diabetic complications and that these parameters can be used as predictive markers in the diagnosis of prediabetic patients.

Blood group antigens are considered among hereditary indicators, playing a vital role in understanding genetics and disease susceptibility.[25] In recent years, the relationship between blood types and T2DM has been investigated to identify susceptible groups. Although some studies have reported conflicting results, B and O blood types have been associated with increased and decreased risk of developing T2DM, respectively.[26, 27] In our study, no differences were found in blood types between the patients with T2DM and the control group. However, when the blood groups and inflammation markers were compared in the patient group with T2DM, platelet counts were found to be statistically significantly higher in individuals with group B compared to those with group O. We thought that these results were associated with a higher risk of inflammation in patients with B blood group compared to O blood group among T2DM patients. Although studies have reported different results regarding the relationship between susceptibility to inflammatory diseases and ABO blood group antigens, it has been shown that the risk of developing thromboembolic and cardiovascular disease is lower in people with O blood group compared to other blood groups[28].

Our study is not free of limitations. The study sample is not large because of the inclusion of only newly diagnosed diabetic patients in the study and the strict exclusion criteria. Another limitation of the study is the retrospective design.

Conclusions

In conclusion, we suggest that the inflammatory hematological parameters in newly diagnosed T2DM patients and the high MHR and SII ratios indicate the presence of chronic systemic inflammation in patients before the development of complications and that such parameters and ratios may lead to the diagnosis and starting preventive treatment in prediabetic patients. We think that, among newly diagnosed T2DM patients, higher platelet counts in individuals with B blood group compared to individuals with group O are associated with an increased risk of inflammation and that comprehensive studies with large samples will contribute further to the early diagnosis and the prediction of prognosis.

Compliance with Ethical Standards

Ethics committee approval of the study was obtained from the Health Sciences University Dışkapı Yıldırım Beyazıt Training and Research Hospital Ethics Committee (Decision no: 01-11-2021/123/04).

Conflict of Interest

The author declares no conflicts of interest.

Author Contribution

OS: Study idea, hypothesis, study design; Material preparation, data collection and analysis; Writing the article; Critical review and publication process

Financial Disclosure

None

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Araştırma Makalesi | Research Article

ÇOCUKLAR İÇİN ÖZEL GEREKSİNİM RAPORU (ÇÖZGER)'NA BAŞVURAN OLGULARIN PSİKİYATRİK TANI VE ÖZEL GEREKSİNİM DÜZEYLERİNİN ÖNCEKİ YÖNETMELİKTEKİ TANI VE ÖZÜR ORANLARI İLE KARŞILAŞTIRILMASI: RETROSPEKTİF ÇALIŞMA

COMPARISON OF PSYCHIATRIC DIAGNOSIS AND SPECIAL NEEDS LEVELS OF CASES WHO APPLIED TO THE SPECIAL NEED REPORTS FOR CHILDREN (SNRFC) WITH THE DIAGNOSIS AND DISABILITY RATES IN PREVIOUS REGULATION: A RETROSPECTIVE STUDY

Rahime Duygu Temeltürk[1*], Sabide Duygu Uygun[1], Merve Canlı[2], Ayşegül Efe[2], Yusuf Gürel[3], Miray Çetinkaya[2], Fatma Hülya Çakmak[4]

[1]Ankara Üniversitesi Tıp Fakültesi, Çocuk ve Ergen Ruh Sağlığı ve Hastalıkları Anabilim Dalı, Ankara, Türkiye. [2]Sağlık Bilimleri Üniversitesi, Dr. Sami Ulus Kadın Doğum Çocuk Sağlığı ve Hastalıkları Eğitim ve Araştırma Hastanesi, Çocuk ve Ergen Ruh Sağlığı ve Hastalıkları Bölümü, Ankara, Türkiye. [3]Sağlık Bilimleri Üniversitesi, Konya Beyhekim Eğitim ve Araştırma Hastanesi, Çocuk ve Ergen Ruh Sağlığı ve Hastalıkları Bölümü, Konya, Türkiye. [4]Beylikdüzü Devlet Hastanesi, Çocuk ve Ergen Ruh Sağlığı ve Hastalıkları Bölümü, İstanbul, Türkiye.

ÖZ

Amaç: Bu çalışmada Çocuklar İçin Özel Gereksinim Raporu (ÇÖZGER)'na başvuran olguların sosyodemografik ve klinik özelliklerinin incelenmesi, psikiyatrik tanı ve özel gereksinim düzeylerinin eski yönetmelik ile karşılaştırılması amaçlanmıştır.

Yöntem: Çalışmamızda, Haziran 2019-Aralık 2019 tarihleri arasında Dr. Sami Ulus Kadın Doğum, Çocuk Sağlığı ve Hastalıkları Eğitim Araştırma Hastanesi sağlık kuruluna ÇÖZGER için başvuran ve psikiyatri ile ilgili herhangi bir alandan özel gereksinimi bulunan 6-18 yaş arasındaki çocuk ve ergenin dosya verileri geriye dönük olarak değerlendirilmiştir.

Bulgular: Çalışmaya %38'i (n=474) kız, %62'si (n=772) erkek olan 1246 olgu dahil edildi. Ortalama yaş 12,1±4,6 idi. Olguların %58 (n=723)'inde gecikmiş dönüm noktası, en sık olarak Hafif Düzeyde Zihinsel Yetersizlik (ZY) saptandı (n=459; 36,8%). Olguların %19,8 (n=247)'inde en az bir psikiyatri dışı alanda özel gereksinim varlığı tespit edilmiş olup en sık olarak hareket sistemi ve sinir sistemi alanlarından olduğu belirlendi (sırasıyla, n=156 ve n=88). ÇÖZGER sonrasında, eski sistemine göre Hafif Düzeyde ZY olgularında anlamlı bir azalma olduğu ($\chi2$=19,41; p<0,001), Orta Düzeyde ZY, Ağır Düzeyde ZY ve Dil-konuşma bozuklukları tanılarının anlamlı düzeyde daha fazla belirtildiği (sırasıyla $\chi2$=5,63 p<0,05; $\chi2$=5,14 p<0,05; $\chi2$=8,16 p<0,01) bulundu. ÇÖZGER'de %20-39 ve %90-99 engel oranı aralığında anlamlı artışlar olduğu gözlendi (sırasıyla, $\chi2$=105,60; p<0,001 ve $\chi2$=159,00; p<0,001).

Sonuç: Sağlık kurulu için ÇÖZGER'e başvuran çocuk ve ergenlerle ilgili tanımlayıcı verilerin belirlenmesi gerek psikiyatristlere gerek de diğer branşlarda çalışan hekimlere klinik pratikte önemli yararlar sağlayacaktır. ÇÖZGER'e geçiş sürecinin değerlendirildiği, ülkemizde farklı merkezlerin dahil edildiği daha geniş örneklemli ileri araştırmalara ihtiyaç vardır.

Anahtar kelimeler: Çocuk psikiyatrisi, sağlık kurulu, psikiyatrik tanı, özel gereksinim

ABSTRACT

Objective: In this study, it was aimed to examine the sociodemographic and clinical characteristics of cases who applied to Special Need Reports for Children (SNRFC), and to compare psychiatric diagnoses and special needs levels with previous regulation.

Methods: Chart reviews of children and adolescents aged between 6-18 years old who applied to Dr. Sami Ulus Maternity and Child Health and Diseases Training and Research Hospital Medical Board, and had special needs from any sections related to psychiatry on June 2019-December 2019, were analyzed retrospectively.

Results: The study included 1246 cases whose 38%(n=474) were female and 62%(n=772) were male. The mean age was 12.1±4.6.Delayed milestone was determined in 58% of cases(n=723), Mild Intellectual Disability(ID) was found most frequently found(n=459; 36.8%).Special needs in at least one field other than psychiatry was detected in 19.8%(n=247) of the cases,the most common diagnoses were specified in physical medicine and rehabilitation and neurology areas(n=156 and n=88, respectively).After SNRFC, there was a significant reduction of cases diagnosed with Mild ID compared to the old regulation($\chi2$=19.41; p<0.001),whereas the diagnoses of Moderate ID,Severe ID and Language-Speech Disorders were significantly higher($\chi2$=5.63 p<0.05; $\chi2$=5.14 p<0.05; $\chi2$=8.16 p<0.01, respectively).Significant increases were observed in range of 20-39% and 90-99% disability ratios in SNRFC($\chi2$=105.60; p<0.001 and $\chi2$=159.00; p<0.001, respectively).

Conclusion: Determining the descriptive data about children and adolescents who applied to SNRFC will provide important benefits in clinical practice both for psychiatrists and physicians working in other specialties.Further studies with larger samples from different centers are needed to assess the transition to SNRFC.

Keywords: Child psychiatry, medical board, psychiatric diagnosis, special needs

*** İletişim kurulacak yazar/Corresponding author:** Rahime Duygu Temeltürk; Ankara Üniversitesi Tıp Fakültesi, Çocuk ve Ergen Ruh Sağlığı ve Hastalıkları Anabilim Dalı, Balkiraz Mahallesi, Tıp Fakültesi Caddesi, No:1/4, 06620, Mamak, Ankara, Türkiye
Telefon/Phone: +90 (312) 595 64 99 **e-posta/e-mail:** rduygukaydok@gmail.com

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Giriş

Özel gereksinimi olan birey, bedensel, zihinsel, ruhsal ve sosyal özelliklerinde işlev kaybı veya bozukluğu sonucu normal yaşamın gereklerine uymayan ve genel eğitim hizmetlerinden yararlanamayan kişiler olarak tanımlanmaktadır.[1,2] Bu bireylerin tanımlanmasında, yaşanılan dönemin sosyokültürel, ekonomik, siyasi özellikleri ile dünyada yaşanan teknolojik ve bilimsel gelişmelere paralel olarak çeşitli terimlerin kullanıldığı bilinmektedir. Geçmişte "anormal", "ayrıcalıklı", "engelli", "özürlü", "yetersiz", "özel eğitime muhtaç" gibi kavramlar kullanılırken günümüzde "özel gereksinimi olan birey" terimi kullanılmaktadır[1]. Dünya Sağlık Örgütü, Küresel Hastalık Yükü (2004) verilerine göre dünya nüfusunun %15,3'ünün, 0-14 yaş grubundaki çocukların ise %5,2'sinin "engelli" olduğunu bildirmiştir.[3] Türkiye Engelliler Araştırması'nda ise, ülkemizde tüm nüfusun %12,29'unun, çocuk ve genç yaş grubu nüfusun %8,78'inin "özürlü" olduğu belirtilmiştir.[4]

Özel gereksinimi olan bireylerin uyum becerilerinin yetersizliği ile günlük gereksinimlerini karşılamadaki güçlüklerinden ötürü korunma, bakım, rehabilitasyon, danışmanlık ve eğitim hizmetlerine ihtiyaç duydukları bilinmektedir. Çocuk ve genç psikiyatrisi alanında özel gereksinimli çocuklara, sosyal, eğitsel ve ekonomik haklardan yararlanmaları için sağlık kurulu raporu düzenlenmektedir. Ülkemizde 2006 yılından beri sağlık kurulu raporlarının düzenlenmesinde "Özürlülük Ölçütü, Sınıflandırılması ve Özürlülere Verilecek Sağlık Kurulu Raporları Hakkında Yönetmelik" esas alınmıştır.[5] Çocuk ve erişkinler için aynı şekilde düzenlenen bu rapor sistemi, 20.02.2019 tarihinde yürürlüğe giren "Çocuklar İçin Özel Gereksinim Değerlendirmesi Hakkında Yönetmelik" ile kaldırılmış ve çocuklar için ayrı bir rapor olan "Çocuklar İçin Özel Gereksinim Raporu (ÇÖZGER)" kullanılmaya başlanmıştır.[6]

ÇÖZGER ile birlikte rapor sisteminde birtakım değişiklikler yapılmıştır. Eski yönetmelikteki özürlü sağlık kurulu raporlarında her bir sisteme ilişkin yüzde (%) şeklinde belirtilen özür oranları ÇÖZGER'de özel gereksinim alanlarına ait özel gereksinim düzeyleri olarak belirtilmiştir. ÇÖZGER'deki özel gereksinim düzeyleri, "Özel gereksinimi vardır (ÖGV)" dan başlayarak "Özel koşul gereksinimi vardır (ÖKGV)" a kadar uzanan yedi dereceden oluşmaktadır. ÇÖZGER sisteminin yüzde (%) engel oranı içeren diğer mevzuatlara uyumu arandığında özel gereksinim düzeylerinin karşılık geldiği engel oranları da yönetmelikte yer almaktadır. Buna göre ÖGV için %20-39, hafif düzeyde ÖGV için %40-49, Orta düzeyde ÖGV için %50-59, İleri düzeyde ÖGV için %60-69, Çok ileri düzeyde ÖGV için %70-79, belirgin ÖGV için %80-89 ve ÖKGV için %90-99 olarak belirtilmiştir. Çocuk ve ergen psikiyatrisi ile ilişkili olarak ÇÖZGER'de bilişsel gelişim alanı, çocuk ve genç psikiyatrisi alanı ve dil-konuşma-iletişim gelişimi alanı olmak üzere üç özel gereksinim alanı tanımlanmıştır. 'Bilişsel Gelişim Alanı'nda bilişsel gelişim gerilikleri ve zihinsel yetersizlikler; 'Çocuk ve Genç Psikiyatrisi Alanı'nda şizofreni ve diğer psikotik bozukluklar, Otizm Spektrum Bozukluğu (OSB), organik beyin hasarına bağlı bilişsel, ruhsal bozukluklar, duygudurum bozuklukları, anksiyete bozuklukları ve Özgül Öğrenme Bozukluğu (ÖÖB); Dil-Konuşma-İletişim Alanı'nda alıcı ve/veya ifade edici dil gelişim gerilikleri, konuşma sesi bozuklukları, akıcılık bozukluğu, sosyal iletişim bozukluğu, ses bozuklukları ve işaret dili ile iletişim kurma yer almaktadır. Her bir alana özgü tanılar ICD-10 (Uluslararası Hastalık Sınıflandırması) kodu ile belirtilmektedir. Bilişsel gelişim alanında "zeka geriliği" ya da "mental motor retardasyon" terimi yerine "gecikmiş dönüm noktası" ifadesinin kullanılması gerektiğinin altı çizilmektedir. Ayrıca ÇÖZGER öncesi sistemde Kulak Burun Boğaz alanında raporlanan dil ve konuşma bozukluklarının ÇÖZGER'de çocuk ve ergen psikiyatrisi ile ilişkili alanlara dahil edildiği görülmektedir.[6]

Alanyazın incelendiğinde, ÇÖZGER'e geçiş sonrasındaki yenilikleri çocuk ve ergen psikiyatrisi alanı içinde değerlendiren üç çalışmaya ulaşılmıştır. Bunlardan ilki Bolu'da bir üniversite hastanesine ÇÖZGER için başvuran hastaların sosyodemografik ve klinik özelliklerinin değerlendirildiği bir araştırmadır.[7] Öztürk ve Kayhan (2020)[7] tarafından yapılan bu çalışmada, ÇÖZGER'deki psikiyatrik tanılar arasında en sık gecikmiş dönüm noktası tanısının olduğu, bu olgular içinde de en sık olarak hafif düzeyde gecikme (BGG) tanısına rastlandığı bulunmuştur. Bu olguların yaklaşık yarısında ek tıbbi hastalık saptandığı, sistem açısından en sık olarak nörolojik hastalıkların görüldüğü belirtilmiştir. İkinci araştırmada, İstanbul'da bir eğitim araştırma hastanesi sağlık kuruluna başvuran ve ÇÖZGER hekimi tarafından çocuk ve ergen psikiyatrisine yönlendirilen olguların psikiyatrik tanı ve özel gereksinim düzeyleri önceki yönetmelikte belirtilen tanı ve özür oranlarıyla karşılaştırılmıştır.[8] Yıldız ve Tarakçıoğlu (2020)[8], bu çalışmada, çocuk psikiyatrisi özel gereksinim alanları içinde sıklık olarak sırasıyla çocuk ve genç psikiyatrisi alanı, bilişsel gelişim alanı ve dil-konuşma-iletişim alanı olarak bulmuşladır. En sık ÖÖB tanısının saptandığını, bunu hafif düzeyde BGG'nin takip ettiği, en sık karşılaşılan eski tanının ise yine hafif düzeyde BGG olduğunu belirtmişlerdir. Ayrıca geçmiş raporlarında OSB ve orta düzeyde BGG tanısı alanların yeni yönetmeliğe göre aldıkları tanılara karşılık gelen özür oranlarının yükseldiği saptanmıştır. Son çalışma ise Bursa'da çocuk hastalıkları hastanesine başvuran olguların sosyodemografik özellikleri ile ÇÖZGER öncesi-sonrası rapor karşılaştırmaları değerlendirilmiştir[9]. Güller ve Yaylacı (2021)[9] bu çalışma ile ÇÖZGER sonrası %90-99 engel oranı aralığında verilen raporların oranında anlamlı düzeyde artış olduğuna dikkati çekmişlerdir. Her üç araştırmada da 0-18 yaş grubundaki ÇÖZGER'e başvuran tüm olgular incelenmiştir.

Ülkemizde çocuk ve ergen yaş grubunda son yıllarda artan psikiyatri başvuruları ve sağlık kurulu raporları kapsamında artan psikiyatri konsültasyonları düşünüldüğünde, psikiyatrik tanılamanın ve gereksinim düzeyine göre yönlendirmelerin doğru ve eksiksiz bir şekilde yapılması oldukça önemlidir. Bu kapsamda en son yürürlüğe giren ve yaygın bir şekilde uygulanan ÇÖZGER'de yer alan psikiyatri ile ilişkili alanlar ve gereksinim düzeyleri hakkında yapılacak çalışmalara

gereksinim olduğu aşikardır. Son yıllarda bu konuda yapılan araştırmaların arttığı görülse de Ankara ilinde yapılan herhangi bir çalışmaya rastlanmamıştır. Araştırmamızın Ankara'nın pek çok bölgesinden ve çevre illerden gelen pediyatrik popülasyona hizmet veren ÇÖZGER vermeye yetkili bir hastanede yapıldığı düşünüldüğünde, çalışmamızın sonuçlarının alanyazına önemli katkılar sağlayacağı öngörülmektedir. Bu çalışmada, ÇÖZGER için başvuran ve çocuk ve genç psikiyatrisi alanında özel gereksinimi olan olguların klinik özellikleri, psikiyatrik tanıları, eş tanılarının incelenmesi ve ÇÖZGER'e göre belirlenen tanı ve gereksinim düzeylerinin eski rapor sistemindeki tanı ve özür oranlarıyla karşılaştırılması amaçlanmıştır.

