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İstanbul Tıp Fakültesi Dergisi

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İstanbul Tıp Fakültesi Dergisi

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Case Report	1000	200	15	2	10 or total of 20 images
Technical Note	1500	No abstract	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	1	1

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All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

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Book section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR,

editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a single author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or technical report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study KidneyInt: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktivitelerive Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. SağlıkBilimleriEnstitüsü, DoktoraTezi. 2007.

Manuscripts accepted for publication, not published yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub ahead of print articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. DiagnIntervRadiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts published in electronic format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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CASE REPORT

RESEARCH / ARAŞTIRMA
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PREDICTIVE RELEVANCE OF DIFFERENT CLINICAL AND LABORATORY FINDINGS FOR HIGHER MORTALITY IN PATIENTS WITH COVID-19 IN A SINGLE CENTER COHORT: NEUTROPHIL/ LYMPHOCYTE RATIO, HIGH CRP, GGT AND CREATININE LEVELS ARE ASSOCIATED WITH HIGH MORTALITY

COVID-19'DA KLİNİK VE LABORATUVAR BULGULARININ MORTALİTE GÖSTERGESİ OLARAK DEĞERİ, TEK MERKEZ KOHORT ÇALIŞMASI, YÜKSEK NÖTROFİL/LENFOSİT ORANI, CRP, GGT, KREATİNİN DEĞERLERİ VE ARTMIŞ MORTALİTE RİSKİ

Oğuz EVLİCE[1], Fatih KUŞ[2], Özlem ARIK[3], Murat BEKTAŞ[4]

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ABSTRACT

Objective: Early detection of mortality risk is important in patients diagnosed with of coronavirus disease 2019 (COVID-19). Therefore, we aimed to evaluate the predictive value of different clinical and laboratory parameters in disease severity and mortality in patients with COVID-19.

Materials and Methods: Patients admitted to hospital with a diagnosis of COVID-19 were evaluated retrospectively. The patients' admission date, discharge date, intensive care transfer/ death date, contact history, smoking, symptoms at the time admission, vital markers at admission, and laboratory parameters were recorded.

Results: The study included a total of 347 patients, of whom 168 (48.4%) were female. The mean age of the patients was 59.69±16.87 (14-97) years, while 40.9% (n=142) were aged over 65 years. Overall, 10.1% (n=35) of the patients required transfer to an intensive care unit and 8.4% (n=29) were deceased. When clinical parameters were evaluated at the time of admission, oxygen saturation was found to be lower in the group that died (79.51±6.95), compared to the survivors (88.78±6.11) (p<0.001). Additionally, male gender (p=0.05), advanced age (p<0.001),

ÖZET

Amaç: Koronavirüs hastalığı 2019 (COVID-19) tanısı ile takip edilen hastalarda mortalite riskinin erken tespiti önemlidir. COVID-19'da farklı klinik ve laboratuvar parametrelerin hastalık şiddeti ve mortalite göstergesi olarak değerinin saptanması hedeflenmiştir.

Gereç ve Yöntem: COVID-19 tanısı ile hastaneye yatırılan hastalar retrospektif olarak değerlendirilmiştir. Hastaların yatış tarihi, taburculuk tarihi, yoğun bakıma sevk ve ölüm tarihleri, başvuru sırasındaki semptomları, başvuru anındaki klinik ve laboratuvar parametreleri kaydedilmiştir.

Bulgular: Çalışmaya 168'i (%48,4) kadın olmak üzere toplam 347 hasta dahil edildi. Hastaların yaş ortalaması 59,69±16,87 (14-97) iken, %40,9'u (n=142) 65 yaşın üzerindeydi. Hastaların %10,1'i (n=35) yoğun bakım ünitesine transfer edildi ve %8.4'ü (n=29) öldü. Başvuru anındaki klinik paremetreler değerlendirildiğinde, oksijen saturasyonu ölen grupta (79,51±6,95) sağ kalanlara göre daha düşüktü (88,78±6,11) (p<0,001). Erkek cinsiyet (p=0,05), ileri yaş (p<0,001), pozitif PCR sonucu (p=0,036), şiddetli toraks BT tutulumu (p<0,001) ve en az bir komorbidite varlığı (p=0,003) mortalite grubunda daha fazlaydı. Çok değişkenli analizlerde

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positive PCR result (p=0.036), congestive heart failure (p=0.044), severe COVID-19 involvement on thorax CT (p<0.001), and presence of at least one comorbidity (p=0.003) were observed at a higher rate in the mortality group. In the multivariate analyses, increased values of the NLR (HR: 1.04, 95% CI: 1.00-1.08), creatinine (OR: 1.37, 95% CI: 1.13-1.66), CRP (=-0.18, OR: 0.98, 95% CI: 0.97-0.99), GGT (OR: 1.006, 95% CI: 1.001-1.012), age (OR: 5.67, 95% CI: 2.24-14.38), male gender (OR: 2.38, 95% CI: 0.98-5.75), and presence of any comorbidity (OR: 5.23, 95% CI: 2.08-13.13) were associated with mortality.

Conclusions: Several clinical and laboratory parameters, such as advanced age, male gender, presence of any comorbidity, and NLR, GGT, CRP and creatinine levels at the time of admission can predict mortality in COVID-19 patients. These parameters obtained at the time of admission can contribute to the reduction of mortality through a closer clinical and laboratory follow-up in these patients.

Keywords: COVID-19, mortality, mortality risk factors, gamma-glutamyl transferase, neutrophil/lymphocyte ratio, SARS-CoV-2

artmış nötrofil/lenfosit oranı (HR: 1,04, %95 GA: 1,00-1,08), kreatinin (OR: 1,37, %95 CI: 1,13-1,66), CRP (=-0,18, OR: 0,98, 95) % GA: 0,97-0,99), GGT (OR: 1,006, %95 GA: 1,001-1,012), yaş (OR: 5,67, %95 GA: 2,24-14,38), erkek cinsiyet (OR: 2,38, %95 GA: 0,98 -5,75) ve komorbidite varlığı (OR: 5,23, %95 GA: 2,08-13,13) mortalite ile ilişkili bulundu.

Sonuç: COVID-19'da ileri yaş, erkek cinsiyet, komorbidite varlığı ve başvuru anındaki artmış NLR, GGT, CRP ve kreatinin değerleri gibi çeşitli klinik ve laboratuvar parametreler mortaliteyi öngörmede yardımcı olabilir. Başvuru anında elde edilen bu parametrelerle belirlenen hastaların daha yakın klinik ve laboratuvar takibi mortalitenin azaltılmasına katkı sağlayacaktır.

Anahtar Kelimeler: COVID-19, mortalite, mortalite risk faktörleri, gama-glutamil transferaz, nötrofil/lenfosit oranı, SARS-CoV-2

INTRODUCTION

In November 2019, a new coronavirus called "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) was identified in Wuhan, China (1, 2). Coronavirus disease 2019 (COVID-19) caused by the virus can lead to a wide clinical variability from mild diseases to acute respiratory failure and death (3). With the emergence of SARS-CoV-2 in Wuhan and its spread across the world, COVID-19 has become a global problem and resulted in the deaths of millions of people. Several risk factors, such as male gender, advanced age, and diabetes have been associated with high mortality in patients with COVID-19 in previous studies (4, 5). In addition, there have been some efforts to determine biomarkers that can indicate prognosis and mortality related to the disease. Laboratory parameters, such as IL-6, D-dimer, C-reactive protein (CRP), and absolute lymphocyte count are some of these biomarkers that have been found to have prognostic significance (4–6).

Neutrophil/lymphocyte ratio (NLR) can be easily calculated by dividing the absolute neutrophil count by the absolute lymphocyte count in routine blood tests and appears to be a useful biomarker because it is applicable in almost every laboratory. In a study by Liu et al., NLR was identified as an independent risk factor of mortality in patients followed up in hospital with a diagnosis of COVID-19, as well as other routine laboratory parameters (7).

In this study, we aimed to evaluate the prognostic importance of routine laboratory parameters and NLR in the prediction of severe disease and mortality in patients with COVID-19.

MATERIALS AND METHODS

Study design and participants

Patients over 18 years who presented to our hospital and received a probable or definitive diagnosis of COVID-19 between July 1, 2020 and October 1, 2020 were included in the study. Cases were defined according to the World Health Organization definitions (8). A positive result in the SARS-CoV-2 real-time reverse-transcription polymerase chain reaction (RT-PCR) test of a respiratory tract sample was defined as a definite diagnosis, while appearance consistent with viral pneumonia in thoracic computed tomography (CT) together with appropriate clinical findings was accepted as a probable case despite a negative SARS-CoV-2 RT-PCR test.

All the patients were treated with hydroxychloroquine and/or favipiravir. Dexamethasone 8 mg or equivalent was applied to patients with an oxygen saturation (SpO_2) of <90% at the time of admission and/or during follow-up. The patients who developed secondary bacterial infections were evaluated on daily rounds and treated with antibiotics, if necessary.

The study was approved by the local ethics committee of Agri Education and Research Hospital (Date: 11.12.2020, No: 29).

Data collection

Demographic data, accompanying diseases, laboratory and lung CT findings of the patients were retrospectively obtained from the hospital information system. For the patients who were transferred to other intensive care unit (ICU) centers, the mortality status was screened for using the National Death Notification System.

The patients' admission date, discharge date, ICU transfer/death date, contact history, smoking, symptoms during admission, vital markers at admission, complete blood count, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, lactate dehydrogenase (LDH), creatine kinase (CK), D-dimer, ferritin, troponin and CRP values, the highest values of ferritin and CRP and the lowest lymphocyte count during the follow-up, and lymphocyte count at discharge were recorded. Hydroxychloroquine, favipiravir and steroid use for the treatment of COVID-19 and antibiotic use for the treatment of secondary infections were also noted. In thorax CT, cases with ground glass densities of over three foci or greater than 3 cm diameter or the presence of consolidation were classified as moderate pneumonia, and those with the involvement of all lobes in both lungs and at least three lesions larger than 3 cm in diameter were classified as severe pneumonia (9).

Statistical analysis

All the data were analyzed using the Statistical Package for the Social Sciences (SPSS) software package for Windows (v 21.0; IBM, Armonk, NY, USA). Individual and aggregated data were summarized using descriptive statistics, including mean, standard deviation and median (min-max) values, frequency distributions and percentages. The normality of data distribution was verified with the Kolmogorov-Smirnov test. Comparison of the variables with a normal distribution was performed with Student's t-test. Variables which were not normally distributed were compared between the groups using the Mann Whitney U and Kruskal-Wallis tests. Evaluation of categorical variables was performed with the chi-square test. Correlation analysis was performed using the Pearson or Spearman test according to the normality of data distribution. p values of <0.05 were considered statistically significant.

RESULTS

Demographic features

The study included a total of 347 patients, of whom 168 (48.4%) were women. The mean age of the patients was 59.69±16.87 (14-97) years, while 40.9% (n=142) were over 65 years. Of the patients, 30.8% (n=107) had a history of

Table 1: Baseline clinical features of the patients hospitalized with COVID-19

	Clinical variables	Number (n)	Percent (%)
Gender	Male	179	51.5
	Female	168	49.5
Age (year)	<65	205	59
	≥65	142	41
PCR status	Negative	96	27.6
	Positive	251	72.3
ICU admission	No	312	90
	Yes	35	10
Tobacco use	Absent	307	89
	Present	30	11
HT	Absent	200	57.7
	Present	147	42.3
DM	Absent	258	74.4
	Present	89	25.6
COPD/asthma	Absent	281	81
	Present	66	19
Coronary artery disease	Absent	297	85.6
	Present	50	14.4
Malignancy	Absent	344	99.2
	Present	3	0.8
Acute renal failure	Absent	340	98
	Present	7	2
Hydroxychloroquine treatment	Absent	184	53.1
	Present	163	46.9%
Favipiravir treatment	Absent	100	28.8%
	Present	247	71.2%

PCR: Polymerase chain reaction, ICU: Intensive care unit, HT: Hypertension, DM: Diabetes mellitus, COPD: Chronic obstructive pulmonary disease

contact with a positive COVID-19 case and 72% (n=250) had a positive COVID-19 RT-PCR test. The most common complaint was fatigue/myalgia at a rate of 71.2% (n=247), followed by cough (59.4%), shortness of breath (53.6%), fever (36.0%), and headache (18.4%). When the risk factors of the patients were evaluated, the presence of at least one comorbidity was observed in 13.8% (n=48) of the patients. The baseline clinical and laboratory parameters of the patients are summarized in Table 1.

Overall, 10.1% (n=35) of the patients required transfer to ICU and 8.4% (n=29) died. When the clinical parameters were evaluated at the time of admission, oxygen saturation was found to be lower in the mortality group (79.51±6.95) compared to the survivors (88.78±6.11) (p<0.001). In the univariate analysis, male gender (p=0.054), advanced age (p<0.001), a positive PCR result (p=0.036), congestive heart failure (p=0.044), severe COVID-19 involvement in thorax CT (p<0.001), and presence of at least one comorbidity (p=0.003) were observed at a significantly higher rate among the patients that developed mortality. The comparison of the clinical and laboratory parameters of the patients is presented in Table 2.

Laboratory parameters at the time of admission

There was a statistically significant increase in the leukocyte, neutrophil and NLR values among the patients who were deceased (p=0.03, 0.03, and 0.001, respectively). Furthermore, the serum urea (p<0.001), creatinine (p<0.001), LDH (p<0.001), AST (p<0.001), ALT (p=0.02), GGT (p=0.046), D-dimer (p=0.002) and troponin (p<0.001) values were significantly higher in the mortality group compared to the survivors. Additionally, the mortality group had increased CRP (104.2 vs 57.6 mg/L; p=0.001) and ferritin (589.5 vs 375.0 ng/mL) levels but lower lymphocyte (0.95 vs 1.4x109/L) values (p=0.001, <0.001, and =0.001, respectively) (Table 2).