Yöntem

Ankara Dr. Sami Ulus Kadın Doğum ve Çocuk Sağlığı ve Hastalıkları Eğitim ve Araştırma Hastanesi Etik Kurulu onayı sonrasında (Protokol No: E-20/11-031), 01.06.2019-01.12.2019 tarihleri arasında hastane sağlık kuruluna ÇÖZGER tekrarı için başvuran ve ÇÖZGER'de psikiyatri ile ilgili herhangi bir alandan (bilişsel gelişim, çocuk ve genç psikiyatrisi, dil-konuşma-iletişim alanları) özel gereksinimi bulunan 6-18 yaş arasındaki çocuklar çalışmaya dahil edilmiştir. Bu olguların geriye dönük olarak sosyodemografik (yaş, cinsiyet, eğitim durumu, sosyoekonomik düzey) ve klinik (psikiyatrik bozukluklar, eş tıbbi hastalık) özellikleri, ÇÖZGER öncesi ve sonrası psikiyatrik tanıları, eski rapor sistemindeki özür oranları, ÇÖZGER'de belirtilen özel gereksinim alanları ve düzeyleri incelenmiştir.

Sağlık kurulu heyetine başvuran çocuk ve ergenler ÇÖZGER yetkili hekimi (çocuk sağlığı ve hastalıkları uzmanı) tarafından değerlendirildikten sonra diğer ilgili bölümlere yönlendirilmektedir. Bu bölümler göz, kulak-burun-boğaz, çocuk nörolojisi, fiziksel tıp ve rehabilitasyon, ortopedi, çocuk cerrahisi, tıbbi genetik ve çocuk ve ergen psikiyatrisidir. Raporlar düzenlenirken 20.02.2019 tarihli 30692 sayılı Resmi Gazete'de yayınlanan ÇÖZGER yönetmeliği esas alınmakta ve değerlendirmeler sonucunda özel gereksinim alanları ve düzeyleri belirlenmektedir.

Sosyoekonomik düzeyi sınıflandırmak için Hollingshead-Redlich ölçeği kullanılmıştır.[10] Ölçekte beş ayrı sosyoekonomik-sosyokültürel düzey (SED) tanımlanmıştır; değerlendirmede anne ya da babadan en yüksek düzeyde olanın durumu esas alınmıştır. "Varlıklı, eğitimli toplumsal katmanda aile" "1", "üniversite eğitimi almış, meslek sahibi ya da yüksek idari konumda anne-baba" "2", "küçük iş adamı, memur ya da vasıflı işçi, lise mezunu anne-baba" "3", "yarı vasıflı işçi, lise düzeyinin altında eğitimli anne-baba" "4", "yarı vasıflı işçi, eğitimsiz, ilkokul düzeyinde eğitimli anne-baba" "5" olarak kodlanmıştır. Çalışmamızda, bu ölçeğe göre 1-2 yüksek, 3 orta, 4-5 düşük SED olarak tanımlanmıştır.

ÇÖZGER düzenlenmesi sırasında, psikiyatrik tanılar çocuk ve ergen psikiyatri uzmanları tarafından DSM-5 temel alınarak yapılan klinik görüşmeler sonucunda konmuştur. Ayrıca standart değerlendirme araçları ile tanılar desteklenmiştir. Bu kapsamda 6 yaşından büyük çocukların zeka düzeyini belirlemek amacıyla, kliniğimizde çalışan deneyimli ve sertifikalı klinik psikologlar tarafından Wechsler Çocuklar için Zeka Ölçeği (WÇZÖ-R) uygulanmış, ÖÖB düşünülen olgularda ise Özgül Öğrenme Güçlüğü bataryası kullanılmıştır.

Wechsler Çocuklar için Zeka Ölçeği Geliştirilmiş Formu (WÇZÖ-R): Wechsler tarafından 1949'da geliştirilen ve 1974 yılında gözden geçirilmiş formu oluşturulmuştur.[11] WÇZÖ-R'nin Türkçe standardizasyonu, geçerlik ve güvenirlik çalışması Savaşır ve Şahin tarafından yapılmıştır.[12] Bu zeka testi sözel ve performans becerilerini içeren 12 alttestten oluşmakta ve bunlara göre sözel, performans ve toplam zeka puanı hesaplanmaktadır.

Özgül Öğrenme Güçlüğü Bataryası: ÖÖG tanısını desteklemek amacıyla Korkmazlar (1993)[13]'ın kullandığı, sonrasında Erden ve Kurdoğlu (2003)[14]'nun bazı testler ekleyerek genişlettiği klinik bataryadır. Okuma testi, yazma testi, alfabenin sırasıyla ve küçük harflerle yazılması, sınıf düzeyine göre toplama ve çarpım tablosu soruları, aylar ve günler ile öncelik-sonralık ilişkilerinin sorgulanması, Gesell gelişim figürleri, saat çizme testi, Harris lateralleşme testi olmak üzere 9 alttestten oluşmaktadır.[15]

ÇÖZGER'de "Bilişsel Gelişim Alanı"nda yer alan "bilişsel gelişimde gecikme" kategorileri işlevsel değerlendirmelerde bilişsel gelişim düzeyi "(-1.5 SD) ile (-2 SD) arasında", "(-2 SD) ile (-3 SD) arasında" ve "(-3 SD) altında" şeklinde üçe ayrılmıştır.[6] ÇÖZGER kapsamında değerlendirilen olgular, psikiyatrik muayene ve psikometrik değerlendirmeler (WISC-R) sonucunda çocuk ve ergen ruh sağlığı ve hastalıkları uzmanları tarafından "Hafif, Orta ve Ağır Düzeyde Zihinsel Yetersizlik" şeklinde tanılanmaktadır.[16] Standart zeka değerlendirme araçlarına göre belirlenen zeka puanları ve klinik değerlendirme sonucundaki işlevsellik düzeyine göre normal dağılımın "(-2 SD) ile (-3 SD) arasında" olan olgular Hafif Düzeyde Zihinsel Yetersizlik (ZY) (zeka puanı olarak 50-70) tanısı almaktadır. Buna göre, (-3 SD) altındaki değerler (zeka puanı olarak 50 ve altı) ise Orta Düzeyde ZY ve sonrasına (Ağır) karşılık gelmektedir. Bu nedenle çalışmamızda, bilişsel değerlendirmeler sonucunda gelişim düzeyi "(-2 SD) ile (-3 SD) arasında" olan olgular "Hafif Düzeyde ZY", "(-3 SD) altında" olanlar ise "Orta-Ağır Düzeyde ZY" şeklinde tanılanmıştır. Hafif Düzeyde ZY tanısı, ÇÖZGER yönetmeliğinde yer alan 'Bilişsel Gelişim Alanı' kategorisindeki değerlendirmesi 'İşlevsel değerlendirmede bilişsel gelişim (-2 SD) altındadır.' ile uyumludur.[6] Bu çalışmada Hafif Düzeyde ZY tanılı çocukların özel gereksinim düzeyleri 'Hafif'ten 'Çok İleri'ye doğru bir sınıflama içerisinden çocuğun günlük işler, sosyal ve akademik alanlardaki işlevselliği göz önünde bulundurularak psikiyatrik muayene ile klinik değerlendirme sonucunda belirlenmiştir. Orta ve Ağır Düzeyde ZY tanısı ise ÇÖZGER yönetmeliğinde yer alan 'Bilişsel Gelişim Alanı' kategorisindeki 'Özel Koşul

Gereksinimi Vardır (ÖKGV).' değerlendirmesi 'İşlevsel değerlendirmede bilişsel gelişim (-3 SD) altındadır.' ile uyumludur.[6] Bu nedenle bu tanılar için özel gereksinim düzeyi ÖKGV şeklinde belirlenmiştir.

İstatistiksel Analiz

Verilerin istatistiksel analizinde SPSS 23.0 paket program kullanıldı. Tanımlayıcı veriler kategorik değişkenler için sayı ve yüzde olarak, sayısal veriler için ortalama ve standart sapma olarak gösterildi. Bağımsız gruplar arasında kategorik değişkenlerin karşılaştırılmasında ki-kare testi, bağımlı gruplarda McNemar testi kullanıldı. Sayısal verilerin normal dağılım gösterip göstermediği Kolmogorow-Smirnov testi ile incelendi ve bağımsız iki grup karşılaştırmasında Student t testi uygulandı. Tüm karşılaştırmalar çift yönlü yapıldı ve anlamlılık için p değeri 0.05 olarak alındı.

Bulgular

Çalışmaya dahil edilen 1246 olgunun %38'i (n=474) kız, %62'si (n=772) erkekti. Yaş ortalaması önceki sağlık kuruluna başvurduklarında 10 ± 2,4 (4,7-15,7), ÇÖZGER sırasında 12,1 ± 4,6 (7,1-17,7) olarak hesaplandı. Olguların hepsinin mevcut raporu bulunmakla birlikte, %97,9'unun (n=1220) önceki raporunu yenilemek, %2,1'inin (n= 26) ise raporuna itiraz etmek amacıyla başvurduğu saptandı. Ailelerin sosyoekonomik düzeyleri incelendiğinde %62,7'sinin (n=782) düşük, %35,4'ünün orta (n=442), %1,7'sinin yüksek (n=22) düzeyde olduğu belirlendi. ÇÖZGER'e başvuran olguların %3,1 (n=39)'i yabancı uyruklu olup, bu olguların 18 (%46,1)'i Irak, 16 (%41)'sı Suriye, 3 (%7,7)'ü Afganistan, 2 (%5,1)'si İran vatandaşıydı.

ÇÖZGER'de çocuk ve genç psikiyatrisi kapsamında özel gereksinim alanları incelendiğinde, bilişsel gelişim alanında %58 (n=723), çocuk ve genç psikiyatrisi alanında %38,5 (n=480), dil-konuşma-iletişim gelişimi alanında %8 (n=100) olguda özel gereksinim varlığı saptandı. Çocuk ve genç psikiyatrisi alanında yer alan tanılar incelendiğinde, tüm olguların %33,4 (n=417)'ünde ÖÖB, %5 (n=63)'inde OSB tanısı olduğu bulundu. ÇÖZGER kapsamında psikiyatrik tanılar incelendiğinde, en sık olarak %58 (n=723) ile gecikmiş dönüm noktası (sınırda zihinsel işlevsellik ve zihinsel yetersizlikler), bu tanı kategorisi altında da en sık Hafif Düzeyde ZY saptanmış olup tanı dağılımı Tablo 1'de gösterilmiştir.

ÇÖZGER'de yer alan tanıların cinsiyet dağılımlarına bakıldığında, ÖÖG ve OSB tanılarının erkeklerde, Hafif Düzeyde ZY tanısının ise kızlarda anlamlı olarak daha yüksek oranda görüldüğü bulunmuştur (Tablo 1).

Tablo 1. ÇÖZGER çocuk ve genç psikiyatrisi alanındaki tanılar ve cinsiyetler arası dağılımları

Tanılar	**Toplam** n (%)	**Kız** n (%)	**Erkek** n (%)	*p*	*χ2*
Hafif Düzeyde ZY	459 (36,8)	193 (40,7)	266 (34,5)	**0,026***	**4,94**
ÖÖB	417 (33,4)	142 (30,0)	275 (35,6)	**0,040***	**4,23**
Sınırda Zihinsel İşlevsellik	120 (9,63)	54 (11,3)	66 (8,5)	0,102	2,67
Dil-Konuşma-İletişim Bozuklukları	100 (8)	30 (6,3)	70 (9,1)	0,080	2,98
Orta Düzeyde ZY	97 (7,7)	41 (8,6)	56 (7,3)	0,372	0,79
OSB	63 (5)	10 (2,1)	53 (6,9)	**<0,001*****	**13,83**
Ağır Düzeyde ZY	47 (3,7)	20 (4,2)	27 (3,5)	0,516	0,42

ÇÖZGER: Çocuklar için özel gereksinim raporu, ZY: Zihinsel yetersizlik; ÖÖB: Özgül öğrenme bozukluğu; OSB: Otizm spektrum bozukluğu
McNemar Test
** p<0,05, ** p<0,01, *** p<0,001*

Toplam 1246 olgunun 233'ünde (%18,6) birden fazla alanda özel gereksinim düzeyi saptanmıştır. Bu olguların 134'ünün (% 57,5) Bilişsel Gelişim Alanı ve Çocuk ve Genç Psikiyatrisi Alanı'ndan, 76'sının (%32,6) Bilişsel Gelişim Alanı ve Dil-Konuşma-İletişim Alanı'ndan, 15'inin (%6,4) Çocuk ve Genç Psikiyatrisi Alanı ve Dil-Konuşma-İletişim Alanı'ndan, 8'inin (3,4) her üç alandan da özel gereksinimi olduğu bulunmuştur. ÇÖZGER'de yer alan tanılar incelendiğinde, OSB olgularının (n=63) 44'ünde (%69,8) ZY ek tanısının olduğu tespit edilmiştir. Bunların 30'u (%68,1) Hafif Düzeyde, 8'i (18,1) Orta Düzeyde, 6'sı (%13,6) Ağır Düzeyde ZY tanısı almıştır. Doksan olguda Sınırda Zihinsel İşlevsellik ve Özgül Öğrenme Bozukluğu tanılarının birlikte belirtildiği saptanmıştır. Üç alandan da özel gereksinimi bulunan olguların tümüne Sınırda Zihinsel İşlevsellik, Özgül Öğrenme Bozukluğu ve Konuşma Bozukluğu tanılarının konduğu bulunmuştur. Dil-konuşma ve iletişim alanındaki tanıların sıklıkla birincil tanılara eşlik ettiği ve raporlarda yer aldığı görülmüştür. ÇÖZGER öncesi raporda ÖÖB tanısı alanların %2,6'sında (n=11), Hafif Düzeyde ZY tanısı alanların %8,1'inde (n=41), Orta Düzeyde ZY tanısı alanların %14,4'ünde (n=12) dil ve konuşma bozuklukları saptanmıştır. ÇÖZGER'de ise bu oranlar sırasıyla, %3,5 (n= 15), %10,4 (n=48), %18,5 (n=18) şeklindedir. ÇÖZGER kapsamında birincil tanının dil-konuşma bozukluğu olduğu olgular da dahil edildiğinde toplamda 100 olgunun %20 (n=20)'sinde dil bozukluğu, %56 (n=56)'sında konuşma sesi bozukluğu (artikülasyon bozukluğu/fonolojik bozukluk), %18 (n=18)'inde akıcılık bozukluğu (kekemelik), %4 (n=4)'ünde sosyal iletişim bozukluğu saptanmış, %2 (n=2)'sinde ise işaret dili ile iletişim kurması gereken çocuklar kategorisinin altında olarak raporlanmıştır.

ÇÖZGER kapsamında belirtilmeyen psikiyatrik tanılar incelendiğinde, tüm örneklemin (n=1246) %52,3'ünde (n=652) en az bir psikiyatrik tanının eşlik ettiği bulunmuştur. Bu olguların %86,6'sında (n=565) DEHB, %11,8'inde (n=77) Anksiyete Bozuklukları, %7'sinde (n=46) KOKGB, %5'inde (n=33) Davranım Bozukluğu, %0,61'inde (n=4) Depresif Bozukluk, %0,9'unda (n=6) Dışa Atım Bozukluğu, %0,7'sinde (n=5) Tik Bozukluğu, %0,1'inde (n=1) Madde Kullanım Bozukluğu saptanmıştır. ÇÖZGER öncesi düzenlenen raporda ve ÇÖZGER'de yer alan psikiyatrik tanılar incelendiğinde, her ikisinde de en sık olarak Hafif Düzeyde ZY tanısının olduğu gözlenmiştir. Eski sağlık kurulu raporunda belirtilen tanılar, özür oranları ile ÇÖZGER tanıları, özel gereksinim düzeyleri ve bunlara karşılık gelen özür oranları Tablo 2'de sunulmuştur. ÇÖZGER sonrasında, önceki rapor sistemine göre Hafif Düzeyde ZY olgularında anlamlı bir azalma olduğu görülmüştür ($\chi 2$=19,41; p<0,001). Eski ve yeni rapor sistemine göre ÖÖB, Sınırda Zihinsel İşlevsellik ve OSB olguları karşılaştırıldığında anlamlı bir farklılık gözlenmemiştir (sırasıyla $\chi 2$=1,11 p=0,740; $\chi 2$=3,20 p=0.07; p=0,740; $\chi 2$=1,33 p=0,250). Önceki raporlara kıyasla ÇÖZGER raporlarında, Orta Düzeyde ZY, Ağır Düzeyde ZY ve Dil-konuşma bozuklukları tanılarının anlamlı düzeyde daha fazla belirtildiği bulunmuştur (sırasıyla $\chi 2$=5,63 p=0,018; $\chi 2$=5,14 p=0,016; $\chi 2$=8,16 p=0,004).

Tablo 2. ÇÖZGER öncesinde kategorize edilen tanılar ve özür oranları ile ÇÖZGER tanıları ve özel gereksinim düzeyleri ve özür oranı karşılıklarının karşılaştırılması

ÇÖZGER öncesi tanı (n)	Özür Oranı	ÇÖZGER tanı (n)	Özel gereksinim düzeyi (n)	ÇÖZGER'deki özür oranı karşılığı
Hafif Düzeyde ZY (506)	%50	Hafif Düzeyde ZY (459)	Hafif ÖGV (113)	%40-49
			Orta ÖGV (256)	%50-59
			İleri ÖGV (34)	%60-69
			Çok İleri ÖGV (8)	%70-79
ÖÖB (421)	%20 veya %30	ÖÖB (417)	ÖGV (417)	%20-39
Sınırda Zihinsel İşlevsellik (115)	%25	Sınırda Zihinsel İşlevsellik (120)	ÖGV (120)	%20-39
Orta Düzeyde ZY (83)	%70	Orta Düzeyde ZY (97)	Çok İleri ÖGV (3)	%70-79
			ÖKGV (76)	%90-99
Dil-Konuşma Bozuklukları (79)	%20-40	Artikülasyon (56)	ÖGV (56)	%20-39
		Dil Bozukluğu (20)	ÖGV (19)	%20-39
			Belirgin ÖGV (1)	%80-89
		Kekemelik (18)	ÖGV (18)	%20-39
		Sosyal İletişim (4)	ÖGV (4)	%20-39
		İşaret dili (2)	ÖKGV (2)	%90-99
OSB (66)	%40 veya %80	OSB (63)	ÖKGV (63)	%90-99
Ağır Düzeyde ZY (40)	%90	Ağır Düzeyde ZY (47)	ÖKGV (47)	%90-99

ÇÖZGER: Çocuklar için özel gereksinim raporu; ZY: Zihinsel yetersizlik; ÖÖB: Özgül öğrenme bozukluğu; OSB: Otizm spektrum bozukluğu; ÖGV: Özel gereksinimi vardır; ÖKGV: Özel koşul gereksinimi vardır

ÇÖZGER öncesi raporlarda belirtilen özür oranları ile ÇÖZGER'deki özel gereksinim düzeylerine karşılık gelen oranlar tek tek karşılaştırıldığında (ÇÖZGER Ek 3'te bulunan Mevzuatla Uyum Arandığında Kullanılacak Tablo'ya göre)[6], ÇÖZGER'e geçişle birlikte %20-39 (ÖGV), %90-99 (ÖKGV) aralığında anlamlı artışlar, %50-59 (Orta ÖGV), %70-79 (Çok İleri ÖGV) ve %80-89 (Belirgin ÖGV) aralığında anlamlı azalmalar bulunmuştur (Tablo 3).