In the correlation analysis, NLR was slightly positively correlated with age (r=0.173, p=0.001) and CRP (r=0.106, p=0.049) and moderately negatively correlated with SpO_2 (r=-0.437, p<0.001). A mild negative correlation was observed between CRP and SpO_2 (r=-0.152, p=0.005) and a moderate positive correlation between CRP and GGT (r=0.325, p<0.001). There was no correlation between the remaining clinical and laboratory parameters.

Evaluation of risk factors for mortality

In the multivariate analyses (forward logistic regression method), increased NLR [odds ratio (OR): 1.034, 95% confidence interval (CI): 1.003-1.066], advanced patient age (OR: 1.097, 95% CI: 1.054-1.14), presence of any comorbidity (OR: 12.74, 95% CI: 3.36-48.3), male gender (OR: 3.48, 95% CI: 1.28-9.47), and high creatinine (OR: 1.37, 95% CI: 1.13-1.66), CRP (=0.018, OR: 1.018, 95% CI: 1.005-1.032) and GGT (OR: 1.006, 95% CI: 1.001-1.012) levels were associated with mortality (Table 3). Although the serum ferritin, BUN, ALT, AST, D-dimer and troponin levels were higher in the mortality group according to the univariate analysis, none of these parameters were associated with mortality in the multivariate analysis.

DISCUSSION

In this study, the rates of ICU admission (10.1%) and mortality (8.4%) were found to be lower compared to previous studies. In a study by Liu et al. including 245 patients, the mortality rate was reported to be 13.4% (7), and in a multicenter study with 1,859 patients, it was determined as 11.1% (10).

Several risk factors associated with a severe disease course and high mortality have been previously described in patients with COVID-19. In a multicenter retrospective study, several parameters such as age, number of comorbidities, cancer history, shortness of breath, change of consciousness, radiological involvement, and elevated laboratory parameters (NLR, LDH, and direct bilirubin) were included in risk scoring to evaluate the risk of progression to critical illness in patients hospitalized with COVID-19. It was stated that this scoring, performed at the time of hospital admission, could predict progression to critical illness (11). In a study conducted in China, advanced age, male gender, and hypertension were found to be associated with mortality (12). Similarly, in another study with 3,988 patients, advanced age, male gender, and the presence of chronic obstructive pulmonary disease and diabetes mellitus as comorbidities were identified as risk factors for higher mortality (13). Similarly, in the current study, older age, presence of at least one comorbidity, and male gender were associated with higher mortality.

When the laboratory parameters were evaluated, NLR, serum CRP and ferritin levels were higher among the mortality group than the survivors. NLR and increased inflammatory markers and their association with poor prognosis have also been defined in many diseases, such as liver cirrhosis and cerebrovascular events and malignancy (14-18). Additionally, it has been reported that NLR can be used as a supporting finding for the pneumonia severity score in patients followed up with a diagnosis of pneumonia in terms of infectious diseases (19). Neutrophils, along with lymphocytes, play an important role in the response to viral infections (20). Patients diagnosed with COVID-19 who have a high neutrophil count at the time of admission have an up to eight-fold higher risk of progression to severe disease (21). More than one mechanism contributes to the occurrence of lymphopenia in COVID-19. SARS-CoV-2 can cleave lymphocytes by attaching to angiotensin-converting enzyme (ACE) receptors expressed by lymphocytes. The cytokine storm that occurs with the increase in inflammatory mediators can trigger lymphocyte apoptosis. In addition, cytokine acti-

Table 2: Comparison of the clinical and laboratory parameters between the patients with and without mortality

Clinical and laboratory findings (mean±SD)	Total n=347	Non-mortality group n=318 (91.6%)	Mortality group n=29 (8.4%)	p-value
Body temperature (°C)	36.83±0.73	36.83±0.74	36.84±0.65	0.666
Oxygen saturation (SpO_2)	88.00±6.69	88.78±6.11	79.51±6.95	**<0.001**
Systolic BP (mmHg)	119.08±14.89	118.87±14.67	122.10 ±17.08	0.233
Diastolic BP (mmHg)	72.76±10.58	72.76±10.56	72.75±10.98	0.585
Hemoglobin (g/L)	13.69±0.87	13.70±1.91	13.52±1.46	0.279
Platelet (x10^9/L)	193.86±80.33	196.06±82.05	170.40±54.01	0.136*
WBC count (x10^6/L)	7.40±8.97	7.25± 9.24	9.05±4.89	**0.032***
Neutrophil count (x10^9/L)	5.44±0.54	5.27±5.45	7.34±4.87	**0.030***
Lymphocyte count (x10^9/L)	1.36±0.765	1.40±0.78	0.95±0.46	**0.001***
Neutrophil/lymphocyte ratio	5.72±8.78	5.23±8.40	11.03±11.06	**<0.001***
BUN (mg/dL)	46.26±34.46	43.34±31.40	78.27±48.56	**<0.001***
Creatinine (mg/dL)	1.18±1.03	1.13±1.01	1.66±1.06	**<0.001***
Glucose (mg/dL)	135.21±62.96	133.41±59.59	154.53±92.24	0.123*
AST (U/L)	38.29±33.60	33.89±17.36	86.59±88.92	**<0.001***
ALT (U/L)	30.21±28.49	27.94±18.75	55.07±73.14	**0.023***
GGT (IU/L)	49.83±62.28	47.37±53.88	76.72±119.00	**0.046***
LDH (mg/dL)	313.12±146.00	299.26±123.10	465.22±255.80	**<0.001***
CRP (mg/L)	61.53±85.12	57.63±87.29	104.20±34.79	**<0.001***
Ferritin (ng/mL)	329.60±335.80	305.82±317.90	589.52±415.50	**<0.001***
D-dimer (µg/mL)	0.70±1.16	0.70±1.21	0.76±0.64	**0.021***
Troponin (ng/l)	32.54±333.7	9.36±30.17	285.82±1135.40	**<0.001***
	Clinical variables	**Patients alive n (%)**	**Patients exitus n (%)**	**p-value (Odds ratio)**
Gender (n, %)	Male Female	159 (50.0%) 159 (50.0%)	20 (69.0%) 9 (31.0%)	**0.054**
Age (years) (mean±SD)	59.7±16.9	58.34±16.4	74.5±14.4	**<0.001***
Age (n, %)	<65 year ≥65 year	198 (62.3%) 120 (37.7%)	7 (24.1%) 22 (75.9%)	**<0.001 (15.9)**
PCR (n, %)	Negative Positive	93 (29.2%) 225 (70.8%)	3 (10.7%) 25 (89.3%)	**0.03 (4.74)**
Intensive care unit (n, %)	No Yes	302 (95.0%) 16 (5.0%)	10 (34.5%) 19 (65.5%)	**<0.001**
Tobacco use (ever) (n, %)	Absent Present	280 (88.1%) 36 (11.3%)	27 (93.1%) 2 (6.9%)	0.617
Hypertension (n, %)	Absent Present	186 (58.5%) 132 (41.5%)	14 (48.3%) 15 (51.7%)	0.287
Diabetes mellitus (n, %)	Absent Present	236 (74.2%) 82 (25.8%)	22 (75.9%) 7 (24.1%)	0.846
COPD/asthma (n, %)	Absent Present	260 (81.8%) 58 (18.2%)	21 (72.4%) 8 (27.6%)	0.220
Coronary artery disease (n, %)	Absent Present	274 (86.2%) 44 (13.8%)	23 (79.3%) 6 (20.7%)	0.225
Malignancy (n, %)	Absent Present	315 (99.1%) 3 (0.9%)	29 (100.0%) 0 (0.0%)	0.769
CT findings (n, %)	Moderate involvement Severe involvement	229 (73.4%) 83 (26.6%)	9 (31.0%) 20 (69.0%)	**<0.001**
Any comorbidity (n, %)	Absent Present	280 (88.1%) 38 (11.9%)	19 (65.5%) 10 (34.5%)	**0.003 (11.3)**

BP: Blood pressure, WBC: White blood cell, BUN: Blood urea nitrogen, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transferase, LDH: Lactate dehydrogenase, CRP: C-reactive protein, PCR: Polymerase chain reaction, COPD: Chronic obstructive pulmonary disease, CT: Computer tomography, SD: Standard deviation, p<0.05 statistically significant,
*: Mann-Whitney U test=Mortality-Non-mortality

Table 3: Multivariate analysis of mortality-associated factors with the forward logistic regression method

Variables	Bj	OR	95% CI	p-value
Neutrophil/lymphocyte ratio (NLR)	0.033	1.034	**1.003-1.066**	***0.03****
Age (years)	0.093	1.097	**1.054-1.14**	***<0.001****
Gender (male)	1.25	3.48	**1.28-9.47**	***0.015****
Any comorbidity	2.55	12.74	**3.36-48.3**	***<0.001****
Creatinine (mg/dL)	0.314	1.37	**1.13-1.66**	***0.001****
CRP (mg/L)	0.018	1.018	**1.005-1.032**	***0.008****
GGT (IU/L)	0.006	1.006	**1.001-1.012**	***0.022****
Constant	-10.211	0.00		***<0.001****

*: statistically significant at p<0.05. OR: Odds ratio, CI: Confidence interval, CRP: C-reactive protein, GGT: Gamma-glutamyl transferase, Bj: Regression coefficient

vation impairs lymphocyte proliferation and turnover by affecting lymphoid organs (22). In a previous study, it was shown that a decrease in the CD8+ T lymphocyte count and interleukin (IL)-6 was a good prognostic marker of mortality in patients with COVID-19 (23). On the other hand, the functional capacity of lymphocytes decreases with the decrease in the number of lymphocytes, especially in COVID-19 cases with a severe clinical course (24). It has been observed that COVID-19 progresses more severely in patients with a high NLR value (11, 20, 25). In a study by Liu et al. evaluating 245 patients, NLR was found to be an independent risk factor associated with high mortality, especially among men (7). In another observational study with 1,859 COVID-19 patients, mortality was found to have a significant correlation with increased NLR, low platelet count and high creatine and D-dimer values (10). We also observed a significant association between NLR and mortality in our study.

In this study, although several clinical and laboratory parameters, such as severe thorax CT findings, leucocyte and neutrophile counts, and ferritin, CRP, creatinine, ALT, AST, GGT, LDH and troponin levels were higher, lymphocyte levels were lower among the mortality group in the univariate analysis, only advanced age, presence of any comorbidity, and higher NLR, CRP, creatinine and GGT values were determined to be associated with high mortality in the multivariable analysis. This is consistent with previous studies reporting an association between high mortality in COVID-19 and higher ferritin, CRP and D-dimer levels reflecting a greater inflammatory response (26, 27). Additionally, we determined that the correlation between low SpO_2 with CRP and NLR was also compatible with the association between these two parameters and high mortality in our study.

Liver test abnormalities are more frequently observed in severe cases of COVID-19. Liver injury and liver enzyme abnormalities in COVID-19 may be multifactorial and result from the direct pathogenic effects of the virus, adverse drug reactions, higher systemic immune response (cytokine storm), and hypoxia in these patients (28). Elevation of the GGT level and the organ infiltration of IL-6-producing cells are the defining characteristics for patients with the severe COVID-19 (29, 30). In a study by Zhang et al., CRP and NLR were correlated with high GGT levels (29). In our study, CRP but not NLR was correlated with GGT, which is consistent with the above-mentioned study. Higher GGT levels at admission, as well as other liver enzymes have been associated with mortality and ICU admission among COVID-19 patients (31). Furthermore, GGT has been found to be a useful biomarker when combined with other laboratory parameters in COVID-19 (32). The biliary epithelium expresses the ACE-2 receptor, which is the known as the binding site of SARS-CoV-2, while the expression in hepatocytes is possibly much lower, which is consistent with increased GGT rather than transaminase levels among the patients with COVID-19 in our study (33). In addition to GGT elevation, an increased creatinine level has also been associated with poor prognosis and may be an independent risk factor of in-hospital death in patients with COVID-19 (34). Furthermore, higher BUN and/or creatinine levels and NLR are independent predictors of severe disease and higher mortality in patients with COVID-19, which is also in agreement with our findings (35). Liver and kidney dysfunction reflecting multiorgan involvement in COVID-19, possibly due to the direct effect of the SARS-CoV-2 virus or cytokine storm can contribute to the higher mortality associated with the disease.

In conclusion, in COVID-19, at risk patients can be identified in the early period with several simple clinical and laboratory parameters. This evaluation, performed at the time of admission, can contribute to the reduction of mortality through a closer clinical and laboratory follow-up. The hospital mortality rate can be reduced by determining high-risk patients at admission, which would

also facilitate early appropriate treatment in these patients. Controlled prospective studies with larger patient populations are needed to confirm the prediction of mortality in patients with COVID-19.

Limitations of our study

Important limitations of our study are its retrospective observational design and the absence of a control group. The patient group included in our study population may not reflect all COVID-19 patients due to the hospitalization of more severe cases, and this may have caused a bias.

Ethics Committee Approval: This study was approved by the Ethical Committee of the Agri Education and Research Hospital (Date: 11.12.2020, No:29)

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PERSONAL SENSITIVITY, SELF-MEDICATION, MASK USAGE, AND COVID-19 SYMPTOMS IN SUB-SAHARAN AFRICANS

SAHRA ALTI AFRİKALILAR ARASINDA KİŞİSEL DUYARLILIK, KENDİ KENDİNE İLAÇ TEDAVİSİ, MASKE KULLANIMI VE COVID-19 BELİRTİLERİ

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ABSTRACT

Objective: The study objective was to explore the episode of COVID-19 symptoms among sub-Saharan African (SSA) by examining the predicting effect of mask usage, self-medication, and personal sensitivity on the symptoms.

Materials and Methods: In a cross-sectional study in the SSA population, 536 individuals were asked about the episode of COVID-19 symptoms, personal sensitivity, mask usage, and self-medication. "Hierarchical multiple linear regression statistical method" was used to evaluate the data.