Psikiyatri dışındaki tanılar incelendiğinde tüm olguların (n=1246) %23,1 (n=288)'inde en az bir tıbbi hastalığın eşlik ettiği saptanmıştır. Bu olgular incelendiğinde, 165 olguya (%57,2) hareket sistemi alanından tanıların konduğu, 70 tanesinin (%5,61) serebral palsi tanılı olduğu görülmüştür. Bunu 96 olgu ile epilepsi (%7,70) tanısının izlediği, 48 olguda (%3,85) görme işlevi kısıtlılığı/kaybı olduğu, 26 olguya (%2,08) sensörinöral işitme kaybı tanısının konduğu, 25 olgunun (%2,00) Down Sendromlu olduğu, 15 olgunun (1,20) hipotiroidi tanısının olduğu belirlenmiştir. Olguların %19,8 (n=247)'inde en az bir psikiyatri dışı alanda özel gereksinim varlığı bulunmuştur. Tüm olguların (n=1246) %12,52'sinde hareket sistemi alanından özel gereksinim belirtilmiş olup diğer alanlar Tablo 4'te özetlenmiştir.

Yabancı uyruklu olguların (n=39) psikiyatrik tanıları incelendiğinde, 13 olgunun Ağır Düzeyde ZY (%33,3), 12 olgunun Orta Düzeyde ZY (%30,8), 9 olgunun Hafif Düzeyde ZY (%23,0), 3 olgunun OSB (%7,7), 2 olgunun Konuşma Bozukluğu (%5,1), 1 olgunun Sınırda Zihinsel İşlevsellik (%2,6), 1 olgunun ÖÖG (%2,6), tanısını aldığı saptanmıştır. ÇÖZGER özel gereksinim düzeyleri incelendiğinde, 2'sinin ÖGV (%5,1), 1'inin Hafif ÖGV (%2,6), 5'inin Orta ÖGV (%12,8), 2'sinin İleri ÖGV (%5,1), 1'inin Çok İleri ÖGV (%2,6), 28'inin ÖKGV (%71,7) olarak raporlandığı bulunmuştur. Toplam 39 olgunun 29'unun (%74,3) psikiyatri dışı alanlardan da özel gereksinimi olduğu, bu 29 olgunun 25'inin (%86,2) hareket sistemi alanından, 1'inin görme işlevi, 1'inin işitme-kulak burun boğaz, 1'inin sinir sistemi, 1'inin kalıtsal-doğmalık hastalıklar alanlarından tanı aldığı saptanmıştır.

Tablo 3. ÇÖZGER öncesi özür oranları ile ÇÖZGER özel gereksinim düzeylerine karşılık gelen oranların karşılaştırılması

Özür Oranı (Özel Gereksinim Düzeyi)	**ÇÖZGER Öncesi** n (%)	**ÇÖZGER** n (%)	*p*	χ^2
%20-39 (ÖGV)	449 (36,03)	581 (46,62)	**<0,001*** **	**105,60**
%40-49 (Hafif ÖGV)	116 (9,30)	120 (9,63)	0,8383	0,04
%50-59 (Orta ÖGV)	463 (37,15)	284 (22,79)	**<0,001*** **	**132,56**
%60-69 (İleri ÖGV)	43 (3,45)	38 (3,04)	0,644	0,21
%70-79 (Çok İleri ÖGV)	83 (6,66)	12 (0,96)	**<0,001*** **	**52,68**
%80-89 (Belirgin ÖGV)	47 (3,77)	4 (0,32)	**<0,001*** **	**34,58**
%90-99 (ÖKGV)	45 (3,61)	206 (16,53)	**<0,001*** **	**159,00**

ÇÖZGER: ÇÖZGER: Çocuklar için özel gereksinim raporu; ÖGV: Özel gereksinimi vardır; ÖKGV: Özel koşul gereksinimi vardır
McNemar Test
** p<0,05, ** p<0,01, *** p<0,001*

Tablo 4. Özel gereksinim varlığının psikiyatri dışı alanlardaki dağılımları

Psikiyatri Dışı Alanlar	**n (%)**	**Psikiyatri Dışı Alanlar**	**n (%)**
Hareket Gelişimi	156 (12,52)	Sindirim Sistemi	5 (0,40)
Sinir Sistemi	88 (7,06)	Endokrin Sistem	3 (0,24)
Görme İşlevi	48 (3,85)	Hematoloji-Onkoloji	3 (0,24)
İşitme-Kulak Burun Boğaz	26 (2,08)	Metabolizma	2 (0,16)
Kalıtsal-Doğmalık Hastalık	25 (2,00)	Nefroloji	2 (0,16)
Kalp, Dolaşım Sistemi	10 (0,80)	Alerji-İmmünoloji	1 (0,08)

Tartışma

Bu çalışmada, ÇÖZGER yönetmeliğinin yürürlüğe girmesinden sonraki dönemde sağlık kuruluna başvuran ve çocuk ve ergen psikiyatrisi alanından tanı alan olguların psikiyatrik tanıları incelenerek, eski rapor sistemindeki özür oranları ile ÇÖZGER'de belirtilen özel gereksinim alanları ve düzeyleri karşılaştırılmıştır. ÇÖZGER verilerinin psikiyatrik kapsamda değerlendirildiği önceki çalışmalardan[7-9] farklı olarak, araştırmamız Ankara'da yoğun bir şekilde sağlık kurulu raporu verilen bir hastanenin geniş örneklemi üzerinde ve farklı bir yaş grubunda (6 yaş üzeri) yapılmıştır.

Çalışmamızda olguların yaş ortalamaları 12,1 olarak saptanmış olup, sağlık kurulu başvurularının değerlendirildiği diğer çalışmalarda belirlenen yaş ortalamalarından (8,00-9,10)[7-9,17] farklılık göstermektedir. Bu durumun, önceki çalışmaların 0-18 yaş grubunda yapılması, çalışmamızın ise 6-18 yaş arası çocuk ve ergenleri kapsamasından kaynaklandığı düşünülmektedir. Çalışmamıza göre, ÇÖZGER'e başvuran olguların %62'sinin erkek olduğu bulunmuştur. Bu oran, Türkiye İstatistik Kurumu 2010 verileri[18] (%58,6) ve çocuklarda sağlık kurulu raporlarının incelendiği diğer araştırmalar[7,19,20] (%61,6-62,4) ile benzerdir. Sağlık kurulu raporları ile ilgili yapılan araştırmalarda erkek cinsiyetin daha baskın bir şekilde görülme nedeni, bu raporlarda sıklıkla yer alan ZY, OSB, ÖÖB gibi nörogelişimsel psikiyatrik bozuklukların erkeklerde daha yüksek oranda görülmesi ile açıklanabilir.[21] Diğer yandan bu sonuçlar, kızların sağlık hizmetine erişim konusunda daha geri planda kaldıkları veya ihmal edildikleri şeklinde de yorumlanabilir.[19]

Araştırmamız kapsamında olguların en sık olarak bilişsel gelişim, sonra sırasıyla çocuk ve genç psikiyatrisi ve dil-konuşma-iletişim gelişimi alanlarından tanı aldıkları bulunmuştur. Özel gereksinim alanlarındaki bu dağılım, Güller ve Yaylacı'nın çalışmasına benzer niteliktedir.[9] Çalışmamızda, ÇÖZGER'e göre psikiyatrik tanılar değerlendirildiğinde en sık olarak "Gecikmiş dönüm noktası" tanısının konduğu görülmektedir. Ayrıca hem ÇÖZGER öncesi ve hem de ÇÖZGER sonrası sağlık kurulu raporlarında en sık olarak %36,8 ile 'Hafif Düzeyde ZY' tanısı saptanmış olup bu bulgular alanyazındaki diğer çalışmalarla uyumludur[7,17,19,22,23]. Yine önceki çalışmalarla paralel bir şekilde ikinci en sık tanı %33,4 ile ÖÖB olarak bulunmuştur[7,22,23]. Sonrasında ise olguların yaklaşık %10'unda 'Sınırda Zihinsel İşlevsellik' tanısının bulunduğu görülmektedir. Bu oranın önceki çalışmalardan daha fazla saptanmasının[17], araştırmamızdaki örneklem grubunun genel olarak düşük sosyoekonomik düzeye sahip ailelerden oluşmasından ötürü olabileceği düşünülmektedir[24]. Çalışma bulgularımıza göre, olguların yaklaşık %5'inde OSB tanısı saptanmıştır. Öte yandan, sağlık kurulu raporları üzerine yapılan diğer araştırmalarda OSB tanı oranının %12-14 arasında olduğu ifade edilmiştir.[7-9,19,23] Görülen bu farklılık, çalışmamıza dahil edilen çocukların 6 yaş üzerinde olması ve okul çağında ÖÖB tanısının oransal olarak belirgin bir artış göstermesi şeklinde yorumlanabilir.[25] Nitekim, bulgularımıza göre OSB tanı oranı Öztürk ve arkadaşlarının yaptığı çalışmada (2018) 6-18 yaş grubunda belirtilen oran ile benzerdir.[22] Güncel alanyazında, OSB ile Hafif Düzeyde ZY sıklığı benzer oranda bildirilmektedir.[26,27] Ancak mevcut çalışmamız bu verilerle uyuşmamaktadır. Bu durum, OSB tanısına

yönelik ek bir tanı aracı kullanılmamasından kaynaklanıyor olabilir. Ayrıca ek olarak çalışmamızda, OSB'ye yaklaşık %70 oranında ZY tanısının eşlik ettiği bulunmuştur. Okul çağındaki OSB'li çocuklarda %65'e varan oranlarda ZY eş tanısının görüldüğü bildirilmektedir[28]. Bu anlamda sonuçlarımızın alanyazında belirtilen oranlara yakın olduğu görülmektedir.

Tanıların cinsiyetlere göre dağılımı sonuçlarına göre, kızlarda Hafif Düzeyde ZY, erkeklerde ise OSB ve ÖÖB tanılarının daha yüksek oranda bulunduğu dikkati çekmektedir. Yıldız & Tarakçıoğlu (2020) ÇÖZGER kapsamında çocukların tanısal değerlendirmesinde, hafif düzeyde bilişsel gelişimde gecikme tanısının kızlarda, OSB tanısının ise erkeklerde anlamlı olarak daha fazla saptandığını bildirmişlerdir.[8] Kayhan & Öztürk (2020), zihinsel yetersizlikler, OSB ve ÖÖB tanılarının erkeklerde kızlara göre daha sık görüldüğünü bildirmişlerdir[7]. Gümüş & Yürümez (2014), Hafif düzeyde ZY tanısının kızlar, OSB ve ÖÖG tanılarının ise erkeklerde daha yüksek oranda görüldüğünü ifade etmişlerdir[19]. Bu anlamda çalışmamızda OSB ve ÖÖB tanılarının erkeklerde daha yüksek oranda saptanması diğer çalışmaların bulguları ile uyumludur.[7,19] Alanyazında genel olarak zihinsel yetersizliklerin erkeklerde kızlara göre daha yüksek oranda görüldüğü belirtilse de[29], sonuçlar ÇÖZGER'de belirtilen diğer psikiyatrik bozuklukların (ÖÖB, OSB gibi) erkeklerde kızlara göre çok daha yüksek oranda görülmesinden kaynaklanıyor olabilir. Diğer bir deyişle, Hafif düzeyde ZY tanısı açısından cinsiyetler arası karşılaştırma yapıldığında görülen kızlardaki bu oransal yükseklik, aslında kızlarda diğer tanıların görece daha az oranda saptanması ile açıklanabilir.

Dil ve konuşma bozuklukları çocukluk çağının en sık görülen nörogelişimsel bozukluklarından biridir, erkek cinsiyette daha sık görülmektedir.[30] ZY'li çocukların normal gelişim gösteren çocuklara göre dil gelişimlerinin yavaş olduğu, yaşıtlarına göre daha kısa ve basit cümleler kurdukları, artikülasyon bozukluklarının ve kekemeliğin daha sık görüldüğü bilinmektedir.[31,32] Diğer bir deyişle, çocuklarda ZY ve Dil-konuşma bozukluğu birlikteliğine sıkça rastlanmaktadır.[33] Çalışmamızda da benzer şekilde, Hafif ve Orta düzeyde ZY tanılı olgulara sıklıkla Dil ve Konuşma Bozuklukları tanılarının eşlik ettiği bulunmuştur. Bu anlamda, ZY tanısı konan çocukların işitme ve konuşma değerlendirmesinin rutin olarak yapılması ve bu alandaki eksikliklerine yönelik olarak özel eğitime yönlendirilmeleri oldukça önemli ve gereklidir. Ayrıca bu olgular incelendiğinde, en sık olarak konuşma sesi bozukluğu (artikülasyon bozukluğu/fonolojik bozukluk), sonrasında dil bozukluğu ve akıcılık bozukluğu (kekemelik) tanılarının olduğu görülmektedir. Bu sonuçlar, mevcut literatürle uyumludur.[34] Bunların yanı sıra araştırmamızda, ÇÖZGER'de dil ve konuşma bozuklukları tanısının önceki sağlık kurulu raporlarından daha sık oranda belirtildiği dikkati çekmektedir. Görülen bu farklılık, ÇÖZGER öncesinde dil ve konuşma alanı ile ilgili tanıların kulak burun boğaz sistemi altında tanımlanırken, ÇÖZGER ile birlikte bu alandaki değerlendirmenin çocuk ve ergen psikiyatristleri tarafından yapılması ile açıklanabilir.[9]

ÇÖZGER öncesi ve sonrası tanılar karşılaştırıldığında, önceki sağlık kurulu raporunda belirtilen Hafif düzeyde ZY olguların oranında ÇÖZGER ile birlikte azalma olduğu, tersine Orta ve Ağır ZY olgularının oranında ise artma olduğu gözlenmiştir. Bu durum, takipler sırasında olguların kronolojik yaşına göre entelektüel ve/veya günlük yaşam etkinliklerindeki işlevselliklerindeki kısıtlılığın arttığı şeklinde düşünülebilir. Aslında zihinsel yetersizlikler, yaşam boyu süren nörogelişimsel bir bozukluk olarak ele alınsa da, zaman içerisinde bilişsel yetersizliklerin düzeyi ve uyum becerilerinde çevresel değişim taleplerine bağlı olarak görece azalmalar olabilmektedir.[31]

Sağlık kuruluna başvuran olguların çocuk ve ergen psikiyatrisi dışında diğer bölümlerden tanı alma sıklığı %27-57 arasında değişen oranlarda belirtilmekle birlikte[7,17], en fazla nöroloji[7,17,22] ve fizik tedavi ve rehabilitasyon bölümlerinden[8,23] tanı aldıkları vurgulanmaktadır. Benzer şekilde, çalışmamızda olguların yaklaşık %20'sinde psikiyatri dışındaki alanlarda da özel gereksinim varlığının belirtildiği ve bu olguların en sık olarak hareket gelişimi alanından tanı aldığı, bunu sinir sisteminin takip ettiği bulunmuştur. Yine literatürle uyumlu olarak en sık olarak epilepsi ve serebral palsi tanılarının görüldüğü saptanmıştır.[7,17,20]

ÇÖZGER kapsamında değerlendirilen olguların raporda belirtilmeyen diğer psikiyatrik tanıları incelendiğinde, en sık DEHB tanısının görüldüğü, bunu Anksiyete Bozuklukları'nın takip ettiği görülmektedir. Çalışmamızdaki olguların büyük çoğunluğunu ÖÖB ve Hafif düzeyde ZY tanılı çocuklar oluşturmaktadır. Türkiye'de çocuk ve ergenlerde yapılan çalışmalarda ÖÖB'ye eşlik eden DEHB tanısı %40-80 arasında farklı oranlarda belirtilmiştir.[35-37] Ayrıca ÖÖB'li çocukların yaklaşık dörtte birinde Anksiyete Bozukluğu eştanısının görüldüğü bildirilmiştir.[36,38] ZY'lere de sıklıkla DEHB ve Anksiyete Bozuklukları'nın eşlik ettiği bilinmektedir (DEHB- %30, Anksiyete- %7-34).[39] Hafif düzeyde ZY'lerde psikiyatrik eştanıların incelendiği bir çalışmada, bu olguların yaklaşık %65'inde DEHB, %18'inde Anksiyete Bozukluğu saptandığı bulunmuştur.[32] Sonuç olarak, raporda belirtilmeyen diğer psikiyatrik bozukluklar değerlendirildiğinde alanyazınla uyumlu sonuçlar bulunmuştur.

Çalışmamızdaki yabancı uyruklu olguların büyük çoğunluğunu Irak ve Suriye vatandaşları oluşturmaktadır. ÇÖZGER' göre bu olguların psikiyatri alanındaki özel gereksinim raporu düzeyi en sık olarak ÖKGV şeklinde belirtilmiş ve %74,3'ünde ek tıbbi tanı saptanmıştır. Bulgularımızın Kayhan & Öztürk'ün (2020) bir üniversite hastanesine ÇÖZGER için başvuran olguların değerlendirilmesi üzerine yaptığı araştırma ile uyumlu olduğu görülmektedir[7]. Ayrıca bu grupta psikiyatrik tanı olarak en sık ZY tanısının saptanması da mevcut alanyazını destekler niteliktedir.[40]

Çalışmamıza göre, ÇÖZGER'e geçişle birlikte %20-39 (ÖGV) engel oranında anlamlı düzeyde artış olduğu bulunmuştur. Bu sonuç, ÇÖZGER öncesinde Sınırda

Zihinsel İşlevsellik ve Özgül Öğrenme Bozukluğu tanılarının birlikte olduğu olguların engel oranının %40 olması ('Hafif ÖGV'ye karşılık gelmekte), ancak ÇÖZGER'e göre bu iki tanının varlığının ÖGV şeklinde ifade edilmesi şeklinde açıklanabilir. Ayrıca ÇÖZGER sonrasında %90-99 engel oranı olanlarda (ÖKGV) belirgin düzeyde artış saptanmıştır. Bu bulgu, Güller & Yaylacı'nın (2020) çalışma sonuçları ile benzer nitelikte olup bu durumun ÇÖZGER yönetmeliğinde yapılan özel gereksinim düzeyindeki yeni düzenlemelerin sonucu olduğu düşünülmektedir:[9] ÇÖZGER öncesinde Atipik Otizm tanısı ile %40 şeklinde rapor verilen olguların ÇÖZGER'de tek özel gereksinim düzeyi ÖKGV şeklinde belirlenmiştir. Ayrıca benzer şekilde, Orta ve Ağır düzeyde ZY olgularının sayısındaki artış ve öncesinde Orta düzeyde ZY ile %70 engel oranı verilen durumların ÇÖZGER'e geçişle birlikte ÖKGV şeklinde tanımlanması bir diğer neden olabilir. Ancak yapılan bu karşılaştırmalar (engel oranı ile özel gereksinim düzeyi), eş tanıların varlığı ve ek değişkenlerin yoğun olması gibi nedenlerden ötürü sağlıklı sonuç vermeyebilir ve bu durum araştırmamız için bir kısıtlılık oluşturabilmektedir.

Çalışmamızın önemli bir kısıtlılığı, verilerin geriye dönük olarak incelenmesidir. Bu nedenle, bulgularımız uzunlamasına yapılan çalışmalara göre daha az güvenilirdir. Ayrıca bu durumdan kaynaklanan sonuçları etkileyecek olası veri eksiklikleri de kısıtlılık oluşturmaktadır. İkinci olarak çalışmamız yalnızca tek merkezde yürütüldüğünden, sonuçlarımız tüm klinik popülasyonu veya toplum popülasyonunu yansıtmayabilir. Son olarak, çalışmanın bir diğer kısıtlılığı, psikiyatrik tanıların alanda çalışan uzman hekimler tarafından klinik olarak konulması, yapılandırılmış tanı görüşmelerinin kullanılmamış olmasıdır.

Sonuç olarak bu çalışma, en son yürürlüğe giren ve klinikte tüm branşların oldukça sık karşılaştığı ÇÖZGER'e yönelik tanımlayıcı bilgiler sağlamaktadır. Çocuk ve genç psikiyatrisi alanında ÇÖZGER'in getirdiği değişikliklerin incelendiği önceki çalışmaları destekler nitelikte olmakla birlikte, örneklemin Ankara'dan seçilmesi, farklı yaş grubunun dahil edilmesi gibi bazı farklılıklar da barındırmaktadır. Sonuçlarımızın gerek çocuk ve ergen psikiyatristlerine gerek de diğer dallarda çalışan hekimlere klinik pratikte önemli yararlar sağlayacağını düşünmekteyiz. Bu anlamda, ülkemizde farklı merkezlerin dahil edildiği daha geniş örneklemli ileri araştırmalar yazına önemli katkılar sağlayacaktır.

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Yazar Katkısı

RDT, SDU, MC: Çalışma fikri, hipotez, çalışmanın tasarımı; RDT, SDU, MC, AE, YG, MÇ, FHÇ: Veri toplama, kaynak tarama; RDT, SDU, MÇ, AE: Analiz ve yorumlama; Eleştirel inceleme: RDT, SDU, MÇ, FHÇ; RDT, SDU, MC: Yazının son halinin verilmesi ve yayınlanma süreci.

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Araştırma Makalesi | Research Article

ANTINEUTROPHIL CYTOPLASMIC ANTIBODY SEROTYPES MAY AFFECT THE CLINICAL PRESENTATION OF PATIENTS WITH POLYANGIITIS: A SINGLE-CENTER, RETROSPECTIVE CROSS-SECTIONAL STUDY

ANTİNÖTROFİL SİTOPLAZMİK ANTİKOR SEROTİPLERİ GRANÜLOMATÖZ POLİANJİİTLİ HASTALARDA KLİNİK PREZANTASYONU ETKİLEYEBİLİR: TEK MERKEZ, RETROSPEKTİF KESİTSEL ÇALIŞMA

Ayten Yazici[1*], Oznur Sadioglu Cagdas[1], Ali Osman Arisal[2], Ozlem Ozdemir Isik[1], Neslihan Gokcen[2], Ayse Cefle[1]

[1]University of Kocaeli, School of Medicine, Department of Internal Medicine, Division of Rheumatology, Kocaeli, Türkiye. [2]University of Kocaeli, School of Medicine, Department of Internal Medicine, Kocaeli, Türkiye.

ABSTRACT

Objective: Granulomatosis with polyangiitis (GPA) is one of the systemic vasculitis included in antineutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV). Clinical characteristics, ANCA serotypes, and their frequencies vary depending on the type of vasculitis. In this study, we aimed to investigate the link between ANCA serotypes and clinical variables documented at the time of admission.

Methods: This study was a retrospective cross-sectional study including 40 patients with GPA. The demographic, clinical, laboratory, and radiological findings were extracted from medical records. Logistic Regression Analysis was performed to estimate the relationship between ANCA serotypes and other variables.

Results: The mean age was 53.95±12.94 years, and there was a delay of 15.85±23.03 months in diagnosis. While constitutional symptoms were observed in 42% of patients, symptoms related to ear-nose-throat in 70%, lung in 87.5%, kidney in 62.5%, neurological findings in 30%, eye in 25%, skin in 25% and musculoskeletal system findings in 90% were noted. Male gender was seen more frequently in cANCA (60.7%) positive patients; however, pANCA-positive patients were mainly female (83.3%) (p=0.050). Delay in diagnosis was longer (p=0.001) and subglottic stenosis was more common (p<0.001) in pANCA-positive patients. However, the inflammatory response was more severe, and renal involvement (p=0.007) and arthralgia (p=0.048) were higher in cANCA-positive patients.

Conclusion: While renal involvement seemed to be related to cANCA, subglottic stenosis was mostly associated with pANCA in GPA. However, larger studies are needed to make a more accurate conclusion about the effect of ANCA serotypes on diversity in the clinical spectrum.

Keywords: Granulomatosis with polyangiitis, vasculitis, antineutrophil cytoplasmic antibody, renal involvement, subglottic

ÖZ

Amaç: Granülomatöz polianjiit (GPA) antinötrofil sitoplazmik antikor (ANCA) ilişkili vaskülitlerden (AİV) biridir. Vaskülitin tipine bağlı olarak klinik özellikler, ANCA serotipleri ve sıklıkları değişmektedir. Bu çalışmada, ANCA serotipleri ile başvuru anındaki klinik değişkenler arasındaki ilişkinin araştırılması amaçlandı.

Yöntem: Bu retrospektif kesitsel çalışmaya 40 GPA hastası dahil edildi. Demografik, klinik, laboratuar ve radyolojik bulgular hastaların dosyalarından toplandı. ANCA serotipleri ile diğer değişkenlerin ilişkisini değerlendirmek için Logistic Regresyon analizi kullanıldı.

Bulgular: Hastaların ortalama yaşları 53,95±12,94 yıl olup tanıda 15,85±23,03 aylık bir gecikme vardı. Konstitüsyonel semptomlar %42 hastada gözlenirken %70'inde kulak-burun-boğaz, %87,5'inde akciğer, %62,5'inde böbrek, %30'unda nörolojik, %25'inde göz, %25'inde cilt ve %90'ında kas-iskelet sistemi ile ilişkili semptomlar kaydedildi. cANCA pozitif olanlarda erkekler daha sık iken (%60,7) pANCA pozitiflerde kadınlar daha fazla (%83,3) idi (p=0.050). pANCA pozitif hastalarda tanıda gecikme daha uzun (p=0.001) ve subglottik stenoz daha sıktı (p<0.001). Bunun yanında cANCA pozitif hastalarda inflamatuar yanıt daha şiddetli ve böbrek tutulumu (p=0.007) ile artralji (p=0.048) daha fazlaydı.

Sonuç: GPA'da cANCA böbrek tutulumu ile ilişkili görünürken subglottik stenoz daha çok pANCA ile ilişkiliydi. Bununla birlikte ANCA serotiplerinin klinik spektrumdaki çeşitlilik üzerindeki etkisi hakkında daha doğru bir sonuca varmak için daha büyük çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Granülomatöz polyanjiit, vaskulit, antinötrofil sitoplazmik antikor, böbrek tutulumu, subglottic stenoz

[*]**İletişim kurulacak yazar/Corresponding author:** Ayten Yazici; University of Kocaeli, School of Medicine, Department of Internal Medicine, Division of Rheumatology, Baki Komsuoğlu Bulvarı, No:515, Umuttepe Campus, İzmit, Kocaeli, 41001, Türkiye.
Telefon/Phone: +90 (262) 303 75 25 **e-posta/e-mail:** burakdefy@hotmail.com
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Introduction

Granulomatosis with polyangiitis (GPA), also known as Wegener granulomatosis, is an antineutrophil cytoplasmic antibody (ANCA) associated necrotizing vasculitis involving small and medium-sized vessels. The etiology of GPA is not fully understood, but it is thought to be a complex multifactorial process in which genetic predisposition, environmental and immunological factors have a unique role in the etiology of the disease. Although GPA mainly targets the upper and lower respiratory tract and kidney, it can affect other organs and systems.[1,2] The prevalence and annual incidence have been reported as 2.3-146 and 0.4-14.4 cases per one million, respectively.[2] Studies have shown that GPA, which affects men and women equally, is more common in Northern European countries than in other countries.[2-6] It is well known that clinical characteristics, organ involvement, ANCA types and their frequencies vary through ANCA associated vasculitis (AAV) types including GPA, microscopic polyangiitis (MPA), and eosinophilic granulomatous polyangiitis (EGPA). Furthermore, some authors proposed a classification based on types of ANCA as anti-proteinase 3 (PR3) associated vasculitis and anti-myeloperoxidase (MPO) associated vasculitis due to the close relationship between ANCA serotype and disease characteristics, but it is not widely accepted in current literature.[5] Although anti-PR3 (65-75%) is the most frequently detected ANCA serotype in GPA, anti-MPO positivity and ANCA negativity have been reported in 20-30% and 5% of the patients, respectively.[5,7] The aim of the study was to reveal the clinical and laboratory characteristics of GPA patients and to investigate the possible relationship of those with ANCA serotypes.

Methods

Study Design and Patients

The study was designed as a retrospective cross-sectional study. All patients with GPA aged 18-80 years who were followed up in the rheumatology outpatient clinic between 2004 and 2021, and fulfilled the 2012 revised Chapel-Hill consensus criteria were included in the study.[8] Patients with both ANCA serotypes positive or negative, and patients with other vasculitis were excluded. Study protocol was approved by the local ethics committee (ethics approval number: GOKAEK-2021/9.11).

Clinical and Laboratory Assessment

The demographic, clinical, laboratory, and radiological data of the patients at the time of admission to the clinic were retrospectively screened from medical records. In our tertiary university hospital laboratory, ANCA detection is made by indirect immunofluorescence (IIF) while ANCA serotypes are determined by enzyme-linked immunosorbent assay (ELISA). Since PR3 and MPO were not assessed in all patients, the ANCA serotypes were defined as cANCA and/or PR3 and pANCA and/or MPO. Three patients with positive for both ANCA serotypes and three patients with negative ANCA were excluded from the comparisons. Systemic involvement was recorded from patients' database. Accordingly, an otolaryngologist evaluated patients in terms of ear-nose-throat (ENT) involvement with clinical examination. In some patients, head and neck magnetic resonance imaging was performed to evaluate and/or confirm ENT involvement. Lung involvement was assessed by computed tomography. Renal involvement was defined as abnormal urine test including hematuria and/or urine protein greater than 0.5g/24 hours. Neurological involvement was defined as peripheral and central nervous system findings that were assessed by clinical examination, electromyography, and brain magnetic resonance imaging. Similarly, cutaneous and eye involvement were assessed by dermatologist and ophthalmologist. Musculoskeletal system involvement was defined as arthralgia, arthritis, and myalgia. Patients underwent electrocardiography to assess cardiac involvement. Relapse was defined as re-occurrence or new onset of the organ or life-threatening disease.[1]

Statistical Analysis

SPSS for Windows 20.0 version was used for statistical analysis. The normal distribution of numerical variables was evaluated with the Kolmogorov-Smirnov Test. Besides descriptive statistical methods (mean, standard deviation, median and 25-75th percentile, min and max), the independent groups T test or Mann Whitney test were used for continuous variables, in comparison of paired groups. For categorical variables chi-square test and Fisher exact test were used. The relationship between ANCA serotypes and clinical and laboratory findings was determined by Spearman Correlation Analysis, and Logistic Regression Analysis was used as advanced statistics. For the testing of two-sided hypotheses, $p < 0.05$ was considered as sufficient for statistical significance.

Results

The mean age of GPA patients (female/male= 22/18) was 53.95±12.94 (median, min-max=55, 25-75) years. There was 15.85±23.03 (median, min-max=4, 1-180)-month delay in the diagnosis. During a mean follow-up period of 95.03±61.36 (median, min-max=82, 2-360) months, two patients died; one of them due to disease activation and concomitant infection, another one due to COVID-19 infection (5%). 37.5% of patients suffered from at least one relapse during follow-up. At the time of admission, 42% of the patients had constitutional findings. Most patients had several types of involvement. Accordingly, ear-nose-throat involvement, lung involvement, renal involvement, neurological involvement, skin involvement, and musculoskeletal system involvement were detected in 70%, 87.5%, 62.5%, 30%, 25%, 25%, and 90% of patients, respectively. None of patients had cardiac involvement (Table 1).

The number and percentages of patients according to the presence of ANCA positivity and its subtypes are given as following; 3 patients (7.5%) had neither pANCA nor cANCA positivity, 3 patients (7.5%) had positive test results for both pANCA and cANCA, 28 patients (70%) had cANCA/anti-PR3 positivity, finally, 6 patients (15%) had pANCA/anti-MPO positivity (Table 2).

The comparison of only pANCA/anti-MPO positive and only cANCA/anti-PR3 positive groups revealed that female gender was seen more frequently in pANCA/anti-MPO positive patients and male gender was seen more frequently in cANCA/anti-PR3 positive patients (p=0.050). While patients in pANCA/anti-MPO positive group had prolonged time to diagnosis (p=0.001), cANCA/anti-PR3 positive group had higher inflammatory markers including erythrocyte sedimentation rate, C-reactive protein, and thrombocyte count (p<0.001, p=0.006, and p=0.034, respectively). In addition, anemia was more common in cANCA/anti-PR3 positive group than in pANCA/anti-MPO positive group (75% vs 33.3%, p=0.048) (Table 2). Among

Table 1. The comparison of demographic and clinical findings according to ANCA serotypes

	All Patients (n=40)	**cANCA/PR3 (+) Patients (n=28)**	**pANCA/MPO (+) Patients (n=6)**	**p***
Age of onset	46.10±15.02	44.8±17.7	38.2±15.7	0.326[a]
Delay in diagnosis	15.85±23.03	11.4±16.5	45.5±36.4	**0.001**[a]
Gender (Female)	22 (55)	11 (39.3)	5 (83.3)	0.050[b]
Constitutional findings	34 (42)	25 (89.3)	5 (83.3)	0.681[b]
Fever	20 (50)	16 (57.1)	3 (50)	0.749
Weight loss	17 (42.5)	14 (50)	1 (16.7)	0.136
Weakness	34 (85)	25 (89.3)	5 (83.3)	0.681
Anorexia	20 (50)	16 (57.1)	2 (33.3)	0.289
Musculoskeletal involvement	36 (90)	26 (92.9)	4 (66.7)	0.071[b]
Myalgia	32 (80)	24 (85.7)	4 (66.7)	0.267
Arthralgia	27 (67.5)	21 (75)	2 (33.3)	**0.048**
Arthritis	7 (17.5)	6 (21.4)	1 (16.7)	0.793
Ear-nose-throat involvement	28 (70)	19 (67.9)	5 (83.3)	0.450[b]
Mastoiditis	7 (17.5)	6 (21.4)	0	0.211
Otitis	7 (17.5)	6 (21.4)	0	0.211
Sinusitis	11 (27.5)	7 (25)	2 (33.3)	0.675
Septum perforation	2 (5)	1 (3.6)	0	0.638
Subglottic stenosis	6 (15)	1 (3.6)	4 (66.7)	**<0.000**
Pulmonary involvement	35 (87.5)	26 (92.9)	4 (66.7)	0.071
Nodule	27 (67.5)	19 (67.9)	3 (50)	0.406
Cavity	15 (37.5)	11 (39.3)	1 (16.7)	0.293
ILD	14 (35)	13 (46.4)	1 (16.7)	0.179
Alveolar hemorrhage	8 (20)	7 (25)	1 (16.7)	0.662
Pleurisy	1 (2.5)	1 (3.6)	0	0.638
Lymphadenopathy	11 (27.5)	8 (24.2)	1 (16.7)	0.549
Renal involvement	25 (62.5)	21 (75)	1 (16.7)	**0.007**[b]
Neurological involvement	12 (30)	8 (28.6)	1 (16.7)	0.549[b]
Mononeuritis multiplex	1 (2.5)	0	0	NA
Peripheral nervous system	8 (20)	5 (17.9)	0	0.262
Central nervous system	4 (10)	3 (10.7)	1 (16.7)	0.681
Skin involvement	10 (25)	9 (32.1)	0	0.105[b]
Erythema nodosum/nodule	4 (10)	3 (10.7)	0	0.401
Palpable purpura	2 (5)	1 (3.6)	0	0.638
Leukocytoclastic vasculitis	2 (5)	2 (7.1)	0	0.500
Skin ulcer	2 (5)	2 (7.1)	0	0.500
Urticaria	1 (2.5)	1 (3.6)	0	0.638
Eye involvement	10 (25)	7 (25)	1 (16.7)	0.662[b]
Episcleritis/scleritis	6 (15)	5 (17.9)	0	0.324
Uveitis	2 (5)	2 (7.1)	0	0.500
Conjunctivitis	3 (7.5)	3 (10.7)	0	0.401
Gastrointestinal involvement	5 (12.5)	2 (7.1)	2 (33.3)	0.071[b]
Urogenital involvement (orchitis)	1 (2.5)	1 (3.6)	0	0.638[b]
Comorbid Disease	28 (70)	18 (64.3)	4 (66.7)	0.912[b]
Relapse	15 (37.5)	10 (35.7)	3 (50)	0.513[b]
Mortality	2 (5)	1 (3.6)	1 (16.7)	0.216[b]

[a]Values given as mean±standard deviation, Independent Samples T test and One way ANOVA

[b]Values given as n (%), Fisher's Exact test

*The comparisons were made between cANCA/PR3 (+) patients and pANCA/MPO (+) patients

ILD: Interstitial lung disease, ANCA: Antineutrophil cytoplasmic antibody, PR3: Anti-proteinase 3 antibody, MPO: Anti-myeloperoxidase antibody

Table 2. The comparison of laboratory findings at the time of diagnosis according to ANCA serotypes

	All Patients n=40	cANCA/PR3 (+) Patients n=28	pANCA/MPO (+) Patients n=6	p*
Increased ESR	28 (70)	25 (89.3)	0	**0.000**
Increased CRP	30 (75)	24 (85.7)	2 (33.3)	**0.006**
Leukocytosis	26 (65)	19 (67.9)	3 (50)	0.406
Neutrophil	21 (52.5)	15 (53.6)	2 (33.3)	0.325
Lymphopenia	12 (30)	10 (35.7)	1 (16.7)	0.338
Anemia	25 (62.5)	21 (75)	2 (33.3)	**0.048**
Increased RDW	9 (22.5)	7 (25)	0	0.150
Thrombocytosis	15 (37.5)	13 (46.4)	0	**0.034**
Increased Creatinine	10 (25)	7 (25)	1 (16.7)	0.632
Increased LDH	10 (25)	6 (21.4)	2 (33.3)	0.566
RF positivity	13 (32.5)	11 (39.3)	0	0.056
Anti-CCP positivity	2 (5)	2 (0.7)	0	0.458
ANA positivity	4 (10)	3 (10.7)	0	0.392
ANCA positivity	37 (92.5)	28 (100)	6 (100)	1.000
c-ANCA	28 (70)	26 (92.9)	0	
p-ANCA	9 (22.5)	0	6 (100)	
anti-PR3	29 (72.5)	25 (89.3)	1 (16.7)	
anti-MPO	3 (7.5)	0	2 (33.3)	