Results: The personal sensitivity (r=0.245<0.01), taking off face mask in enclosed public places (r=0.255<0.01) and self-medication (r=0.392<0.01) were positively associated with COVID-19 symptoms. Overall, the total predictive effect of self-medication, taking off the mask in public spaces, and personal sensitivity accounted for 21% of the variance in the episode of COVID-19 symptoms of the study population.

Conclusion: Personal sensitivity, mask usage, and self-medication support understanding of the episode of COVID-19 symptoms experienced among the study population. It is important to encourage the use of masks in high-risk areas. To improve post-COVID-19 health policies, self-medication used to decrease the risk of COVID-19 infection and other related public health concerns should be reduced.

Keywords: Self-medication, mask usage, personal sensitivity, COVID-19, Sub-Saharan Africans

ÖZET

Amaç: Çalışmanın amacı, Sahra Altı Afrikalı (SAA) bireylerde kişisel koruyucu ekipman kullanımının ve kendi kendine ilaç tedavisinin, COVID-19 semptomlarının ortaya çıkması üzerine olan etkisini araştırmaktır.

Gereç ve Yöntem: SAA popülasyonunda 536 kişi üzerinde yapılan kesitsel bir ankette COVID-19 semptomları, kişisel duyarlılık, maske kullanımı ve halk arasında benimsenen kendi kendine tedavi yöntemleri değerlendirilerek kaydedildi. İstatistiksel metod olarak "Hiyerarşik çoklu doğrusal regresyon modelleme" kullanıldı.

Bulgular: Kişisel duyarlılık (r=0,245<0,01), halka açık kapalı alanlarda yüz maskesinin çıkarılması (r=0,255<0,01) ve kendi kendine ilaç tedavisi (r=0,392<0,01) COVID-19 semptomları ile pozitif olarak ilişkiliydi. Genel olarak, kendi kendine ilaç tedavisinin, kamusal alanlarda maskeyi çıkarmanın ve kişisel duyarlılığın toplam öngörücü etkisi, çalışma popülasyonunun COVID-19 semptomları epizodundaki varyansın %21'ini oluşturdu.

Sonuç: Kişisel duyarlılık, maske kullanımı ve kendi kendine ilaç tedavisi, çalışma popülasyonunda yaşanan COVID-19 semptomlarının epizodunun anlaşılmasını destekler. Yüksek riskli alanlarda maske kullanımının teşvik edilmesi önemlidir. COVID-19 sonrası sağlık politikalarının iyileştirilmesi için COVID-19 enfeksiyonu riskini ve diğer ilgili halk sağlığı endişelerini azaltmak için kullanılan kendi kendine ilaç tedavisi azaltılmalıdır.

Anahtar Kelimeler: Kendi kendine tedavi, maske kullanımı, bağışıklık, COVID-19, Sahra Altı Afrikalılar

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INTRODUCTION

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the virus responsible for the COVID-19 pandemic, has caused global disruption due to the diseases' spread rate and mortality (1). While global vaccine implementation is underway, there is still concern about Africa's vulnerability to COVID-19 infections due to the weak healthcare systems, insufficient checking and tracing systems, overstretched healthcare facilities, and limited resources (2-5).

Self-medication causes public health concern focusing on the safety of self-administration of drugs, causes antibiotic resistance, and may lead to death (6-9). Meanwhile, mask usage has become a symbol of social responsibility, and it is an essential tool for personal protection against COVID-19 (10-14). Sensitivity is a concept of awareness of risk (15). Considerable evidence has supported the mediating role of sensitivity to the risk of contracting infectious diseases (16-18). While the concept of personal sensitivity to disease remains relevant and has a psychomedical implication, more evidence is needed to establish the influence of COVID-19 infection among vulnerable populations. These indicators may help to understand COVID-19 symptom experience among the study group. Following the World Health Organization (WHO), the apparent incidences of COVID-19 symptoms are dry cough, fever, and tiredness, among others (19). Hence, mask usage (as one of the crucial protection measures), self-medication (that may distort medical action, early detection of COVID-19 and intervention), and personal sensitivity to understand health decisions and risk.

The current study examined the association of personal sensitivity, mask usage, and self-medication on COVID-19 symptoms among a group of SSAs. The study also examined the predictive effects of self-medication, mask usage, and personal sensitivity on the episode of COVID-19 symptoms. The evidence presented supports understanding the three vital domains' influence on unreported COVID-19 symptoms experienced in the study population.

MATERIALS AND METHODS

Study design, population, and data collection

Data from a sample of 536 sub-Saharan Africans were analyzed. A cross-sectional study design was adopted to extract information on the usage of masks and the personal sensitivity to COVID-19 from sub-Saharan Africans in Nigeria, Cameroun, China, South Africa, and others living in Diaspora between 18-24 August 2020. All participants were between 19 years or older (60≥) with access to internet facilities and social media platforms. Data collection was carried out over seven days. A simple snowballing method was used through referral via Facebook, WhatsApp, Instagram, and We Chat (for SSA migrant residents in China).

Episodes of COVID-19 symptoms

The episodes of COVID-19 symptoms were measured using the WHO indicators (22). We solicited information on COVID-19 symptoms experienced by respondents in the last 60 days before the survey. Twelve counts of COVID-19 symptoms were asked in dichotomous (yes/no) questions. Upon collection of data, a reliability test was estimated to establish the reliability of the collected data. The episodes of COVID-19 symptoms reliability test had a Cronbach's coefficient alpha$^2\alpha$ of 0.824 with an analysis of variance significant at 0.001.

Personal sensitivity

The personal sensitivity questionnaire was adopted from existing studies on vulnerability (20, 21). The six items to evaluate participants' sensitivity and the questions were adjusted to fit one's chances of infection and exposure to COVID-19. The six items were ranked with a 5-point Likert type scale ranging from strongly disagree to strongly agree, with a question like "There is a great chance that I will be exposed to chronic disease (19)." The reliability test result of Cronbach's coefficient alpha^2=0.838 with an analysis of variance significant at 0.001.

Control variables

Data on respondents' age, marital status, education attainment were collected. The respondents' ages were grouped into six categories as ≤19, 20-29, 30-39, 40-49, 50-59, 60≥ in the questionnaire. The employment status was a dichotomous question (No=1, Yes=2), while marital status was categorized into five categories, i.e., Single, in partnership but not married, married, divorced or separated, widowed. Information on health-seeking options in the 60 days preceding the survey was asked in three dichotomous questions such as: "visit a hospital", "used prescription drugs", "self-medicated". The use of masks was measured with four basic questions of SSA's daily habit on mask usage in the following form: use of face mask every time, the importance of face mask in the pandemic, mask use in enclosed areas, and perception of the effectiveness of face masks.

Data analysis

Data collected from the questionnaire was extracted into Microsoft Excel and imported into SPSS version 25 for analysis. Descriptive analysis, including mean and standard deviation, was used to measure respondents' demographics and socioeconomic characteristics. To establish the association between variables, we employed correlation matrix analysis to establish an association between demographic/socioeconomic characteristics and mask usage. Similarly, a correlation matrix was used to measure the association between demographic/socioeconomic characteristics of the study population and other research variables. The interpretation of correlation coefficients were small (r=0.10), medium (r=0.30) or large (r=0.50) (22). Hierarchical multiple

regression analysis was used to evaluate the predictive effect of self-medication on COVID-19 symptoms as Model 1. Two additional variables completed the regression analysis, such as the added effects of taking off masks (Model 2) and personal sensitivity (Model 3).

RESULTS

Demographic/socioeconomic characteristics of SSAs study population

The descriptive statistic as shown in Table 1 shows the frequency result for gender as female (55%) and male (45%) with a mean=1.45 (SD=0.49). The distribution of mean and standard deviation of respondents are as follows; ages (2.42±0.85), marital status (1.7±0.96), educational attainment (4.4±0.62), and employment status (1.55±0.50). The details of respondents' demographic and socioeconomic characteristics are presented in Table 1.

The data from Table 2 shows that at least two out of ten (21.5%) of every SSA enumerated visited the hospital in the last 60 days before the survey. Meanwhile, 27.8% had used prescription drugs 60 days before the study, while almost four out of ten had self-medicated. Based on mask usage, at least one in four SSA study populations does not use a face mask every time. However, an insignificant proportion (4.1%) believed that a face mask is not essential during a global pandemic. Overall, about 38.6% reported taking off face masks in public areas such as restaurants, supermarkets, car parks, and religious places, and about 17% concluded that face masks are ineffective.

A comparative association test was estimated for the country of residency and taking off masks in enclosed public areas. Predominantly, a significant number of SSAs who reside in Nigeria (57.1%) take off masks in enclosed public spaces. Similarly, about 15.5% of Cameroon respondents take off masks in enclosed public areas. Therefore, there was an association between the SSA under investigation and taking off of masks in enclosed public areas (X^2=35.622, p<0.001).

Table 1: Demographic/socioeconomics characteristics of Sub-Saharan Africans (n=536)

Variables	%	Mean	SD
Gender		1.45	0.49
Female	55.0		
Male	45.0		
Age		2.42	0.85
≤19	5.6		
20-29	49.1		
30-39	37.1		
40-49	5.4		
50-59	1.5		
60≥	1.3		
Marital status		1.77	0.96
Single	56.5		
In partnership	12.5		
Married	29.1		
Separated/divorced	1.1		
Widowed	0.7		
Education attainment		4.41	0.62
Secondary	7.1		
Tertiary	44.8		
Post graduate	48.1		
Employment status		1.55	0.50
No	44.6		
Yes	55.4		

Medical-care seeking and mask usage among Sub-Saharan Africans (n=536)

Table 2: Medical-care seeking and mask usage among SSAs surveyed (n=536)

Variables	%	Mean	SD
Hospital visit in the last 60 days		1.21	0.411
No	78.5		
Yes	21.5		
Use prescription medication in the last 60 days		1.28	0.448
No	72.2		
Yes	27.8		
Self-medicated in the last 60 days		1.39	0.488
No	61.2		
Yes	38.8		
Use of face mask every time		1.74	0.442
No	26.5		
Yes	73.5		
A face mask is important during a pandemic		1.96	0.199
No	4.1		
Yes	95.9		
Take off the mask in an enclosed public area		1.39	0.487
No	61.4		
Yes	38.6		
A face mask is ineffective		1.18	0.384
No	82.1		
Yes	17.9		

Descriptive analysis by the residency and taking off of masks in enclosed Public Areas of SSAs surveyed (n=536)

Table 3: Cross tabulation by country of residency and taking off of masks in enclosed public areas of Sub-Saharan Africans surveyed (n=536)

Country of residency	Yes (n, %)	No (n, %)	X^2=35.622$^{a/df=6}$
Nigeria	118 (57.1%)	98 (47.3%)	p<0.001
Cameroon	51 (15.5%)	73 (35.3%)	
China	22 (6.7%)	13 (6.3%)	
South-Africa	11 (3.3%)	2 (1%)	
Ghana	10 (3.0%)	1 (0.5%)	
Canada	14 (4.3%)	2 (1%)	
Others	33 (10.3%)	18 (8.7%)	

Bivariate analysis: Correlation matrix

The Pearson correlation coefficient matrix evaluated the association between COVID-19 symptoms, personal sensitivity, face mask usage, and demographic/socioeconomic characteristics of the SSA population surveyed. From Table 4, the personal sensitivity was positively correlated with COVID-19 symptoms (r=0.245<0.01). Using a face mask correlates inversely with COVID-19 symptoms (r=-0.122<0.01). The importance of face masks is positively correlated with COVID-19 symptoms (r=0.096<0.05). Taking off the face mask in enclosed public places was positively associated with COVID-19 symptoms (r=0.255<0.01). The hospital visitation was associated with COVID-19 symptoms (r=0.144<0.01). Similarly, prescription medication was correlated with COVID-19 symptoms (r=0.272<0.01). Lastly, the use of self-medication was moderately correlated with COVID-19 symptoms (r=0.392<0.01).

Education attainment was positively correlated with personal sensitivity (r=0.086<0.05). Use of prescription medication shows positive correlation with personal sensitivity (r=0.088<0.05). The use of self-medication was positively correlated to personal sensitivity (r=0.196<0.01). Gender was negatively correlated with taking off the face mask in enclosed public places (r=-0.108<0.05). Similarly, taking off the face mask in enclosed public places was negatively correlated with the marital status of SSA (r=-0.090<0.05). The age of SSA was negatively correlated with taking off

Table 4: Hierarchical multiple linear regression models assessing the association between COVID-19 symptoms, self-medication, taking off face masks in public, and personal sensitivity to COVID-19

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
COVID-19 symptoms	1													
Personal sensitivity	0.245**	1												
Gender	-0.074	-0.027	1											
Age	-0.022	0.074	0.220**	1										
Marital status	0.023	0.080	0.060	0.530**	1									
Education attainment	-0.031	0.086*	0.158**	0.354**	0.241**	1								
Employment status	-0.012	0.004	0.147**	0.361**	0.358**	0.176**	1							
Frequent face-mask use	-0.122**	-0.008	0.007	0.020	0.055	-0.019	-0.020	1						
The face mask is important	0.096*	-0.015	-0.021	0.049	-0.050	-0.060	-0.053	0.195**	1					
I take off my face mask	0.255**	0.046	-0.108*	-0.185**	-0.090*	-0.062	-0.090*	-0.297**	-0.126**	1				
The face mask is ineffective	0.052	-0.005	-0.011	-0.017	-0.046	0.036	-0.012	-0.105*	-0.320**	0.199**	1			
Hospital visit	0.144**	0.077	0.039	0.038	0.064	0.035	0.039	0.046	0.039	-0.013	0.040	1		
Prescription medication	0.272**	0.088*	-0.033	0.046	0.044	-0.001	0.054	0.052	0.086*	-0.022	-0.051	0.508**	1	
Self-medication	0.392**	0.196**	-0.004	0.035	-0.021	0.029	0.006	-0.121**	-0.009	0.178**	0.037	0.013	0.155**	1

Significant: *: p<0.05, **: p<0.01

the mask in enclosed public areas (r=-0.185<0.01). The employment status was inversely correlated with taking off the mask in an enclosed public area (r=-0.090<0.05).