Values given as n (%), Fisher's Exact test

*The comparisons were made between cANCA/PR3 (+) patients and pANCA/MPO (+) patients

ESH: erythrocyte sedimentation rate, CRP: C-reactive protein, RDW: Red cell distribution width, LDH: Lactate dehydrogenase, RF: Rheumatoid factor, CCP: cyclic citrullinate peptide, ANA: Anti-nuclear antibody, ANCA: Antineutrophil cytoplasmic antibody, PR3: Anti-proteinase 3 antibody, MPO: Anti-myeloperoxidase antibody

Table 3. Logistic regression analysis for ANCA serotypes in patients with GPA

	Univariable Analysis			Multivariable Analysis		
	OR	95% CI	p	OR	95% CI	p
Delay in diagnosis	1.056	1.010-1.105	**0.017**			
Subglottic stenosis	54.000	3.931-741.79	**0.003**	54.000	0.061-0.928	**0.039**
Renal involvement	0.067	0.007-0.672	**0.022**			
Increased ESR	0.000	0.000	0.998			
Increased CRP	0.083	0.011-0.615	**0.015**			

ANCA: antineutrophil cytoplasmic antibody, GPA: Granulomatosis with polyangiitis, CI: Confidence interval, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein

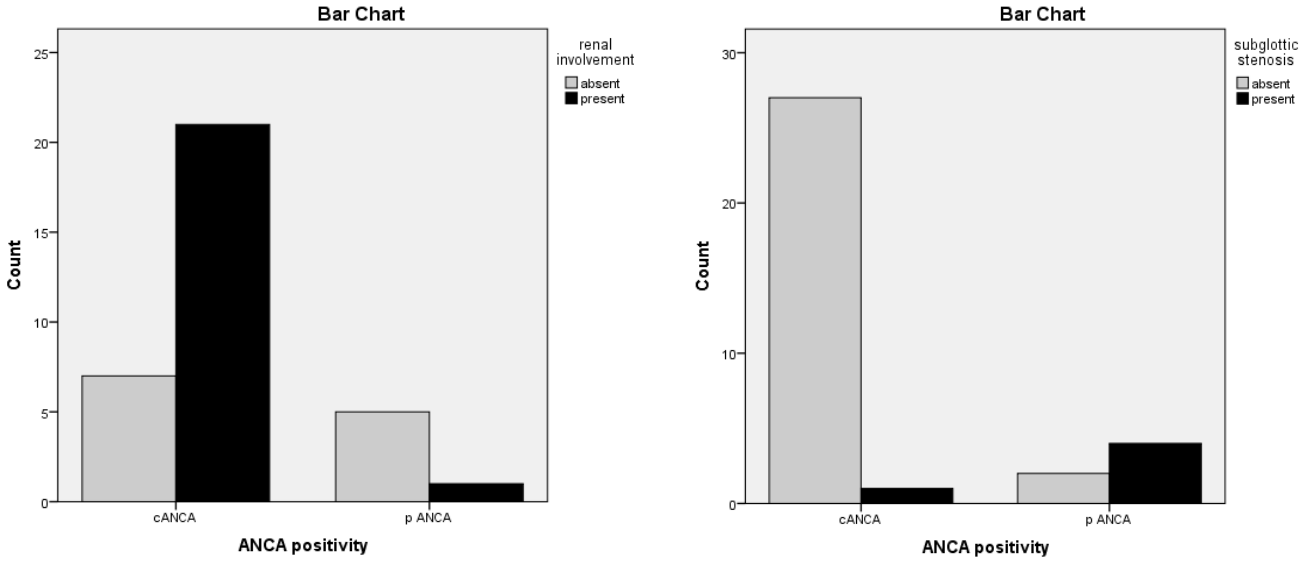

Figure: Frequency of renal involvement and subglottic stenosis according to ANCA serotypes

the clinical findings, while the frequency of subglottic stenosis (66.7% vs 3.6%; p<0.001) was seen higher in pANCA/anti-MPO positive patients, renal involvement (75% vs 16.7%; p=0.007) and arthralgia (75% vs 33.3%; p=0.048) were seen more frequently in those with cANCA/anti-PR3 positivity (Table 1) (Figure). Additionally, there were no correlations between ANCA serotypes and clinical/laboratory findings except delay in diagnosis (r=0.539, p= 0.001), renal involvement (r=-0.465, p=0.006), subglottic stenosis (r=0.679, p<0.001) and elevation of ESR (r=-0.772, p<0.001) and CRP (r=-0.471, p=0.005). However, ANCA serotypes were only associated with subglottic stenosis in logistic regression analysis (Table 3).

In this study, 70% of the patients had at least one comorbid disease (Diabetes Mellitus 15.5%, Hypertension 33.3%) and there was no difference between ANCA serotypes in terms of comorbid disease frequency. Although relapse and mortality rate were seemed to be higher in pANCA/anti-MPO positive patients, comparison of two groups did not reveal statistically significant differences (Table 1). The treatments received by the patients during the follow-up are given in Table 4.

Table 4. Previous and current treatments of patients

	n (%)
Previous treatment	
Cyclophosphamide	22 (55)
Corticosteroid	39 (97.5)
Azathioprine	23 (57.5)
Methotrexate	14 (35)
Rituximab	22 (55)
IVIG	10 (25)
Plasmapheresis	5 (12.5)
Current treatment	
Cyclophosphamide	3 (7.5)
Azathioprine	13 (32.5)
Methotrexate	8 (20)
Rituximab	17 (42.5)

IVIG: Intravenous immunoglobulin

Discussion

GPA is an ANCA-related necrotizing vasculitis with unknown etiology. This study investigated whether there is a relationship between ANCA serotypes and clinical and laboratory findings in patients with GPA. Similar to other systemic autoimmune diseases, GPA frequently causes nonspecific constitutional symptoms (in 60% of the patients), and lesser degree fever (48%), weight loss (28%), and joint findings (38%).[9] Arthralgia and myalgia have been reported in 70% of the patients, and arthritis in 23%.[10] In our study, constitutional findings, fever and arthritis were found in 42% of the patients, similar to previous studies, while weight loss and arthralgia/ myalgia ratio were found to be higher.

GPA is a multisystemic disease, however mostly targets the upper (75-90%) and lower respiratory tracts (60-80%), and kidney (56-80%).[7,9-12] In a cohort study including 502 patients with ANCA associated vasculitis, organ predilection was studied based on ANCA serotypes. Accordingly, the majority of patients with crescentic glomerulonephritis (above 80% of patients) were strongly related anti-MPO ANCA while most of patients with lung cavities and destructive ear-nose-throat involvement (above 80% of patients) were closely associated with anti-PR3 ANCA.[12] In a review article, authors searched the literature to show the differences of clinical, histopathologic, and pathophysiologic variables between anti-PR3 positive and anti-MPO positive patients. As a result, extrarenal organ manifestations and respiratory tracts involvement were frequently observed in anti-PR3 positive patients.[13] Among the upper respiratory tracts, the nasal cavity, and paranasal sinuses are the most commonly involved organs, followed by otologic (35%) and subglottic involvement (16-23%).[14,15] A study conducted by Schirmer et al. showed that anti-MPO positive patients had limited disease and had an increased frequency of subglottic stenosis.[16] Other organs and systems affected by GPA in decreasing fashion are given as following; eyes in 24-33%, mucocutaneous involvement in 25-50%, neurological involvement in 35%, and gastrointestinal involvement in 15%.[6,9-11] In our study, ear-nose-throat organs (70%), lungs (87.5%), renal (62.5%), neurologic (30%), eyes (25%), skin (25%) and gastrointestinal (12.5%) involvement were seen in similar rate compared to previous reports. Moreover, in the present study, we found that arthralgia and renal involvement were more common in patients with anti-PR3 ANCA while subglottic stenosis was more seen in patients with anti-MPO ANCA. Our results were conflicting with the study conducted by Cornec et al.[12] Retrospective cross-sectional study design and small sample size of our study may be the reasons for the conflicting results.

ANCA positivity is associated with disease activity and seen in 80-90% of patients with GPA. Studies have reported about 80% positivity for cANCA and 10% for pANCA subtype.[9,11,17] Although 65-75% of anti-PR3 positivity and 20-30% of anti-MPO positivity have been reported in GPA, anti-PR3 positivity has been reported up to %90 of patients in previous papers, especially during active periods.[5,7,18] In the current study, ANCA positivity was observed in 92.5% of patients while the rates of its serotypes positivity including cANCA, pANCA, anti-PR3, and anti-MPO were found in 70%, 22.5%, 72.5%, and 7.5% of those patients, respectively.

Disease severity, which varies from extensive organ involvement to limited form, affects ANCA positivity ratio. In the study of Stone et al., ANCA (90.6% vs 78.4%) and anti-PR3 (78.1% vs 58.8%) positivity have been found to be associated with severe disease, but not anti-MPO positivity (13.4% vs 8%).[11] In our study, we found that there were significant differences between cANCA and pANCA positive patients in terms of demographic, clinical, and laboratory findings. While cANCA/anti-PR3 positivity was related with male gender, pANCA/anti-MPO positivity was related with female gender. Patients with pANCA/anti-MPO positivity had a higher delay in

diagnosis. Elevated inflammatory markers, anemia, arthralgia and renal involvement were more prominent in the cANCA/anti-PR3 positive group, while subglottic stenosis was more common in pANCA/anti-MPO positive group.

In addition to the different clinical presentation in GPA, MPA and EGPA, the positivity rates of ANCA serotypes are also varies.[5] It has been shown that anti-MPO and anti-PR3 are associated with different organ involvement in AAV. While anti-PR3 positive AAV patients had more upper respiratory tract involvement, kidney is affected more frequently in anti-MPO positive AAV patients. In a previous study, anti-MPO have been found in 80% of AAV patients limited to the kidneys and anti-PR3 have been found in 90% of those with destructive lesions in the nasal septum.[7] On the basis of abovementioned literature, it can be assumed that similar differences in clinical presentation and organ involvement might be observed in GPA patients. Surprisingly, we observed an inverse relationship with ANCA serotypes and organ involvement compared to previous reports regarding AAV patients. Although ANCA serotypes were associated with only subglottic stenosis in logistic regression analysis, cANCA/anti-PR3 positivity was more common in patients with renal involvement, and pANCA/anti-MPO positivity was more common in patients with subglottic stenosis. However, our study has limited number of patients to generalize the present observation to GPA population, and needs to be validated by larger cohorts.

Anti-PR3 and anti-MPO are important prognostic indicators in AAV. Studies have shown that patients with positive anti-PR3 have increased relapse risk than those with positive anti-MPO. In addition, the frequency of relapse has been reported to be lower in those with ANCA negative compared to positive ones.[5,7] During the median 82 months follow-up, 37.5% of our patients had at least one relapse. A trend was seen regarding association of pANCA/anti-MPO positivity with increased risk of relapse but this did not reach statistical significance. Possible explanation for this might be limited numbers of patients in pANCA/anti-MPO positive group.

In GPA, 5-year survival has been reported to be 70-80%, and 10-year survival has been reported as 40% in patients with renal involvement and 60-70% in those without renal involvement.[5,15] In our study, the mortality rate was 5% in the median 82 months follow-up and one of these patients was lost due to the COVID-19 pandemic. The patient who died due to disease activity had pANCA/anti-MPO positivity, and renal lung and neu-rological involvement. These data are not sufficient to mention the effect of the ANCA serotype on prognosis.

The study has some limitations. First, the retrospective study design and small sample size might be the important reasons why we could not show other possible links between ANCA serotypes and clinical presentations in patients with GPA. Second, the lack of uniformity in serotype selection (ELISA or IIF) might cause heterogeneity. The other limitation was that we could not obtain more information about disease activity of patients such as the Birmingham vasculitis activity score, vasculitis damage index, and five factors score. Last but not least, the short follow-up period prevented us from investigating the impact of ANCA serotypes on prognosis. However, this study has also some strengths. First, even though an international consensus recommends that immunoassays can be used as the primary screening method for patients suspected of having ANCA-associated vasculitis, these methods might still not be available in some clinics. Indirect immunofluorescence is a slightly more used method. Thus, the advantage of our study is that we obtain the indirect immunofluorescence results and compare these results with the clinical parameters of patients with GPA. Second, we show the close relationship between pANCA/anti-MPO and subglottic stenosis, which can contribute to the literature due to the lack of data on this involvement.

Conclusions

The main findings of this study could be summarized as presence of association of renal involvement with cANCA /anti-PR3 and sublottic stenosis with pANCA/anti-MPO. However, we have to emphasize that large, multicenter studies are needed to achieve stronger evidence regarding ANCA serotypes and clinical diversity in GPA. Just as classification of GPA on the basis of limited vs multiorgan involvement which helps clinicians to determine prognosis and the best treatment, larger multicenter prospective studies might reveal the answer to the question of is there any prognostic benefit of dividing GPA patients according to ANCA serotypes. Thereby, defining new high-risk subgroups and treating them more aggressively can improve the prognosis in GPA.

Compliance with Ethical Standards

Study protocol was approved by Kocaeli University Local Ethics Committee (ethics approval number: GOKAEK-2021/9.11), and a written informed consent was obtained from each patient.

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None

Conflict of Interest

The authors have declared no conflict of interest.

Author contributions

All authors contributed to the study design and AY, OSC collected the data. AY and OOI analyzed the data and take responsibility for the accuracy of the data analysis. All authors interpreted the data, drafted the manuscript, and critically revised it for important contents. All authors read and approved the final manuscript.

Financial Disclosure

None

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DİYABETLİ OLAN VE OLMAYAN COVID-19 TANILI HASTALARDA CRP/ALBÜMİN ORANININ ARAŞTIRILMASI

INVESTIGATION OF CRP/ALBUMIN RATIO IN PATIENTS WITH AND WITHOUT DIABETES DIAGNOSED WITH COVID-19

Nihal Gökalp Dağtekin [1*], Mehmet Ali Eren [2]

[1] Yavuzeli İlçe Devlet Hastanesi, İç Hastalıkları Bölümü, Gaziantep, Türkiye [2] Harran Üniversitesi Tıp Fakültesi, Endokrinoloji Bölümü, Şanlıurfa, Türkiye

ÖZ

Amaç: Diyabetes mellitusta ortaya çıkan hiperglisemi çeşitli mekanizmalar ile hücresel ve humoral bağışıklık sistemini bozar. Diyabetli hastalarda Covid-19 duyarlılığı artmıştır. C-reaktif protein ve albümin seviyeleri çeşitli infeksiyon ve inflamasyon durumlarından etkilenmektedir. Son yıllarda CRP/albümin oranı yeni bir prognostik skor olarak kullanılmaktadır. Çalışmamızda diyabeti olan ve olmayan Covid-19 hastalarında CRP, albümin ve CRP/albümin oranlarını araştırarak hastalık şiddeti açısından fark olup olmadığını saptamayı amaçladık.

Yöntem: Çalışmaya Covid-19 tanılı 40 diyabeti olan, 32 diyabet tanısı olmayan hastalar dahil edildi. Romatolojik hastalık, kanser ve organ yetmezliği, gebe, Covid-19 dışında eşlik eden enfeksiyonu olanlar dahil edilmedi. Çalışmaya dahil edilenlerin kayıtları geçmişe yönelik olarak taranarak cinsiyet, yaş, CRP, albümin, lökosit, lenfosit, nötrofil, platelet, hemoglobin, MPV değerleri kaydedildi.

Bulgular: Ortalama CRP düzeyi diyabeti olan grupta (6.54 ± 4.12 mg/dl) diyabeti olmayan gruba göre (2.94 ± 2.50 mg/dl) anlamlı şekilde yüksek bulundu (p<0.001). Ortalama CRP/albümin oranı diyabeti olan grupta (1.76 ± 1.21) diyabeti olmayan gruba göre (0.75 ± 0.64) anlamlı yüksekti (p<0.001). Ortalama albümin düzeyi açısından diyabeti olan grup (3.89 ± 0.43 g/dl) ve diyabeti olmayan grup (4.05 ± 0.38 g/dl) arasında anlamlı fark saptanmadı (p=0.11).

Sonuç: CRP/albümin oranı enfeksiyon şiddetini gösteren bir belirteç olarak sıklıkla kullanılmaktadır. Ancak Covid-19 hastalığının diyabeti olan ve olmayan kişilerde şiddetini göstermek için CRP değerinden daha üstün olduğu gösterilememiştir. Diyabet hastalığı olanlarda Covid-19 enfeksiyonu daha fazla akut faz reaktanı yanıtı oluşturmaktadır.

Anahtar Kelimeler: Diyabet, Covid-19, CRP, albümin, CRP/albümin oranı

ABSTRACT

Objective: Hyperglycemia occurring in diabetes mellitus impairs the cellular and humoral immune system through various mechanisms.Patients with diabetes have increased sensitivity to Covid-19. CRP and albumin levels are affected by various infections and inflammations. In recent years, the CRP/albumin ratio has been used as a new prognostic score. In our study, we aimed to determine whether there is a difference in disease severity by investigating the levels of CRP, albumin and CRP/albumin in Covid-19 patients with and without diabetes.

Methods: 40 diabetic patients and 32 patients without a diabetes who had diagnosis of Covid-19 were included in the study. Patients with rheumatological disease, cancer and organ failure, pregnant, and concomitant infections other than Covid-19 were not included. The data of the study were obtained retrospectively, and gender, age, CRP, albumin, leukocyte, lymphocyte, neutrophil, platelet, hemoglobin, MPV values were recorded.

Results: The mean CRP level was found to be significantly higher in the diabetic group (6.54 ± 4.12 mg/dl) compared to the nondiabetic group (2.94 ± 2.50 mg/dl) (p<0.001). The mean CRP/albumin ratio was significantly higher in the diabetic group (1.76 ± 1.21) than in the nondiabetic group (0.75 ± 0.64) (p<0.001). There was no significant difference between the diabetic group (3.89 ± 0.43 g/dl) and the non-diabetic group (4.05 ± 0.38 g/dl) in terms of mean albumin level (p=0.11).

Conclusion: CRP/albumin ratio is frequently used as an indicator of severity of infection. However, it has not been shown to be superior to the CRP value to show the severity of Covid-19 disease in people with and without diabetes. In patients with diabetes, Covid-19 infection produces more acute phase reactant response.

Keywords: Diabetes, Covid-19, CRP, albumin, CRP/albumin ratio

***İletişim kurulacak yazar/Corresponding author:** Nihal Gökalp Dağtekin; Yavuzeli İlçe Devlet Hastanesi, Gaziantep, Türkiye
Telefon/Phone: +90 (0536)4434757 **e-posta/e-mail:** nihalgklp@gmail.com
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Giriş

Diyabetes mellitus çeşitli mekanizmalar ile hipergliseminin ortaya çıktığı heterojen bir metabolik bozukluktur.[1] Diyabetli bireylerde ortaya çıkan immün yetmezlik durumu nedeniyle bu hastalarda meydana gelen enfeksiyonlar önemli bir sorundur. Sitokin üretiminin bozulması, lökosit alım inhibisyonu, patojen tanımadaki kusurlar, nötrofil, makrofaj ve natural killer hücrelerinin disfonksiyonu, antikor ve kompleman inhibisyonu konak savunmasını bozan başlıca mekanizmalardır.[2]

Diyabetli hastalarda Covid-19 enfeksiyonunun daha ciddi seyrettiği ve bu hastalarda akut solunum depresyonu ve çoklu organ yetmezliği gibi ciddi komplikasyonların riskinin arttığı bilinmektedir.[3] Diyabetli hastalarda Covid-19 duyarlılığını arttıran potansiyel mekanizmalar; yüksek afiniteli hücresel bağlanma ve etkili virüs girişi, viral klirensin azalması, azalmış T hücre fonksiyonu, hiperinflamasyon ve sitokin fırtına sendromuna karşı artan duyarlılık, kardiyovasküler hastalık varlığı gibi patolojilerdir.[4] Ayrıca ACE-2 reseptör ekspresyonu diyabetli hastalarda, yüksek glikoz seviyelerine yanıt olarak artar; bu kısmen diyabetli hastaların hastalığa yakalanmaya daha yatkın olduğunu açıklayabilir ve hastanın viral yükünü artırarak enfeksiyonun şiddetli seyrine katkıda bulunabilir.[5]

Hepatositler tarafından sentezlenen C-reaktif protein (CRP), akut faz yanıtını tespit etmek ve dolayısıyla inflamatuar durumların teşhisi ve izlenmesi için en sık kullanılan laboratuvar testlerinden biridir.[6-8] Albümin negatif bir akut faz proteini olup sepsis, enfeksiyon veya travma gibi patolojik durumlarda veya majör cerrahiden sonra düzeyi azalır.[9] CRP/albümin oranı ise inflamasyonun ciddiyeti ve şiddeti ile ilişkili olan yeni bir prognostik skordur; daha yüksek bir oran, daha yüksek bir inflamatuar durumu gösterir.[10-11]

Bu bilgiler ışığında diyabeti olan ve olmayan Covid-19 hastalarında CRP, albümin ve CRP/albümin oranlarını kıyaslayarak, diyabeti olan ve olmayan hasta gruplarında Covid-19 hastalığının şiddeti açısından farklı olup olmadığını saptamayı amaçladık.

Yöntem

Harran Üniversitesi Tıp Fakültesi Hastanesi'nde 09.06.2020-11.02.2021 tarihleri arasında Covid-19 tanısı ile yatarak takip edilen hastalar geriye dönük olarak taranarak diyabeti olan (Grup 1 (n=40)) ve diyabeti olmayanlar (Grup 2 (n=32)) şeklinde çalışmaya dahil edildi. Çalışma için 2.Helsinki bildirgesinde belirtilen özelliklere uygun olarak Harran Üniversitesi Tıp Fakültesi etik kurul onayı alındı (HRU/21.07.14). Romatolojik hastalığı olan, gebe, kanser tanısı ve organ yetmezliği tanısı olanlar ile Covid-19 dışında eşlik eden enfeksiyonu olan hastalar çalışmaya dahil edilmedi. Çalışmaya dahil edilen hastaların yaş ve cinsiyet bilgileri ile yatışları sırasında elde edilen CRP, albümin, lökosit, lenfosit, nötrofil, platelet, hemoglobin değerleri kaydedildi. CRP/albümin oranı CRP değerinin albümin değerine bölünmesi ile hesaplandı.

İstatistiksel analizler SPSS versiyon 22.0 (IBM®, Chicago, ABD) paket programı kullanılarak yapılmıştır. Veriler ortalama ± standart sapma veya sayı (yüzde) şeklinde sunuldu. Değişkenlerin normal dağılımına uygunluğu görsel (histogram ve olasılık grafikleri) ve analitik yöntemler (Shapiro-Wilk testi) kullanılarak hesaplandı. Normal dağılım gösteren sayısal değişkenler için Bağımsız Gruplarda T testi kullanılırken, normal dağılım göstermeyenler için ise Mann Whitney U testi kullanıldı. Nominal veriler Ki-kare testi kullanılarak değerlendirildi. Korelasyon analizlerinde Pearson korelasyon testi kullanılmıştır. Korelasyon katsayısı 0.01-0.19 arasında düşük veya önemsiz korelasyon, 0.20-0.39 arasında zayıf korelasyon, 0.40-0.59 arasında orta derecede korelasyon, 0.60-0.79 arasında yüksek derecede korelasyon, >0.80 ise çok yüksek derecede korelasyon şeklinde kabul edildi. İki grup arasındaki karşılaştırmalarda p değeri 0.05'in altında olduğunda istatistiksel olarak anlamlı kabul edildi.

Bulgular

İki grup arasında cinsiyet (Grup 1 için K/E=16/24, Grup 2 için K/E=18/14,p:0.161) ve ortalama yaş (Grup 1 için 58.02 ± 8.82 yıl, Grup 2 için 55.78 ± 10.64 yıl, p:0.33) açısından anlamlı fark yoktu. Grup 1'de ortalama CRP düzeyi (6.54 ± 4.12 mg/dl vs. 2.94 ± 2.50 mg/dl) ve ortalama CRP/albümin oranı (1.76 ± 1.21 vs. 0.75 ± 0.64) Grup 2'den anlamlı olarak daha yüksekti (her iki p<0.001). Ortalama albümin düzeyi için ise Grup 1 (3.89 ± 0.43 g/dl) ve Grup 2 (4.05 ± 0.38 g/dl) arasında anlamlı olarak farklılık saptanmadı. Ayrıca kan sayım parametreleri açısından da her iki grup arasında anlamlı farklılık saptanmadı (Tablo 1).

Tablo-1. Diyabeti Olan ve Olmayan Gruplar Arasında Klinik ve Laboratuvar Verilerinin Karşılaştırılması

Veriler	Grup 1	Grup 2	P
Cinsiyet (kadın/erkek)	16/24(%40)	18/14(%56,25)	0.161
Yaş (yıl)	58.02 ± 8.82	55.78 ± 10.64	0.33
CRP (mg/dl)	6.54 ± 4.12	2.94 ± 2.50	<0.001
Albümin (g/dl)	3.89 ± 0.43	4.05 ± 0.38	0.11
CRP/albümin	1.76 ± 1.21	0.75 ± 0.64	<0.001
Lökosit ($x10^3$/ul)	6.87 ± 2.87	6.58 ± 2.16	0.64
Lenfosit ($x10^3$/ul)	1.50 ± 1.05	1.42 ± 0.69	0.91
Nötrofil ($x10^3$/ul)	4.68 ± 2.13	4.53 ± 1.95	0.77
Platelet ($x10^3$/ul)	243.88 ± 110.30	231.53 ± 63.91	0.58
Hemoglobin (g/dl)	13.40 ± 2.12	13.95 ± 1.75	0.24

**CRP: C-reaktif protein*

CRP ile CRP/albümin oranı arasında pozitif yönde çok yüksek derecede (r:0.985, p<0.001), albümin arasında negatif yönde orta derecede (r:-0.425, p<0.001), lökosit arasında pozitif yönde zayıf derecede (r:0.298, p:0.011), nötrofil arasında pozitif yönde zayıf derecede (r:0.363, p:0.002) anlamlı korelasyon saptandı. Albümin ile hemoglobin arasında pozitif yönde zayıf derecede anlamlı korelasyon saptandı (r:0.374, p:0.001). CRP/albümin oranı ile albümin arasında negatif yönde orta derecede (r:-0.533, p<0.001), lökosit arasında pozitif yönde zayıf derecede (r:0.296, p:0.012) ve nötrofil arasında pozitif yönde zayıf derecede (r:0.357, p:0.002) anlamlı korelasyon saptandı. Laboratuvar parametreleri arasında korelasyon analizi Tablo 2'de gösterildi.

Tablo-2. Laboratuvar Parametreleri Arasında Korelasyon

Parametre	CRP	Albümin	CRP/Albümin
Yaş	r:0.106 p:0.374	r:0.021 p:0.863	r:0.079 p:0.510
CRP		r:-0.425 p<0.001	r:0.985 p<0.001
Albümin	r:-0.425 p<0.001		r:-0.533 p<0.001
Lökosit	r:0.298 p:0.011	r:-0.115 p:0.336	r:0.296 p:0.012
Lenfosit	r:-0.014 p:0.908	r:0.007 p:0.951	r:-0.003 p:0.980
Nötrofil	r:0.363 p:0.002	r:-0.154 p:0.198	r:0.357 p:0.002
Platelet	r:0.162 p:0.174	r:-0.060 p:0.618	r:0.182 p:0.127
MPV	r:0.133 p:0.266	r:0.029 p:0.808	r:0.128 p:0.283
Hemoglobin	r:-0.131 p:0.271	r:0.374 p:0.001	r:-0.181 p:0.128
CRP/Albümin	r:0.985 p<0.001	r:-0.533 p<0.001	

**CRP: C-reaktif protein*

Tartışma

CRP, akut faz yanıtını tespit etmek ve dolayısıyla inflamatuar durumların teşhisi ve izlenmesi için en sık kullanılan laboratuvar testlerindendir. Hastalığa özgüllüğü olmamasına ve çeşitli hastalık faktörlerinden etkilenmesine rağmen, inflamasyonun klinik belirti ve semptomlarını desteklemek açısından klinisyenlere değerli bilgiler sağlar.[6-8] Ernst ve ark.'nın yaptığı çalışmada, septik artriti olanlarda ortalama CRP değerlerinin hem inflamatuar hem de normal eklemleri olanlara göre anlamlı yüksek olduğu gösterilmiş olup CRP'nin septik eklem varlığını belirlemede faydalı olduğunu tespit etmişlerdir.[12] Hopstaken ve ark.'nın pnömonili hastalarda yaptığı çalışmada CRP değerinin pnömoni ile ilgili herhangi bir semptom ve bulgudan daha yüksek tanısal olasılık oranına sahip olduğu bulunmuştur.[13] Chen ve ark.'nın yaptığı çalışmada Covid-19'lu hastalarda yüksek CRP seviyesinin daha uzun süre hasta yatışı, pnömoni bulgularının şiddeti ve tomografide tutulum ciddiyeti ile pozitif ilişkili olduğu bulunmuştur.[14] Akbariqomi ve ark.'nın yaptığı çalışmada hastaneye yatırılan diyabeti olan ve olmayan Covid-19 tanılı hastalar karşılaştırıldığında, diyabetli hastalarda lökosit ve nötrofil sayısı, C-reaktif protein ve eritrosit sedimantasyon hızı daha yüksek bulunmuştur.[15] Koh ve ark.'nın normoglisemik (HbA1c: ≤%5.6), prediyabet (HbA1c: %5.7-%6.4) ve diyabetli (HbA1c: ≥6.5%) hastalarla yaptığı çalışmada; CRP' nin, diyabet ve şiddetli Covid-19 enfeksiyonu arasındaki ilişkinin kısmi bir aracısı olduğu, diyabetli hastalarda gözlenen yüksek CRP seviyelerinin, diyabetli hastalarda daha fazla antibiyotik ihtiyacı olması ile ilişkinlendirildiği ve diyabette şiddetli Covid-19 patogenezinde inflamasyonun önemli olduğu bulunmuştur.[16] Debi ve ark.'nın yaptığı bir metaanalizde de yukarıdaki çalışmalardaki gibi serum CRP konsantrasyonu, Covid-19'lu diyabetik hastalarda anlamlı olarak daha yüksek bulunmuştur.[17] Bizim çalışmamızda da bu çalışmalara benzer olarak, Covid-19 pnömonisi nedeniyle yatışı yapılan hastaların diyabeti olanlarında ortalama CRP düzeyi olmayanlara göre yüksek bulundu ve bu durum Covid-19'un diyabetli bireylerde daha şiddetli seyrettiğinin bir kanıtıydı.

Albümin negatif bir akut faz proteinidir ve akut hastalık durumlarında hipoalbüminemi sıklıkla gözlenir. Sepsis, enfeksiyon veya travma gibi patolojik durumlarda veya majör cerrahiden sonra, olaydan sonraki 1 hafta içinde serum albümin düzeyi yaklaşık 1-1,5 g/dl azalır.[9] Violi ve ark.'nın Covid-19 tanılı hastalarda yaptığı çalışmada yoğun bakımdaki hastaların %74'ünde serum albümini <3.5g/dl saptanmıştır; albümin <3.5 g/dl olan hastalar, albümin >3,5 g/dl olan hastalar ile karşılaştırıldığında daha yüksek kreatinin, CRP ve d-dimer seviyeleri tespit edilmiştir.[18] Aloisio ve ark.'nın yaptığı bir çalışmada, Covid-19 tanılı hastalarda serumdaki yüksek laktat dehidrojenaz konsantrasyonları ve düşük albümin konsantrasyonlarının daha yüksek ölüm olasılıkları ile önemli ölçüde ilişkili olduğu bulunmuştur.[19] Biz çalışmamızda yatış sırasında bakılan laboratuvar sonuçlarını dikkate aldık. Bu nedenle ortalama albümin seviyeleri her iki grup için düşük saptanmadı ve iki grup arasında bir fark gözlenmedi.

CRP/albümin oranı, inflamasyonun ciddiyeti ve şiddeti ile ilişkili olan yeni bir prognostik skordur.[10] Zhou ve ark.'nın yaptığı çalışmada CRP/albümin oranının Crohn hastalığında mukozal iyileşmeyi göstermede trombosit/albümin oranı, nötrofil-lenfosit oranı ve trombosit-lenfosit oranına kıyasla daha duyarlı olduğu tespit edilmiştir.[20] Kim ve ark.'nın şiddetli sepsis ve septik şok hastalarında yaptığı çalışmada yatıştan 72 saat sonraki CRP/albümin oranı yüksek olanların daha düşük olanlara kıyasla anlamlı olarak daha yüksek 180 günlük ölüm oranına sahip olduğu gösterilmiştir.[21] Torun ve ark.'nın Covid-19 tanılı hastalarda yaptığı çalışmada; CRP/albümin oranı, fibrinojen/albümin oranı ve

nötrofil/lenfosit oranının Covid-19'un şiddetini tahmin etmek için kullanılabileceği ve bunların arasında CRP/albümin oranının şiddetli Covid-19'un en iyi öngörücüsü olduğu vurgulanmıştır.[22] Bayrak ve ark.'nın yaptığı çalışmada, kontrol grubuna kıyasla en az bir komplikasyonu olan diyabetik hastalarda serum CRP/albümin oranı anlamlı derecede yüksek bulunmuştur.[23] Eren ve ark.'nın Tip 2 diyabetli diyabetik ayak ülseri olan hastalarda yaptığı çalışmada, osteomiyeliti olan hastalarda osteomiyeliti olmayan hastalara kıyasla CRP/albümin oranı daha yüksek bulunmuştur.[24] Biz de çalışmamızda ortalama CRP/albümin oranını literatürdeki çalışmalara benzer olarak diyabetli grupta anlamlı olarak yüksek saptadık. Fakat iki grubun ortalama albümin düzeyleri arasında anlamlı fark bulunmadığından bu sonucun sebebi CRP düzeyleri arasındaki farktır. Dolayısıyla yatış sırasında, henüz albümin düzeyleri etkilenmediği için, inflamasyonun ve prognozun esas belirleyicisinin CRP olduğu söylenebilir.

Diyabetes mellitus en sık bulaşıcı hastalıklara yatkınlık yaratan metabolik bozukluklardan biridir. Hiperglisemi bozulmuş nötrofil kemotaksisi, azalmış fagositoz ve bakterisidal aktivite, değişmiş kemokin üretimi ile hücresel ve humoral bağışıklık sisteminin düzensizliği ile ilişkilidir.[2] Ayrıca nöropati ve vasküler hasar, diyabetlilerde yaraların ilerlemesine yol açar ve iyileşmelerini engeller. Özellikle alt ekstremitelerdeki deri ve yumuşak doku enfeksiyonları, rinoserebralmukormikoz, invaziv dış kulak iltihabı ve idrar yolu enfeksiyonları tipik olarak diyabetes mellitus ile yakın ilişkilidir ve daha sık gözlenir.[25] Diyabetli bireylerde enfeksiyonlar nadir görülen bakteriler, atipik seyirler ve sıklıkla eşlik eden komplikasyonlar nedeniyle hem tanıda gecikmeye hem de tedavide zorluklara yol açar.[26] Genellikle bu hastalarda enfeksiyonlar hızla ilerleyebildiğinden acil müdahale ve geniş spektrumlu parenteral antimikrobiyal tedavi gerektirir.[25] Muller ve ark.'nın yaptığı çalışmada; tip 1 ve tip 2 diyabetli hastaların diyabeti olmayanlara göre alt solunum yolu enfeksiyonları, idrar yolu enfeksiyonları ve deri veya mukozal membran enfeksiyonları açısından daha yüksek risk altında olduğu bulunmuştur.[27] Bonadio ve arkadaşlarının yaptığı çalışmada; diyabetiklerde idrar yolu enfeksiyonunun eradikasyonunun diyabetik olmayanlara göre daha zor olduğu tespit edilmiştir.[28] Carton ve ark.'nın yaptığı çalışmada; diyabetik hastalarda diyabetik olmayanlara göre daha yüksek oranda üriner kaynaklı, toplum kökenli ve E. coli'ye bağlı bakteriyemi insidansı bulunmuştur.[29] Cooper ve ark.'nın yaptığı çalışmada, Staphylococcus aureus bakteriyemisi olan diyabetik hastaların, birincil odak varlığında endokardit olması diyabetik olmayanlara göre daha olası bulunmuştur.[30] Torres ve ark.'nın yaptığı bir başka çalışmada, diyabetli hastalarda toplum kökenli pnömoni için 1,4'e kadar ve invaziv pnömokok hastalığı için 1,4 ile 4,6 arasında değişen bir risk artışı tespit edilmiştir.[31] Zhu ve ark.'nın hastanede yatan 7300 Covid-19 hastası arasında yaptıkları bir başka çalışmada tip 2 diyabetli bireylerin diyabetik olmayan bireylere göre daha fazla tıbbi müdahaleye ihtiyaç duyduğu ve önemli ölçüde daha yüksek mortaliteye ve çoklu organ hasarına sahip olduğu bulunmuştur. Ayrıca, iyi kontrol edilen kan şekerinin, kötü kontrollü kan şekeri olan bireylere kıyasla belirgin şekilde daha düşük ölüm oranı ile ilişkili olduğu bulunmuştur.[32] Biz de çalışmamızda bu çalışmalara benzer olarak Covid-19 hastalığının diyabetik hastalarda daha fazla akut faz reaktanı yanıtı oluşturduğunu ve daha ağır seyrettiğini tespit ettik. Guo ve ark.'nın yaptığı bir başka çalışmada serum IL-6, CRP, serum ferritin ve d-dimer gibi inflamasyonla ilişkili biyobelirteçlerin seviyelerinin ve mutlak nötrofil sayısının, diyabetik hastalarda diyabetik olmayanlara kıyasla anlamlı olarak daha yüksek, mutlak lenfosit sayısının ise daha düşük olduğu bulunmuştur. Yazarlar bu sonuçların diyabetli hastaların sitokin fırtınası oluşturmaya daha duyarlı olduğu şeklinde yorumlanabileceğini belirtmişlerdir.[33] Bizim sonuçlarımızda kan sayım değerleri açısından diyabetli olanlar ve olmayanlar arasında fark saptanmadı. Bu durum hastaların en yüksek nötrofil veya en düşük lenfosit sayısının dikkate alınmaması ile ilişkili olabilir. Ancak yine de diyabet hastalığı olanlarda olmayanlara göre Covid-19 enfeksiyonunun CRP düzeyi ile gösterilmiş olan daha fazla akut faz reaktanı yanıtı oluşturduğunu tespit ettik.