The "face mask is important" shows a moderate positive correlation with frequent usage of face mask (r=0.195<0.01), while frequent use of face masks show a negative correlation with taking off face mask in enclosed public places (r=-0.297<0.01). Meanwhile, "the face mask is ineffective" returned a negative correlation with the use of a face mask every time (r=-0.105<0.05). The use of self-medication was negatively correlated with the consistent use of face masks (r=-0.121<0.01. Taking off the mask in enclosed public places was negatively correlated with face mask is important (r=-0.126<0.01), and "face mask is ineffective" is negatively correlated with taking off the mask in public enclosed places (r=-0.320<0.01). The use of prescription medication was positively correlated with face mask is important (r=0.086<0.05). Face masks ineffective were positively associated with taking off masks in enclosed public places (r=0.199<0.01); meanwhile, the use of self-medication was positively correlated with taking off face masks in enclosed public places (r=0.178<0.01). Hospital visit was associated with the use of prescription medication (r=0.508<0.01)

Regression model

The multiple linear regressions were calculated to predict an episode of COVID-19 symptoms based on self-medication, taking off face masks in public, and personal sensitivity to COVID-19 infection. As presented in Table 5, the results show that the use of self-medication was significantly associated (Adjusted R^2=0.15) with an episode of COVID-19 symptoms. A different model (Model 2) exploring the predictive effect of taking off the mask in an enclosed public environment (ß=0.35, p<0.001) shows a significant association (Change in R^2=0.035) to the model predicting the episode of COVID-19 symptoms (Adjusted R^2=0.19). The third model (Model 3) of personal sensitivity to COVID-19 shows a significant additional effect to the model predicting the episode of COVID-19 symptoms among the study population (Adjusted R^2=0.21). Overall, the regression model highlighted that an estimation of 21% of the variance in the episode of COVID-19 symptoms is accounted for by self-medication, taking off face masks, and personal sensitivity.

DISCUSSION

The current study explored the effect of self-medication, mask usage, and personal sensitivity on the COVID-19 symptoms among surveyed SSAs. The evidence presented in this study supports the growing concern on the risk of self-medication, mask usage, and personal sensitivity as a determinant of risky health behaviors and adverse disease experiences globally. However, evidence has independently assessed risks, mask usages, and self-medication in COVID-19 infection exposure (23-25). However, this study evaluated the three domains to aggregate for their influence on COVID-19 symptoms among the study population.

The finding from the analysis shows that there is a direct association between personal sensitivity and COVID-19 symptoms. Increased personal sensitivity towards being infected with COVID-19 results in increased symptoms,

Table 5: Hierarchical multiple linear regression models assessing the association between COVID-19 symptoms, self-medication, taking off face masks in public, and personal sensitivity to COVID-19

	Model 1			Model 2			Model 3		
	B	**β**	**p**	**B**	**β**	**p**	**B**	**β**	**p**
Constant	11.386		<0.001	10.206		<0.001	9.356		<0.001
Self-medication	2.123	0.392	<0.001	1.938	0.358	<0.001	1.757	0.324	<0.001
Mask off in public				1.036	0.191	<0.001	1.025	0.189	<0.001
Personal sensitivity							0.100	0.173	<0.001
Model fit Indices									
Adjusted R^2=	0.15			0.19			0.21		
ΔF(df1,df2), p-value:	**ΔF(1,530)= 96.118,** p<0.001			**ΔF(1,529)= 30.084,** p<0.001			**ΔF(1,528)= 17.287,** p<0.001		
*Change in R^2	**0.154**			**0.035**			**0.029**		
* Change in F, p-value	**96.118, p<0.001**			**23.005,** p<0.001			19.420, p<0.001		

Dependent variable: Episode of COVID-19 Symptoms
Model 1. Predictors: Constant, Self-Medication
Model 2. Predictors: Constant, Self-Medication, Taking Off Mask in Public
Model 3. Predictors: Constant, Self-Medication, Taking Off Mask in Public, Personal sensitivity

supporting other studies that perceived risk aggravates fear, stress, and worry about getting infected with COVID-19 (26). The more adherence to face masks used, the lesser the episode of COVID-19 symptoms reported, which agrees with the evidence that mask use reduces the risk of COVID-19 disease (12). However, with the non-use of masks in enclosed public places, there is a relative increase in COVID-19 symptoms experienced. The results also suggested self-medicating was associated with the COVID-19 symptoms experienced. Like other studies on risk to diseases, the increasing level of education of SSAs suggested a relative increase in the sensitivity to COVID-19 exposure (27, 28).

Self-medication practice among the study population increased COVID-19 symptoms. While taking off masks in enclosed public spaces was added to self-medication in Model 2, there is evidence of increased COVID-19 symptoms. Overall, the predictive effect of self-medication, taking off masks, and personal sensitivity explains a significant proportion of reported COVID-19 symptoms of SSAs under investigation. However, a substantial proportion of the COVID-19 symptoms reported by the study population was demonstrated by self-medication based on the result from the first model. Although there were added effects of improper mask usage and personal sensitivity on the personal sensitivity and COVID-19 symptoms, they added 3.5% and 2.9%, respectively, to the variance in the symptoms experienced.

Thus, given the negating influence of self-medication, mask usage, and personal sensitivity on the COVID-19 symptoms evidenced in this study, it becomes vital to curb self-medication, inadequate mask practice, and personal sensitivity beyond the current pandemic. The evidence on adverse outcomes from self-medication is quite broad to ignore the consequences. Research has likened self-medication to individual playing doctors and, in turn, is linked to substance abuse and poor medical literacy, which significantly endangered physical and mental health (29, 30). Before the pandemic in Europe, self-medication practice was associated with longstanding illness among the elderly and the severity of diseases (31, 32). With the emergence of the pandemic, the self-medication practice also underlines the problem in the healthcare systems for the safe management of diseases and substance/drug abuses. Self-medication can increase unreported COVID-19 cases, aggravate infections due to no testing and reporting of symptoms to medical doctors, and reduce the effect of medical intervention.

In psychomedical realities, sensitivity requires extensive research and attention for the groups liable to be victims during outbreaks of infectious diseases. The mental health vulnerabilities evidenced in the COVID-19 pandemic is enormous (33). While the evidence in this study as pointed to personal sensitivity to getting infected with COVID-19 increases and predicts the symptoms, a proactive measure should therefore be adopted to reduce mental health vulnerabilities transcending to reduce belief of associated risks of getting infected.

Research strength and limitations

While mask usage, personal sensitivity, and self-medication have been explored in the context of the COVID-19 pandemic independently, no empirical evidence has adopted these three domains together conceptualized around COVID-19. Thus, the empirical evidence presented in this study presents a template for future research and potential intervention for the post-COVID-19 pandemic and guidance for intervention in any future pandemic. Policy and intervention for monitoring access to the over-the-counter pharmaceutical drug become necessary moving forward post-COVID-19 pandemic implementation. Strict global control of prescription medication and drugs will yield the desired result if policies are implemented at the community level to curtail self-medication. Mental health policies should be put in place post-COVID-19 pandemic to reduce the mental health effect of declaring a disease outbreak that may reduce the personal sensitivity to contracting the disease.

Numerous limitations hamper the generalization of our research findings, in particular, the small sample size of SSAs explored in making inferences about the episode of COVID-19 symptoms, self-medication, mask usages, and personal sensitivity. Given the coverage of the SSA participants, several countries are omitted from this research; the result should be interpreted accordingly. Based on findings and given the association and predictability of the variables investigated, it suffices that extensive study should be carried out by adopting a larger sample size.

CONCLUSION

Evidence of self-medication, poor mask usage, and personal sensitivity was reported in the survey as associated with increased symptoms in the study population. It further suggests the minimal hospital visit during the pandemic and risky health practices. These results call for continuous effort in ensuring good health practices among the global population until there is evidence of extermination of COVID-19. Steps towards the reduction of self-medication should be targeted to reduce the associated risks. More importantly, combating a global pandemic is a joint effort that requires all and sundry to engage in safe and healthy practices. Therefore, measures should be implemented to support Strict global control of prescription medication and drugs to reduce the mental health effect of declaring disease outbreaks.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Ethical Committee of the Nile University of Nigeria, Abuja (Date: 15.07.2020, No: NUN/DR/ERB/20/025).

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HOW THE FREQUENCY OF PAIN IN THE EARLY STAGES OF PARKINSON'S DISEASE AFFECTS THE SPEED OF DIAGNOSIS

PARKİNSON HASTALIĞI BAŞLANGICINDA GÖRÜLEN AĞRI SIKLIĞI VE TANI SÜRESİNE ETKİSİ

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ABSTRACT

Objective: The fact that non-motor symptoms such as pain in Parkinson's disease (PD) are more associated with musculoskeletal diseases (MSD) suggests that there may be delays in diagnosis. The aim of this study was to review the first symptoms of PD, especially pain, while at the same time reviewing the medical specialists to whom patients first went and examining the effects of these parameters on time to diagnosis and treatment.

Materials and Methods: Patients with PD were included. The patients were evaluated in terms of clinical features, initial complaints and onset time, presence and type of pain, the medical specialist they first applied to, and time to diagnosis.

Results: Eighty-six patients were included (42 female, 44 male). The first complaints were bradykinesia, tremor, tremor and bradykinesia, shoulder pain, tremor and painful cramps. These complaints started 10.1±5.22 years previously, and the diagnosis of PD was made 8.56±4.87 years previously on average. The first specialist departments to which patients with these complaints applied were Neurology (n=34), Physical Therapy and Rehabilitation (n=34), Neurosurgery (n=10), and Orthopedics (n=8). The first admission to Neurology was 8.7±4.85 years previously. Pain complaints started 7.2±6.69 years before the first admission in 56 patients. Musculoskeletal pain was 86%, dystonic pain was 25%, central pain and neuropathic pain were 11% each in the group of patients who had experienced pain.

Conclusion: PD can be confused with MSD due to findings such as pain and rigidity, which may cause a delay in diagnosis and treatment. This delay can be prevented if non-neurology

ÖZET

Amaç: Parkinson hastalığında (PH) ağrı gibi non-motor bulguların kas-iskelet sistemi hastalıkları ile daha çok ilişkilendirilmesi tanıda gecikmeler yaşanabileceğini düşündürmektedir. Bu çalışmada PH'de ağrı başta olmak üzere ilk semptomların ve başvurulan branşların gözden geçirilmesi ve bu başvuruların tanı ve tedavi süresine etkisini incelemek amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya PH tanılı hastalar dahil edildi. Hastalar klinik özellikleri, ilk yakınmaları ve yakınma başlangıç zamanı, ağrı yakınmasının varlığı ve tipi, ilk başvurdukları branş ve tanı süreleri yönünden değerlendirildi.

Bulgular: Çalışmaya 86 hasta dahil edildi (42'si kadın, 44'ü erkek). İlk yakınma hareketlerde yavaşlık, tremor, tremor ve hareketlerde yavaşlık, omuz ağrısı, tremor ve ağrılı kasılma idi. Hastaların ilk yakınmaları 10,1±5,22 yıl önce başlamıştı, PH tanısı ise ortalama 8,56±4,87 yıl önce konmuştu. Yakınmaları nedeniyle ilk başvurulan branş 34 hastada Nöroloji, 34 hastada Fizik Tedavi ve Rehabilitasyon, 10 hastada Beyin ve Sinir Cerrahisi, sekiz hastada Ortopedi idi. Nörolojiye ilk başvuru 8,7±4,85 yıl önce idi. Elli altı hastada ilk başvurudan 7,2±6,69 yıl önce ağrı yakınması başlamıştı. Ağrılı hasta grubunda kas-iskelet ağrısı %86, distonik ağrı %25, santral ağrı ve nöropatik ağrının her biri %11 oranındaydı.

Sonuç: Parkinson hastalığı ağrı ve katılık gibi bulgular nedeniyle kas-iskelet sistemi hastalıkları ile karışabilmekte, bu durum tanı ve tedavi süresinin gecikmesine neden olabilmektedir. Nöroloji dışı branşlara hastalık hakkında daha detaylı eğitim verilmesi, halkın hastalık bulguları konusunda bilinçlenmesi bu gecikmenin önüne geçebilir.

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specialists are provided with more detailed training about the disease, and if public awareness is raised about the signs and symptoms of PD.

Keywords: Parkinson's disease, pain, non-motor symptoms, musculoskeletal symptoms

Anahtar Kelimeler: Parkinson hastalığı, ağrı, non-motor semptomlar, kas-iskelet semptomları

INTRODUCTION

Parkinson's disease (PD) is a progressive disease and of all neurodegenerative diseases it is the second most common. Non-motor symptoms such as sleep disorders, pain, autonomic findings, anosmia, depression, anxiety, apathy, and cognitive impairment can be observed much before motor symptoms (1-3). Early diagnosis and treatment increase the quality of life (QoL) and reduce the burden of caregivers (4, 5).

Population-based studies show that a significant proportion of patients diagnosed with PD are diagnosed late (6). Clinicopathological studies have shown that erroneous diagnoses can be made even in patients examined by a movement disorders specialist (7). Therefore, it is essential to recognize the non-motor symptoms as well as the motor symptoms of the disease. The prevalence of pain in PD is between 40-85%, and pain typically appears in five categories as musculoskeletal pain, dystonic pain, central/primary pain, radicular/neuropathic pain, and akathisia (8). The fact that the complaint of pain is sometimes seen before the motor findings or in the very early stages of the disease may cause diagnostic difficulties in this patient group. Another condition that causes a delay in diagnosis is the tendency of patients to apply to medical specialties other than neurology for these complaints. The most important determinants of early diagnosis and access to treatment are factors such as the occurrence of the disease in the younger patient group where it is seen more rarely, the presence of early non-motor/obscure motor findings, and knowledge of which physician the patient was first examined by in regard to PD.

The aim of this study was to review the first symptoms of PD, especially pain, while at the same time reviewing the medical specialists by whom the patients were examined and analysing the effects of these parameters on time to diagnosis and treatment.

MATERIAL AND METHOD

Patient selection and data collection

Patients diagnosed with PD according to the diagnostic criteria provided by the Movement Disorders Society (MDS) and who were followed up in the Movement Disorders outpatient clinics of the Neurology Department of two tertiary care centers were included in the study. Patients with parkinsonism due to secondary causes were excluded (9). The files of 86 patients with PD who had applied to the Movement Disorders outpatient clinic in the previous three months were reviewed retrospectively. The patients were evaluated based on their clinical and demographic characteristics, non-motor findings, initial complaints, onset date, pain complaints and type of pain, the medical specialty they first applied to, and time to diagnosis and treatment. Pain type was divided into five categories, namely musculoskeletal pain, dystonic pain, central/primary pain, radicular/neuropathic pain, and akathisia (8). Depending on its location the pain was categorized as either shoulder pain, low back pain, or cramp-like pain in the extremities. Informed consent was obtained from the patients. Ethics committee approval of the study was obtained from the Istanbul Faculty of Medicine Ethics Committee (Date: 22.02.2021, No: 94690).

Statistical analysis

In order to evaluate the demographic and clinical characteristics of the study cohort, independent sample t-test was used for continuous variables, and chi-square test was used for categorical variables. Categorical variables were presented as numbers and percentages, and continuous variables as mean and standard deviation. Data analysis was performed using the SPSS 23.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Of the 86 patients included in the study, 42 were female, and 44 were male. The mean age of the patients was 61.4±9.94 years. The first complaint was bradykinesia in 36 patients, tremor in 24 patients, tremor and bradykinesia in eight patients, shoulder pain in 16 patients, tremor and painful cramps in two patients. The first complaints of the patients started 10.1±5.22 years previously, and the diagnosis of PD was made 8.56±4.87 years earlier on average. The first specialty to which patients with these complaints applied was Neurology in 34 patients, Physical Therapy and Rehabilitation in 34 patients, Neurosurgery in 10 patients, and Orthopedics in eight patients (Figure 1). The first admission to Neurology was 8.7±4.85 years previously. Of the patients whose first admission was a non-neurology specialty, 38 (73%) of them were admitted to the Neurology outpatient clinic on their own because of worsened complaints, and 14 (27%) of them were referred to the Neurology outpatient clinic by another physician.

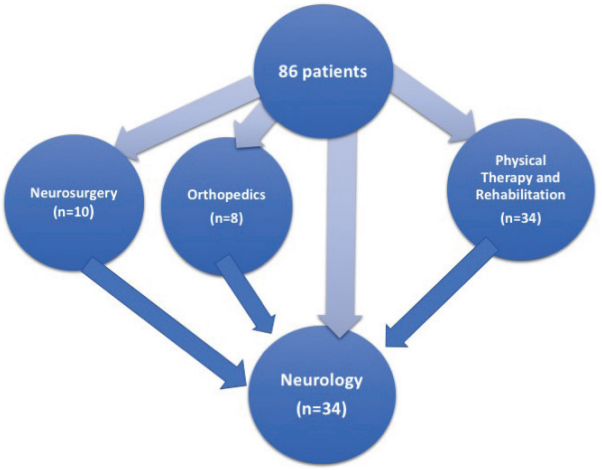

Figure 1: The medical specialties to which the patients applied. The first applied specialties are shown with light blue arrows and the second with dark blue arrows (The number of initially applied patients is given in parentheses).

Table 1: Non-motor findings seen in patients

	Number of patients (n=86)	%
Pain	56	65.1
Constipation	54	62.8
RSBD*	37	43
Hyposmia/anosmia	21	24.4
Urinary problem**	18	21
Daytime sleepiness/sleep disorder	15	17.4
Mood change/apathy	3	3.5
Orthostasis	2	2.3
Mild cognitive impairment	2	2.3

*: REM sleep behavior disorder, **: Urinary incontinence (urgency)/ nocturia

All patients had at least one non-motor symptom other than pain, and these findings started a mean of 4.2±4.23 years before the onset of their symptoms (Table 1).

Pain complaints started 7.2±6.69 years before the first admission in 56 patients (65.1%). Thirty (54%) of the patients with pain complaints were female, 26 were male (46%), and the mean age was 60.6±10.44 years. Although more than one type of pain could be seen in the same person, the predominant pain was shoulder pain in 34 patients, low-back pain and rigidity in 14 patients, and cramp-like pain in the upper and lower extremities in eight patients. All of the patients had received medical treatment and physiotherapy for these reasons. Twenty-two (65%) of the patients with shoulder pain, 4 (29%) of the patients with low back pain, and 4 (50%) of the patients with cramp-like pain were male. In 13 (38%) patients with shoulder pain, the pain was ipsilateral to where parkinsonism was dominant, and it was contralateral in four (12%). The remaining patients could not fully express the side of the pain. When the pain type was divided into musculoskeletal pain, dystonic pain, radicular/neuropathic pain, central pain, and akathisia, it was seen that there could be more than one pain type in the same patient. Regarding pain types, 48 (56%) had musculoskeletal pain, 14 (16%) had dystonic pain, 6 (7%) had central pain, and 6 (7%) had neuropathic pain. Considering the distribution in the group of patients who had pain, musculoskeletal pain was 86%, dystonic pain was 25%, central pain and neuropathic pain were 11% each (Figure 2). In our patient group, akathisia was not described. The mean age of the patients with dystonic pain was significantly younger than that of the other patients (50.1±6.22 vs. 63.9±8.79, p<0.001).

Fifty-eight patients (67%) had akinetic-rigid and 28 patients (33%) had tremor-dominant PD. Twenty-two (38%) of the akinetic-rigid patients and 12 (43%) of the tremor-dominant patients initially applied to neurology. While the time between the first visit to a specialist and the

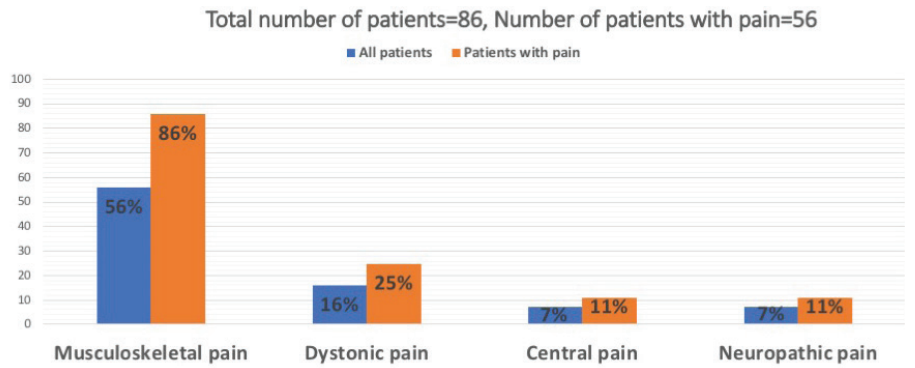

Figure 2: Distribution of pain types in all patients and patient group with pain

first neurology admission in akinetic-rigid patients was 1.6±1.01 years, it was 0.8±0.79 in tremor dominant patients (p<0.001). The mean levodopa equivalent dose used was 1204±448 mg. In all patients, the complaint of pain regressed with oral antiparkinsonian therapy. When we questioned our patients retrospectively, 21 patients (37.5%) with pain stated that the pain had been more restrictive than other complaints in the pre-diagnosis period of the disease.

DISCUSSION

Pain, like other non-motor findings, can frequently be encountered before motor complaints in PD, both at the onset and during the course of the disease, and this has a serious impact on correct diagnosis, time of access to treatment, and QoL of patients. This study shows that patients tend to associate the pain they experience before or in the early period of their illness with motor complaints and that this leads them to apply to non-neurology specialties. The study also shows that physicians from non-neurology specialties are not familiar with the non-motor symptoms of PD, leading to a delay in diagnosis.

According to current diagnostic criteria, PD is clinically diagnosed mainly when motor findings appear, but there is a prodromal phase in which non-motor findings begin to appear, ranging from 5 to 20 years before diagnosis (10-12). It is essential to question prodromal findings such as REM sleep behavior disorder, cognitive disorder, olfactory disorder, incontinence, constipation, prolonged daytime sleepiness, depression and anxiety, erectile dysfunction, and orthostatic hypotension in suspected patients, and to conduct diagnostic investigations in necessary patients in order to make a correct diagnosis in the early period. According to the MDS criteria for prodromal PD, a Unified Parkinson's Disease Rating Scale (UPDRS) score >3 excluding action tremor or an MDS-UPDRS score >6 excluding action and postural tremor is a clinical motor marker for prodromal PD, and it indicates that the diagnosis can be made in a period when motor findings are not excessive (12). In one study, 109 newly diagnosed and untreated PD patients and 107 controls were surveyed, and unexplained pain in various parts of the body that started 2-10 years before the onset of motor symptoms was reported in PD patients (13). In another study, which included 436 PD patients, it was reported that 21% of the patients had prodromal non-motor symptoms and that the most common symptom was pain (14). This suggests that disease progression may be caudo-rostral and that locus ceruleus and raphe nucleus involvement is associated with premotor pain before involvement of the substantia nigra associated with motor findings (15). In addition to being an early symptom and a prodromal symptom, it has been reported that pain is also common in the later stages of the disease (16). In our patient group, pain was the most common complaint (65.1%) among the non-motor symptoms that started before admission, and it started 7.2±6.69 years before the first admission, similar to the literature (12).

Studies have shown that pain as a premotor symptom is seen with a frequency of 30-95% in patients with PD (17-19). Depending on the severity, the first or predominant complaint of the patients may be musculoskeletal pain, and it may occur in association with parkinsonism, that is, with motor complaints such as rigidity and akinesia (20, 21). In the entity that is accepted as central parkinsonian pain, there are criteria such as the onset of pain before the disease, being ipsilateral to where parkinsonism is prominent, and the absence of any other reasons for the pain. Its estimated prevalence has been reported as 4.5-22% (17, 19, 22, 23). In one study, musculoskeletal pain was found to be 70%, followed by dystonic pain (40%), radicular-neuropathic pain (20%), and central pain (10%) (24). In another study, musculoskeletal pain was found to be 41%, followed by radicular-neuropathic pain (27%), central pain (22%), and dystonic pain (17%) (19). As can be seen, the most common pain subtype is musculoskeletal pain, and it was reported in a recent review that shoulder, low back, neck, knee, and hip pain were prominent (19,24-26). In a study conducted on 198 patients with PD by Bonenfant et al., body pain was found to be 74.2% regardless of its type and distribution. In addition, studies have reported that 30-71% of patients complain of more than one type of pain (19, 24, 27, 28). In a study conducted in Turkiye the most common type of pain was musculoskeletal pain (44.4%), followed by dystonic pain (19.1%) (29). In our patient group, the high number of patients presenting with primary complaints of pain was remarkable (n=56, 65.1%). Similar to the literature, the most common pain type was musculoskeletal pain, and the second most common was dystonic pain.

In a study conducted by Kim et al. on 400 PD patients and 138 age- and sex-matched controls, there was no significant difference in musculoskeletal problems limiting the activities of daily living (ADL) for both groups (30). However, they reported that 31.8% of PD patients had more restricted ADLs due to musculoskeletal problems than parkinsonism symptoms. In our patient group, 37.5% of patients with pain also stated that the pain was more restrictive than that of other complaints. In the same study, it was reported that musculoskeletal complaints were more common in women and elderly patients in the PD group, similar to the normal population. In our study, while the complaint of pain was more common in women (54%), there was no significant age difference between the group of patients which had experienced pain and the group that had not, unlike what was reported in the literature. Previous studies found that the laterality of the

complaints and parkinsonism were same in 30% of PD patients with musculoskeletal problems, different in 9%, and partially the same in the remaining (30). One study found that shoulder pain may occur as the first clinical manifestation, especially in cases of akinesia (31). Consistent with these findings, in our patient group, the number of patients with ipsilateral shoulder pain and parkinsonism was higher than those with contralateral shoulder pain and parkinsonism (38% vs. 12%). It is thought that parkinsonism and ipsilateral pain make the diagnosis difficult, and this finding is notable as the first clinical manifestation.

One of the most common problems experienced by PD patients is pain caused by a frozen shoulder which may occur as a sign of the disease. In a previous study, shoulder complaints (43% vs. 23%) and history of frozen shoulder (12.7% vs. 1.7%) were found to be considerably higher in PD patients compared to controls, and one study found that frozen shoulder is the first symptom of the disease in 8% of the patients (31). Another study also showed that shoulder pain was more common in PD patients (80% vs. 40%) compared to controls (32). Although it is thought that UPDRS, rigidity, and long disease duration increase shoulder complaints, shoulder complaints at the onset and before the disease are significant enough not to be ignored (33). In our study, 34 patients had shoulder pain that started before their admission (39.5%) and the first complaint of 16 patients was shoulder pain (18.6%). For early diagnosis, it is essential to know that this condition, which is thought to increase in frequency with immobilization due to rigidity and akinesia, may be one of the first symptoms of the disease.

Low back pain, another common complaint, was higher in PD patients than controls in various studies (34, 35). This has been reported more frequently at the onset of the disease, especially in women, and has been expressed to be a matter of concern (36). This condition, accompanied by more radicular pain than in the normal population, is thought to increase due to pathologies such as parkinsonism-related postural disorder, increased muscle tone, and rigidity. In our study, it was observed that 14 patients (16.3%) had a history of low back pain and that they were treated with different diagnoses. Consistent with the literature, the frequency of low back pain is higher in female patients (36).