Sonuç olarak, diyabet enfeksiyon riskinin ve şiddetinin artmasına neden olan metabolik bir bozukluktur. CRP/albümin oranı enfeksiyon şiddetini gösteren bir belirteç olarak sıklıkla kullanılmaktadır. Ancak Covid-19 hastalığının diyabeti olan ve olmayan kişilerde şiddetini göstermek için yatış anında bakılan değerler göz önüne alındığında CRP değerinden daha üstün olduğu gösterilememiştir. Hastalık seyri sırasındaki değerlerin önemini saptamak için farklı dizayn edilmiş daha geniş çalışmalara ihtiyaç vardır. Ayrıca diyabet hastalığı olanlarda Covid-19 enfeksiyonu daha fazla akut faz reaktanı yanıtı oluşturmaktadır ve diyabetli hastalar bu nedenle daha dikkatli ve yakından takip edilmelidir.

Etik Standartlara Uygunluk

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Case Report | Olgu Sunumu

RARE CAUSE OF SMALL BOWEL OBSTRUCTION: WILKIE'S SYNDROME, A CASE REPORT

İNCE BARSAK OBSTRÜKSİYONUNDA NADİR BİR ETYOLOJİ: WİLKİE SENDROMU, OLGU SUNUMU

Ramazan Serdar Arslan[1*], Mehmet Ali Semsit[2] Suleyman Diker[3]

[1]Servergazi State Hospital, Department of General Surgery, Denizli, Türkiye. [2]Torbalı State Hospital, Department of General Surgery, İzmir, Türkiye. [3]Usak Resarch and Training Hospital, Department of Internal Medicine, Usak, Türkiye

ABSTRACT

Mechanical blockage of the bowel frequently causes abdominal pain, nausea, vomiting, and distention. Post-surgical adhesions are the most common etiology. Wilkie syndrome develops as a result of compression of the third part of the duodenum between the aorta and the superior mesenteric artery, and it is extremely rare. The patients are mostly cachectic due to inadequate nutrition. Surgical intervention, especially duodenojejunostomy, is a preferred method in the treatment of the disease. A multidisciplinary approach is important in the diagnosis, treatment, and management of the disease.

Keywords: Wilkie syndrome, superior mesenteric artery syndrome, duodenojejunostomy, duodenal obstruction

ÖZ

Mekanik barsak obstrüksiyonunda sıklıkla karın ağrısı, mide bulantısı, kusma ve distansiyon görülür. Sıklıkla operasyon sonrası yapışıklıklara sekonder olarak görülür. Wilkie sendromu duodenum üçüncü kısmının aort ve superior mezenterik arter arasında sıkışması sonucunda oluşan, ender görülen, mekanik barsak obstrüksiyon sebeplerinden biridir. Hastalar bulantı, kusma, yetersiz beslenme nedeniyle çoğunlukla kaşektiktir. Cerrahi girişim, özellikle duodenojejunostomi, hastalığın tedavisinde tercih edilen bir yöntemdir. Multidisipliner yaklaşım hastalığın tanı, tedavi ve yönetiminde önemlidir.

Anahtar Kelimeler: Wilkie Sendromu, süperior mezenter arter sendromu, duodenojejunostomi, duodenal obstrüksiyon

***İletişim kurulacak yazar/Corresponding author:** Ramazan Serdar Arslan; Servergazi State Hospital, Department of General Surgery, Denizli, Türkiye.
Telefon/Phone: +90 (505) 5917091 **e-posta/e-mail:** r.serdar.arslan@gmail.com
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Introduction

The signs and symptoms of Wilkie's syndrome (WS), an extremely rare condition, include nausea, bilious vomiting, postprandial epigastric discomfort, anorexia, and weight loss.[1] It is also referred to in the literature as “cast syndrome”, “arterio-mesenteric duodenal compression syndrome”, “superior mesenteric artery syndrome”, and “chronic duodenal ileus”.[1,2] WS incidence as determined by radiological investigations It ranges from 0.2% to 0.78%.[3] The incidence in the general population has been estimated to range from 0.0024% to 0.34%.[1-3] The prevalence of having WS is between 0.0024% and 0.3%.[3] Although it can occur at any age, it more frequently affects children and teenagers. Most individuals receive their first diagnosis between their first and fourth decades. The female-to-male ratio by gender is 3:2. No racial propensity is mentioned. Patients who have undergone surgical correction for scoliosis, congenitally short or hypertrophic Treitz ligament, duodenal malrotation, or an aortic aneurysm usually experience it. Numerous studies in the literature have reported a familial predisposition in the literature.[4] The pathogenesis is due to compression of the third segment of the duodenum between the superior mesenteric artery and the abdominal aorta. The aorta mesenteric angle narrowing, which is 20° (normal: 38-65°), the decrease in aortomesenteric distance, which is 10 mm (normal: 10-28 mm), and proximal duodenal dilatation are significant in the diagnosis of WS.[1-4] Surgery is recommended when conservative treatment fails. Although laparoscopic duodenojejunostomies are currently performed, open duodenojejunostomies may be preferred. In this article, we present a 54-year-old male patient with WS that we treated with a duodenojejunostomy.

Case Report

A 54-year-old man who had been experiencing postprandial epigastric pain, nausea, bilious vomiting, loss of appetite, and gradual weight loss for six months was admitted to our outpatient clinic. It was found that the patient. Who had a history of diabetes mellitus, did not have his endocrinology outpatient controls and had irregular drug use. He was cachectic, and his body mass index was 15.9 kg/m^2. Abdominal examination showed abdominal distension and mild tenderness in the epigastric area. In laboratory examinations glucose: 237 (70-105 mg/dL), creatinin: 2.91(0.7-1.3 mg/dL), total protein: 49.9 (63-86 g/L), albumin: 28.5 (35-55 g/L), amylase: 159 (25-125 U/L), lipase: 123 (13-60 U/L), c reactive protein: 34.5 (0.1-5 mg/L), WBC: 12.5 (4-10.5 10^3/µL). A nasogastric catheter was inserted into the patient, and 4.5 liters of bile content were drained. X-rays of the abdomen and lungs revealed that the stomach was ptotic to the pelvis (Figure 1). In the gastroscopy, alkaline reflux, erythematous pangastritis, and an enlarged stomach were observed. While the duodenal bulbus was normal, enlargement was observed in the second and third parts of the duodenum. No stricture or obstructive pathology was observed (Fujinon EG 530, Japan) (Figure 2). An intravenous and oral contrast-enhanced abdominal computerized tomography showed gastric pitozis, dilatation of the second part of the duodenum, and compression of the third part (Figure 3).

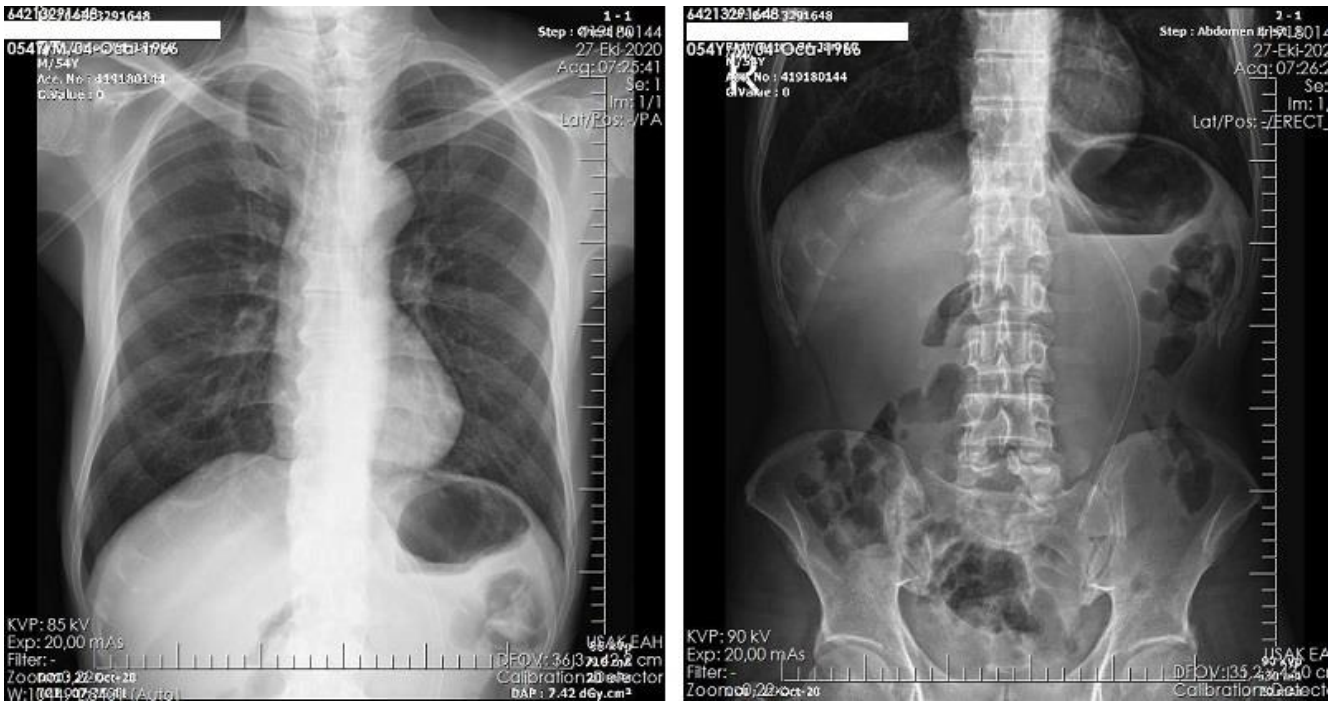

Figure 1. Abdominal and lung x-rays

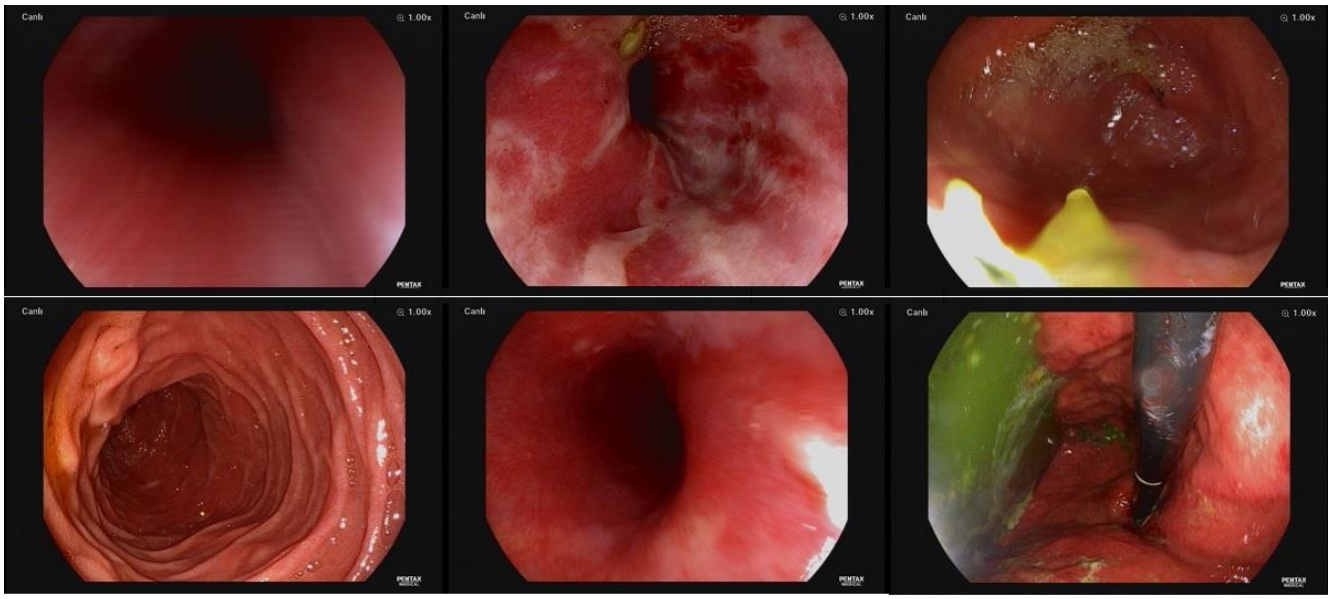

Figure 2. Upper gastrointestial system endoscopy findings

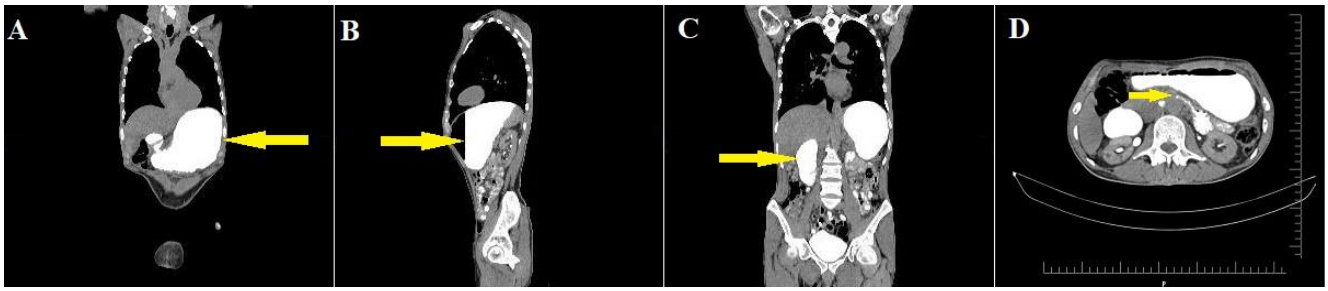

Figure 3. Oral contrast-enhanced abdominal computerized tomography

Aorto-mesenteric angle of 19.5° and the aorto-mesenteric distance of < 1 cm (0.53) (Figure 4). Wilkie's syndrome was diagnosed. After two weeks of total parenteral nutrition therapy, the patient was taken to conservative management therapy. He was discharged home with nasojejunal feeding, given a prescription for erythematous pangastritis. After three weeks of observation, the patient refused to continue her nasojejunal nutrition. The patient was prepared for surgery. During the operation, it was observed that the stomach was ptotic. Duodenum was exposed with Kocher maneuver. It was observed that the duodenum was dilated from the third part towards the proximal (Figure 5). Retrocolic side to side stapled duodenojejunostomy (60 mm) was done between the 3rd part of the duodenum and jejunum at 40 cm from Treitz (Figure 6). On the 5th postoperative day, gastrographin passage radiography was taken. No leak was observed and passage from duodenum to jejunum was detected (Figure 7). The patient recovered smoothly and was discharged home on the 14th postoperative day. In the sixth month control, it was detected that the patient received fifteen kilograms.