It has been shown that the frequency of cramp-like pain increases in Parkinson's patients (23, 37). In our study, it was observed that eight of the patients had cramp-like pain in the arms and legs at the onset of the disease. It shows that cramp-like pain, which may be associated with rigidity, akinesia, motor off periods, and dystonia, can be seen in the early period, and other signs and symptoms of the disease should be carefully screened.

As can be seen, PD may initially manifest itself through pain and this may lead the clinician to misdiagnosis and mistreatment (38). The use of standardized diagnostic criteria, such as the UK Brain Bank Criteria with a diagnostic specificity of 98.6% and a sensitivity of 91.1%, may increase the accuracy of clinical diagnosis. However, clinicopathological studies have shown that the rate of misdiagnosis can be up to 24%, even in patients examined by a movement disorders specialist (7, 39, 40). For this reason, when symptoms start with pain and other non-motor symptoms, these patients can be evaluated by physicians of different specialties, and therefore, delays in diagnosis can be experienced. In our study, it is noteworthy that the first complaints of the patients started at the time that they applied to a physician which was 10.1±5.22 years previous to the date of our study, but the date of diagnosis and start of treatment occurred 8.56±4.87 years before the date of this study. The first admission of 61% of the patients was to specialties other than neurology, and only 27% were referred to neurology by another specialty because their complaints increased and treatment response was poor, and the remaining patients applied of their own initiative. In addition, the diagnosis of akinetic-rigid patients was delayed more than that of tremor dominant patients ($p<0.001$), which suggested that akinesia, as a PD symptom, was less easily recognized both by physicians and the public than tremor symptoms. For this reason, increasing the training of related specialties other than neurology on PD and increasing public awareness about the disease are essential for early diagnosis and treatment.

One of the limitations of this study is the inability to apply pain scales and parkinsonism scales to newly diagnosed individuals at the time of admission due to its retrospective nature. Further studies will be more beneficial if planned in this way. In addition, osteoporosis, primarily due to immobility and vitamin D deficiency due to patients not often being outside, could not be investigated as a cause of pain. Depression and anxiety, which are known to increase pain perception, could not be evaluated with objective scales since it is a retrospective study. Another limitation of the study is the absence of a control group. Without a control group a clear conclusion could not be reached regarding the frequency of pain in people with PD compared with other members of the population. Finally, the female to male ratio is greater in our study compared to that in the general population. This might have an effect on the results.

CONCLUSION

Patients with PD experience pain with considerable frequency at the beginning as well as during the course of the illness and this may indicate that the prodromal phase comes before the motor period. However, the di-

agnosis may be overlooked in patients with obscure findings, and thus the patient's access to treatment might be delayed. For this reason, providing more detailed training on PD to non-Neurology specialties such as Physical Therapy and Rehabilitation, Orthopedics, Internal Medicine, and Neurosurgery will facilitate the patient's access to the correct diagnosis and treatment and increase their QoL. In addition, educating the public on PD will lead to correct admissions. Early diagnosis is also essential in prolonging the early phase of the disease and preventing motor findings when disease-modifying treatments are available.

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GJB2-RELATED NON-SYNDROMIC HEARING LOSS VARIANTS' SPECTRUM AND THEIR FREQUENCY IN TURKISH POPULATION

GJB2 İLİŞKİLİ NON-SENDROMİK İŞİTME KAYBI VARYANTLARININ SPEKTRUMU VE TÜRK TOPLUMUNDAKİ SIKLIKLARI

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ABSTRACT

Objective: Hearing loss (HL) is one of the most prevalent chronic conditions in children and has consequences in speech, language, education, and social functioning which impede the quality of life. Due to the major involvement of the genetic factors in HL, especially non-syndromic HL (NSHL), genetic diagnosis and genetic counseling have a major impact on early management of the affected individuals and their families. Herein, we report the *GJB2* gene variants and their frequencies in NSHL cohort at a tertiary health center between 2002-2021 to contribute for the future genetic counseling of Turkish NSHL patients.

Materials and Methods: Two exons of the *GJB2* gene were amplified in 402 NSHL patients by two separate PCR reactions and sequenced using the Sanger technique.

Results: We found 13 different *GJB2* variants in 35% (141/402) of the patients with NSHL. 53.9% were homozygous and 33.3% were compound heterozygous for the most common (59.21%) variant, c.35delG. Approximately 13% of the patients were found to carry the variants in the heterozygous state. The most frequent *GJB2* variant c.35delG was followed by c.71G>A (6.38%), c.-23+1G>A (3.54%) and c.233delG (2.48%). We found heterozygous p.Asp50Glu (c.150C>A) alteration in four of eight patients with keratitis, ichthyosis, deafness (KID) and palmoplantar keratoderma (PPK) syndrome.

Conclusion: Our results further emphasize the well-known prevalance of the *GJB2* c.35delG alteration being the most pre-

ÖZET

Amaç: İşitme kaybı, çocukluk çağındaki en önemli kronik sağlık sorunlarından biridir ve yaşam kalitesini konuşma, eğitim ve sosyal ilişki sorunlarına yol açarak azaltır. Özellikle non-sendromik işitme kaybında genetik faktörlerin rolü etkilenmiş kişi ve ailelerinin genetik tanı ve genetik danışma aşamalarında doğru yönlendirilmesi açısından kilit bir rol oynar. Bu nedenle, non-sendromik işitme kaybı olan hasta ve ailelerinin önümüzdeki yıllarda genetik tanı ve danışmasına katkıda bulunmak amacıyla, bu çalışmada, 2002-2021 yılları arasında sinirsel tip işitme kaybı tanısı alan hastalardaki *GJB2* gen varyantlarını ve sıklıklarını sunmaya çalıştık.

Gereç ve Yöntem: *GJB2* geninin iki ekzonu, 402 hasta DNA'sında iki ayrı PCR ile çoğaltıldı ve Sanger yöntemi ile dizilendi.

Bulgular: Non-sendromik işitme kaybı olan olguların %35'inde (141/402) *GJB2* geninde 13 farklı değişim saptadık. Hastaların %53,9'u en yaygın (%59,21) varyant olan c.35delG değişimini homozigot taşırken, %33,3'ü birleşik heterozigot olarak taşıyordu. Yaklaşık %13'ünde ise değişim heterozigot olarak belirlendi. Çalışma grubumuzda en yaygın *GJB2* varyantı olan c.35delG değişimini sırasıyla c.71G>A (%6,38), c.-23+1G>A (%3,54) ve c.233delG (%2,48) değişimleri izlemiştir. Keratit-ihtiyoz-sağırlık (KID) ve palmoplantar keratoderma (PPK) sendromu tanılı sekiz hastanın dördünde heterozigot p.Asp50Glu (c.150C>A) değişimi saptandı.

Sonuç: Sonuçlarımız, Türkiye'deki non-sendromik işitme kaybı hastalarındaki c.35delG varyantının uzun zamandır bilinen baskınlığını bir kez daha göstermektedir. Ayrıca, tek mutant alel

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dominant variant in the Turkish NSHL patients. The high rate of mono-allelic state could be considered as coincidental due to high allelic heterogeneity of NSHL, or possibly suggestive for digenic inheritance.

Keywords: Sensorineural hearing loss, *GJB2* gene, c.35delG alteration, mutation frequency

saptanan hastaların oranı, non-sendromik işitme kaybının alelik heterojenitesi nedeniyle rastlantısal olarak değerlendirilebileceği gibi, digenik kalıtımı da düşündürebilir.

Anahtar Kelimeler: Sinirsel tip işitme kaybı, *GJB2* geni, c.35delG değişimi, mutasyon sıklığı

INTRODUCTION

Hearing loss (HL) is one of the important health problems with social and psychological outcomes. Though both environmental and genetic factors are involved in the etiology of HL, hereditary factors are responsible for more than 70% of the cases. HL is classified as syndromic and non-syndromic, depending on the presence or the absence of accompanying findings, respectively. Non-syndromic HL (NSHL), which constitutes most of the cases (70-80%), is further subdivided by mode of inheritance as autosomal recessive, autosomal dominant, X-linked, and mitochondrial HL. Autosomal recessive NSHL (ARNSHL) constitutes 80% of NSHL (1). Bi-allelic variants in any one of the identified over 100 genes are known to cause ARNSHL. This considerable number of genes responsible for hearing loss shows the hearing mechanism's complex structure which involves channel proteins, integral membrane protein, adhesion molecules, enzymes and extracellular matrix components (2). However, the most frequent mutations causative for ARNSHL are found in the *GJB2* gene.

The *GJB2* (*CX26*, GenBank M86849, OMIM: *121011) gene encodes connexin protein providing a chemical connection of the cell with neighboring cells or extracellular space. The main task of GJB2 in the hearing system is to modulate the potassium ion recycling which is required for the action potentials of hair cells in the organ of Corti (3-6). Generally, loss-of-function mutations of the *GJB2* gene cause autosomal recessive non-syndromic hearing loss (ARNSHL). In contrast, gain-of-function mutations of the *GJB2* gene are responsible for the autosomal dominant keratitis, ichthyosis, deafness (KID) or palmoplantar keratoderma (PPK) syndrome, characterized by extensive hyperkeratotic lesions in the skin, keratitis leading to a loss of visual acuity and profound progressive deafness (7).

Diversity in the clinical manifestations of *GJB2* variants can mainly explain the genotype-phenotype correlation. The nonsense mutations causing early truncation and missense mutations preventing the formation of gap junctions may be responsible for profound hearing loss. Some other particular alterations do not impair the formation of functional gap junctions but reduce conductance levels and alter gating properties, causing mild or moderate hearing loss (8-10). On the other hand, *GJB2* mutations like p.Asp50Glu (c.150C>A), p.Gly12Arg (c.34G>C), p.Ala40Val (c.119C>T), and p.Gly45Glu (c.134G>A) which are associated with KID/PPK do not seem to impair the gap junction formation, but they mildly affect gap junction channel properties (11, 12).

ARNSHL's prevalence depends on the frequency of carriers in the population. Knowing the frequency and types of mutation in a population allows early therapeutic intervention. As a result of recent developments in genetic technology, the diagnostic approach to patients with hearing loss now includes Whole Exome Sequencing (WES) after scanning of the *GJB2* gene. Thus, for the appropriate implementation of the *GJB2* gene scanning in a population, the variant frequency data may need to be updated. In this regard, we aimed to investigate the frequency and spectrum of *GJB2* gene variants in our NSHL patients diagnosed with *GJB2*-related disease (ARNSHL).

MATERIALS AND METHODS

The bilateral hearing-loss patients with clinical findings that suggest a possible traumatic or infectious causative, and unilateral hearing-loss patients were excluded from the study. The patients with a syndromic form of hearing loss, except for those with KID or PPK, were also excluded.

Peripheral blood samples of 2 ml were collected upon approval of the patients and families for genetic testing. DNA isolations were performed by using commercial kits according to the instructions (Mammalian Blood and Cells and Tissue DNA Isolation Kit, Roche). Two exons of the *GJB2* gene were amplified by three separate PCR reactions using specific primers (Table 1). To perform an efficient sequencing, the second exon of the *GJB2* gene, which has a relatively large size (681 bp), was amplified and sequenced with two separate PCRs. All PCR reactions were carried out with 2.5 mM $MgCl_2$, 0.2 µM of each primers, 0.2 µM of each dNTP, 1U *Taq* DNA polymerase (Thermo Fisher Scientific) and 100 ng genomic DNA. Purification of the PCR products was performed with Exonuclease and Alkaline Phosphatase enzymes (Thermo Fisher Scientific). Sanger sequencing reactions were carried out with an automated sequencer (ABI 3500). An analysis of sequencing data was performed with the SeqScape software (SeqScape v3.0) using the *GJB2* reference requence (NM_004004.6) fetched from the USCS Genome Browser (https://genome-euro.ucsc.edu/). The study was approved by the Ethics Committee of Istanbul University, Istanbul Faculty of Medicine (Date: 22.01.2021, No: 108).

Table 1: Primers used to amplify non-coding and coding two exons of *GJB2* gene

Primer	Exon	Sequence	Lenghth (bp)	Tm (°C)	Expected PCR product size (bp)
GJB2_F1	1	5′-GTGCGGTTAAAAGGCGCCA-3′	19	66.4	265
GJB2_R1		5′-GGCAACCGCTCTGGGTCT-3′	18	63.8	
GJB2_F2-I	2	5′-CTCCCTGTTCTGTCCTAGCT-3′	20	56.2	840
GJB2_R2_I		5′-GACTGAGCCTTGACAGCTGA-3′	20	59.3	
GJB2_F2-II	2	5′-CTCCCTGTTCTGTCCTAGCT-3′	20	56.2	804
GJB2_R2-II		5′-CCCTCTCATGCTGTCTATTTC-3′	21	56.5	

bp: Base pair, Tm: Melting temparature

RESULTS

Among 402 families with at least one member clinically diagnosed with ARNSHL based on pedigree, 35% (141/402) were found to carry the pathogenic variant in the *GJB2* gene. Of these, 53.9% were homozygous, and 33.3% were compound heterozygous for the identified variants. Approximately 13% of the patients were found to carry a single (mono-allelic) *GJB2* variant (Table 2).

We identified 13 different *GJB2* gene variants in our cohort (Table 2). The *GJB2* gene variants most frequently observed were c.35delG (59.2%), c.71G>A (6.38%), c.-23+1G>A (3.54%), c.233delC (2.48%) and c.358_360delGAG (1.77%). Five variants (c.35delG, c.71G>A, c.-23+1G>A, c.358_360delGAG and c.233delC) were found in the homozygous state, seven were found to be compound heterozygotes, c.35delG being the most frequent one. One patient was found to be compound heterozygous, involving different alleles other than c.35delG. Eighteen (12.7%) of the patients were found to carry a single *GJB2* variant, most frequently (55%) being c.35delG.