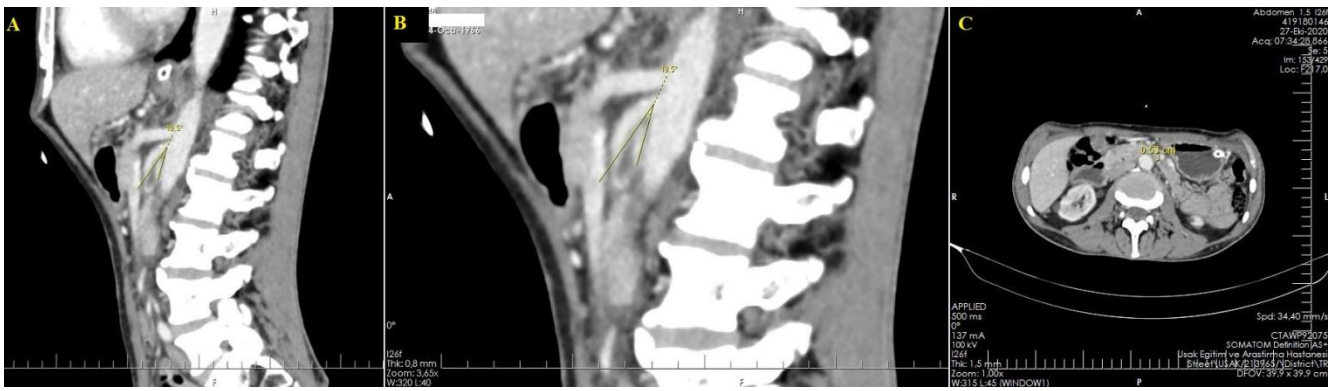

Figure 4. Aorto-mesenteric angle and the aorto-mesenteric distance

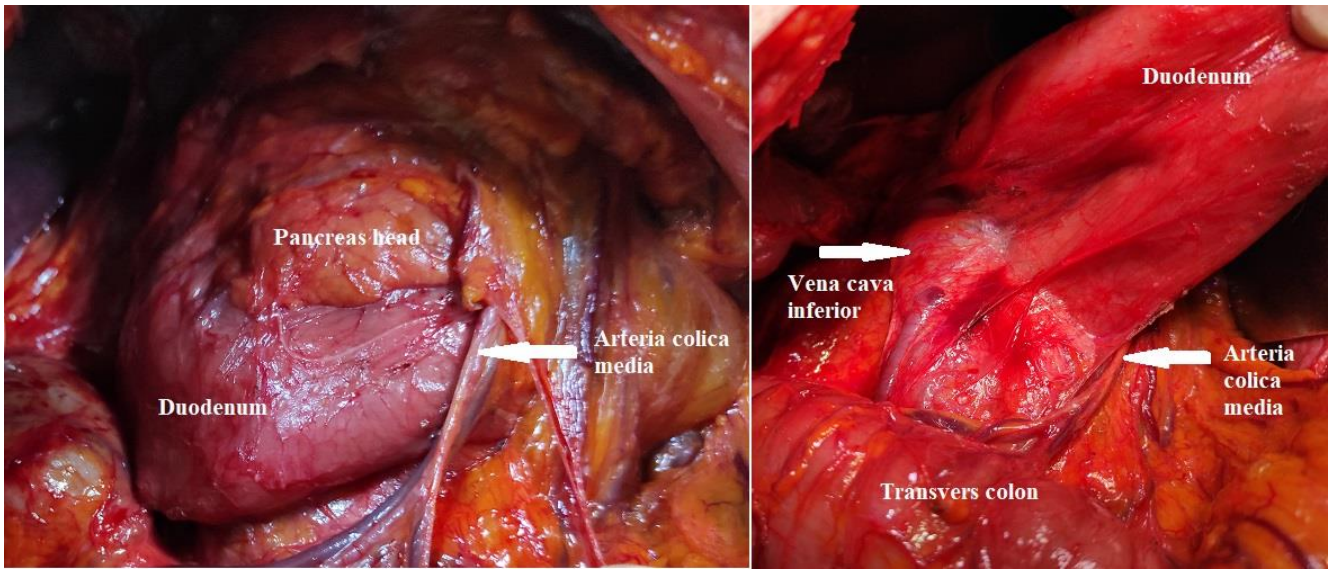

Figure 5. Operation findings

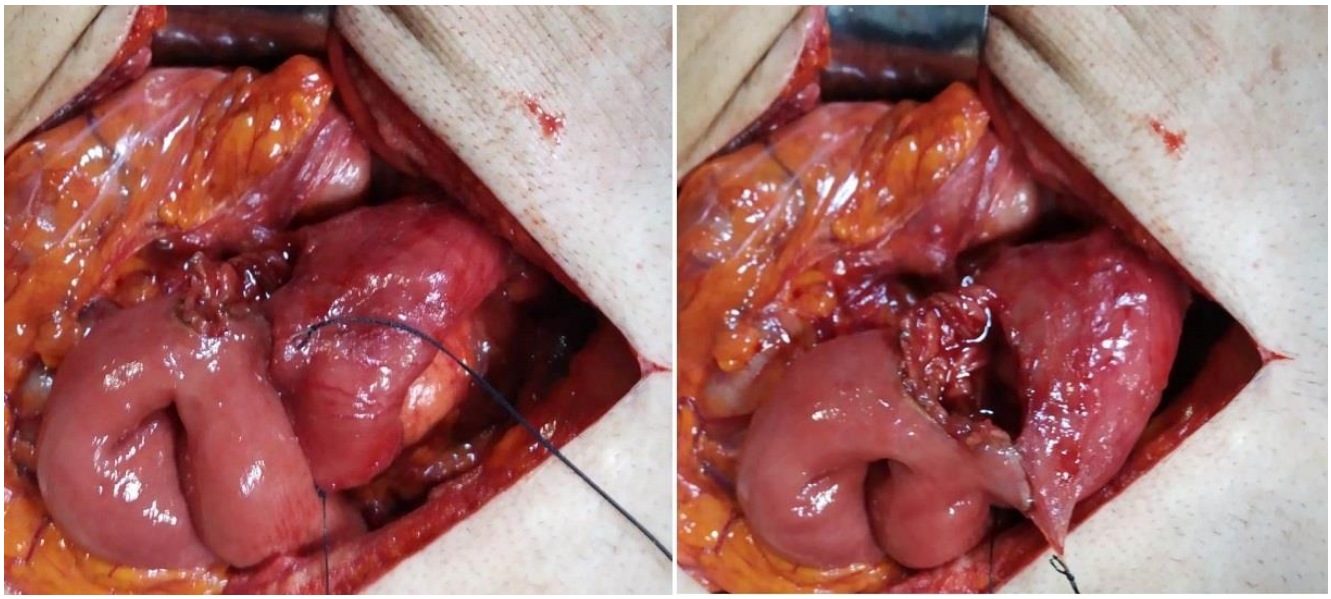

Figure 6. Retrocolic side to side stapled duodenojejunostomy

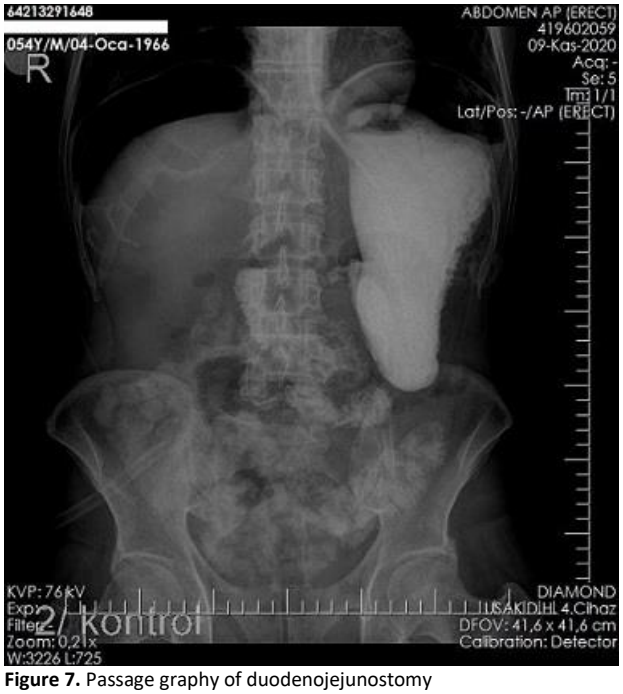

Figure 7. Passage graphy of duodenojejunostomy

Discussion

The small bowel mechanical obstruction symptoms in WS are present. Clinical, endoscopic, and radiologic findings are crucial for diagnosis. In the differential diagnosis, it is important to consider pancreatic neoplasm, duodenal or small intestine tumors, gastric antral neoplasm, and duodenal stricture brought on by inflammatory bowel disease. In patients with Wilkie's syndrome, abdominal computed tomography with water-soluble contrast scans of the upper gastrointestinal tractus reveal dilated stomach, proximal duodenum, and visibly compressed third section of duodenum.[3] The normal aorto-mesenteric angle (AMA) and distance are 25°-60° and 10-20 mm, respectively. We measure AMA 19.5° and distance < 1 cm (0.53). WS can be treated medically or surgically. Medical treatment such as bowel rest, fluid replacement, parenteral nutrition, restoration of electrolyte imbalance, and nasojejunal feeding may be effective in acute situations.[5] In our case, we started the treatment conservatively, but the operation decision was

made because the patient could not tolerate and refused to be fed with a nasojejunal tube. There are three options for the surgical treatment of WS: Strong procedure, gastrojejunostomy, and duodenojejunostomy. In the Strong procedure, it is aimed to widen the distance between the duodenum and aorta by dividing the Treitz ligament without disrupting the intestinal integrity.[6] Gastric decompression is provided by gastrojejunostomy; however, it may not be adequate to relieve duodenal obstruction, necessitating a subsequent procedure.[7] The most popular surgical technique, with a success rate of up to 80-90%, is an open or laparoscopic duodenojejunostomy.[8,9] For the patient, open surgery was preferable. Gastroparesis and gastric atony are significant issues in phytotic stomachs following surgery. To ensure postoperative motility in our situation, we maintained administering metoclopramide intravenously for ten days and neotigmine methylsulfate for two days.

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Compliance with Ethical Standards

Written informed consent was obtained from the patient for the publication of this case report.

Conflict of Interest

All author declared no potential conflict of interest with respect to research of this article.

Author Contribution

All authors contributed equally to this article.

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Case Report | Olgu Sunumu

A CASE OF PURE AUTONOMIC FAILURE PRESENTING WITH SYNCOPE; HYPOTENSION AND HYPERTENSION ATTACKS

SENKOP İLE BELİRTİLEN BİR SAF OTONOM BAŞARISIZLIK OLGUSU; HİPOTANSİYON VE HİPERTANSİYON ATAKLARI

Şenol Coşkun*[1], Müzeyyen Simay Karakullukçu[2], Tayfun Şahin[2]

[1]Acibadem Hospital, Cardiology Clinic, Bursa, Türkiye. [2]Kocaeli University Faculty of Medicine, Department of Cardiology, Kocaeli, Türkiye.

ABSTRACT

A 67-years old female patient was admitted to a cardiology specialist with the complaints of hypertension, syncope, and presyncope attacks for the last one year. While blood and urine tests were normal, follow-up of the patient's vital signs showed hypotensive attacks with presyncope and dizziness. Cranial Magnetic Resonance Imaging (MRI), carotid and vertebral artery color Doppler Ultrasonography (Doppler USG), Electroencephalography (EEG) ve Electromyography (EMG) were performed. Despite other tests being normal, cranial MRI showed white matter damage. The patient is still in routine follow-up.

It may be effective to apply different treatments for patients' syncope and other demonstrative and disability symptoms in pure autonomic failure (PAF).

Keywords: Pure autonomic failure, Syncope, Hypotension, Hypertension attacks

ÖZ

Altmış yedi yaşında kadın hasta son bir yıldır hipertansiyon, senkop ve presenkop atakları nedeniyle kardiyoloji uzmanına başvurdu. Kan ve idrar tetkikleri normal iken hastanın vital bulgu takibinde, presenkop ve baş dönmesi ile birlikte hipotansif atakları görüldü. Kranial MRI, karotis ve vertebral arter renkli Doppler Ultrasonografi (Doppler USG, Elektroensefalografi (EEG) ve Elektromiyografi (EMG) yapıldı. Diğer testlerin normal olmasına rağmen, kranyal Manyetik Rezonans Görüntüleme (MRG)'de beyinde beyaz cevher hasarı saptandı. Hastanın rutin takibi devam etmektedir.

Saf otonomik yetmezlikte, hastaların senkop ve diğer belirtileri için farklı tedavi seçenekleri uygulamak etkili olabilir.

Anahtar Kelimeler: Saf otonomik bozukluk, Senkop, Hipotansiyon, Hipertansiyon atakları

***İletişim kurulacak yazar/Corresponding author:** Senol Coskun; [1]Acibadem Hospital, Cardiology Clinic, Bursa, Turkiye.
Telefon/Phone: +90 (505) 5917091 **e-posta/e-mail:**scoskun2@yahoo.com
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Introduction

Pure autonomic failure (PAF) is not a well-known cause of orthostatic hypotension. A neurodegenerative disorder of autonomic nervous system clinically characterized by orthostatic hypotension was first described in 1925.[1] Bannister and Oppenheimer described a new entity in 1982 showing autonomic failure without other neurological symptoms of the Shy-Dragger Syndrome (also cerebellar ataxia, parkinsonism, and upper and lower motor neuron symptoms with orthostatic hypotension).[2] The hallmark of orthostatic hypotension is failure of releasing norepinephrine upon standing up. Dysfunction or loss of peripheral sympathetic nerves leads to impaired secretion of norepinephrine and other catecholamines. Conversely, patients usually have normal blood pressure while seating and sometimes have high blood pressure while lying down. Approximately, half of the patients have concomitant supine hypertension.[3] The cerebral white matter damage and left ventricular hypertrophy can accompany hypertension and may lead to end organ damage.[4,5] When the diagnosis is established as PAF for the patients presenting with orthostatic hypotension, several different treatments may be beneficial for syncope and other demonstrative and incapacitating symptoms.

Case Report

A 67-years old female patient was admitted to a cardiology specialist for hypertension, syncope, and presyncope attacks for the last 1 year. The patient did not have any known disease except hypertension with no medical treatment. Investigations for the etiology of her hypertension attacks, syncope, and presyncope were carried out. Ambulatory 24 hours cardiac rhythm and blood pressure monitoring showed slight sinus bradycardia at night (lowest 47 bpm) and hypertensive attacks. Transthoracic ECG revealed normal left ventricle ejection fraction with left ventricle hypertrophy. Because of the hypertensive attacks, hormone tests (Thyroid Stimulating Hormone; TSH, Adrenocorticotropic hormone; ACTH, cortisol, renin) and norepinephrine and metanephrine levels in 24 hours urine test were also performed. While all blood and urine tests were normal, follow-up of the patient's vital signs showed hypotensive attacks with presyncope and dizziness. Patient faints upon standing up. Moreover, when asked in details patients admitted having symptoms like frequent constipation and urinary incontinence. Neurology specialist also consulted the patient for the neurological causes of syncope. Cranial MRI, carotid and vertebral artery color Doppler USG, EEG, and EMG were performed. Despite other tests being normal, cranial MRI showed white matter damage (Figure 1). Sympathetic nervous system nuclear imaging as 123I-metaiodobenzylguanidine single-photon emission computer tomography (123I-MIBG-SPECT) was planned with the suspicious diagnosis of pure autonomic neuropathy and multisystem atrophy.

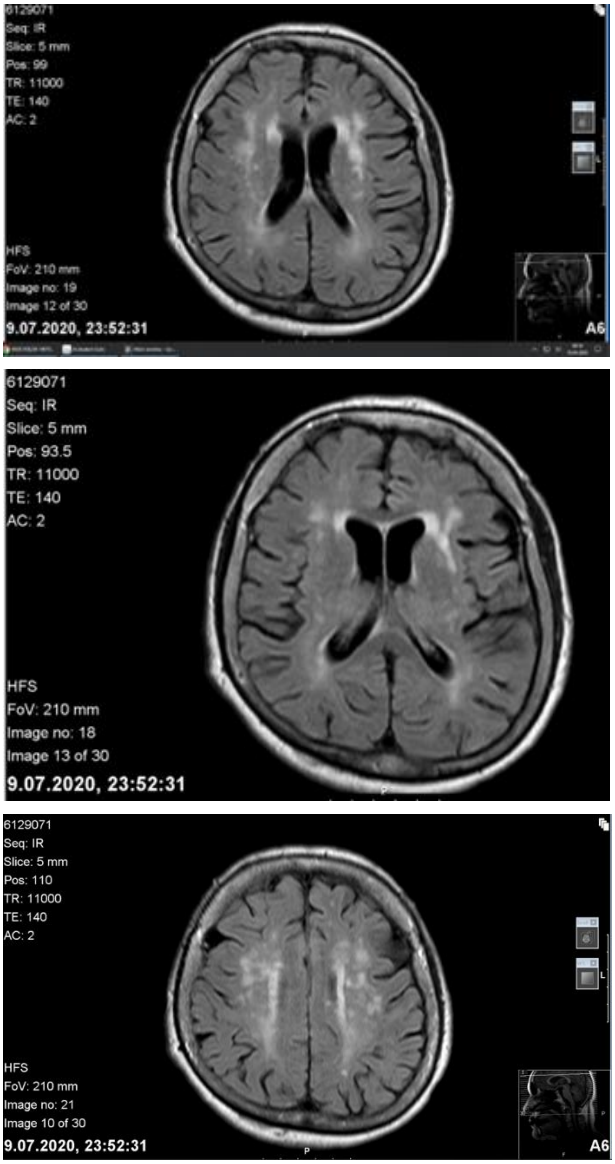

Figure 1. Flair images showing ischemic white matter lesions

The patient was planned to be referred to another hospital after discharging due to the unavailability of access to contrast media for scintigraphy in our hospital. Before the discharge of the patient, she was put on fludrocortisone as a treatment after which no more presyncope and syncope with hypotension attacks were seen. Cardiac sympathetic denervation at 123I-MIBG-SPECT was in line with the diagnosis of pure autonomic neuropathy (Figure 2). The patient did not have any neurological signs of parkinsonism, ataxia, or motor neuron symptoms. The patient is still in routine follow-up without any new complaints.

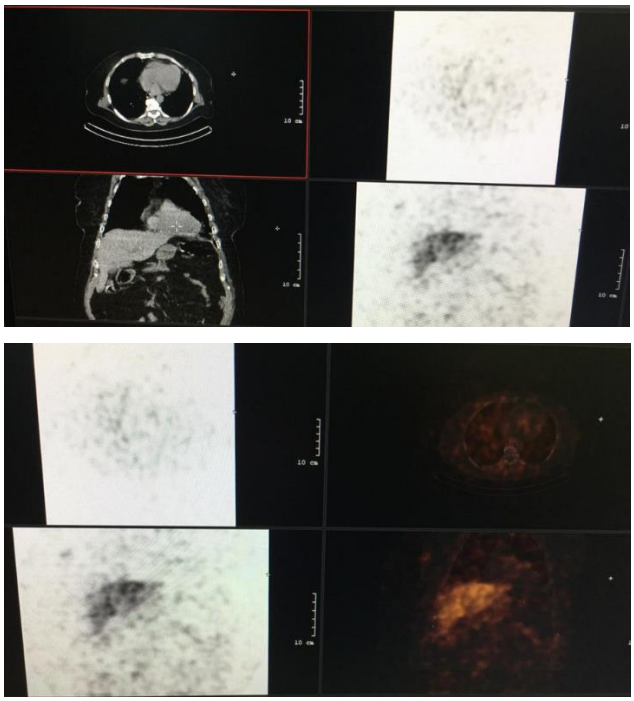

Figure 2. Pure autonomic neuropathy imaging with Spect 123I MIBG spect

Discussion

Autonomy PAF is a rare, sporadic, neurodegenerative disorder of the autonomic nervous system. Its main clinical symptoms are neurogenic orthostatic hypotension and urinary and gastrointestinal autonomic dysfunctions.

Orthostatic hypotension is defined as a reduction in systolic blood pressure by at least 20 mmHg or diastolic blood pressure of 10 mmHg within 3 minutes of standing or 60-degree head-up tilt.[6] When supine hypertension present greater than 140 mmHg or diastolic blood pressure than 90mmhg, then 30-mmHg systolic blood pressure reduction than 30mmhg is presumed more appropriate to meet the diagnosis of orthostatic hypotension.

Mechanically, orthostatic hypotension is related to venous pooling on lower extremities and, splanchnic vascular beds (approximately 300-1000 ml) on standing, which then leads to a reduction in venous return to the heart. Cardiac and stroke volumes are also reduced. In healthy individuals increase in cardiac contractility and heart rate leads to an increase in sympathetic outflow through baroreflex. In PAF, inadequate sympathetic response to standing is the leading cause of orthostatic hypotension. However, differential diagnosis of PAF is tough. It includes non-neurogenic orthostatic hypotension, syncope, neurogenic orthostatic hypotension, autonomic neuropathies, and inherited disorders. If the patient has syncope with hypotensive and hypertensive attacks and systolic blood pressure decrease on standing, interrogation of other symptoms like urgency, anhidrosis, dizziness, constipation, and incontinence may be helpful for the suspicion of PAF.[7] Tilt table test, cardiac sympathetic innervations imaging like ^{123}I-MIBG-SPECT and standing up blood pressure testing will set the diagnosis of PAF. Once the diagnosis is established, the treatment options may improve the quality of life of the patients.

Several strategies ranging from non-pharmacological measurements to medical treatment are available in PAF. Non-pharmacological measurements include an increase in fluid and salt intake, avoiding maneuvers increasing intrathoracic pressure (coughing, straining, etc.), encouraging isotonic exercise, and gradual movement change with postural change. On the other hand, medical treatment include fludrocortisone, midodrine, and another sympathomimetic agent, erythropoietin, caffeine, clonidine, droxidopa, and beta-blockers (with intrinsic sympathomimetic activity).

Conclusion

To increase the awareness of PAF, we here presented a PAF patient who responded to the first line therapy with 0,1 mg fludrocortisone which is available in our country. We would like to point out the use of ^{123}I-MIBG-SPECT imaging to test sympathetic innervations. Consideration of medical and non-pharmacological treatment options during careful follow-up of PAF patients may increase their quality of life.

Compliance with Ethical Standards

Written informed consent was obtained from the patient for the publication of this case report.

Conflict of Interest

All author declared no potential conflict of interest with respect to research of this article.

Author Contribution

All authors contributed equally to this article.

Financial Disclosure

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