Allele (Figure 1) and genotype frequencies (Table 3) consistently showed that c.35delG variant predominates the cohort either in homozygous or in compound heterozygous state. The c.71G>A variant was the second most frequent one.

In eight patients clinically diagnosed with KID syndrome four were found to carry c.150C>A (p. Asp50Glu).

Table 2: Patient allele counts of *GJB2* variants revealed in the NSHL cohort

Mutation	Patient counts			Allele count	Allele frequency	Reference
	Homozygous	Compound heterozygous	Mono-allelic			
c.35delG (p.G12Vfs*2)	67	23	10	167	76.82	13
c.71G>A (p.W24*)	3	12	0	18	8.19	14
c.-23+1G>A	2	3	3	10	4.56	15
c.233delC (p.L79Cfs*3)	2	3	0	7	3.18	16
c.358_360delGAG (p.E120del)	2	0	1	5	2.73	17
c.167delT (p.L56Rfs*26)	0	2	0	2	0.91	18
c.269T>C (p.L90P)	0	1	1	2	0.91	13
c.327_328delGG (p.E110Dfs*4)	0	1	0	1	0.45	19
c.439G>T (p.E147*)	0	1	0	1	0.45	20
c.94C>T (p.R32C)	0	1	0	1	0.45	21
c.487A>G (p.M163V)	0	0	1	1	0.45	22
c.551G>C (p.R184P)	0	0	1	1	0.45	18
c.239A>G (p.Q80R)	0	0	1	1	0.45	33
Total	76 (53.91%)	50 (33.33%)	18 (12.76%)	220		

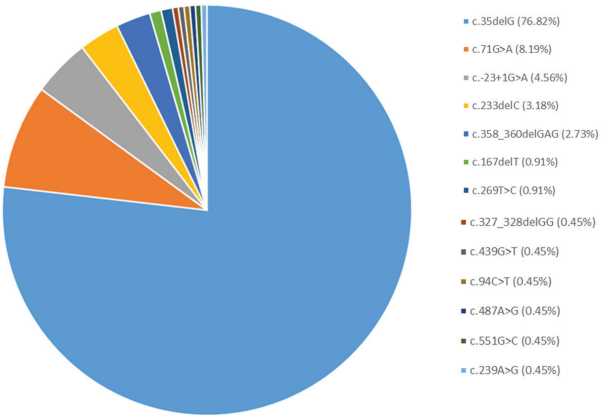

Figure 1: Allele frequency of *GJB2* variants identified in the cohort.

neighboring countries. The most frequent variant was c.35delG, which was identified in more than half of our patients. This mutation is the most common pathogenic variant found among Caucasians, Mediterraneans, and different European populations (12, 23-27). Though c.35delG was reported as the most frequent alteration by different studies in various countries, its frequency was not the same among populations. The variable frequency of c.35delG among different populations can be explained by the founder effect. Many studies suggest that the c.35delG allele originated from South Europe, Italy (28). Interestingly, the frequency of this variant displays a geographic gradient as observed by its frequency decrease from Southern to Northern Europe and from Western to Eastern Asia (29). This geographic gradient can be

Table 3: Patients with different *GJB2* allele genotypes in the cohort

Genotype (HGVS*)	Zygosity	Patient count (%)
c.[35delG];[35delG]	Homozygous	67 (56.78)
c.[35delG];[71G>A]	Compound Heterozygous	12 (10.17)
c.[35delG];[35=]	Heterozygous	10 (8.47)
c.[35delG];[233delC]	Compound Heterozygous	3 (2.54)
c.[71G>A];[71G>A]	Homozygous	3 (2.54)
c.[35delG];[-23+1G>A]	Compound Heterozygous	3 (2.54)
c.[-23+1G>A];[-23=]	Heterozygous	3 (2.54)
c.[358_360delGAG];[358_360delGAG]	Homozygous	2 (1.69)
c.[35delG];[167delT]	Compound Heterozygous	2 (1.69)
c.[233delC];[233delC]	Homozygous	2 (1.69)
c.[-23+1G>A];[-23+1G>A]	Homozygous	2 (1.69)
c.[358_360delGAG];[358_360=]	Heterozygous	1 (0.85)
c.[35delG];[327_328delGG]	Compound Heterozygous	1 (0.85)
c.[35delG];[439G>T]	Compound Heterozygous	1 (0.85)
c.[35delG];[94C>T]	Compound Heterozygous	1 (0.85)
c.[269T>C];[358_360 delGAG]	Compound Heterozygous	1 (0.85)
c.[269T>C];[269=]	Heterozygous	1 (0.85)
c.[487A>G];[487=]	Heterozygous	1 (0.85)
c.[551G>C];[551=]	Heterozygous	1 (0.85)
c.[239A>G];[239=]	Heterozygous	1 (0.85)

*: Human Genome Variation Society (http://varnomen.hgvs.org/recommendations/DNA/)

DISCUSSION

Here we report, the variant spectrum and frequency of the *GJB2* gene in NSHL patients with *GJB2*-related entities (ARNSHL or KID/PPK). Our results were consistent with the previous studies conducted in Turkiye or in

observed even in some individual countries. In Iran, for instance, the frequency of the c.35delG is highest in the North-West and lowest in the South-East populations, consistent with the findings of neighboring countries like Turkiye and Pakistan (30). A similar frequency range for

c.35delG variant has been observed in different cities of Turkiye ranging from 5% to 53% (31).

HL is a health problem, although being nonlethal it poses life quality issues. Though the recent developments in HL treatment partly begin to overcome these limitations, the psychological outcomes of the disease can not be ignored. The current solutions to this problem include early diagnosis, management, follow-up, educational and social support for the families, genetic counseling, and possible cochlear implantation. Due to the high rate of heredity in HL, genetic counseling has a major impact on new cases before genetic testing. On the other hand, the high ratio of autosomal recessive inheritance calls attention to consanguineous marriages. Many studies suggest that the variability in the frequency of some *GJB2* variants in different populations resulted from the founder effect for the frequent variants and consanguineous marriages for the rare variants (15, 29, 31). Within our cohort, five alterations in a homozygous state were consistent with their high frequency of consanguineous marriages in the population from Turkiye. As a result of high allele frequency, c.35delG was found in 87% and 46% of the patients at homozygous and compound heterozygous state, respectively. These ratios may clinically have importance because c.35delG mutation has been shown to have significantly severe hearing impairment in homozygous patients, compared with 35delG/non-35delG compound heterozygotes (8). Interestingly, one of our patients who was in a compound heterozygous state had two different mutations (c.[269T>C;358_360delGAG]) other than the most frequent one (c.35delG). Taken together with the high ratio of compound heterozygosity, this finding suggests that ARNSHL is a health problem not restricted to consanguineous marriages.

Some *GJB2* mutations reported previously from Turkiye were not found in our patient cohort (32). These variants include c.360_362delGAT (p.delE120), c.310_323del14, c.299_300delAT, c.517C>T (p.P173S) and c.238C>A (p.Q80K). This discrepancy supports the idea that the *GJB2* variant spectrum and frequencies vary by geographic origins and the size of the cohorts. Even in the same cohort, the spectrum of variants can fluctuate by the method used and by the size of the cohort. For instance, compared to our previous report, we observed in this present study an additional ten rare variants and one frequent variant (c.-23+1G>A) in the *GJB2* gene due to the inclusion of the non-coding exon of *GJB2* and the analysis of a higher number of patients (33).

The finding that 12.7% of our patients had a single mutant allele of the *GJB2* gene suggests some possibilities, like the presence of a second mutation that might be located in a non-coding or regulatory regions of the *GJB2* gene. Another possibility is that the bi-allelic mutations responsible for the disease might be located in a different gene other than the *GJB2*. In the latter case, the mutation we found in the *GJB2* gene might be just a coincidental variant carried by the patient. However, digenic inheritance involving the *GJB2* gene should also be considered to explain mono-allelic *GJB2* gene variants in the patients. Digenic inheritance in ARNSHL was reported by previous studies (34, 35). Mono-allelic variant carriers of the *GJB2* gene were shown to cause ARNSHL with the presence of other mono-allelic variants in another gene like *GJB6*, *GJB3*, *MITF*, *TMPRSS3*, *GJB4*, *GJA1*, and *GJC3* (36-40). However, there are conflicting data regarding *TMPRSS3/GJB2* digenic inheritance (41). Despite all the supporting publications, the molecular mechanism underlying the digenic ARNSHL remains to be elucidated. Considering all these data, we can at least say that, to elucidate the molecular etiology of ARNSHL in the patients with mono-allelic *GJB2* mutation, digenic inheritance should be kept in mind. For such patients, exome sequencing is presently recommended for genetic diagnosis. However, to confirm the digenic inheritance, a segregation analysis of the candidate variants in the patient's family is also needed. Besides, digenic inheritance carries a possibility of a lead to an incorrect exclusion of the variant in segregation analysis.

In addition to the patients with ARNSHL, we analyzed the *GJB2* gene in the patients with KID/PPK syndrome. Of eight patients clinically diagnosed with KID/PPK, four were found to carry p.D50N (c.150C>A) variant in heterozygous state. Though this study covered only the *GJB2* gene, variants in other connexin genes like *GJB6* (CX30), *GJB4* (CX30.3), *GJB3* (CX31), and *GJA1* (CX43) that are expressed in epidermis and appendages, are known to cause skin disorders (42). However, only *GJB6* variants are known to cause KID/PPK or an overlapping Clouston syndrome (43). Like *GJB2*, *GJB6* also has allelic heterogeneity, and pathogenic *GJB6* variants can cause autosomal recessive and dominant deafness or type 2 ectodermal dysplasia 2 (Clouston type). Therefore, sequencing of the *GJB6* gene should be included in the diagnostic algorithm of the HL patients with skin lesions.

The *GJB2* gene has two exons, and most pathogenic *GJB2* variants, including c.35delG, are located in the second exon of the gene. Therefore, sequencing of this exon is expected to detect most of the causative variants. Consistent with previous studies, sequencing of the second exon was sufficient for the diagnosis of most patients. However, our patients' third most frequent mutation (c.-23+1G>A) is located in the splicing site of the non-coding first exon. Sequencing of this non-coding exon is also required to increase the diagnostic yield of the patients with ARNSHL.

In summary, sequencing only the coding exons of the *GJB2* gene would lead to molecular diagnosis in approximately 33% of the patients. Further, including the non-coding exon would yield an additional 2% diagnostic rate. To identify pathogenic variants in the remaining 65% of the cases, it seems necessary to implement NGS techniques, due to the possible digenic inheritance and multigenic etiology of NSHL. Increasing the availability and decreasing the cost of the NGS have made it the most favorable technique for the diagnosis of genetic diseases in the past years. Depending on the *GJB2* mutation spectrum and frequency in a population, NGS seems to be a recommendable method after excluding the most frequent mutations by Sanger sequencing. It should be noted that the NGS technique may reveal some coincidental variants that could be confusing for the clinician.

The efficiency of NGS in the diagnosis of HL depends both on the cohort and the covered genes. Depending on the patient selection criteria (like ethnicity, with or without positive family history), a positive diagnostic rate was reported to range from 10 to 83% (44). Similarly, in a large multiethnic cohort including 1119 unrelated patients who were tested with NGS gene panel (targeted genomic enrichment and massively parallel sequencing), it was shown that screening of 89 genes increased the diagnostic rate by 2% compared to the screening of 66 genes. This difference was stated to account for 4% of all positive diagnoses (45). Considering the remarkable contribution rate (18%) of gross copy number changes in hearing loss, we can deduce that the MLPA technique may also need to be implemented for the diagnostic algorithm of NSHL (35).

In conclusion, our update on NSHL cohort for *GJB2*-related entities has supported the previous knowledge about the most frequent *GJB2* pathogenic variants and revealed the possibility of compound heterozygosity of the rare variants and potential digenic inheritance.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Ethics Committee of Istanbul University, Istanbul Faculty of Medicine (Date: 22.01.2021, No: 108).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- Z.O.U., B.W.; Data Acquisition- H.K., A.D.A.; Data Analysis/Interpretation- Z.O.U., V.K., F.T., Ç.G.; Drafting Manuscript- Ç.G., Z.O.U.; Critical Revision of Manuscript- Z.O.U., H.K., Ç.G.; Approval and Accountability- Z.O.U., H.K., B.W., Ç.G., A.D.A., V.K., F.T.

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PERINATAL OUTCOMES AND PROGNOSTIC FACTORS IN EARLY AND LATE-ONSET FETAL GROWTH RESTRICTION

ERKEN VE GEÇ BAŞLANGIÇLI FETAL GELİŞİM KISITLILIĞINDA PERİNATAL SONUÇLAR VE PROGNOSTİK FAKTÖRLER

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ABSTRACT

Objective: To evaluate the obstetric and perinatal outcomes of fetuses with early (EO) and late-onset (LO) fetal growth restriction (FGR), and to explore the prognostic factors on perinatal survival and adverse perinatal outcome.

Materials and Methods: We retrospectively reviewed 105 EO- and 55 LO-FGR singleton pregnancies. Umbilical artery (UA), middle cerebral artery (MCA) and ductus venosus (DV) Doppler parameters and cerebroplacental ratio (CPR) were assessed. Prognostic significance of gestational age at delivery, birth weight and Doppler parameters were evaluated.

Results: Gestational age at delivery greater than 27 weeks (sensitivity 87.5%, specificity 76%) and birth weight of 665 g (sensitivity 88.8%, specificity 92%) provided the best prediction of survival in EO-FGR. Logistic regression analysis of UA absent or reversed end diastolic flow (EDF), abnormal DV Doppler, and absent/reversed DV a-wave revealed Odds Ratios of 2.57, 6.97, 4.51 and 8.75 respectively for perinatal mortality in EO-FGR. The incidence of CPR below the 5th percentile was significantly higher in LO-FGR pregnancies with the composite adverse outcome than normal outcome ($p<0.001$).

Conclusion: Gestational age at delivery and birth weight are the strongest predictors of perinatal mortality in EO-FGR. In LO-FGR, CPR <5th percentile is associated with an increased risk of delivery complications.

Keywords: Early-onset fetal growth restriction, late-onset fetal growth restriction, perinatal outcome, Doppler parameters

ÖZET

Amaç: Erken (EB) ve geç (GB) başlangıçlı fetal gelişim kısıtlılığı (FGK) olgularında obstetrik ve perinatal sonuçların değerlendirilmesi ve perinatal sağ kalım ile olumsuz perinatal sonuçlar üzerine etkili prognostik faktörlerin saptanması.

Gereç ve Yöntem: Tekil 105 EB- ve 55 GB-FGK olan gebelik retrospektif olarak derlendi. Umblikal arter (UA), orta serebral arter (MCA) ve duktus venozus (DV) Doppler parametreleri ile serebroplasental oran (CPR) değerlendirildi. Doğumdaki gebelik haftası, doğum ağırlığı ve Doppler parametrelerinin prognostik anlamı incelendi.

Bulgular: Doğumun 27. gebelik haftasından sonra gerçekleşmesi (duyarlılık %87,5, özgüllük %76) ve doğum ağırlığının 665 gr'ın üzerinde olması (duyarlılık %88,8, özgüllük %92) EB-FGK olgularında en iyi sağ kalım öngörüsünü sağladı. Lojistik regresyon analizinde, UA diyastol sonu akımın (EDF) kaybı ve ters akım olması, anormal DV Doppler ve DV a dalgasının kaybı/ters a dalgası EB-FGK'da perinatal mortalite ile ilişkili bulundu (sırasıyla olasılık oranları %2,57, 6,97, 4,51 ve 8,75). Olumsuz sonuçların eşlik ettiği GB-FGK'da, normal sonuçların izlendiği olgulara kıyasla, CPR'ın 5. persentilin altında olma oranı istatiksel olarak anlamlı bulundu ($p<0,001$).

Sonuç: EB-FGK'da doğumdaki gebelik haftası ve doğum ağırlığı en kuvvetli prediktörlerdir. GB-FGK'da, CPR'ın <5. persentil olması doğum komplikasyonları açısından artmış risk ile ilişkilidir.

Anahtar Kelimeler: Erken başlangıçlı fetal gelişim kısıtlılığı, geç başlangıçlı fetal gelişim kısıtlılığı, perinatal sonuç, Doppler parametreleri

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INTRODUCTION

Fetal growth restriction (FGR) is defined as the inability of the fetus to reach its growth potential with increased risks of perinatal mortality and morbidity (1). FGR is classified as early-onset (EO-) (<32 weeks) or late-onset (LO-) (≥32 weeks) FGR based on the gestational age at diagnosis (1). EO- and LO-FGR seems to be caused by different placental pathologies, where EO-FGR originates from the reduction of villous vascular area; LO-FGR is associated with impaired maturation of the villi with mild placental insufficiency (2, 3). EO-FGR affects 1-2% of births and is frequently associated with preeclampsia, abnormal Doppler indices, fetal hypoxia, and increased perinatal mortality (4). LO-FGR affects 3-5% of births, although associated with a lower risk of fetal hypoxia and abnormal Doppler indices, is related to stillbirth, neonatal morbidity and intrapartum fetal distress (5).

Clinical management of FGR pregnancies mostly relies on optimizing the timing of delivery. Doppler evaluation of uterine artery (UtA), umbilical artery (UA), middle cerebral artery (MCA) and ductus venosus (DV) are commonly used in the management of FGR pregnancies. The major concern in EO-FGR is to prevent fetal death and severe neonatal morbidity, balancing the risks of preterm delivery. The typical pattern of fetal Doppler parameters' deterioration, which guides the timing of delivery, has been shown to be present in EO-FGR (4). Absent or reversed end diastolic flow (EDF) in UA is related to increased risk of fetal demise, and delivery is recommended after 32-34 weeks (6). Abnormal DV Doppler parameters (increased PI, absent or reversed atrial contraction wave (a-wave)) and cardiotocography findings are indications for delivery before 32 weeks (6). LO-FGR is associated with low fetal tolerance to the hypoxic conditions induced by normal labor (5). Cerebroplacental ratio (MCAPI/UAPI, CPR) has been reported to be useful in identifying fetuses with poor perinatal outcome in LO-FGR (7).

The aim of the present study was to evaluate the obstetric and perinatal outcomes of fetuses with EO- and LO-FGR. And also, to explore the significance of prognostic factors on perinatal survival and adverse perinatal outcome in EO- and LO-FGR pregnancies.

MATERIALS AND METHODS

This was an observational retrospective cohort study of 105 EO-FGR and 55 LO-FGR singleton pregnancies conducted at the Maternal-Fetal Unit, between January 2015 and December 2019. Approval for this study was obtained by the institution's ethics committee. Fetuses with chromosomal and structural abnormalities, infections and genetic syndromes were excluded from the study. Gestational age was determined based on last menstrual period and was confirmed with crown-rump length of first trimester ultrasound. EO-FGR was defined when the gestational age at diagnosis was <32 weeks and delivery was ≤34 weeks and the following criteria were present: estimated fetal weight (EFW) or abdominal circumference (AC) below the 3rd percentile for the gestational age or absent EDF UA; EFW or AC below the 10th percentile for the gestational age, associated with a mean UtA PI or UA PI above the 95th percentile for gestational age (1). LO-FGR is defined, when the gestational age at diagnosis is ≥32 weeks and delivery is >34 weeks in the presence of EFW or AC below the 3rd percentile for gestational age; EFW or AC below the 10th percentile for the gestational age, associated with a mean UtA-PI above the 95th percentile for the gestational age, CPR below the 5th percentile for the gestational age (1).

Ultrasound and Doppler assessments were performed using Voluson E10 (GE Medical Systems, USA). Fetal biometric parameters and amniotic fluid index were measured and EFW was calculated using the Hadlock formula (8). Doppler velocity waveforms from UtA, UA, MCA and DV were obtained and measured according to the International Society of Ultrasound in Obstetrics and Gynecology guidelines (9). In all cases the last Doppler evaluation was performed within 24 hours before delivery. Abnormal UtA Doppler was defined as mean PI (right+left UtA PI/2) above the 95th percentiles or the presence of a diastolic notch in both uterine arteries (10). Abnormal UA, MCA and DV Doppler parameters and CPR were defined as UA-PI>95th, MCA-PI<5th, DV-PI>95th and CPR<5th for the gestational age respectively (11-13). The UA was also qualitatively assessed for absent or reversed EDF.

Follow-up ultrasound assessments were carried out at least every 1-2 weeks and up to every day depending on the type and severity of FGR. Timing of delivery was based on evidence from randomized controlled trials considering gestational age, severity of FGR, and results of fetal surveillance (6, 12). In cases of EO-FGR with absent or reversed EDF in UA, delivery was recommended at 32 weeks or sooner if DV Doppler parameters were abnormal (6). Evident cardiotocographic abnormalities such as recurrent late decelerations or loss of variability and maternal condition such as severe preeclampsia were also indications for delivery. Antenatal steroids were administrated for fetal lung maturity in EO-FGR. Cases with LO-FGR were followed weekly or twice weekly with amniotic fluid volume measurement, Doppler examination and cardiotocography. Induction of labor was performed in case of maternal medical complications, decreased amniotic fluid index, fetal movement and gestational age ≥39 weeks.

Demographic data, obstetric, and perinatal outcomes were evaluated. Incidences of preeclampsia (BP>140/90 on two separate occasions and proteinuria (>300 mg/day) arising de novo after the 20th week of pregnancy),

cesarean section (CS), fetal death (death after 22 completed weeks of gestation and before birth), neonatal death (death before 28 completed days after birth), perinatal mortality (obtained as the sum of fetal and neonatal death), 5-min Apgar score <7, birth weight, umbilical artery pH and admission to the neonatal intensive care unit (NICU) were studied. The diagnosis of intrapartum fetal distress was based on abnormal CTG tracing according to the FIGO classification system (14). The composite adverse outcome for LO-FGR was defined as 5-min Apgar score <7, umbilical artery pH<7.20, emergency CS for fetal distress and neonatal admission to special care unit.

Statistical analysis

Non-parametrical Kruskal-Wallis, Mann-Whitney U and chi-square test were used to compare categorical data and One-way ANOVA or Student's t-test were used to compare non-categorical data as appropriate. Association between the different Doppler indices and perinatal mortality were assessed by binary logistic regression analysis and results were reported as odds ratios (ORs) with their 95% confidence intervals (CI). Receiver operating characteristic (ROC) curves and the area under the curve (AUC) and 95% confidence interval (CI) of the ROC curve were analyzed for continuous variables that contribute to perinatal mortality, and predictive cut-offs were determined. Statistical Package for Social Sciences software version 20.0 for Windows (SPSS Inc., Chicago, USA) was used.

RESULTS

The clinical characteristics and pregnancy outcomes of women with EO- and LO-FGR are presented in Table 1. There were no statistically significant differences between the study groups with respect to maternal age, nulliparity and interval from diagnosis to delivery (p>0.05). Incidences of preeclampsia and abnormal uterine artery Doppler waveform were 20% vs 5.5% and 80.9% vs 9.1% in the EO- and LO-FGR groups, (p<0.01) respectively. Incidences of cesarean section rate, 5-min Apgar score <7, and NICU admission were significantly higher in the EO-FGR group (p<0.01). In the EO-FGR group, there were 12 fetal (11.4%) and 15 neonatal deaths (14.3%) with a perinatal mortality rate of 25.7%. No fetal or neonatal death was observed in cases with LO-FGR.

Perinatal outcomes of fetuses according to gestational age at delivery for the EO-FGR group are illustrated in Table 2 and Figure 1. Fetal deaths occurred between 25 and 27 weeks of gestation (mean 25.7±0.6 weeks), and in all these cases, parents had decided against intervention. The mean interval between diagnosis of EO-FGR and fetal death was 3.1±1.6 weeks (range: 3 days to 5 weeks). Of the 12 fetuses which died in utero, all had ab-

Table 1: The clinical characteristics and pregnancy outcomes of women with early-onset and late-onset fetal growth restriction

	Early-onset FGR	Late-onset FGR	p value
n	105	55	
Maternal age (y)	29.3±4.9	28.3±6.5	0.229
Nulliparity	54 (51.4)	25 (45.5)	0.508
Gestational age at diagnosis (weeks)	26.7±3.4	34.9±1.5	0.000
Diagnosis to delivery time (weeks)	2.9±2.7	2.5±1.5	0.209
Preeclampsia	21 (20)	3 (5.5)	0.015
Abnormal uterine artery Doppler	85 (80.9)	5 (9.1)	0.000
Umbilical artery absent/reversed EDF[a]	55 (52.4)	-	0.000
Gestational age at delivery (weeks)	29.7±2.9	37.4±1.2	0.000
Birth weight (g)	948±428	2375±417	0.000
Cesarean section rate	89/93 (95.7)	17 (30.9)	0.000
5-min Apgar score <7	37/93 (39.8)	1 (1.8)	0.000
Umbilical artery pH	7.30±0.10	7.35±0.04	0.008
NICU[b] admission	87/93 (93.5)	9 (16.4)	0.000
Fetal death	12 (11.4)	-	0.009
Neonatal death	15 (14.3)	-	0.002

Data are expressed as mean±standard deviation or n (%) where appropriate
[a]: EDF; end-diastolic flow, [b]: NICU; Neonatal intensive care unit

Table 2: Perinatal outcomes of fetuses according to gestational age at birth for early-onset fetal growth restriction group

	Gestational age at birth (weeks)				
	25-26 weeks	**27-28 weeks**	**29-30 weeks**	**31-32 weeks**	**33-34 weeks**
n	23	16	21	26	19
Fetal death	11 (47.8)	1 (6.3)	0 (0)	0 (0)	0 (0)
Neonatal death	6 (26.1)	6 (37.5)	2 (9.5)	1 (3.8)	0 (0)
Survival	6 (26.1)	9 (56.2)	19 (90.5)	25(96.2)	19 (100)

Data are expressed n (%)

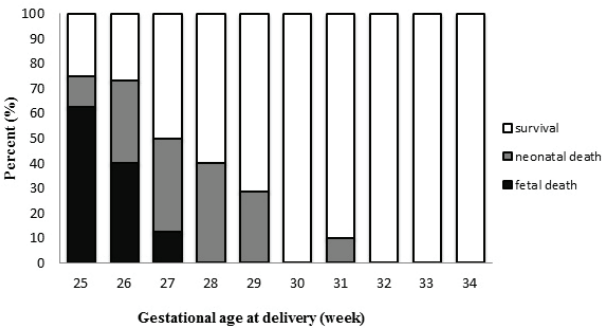

Figure 1: Outcome for fetuses according to gestational age at delivery for early-onset fetal growth restriction group

sent/reversed EDF in UA and 4 (33.3%) had absent/reversed a-wave in DV. In the EO-FGR group, there were 15 neonatal deaths with a mean gestational age at delivery of 27.3±1.5 weeks and mean birth weight of 549±138 g. Causing factors of neonatal deaths were perinatal asphyxia (n=10), RDS (n=2), neonatal sepsis (n=1), and NEC (n=2).

The obstetric characteristics and Doppler features of EO-FGR pregnancies that had perinatal mortality and survival are shown in Table 3. The mean gestational age at diagnosis, delivery and birth weight were significantly higher in pregnancies that survived than with perinatal mortality (p<0.001). ROC curve analysis demonstrated that gestational age greater than 27 weeks (sensitivity 87.5%, specificity 76%, AUC 0.908, p<0.001) and birth

Table 3: The obstetric characteristics and Doppler features of early-onset fetal growth restriction pregnancies that had perinatal mortality and survival

	Perinatal mortality	**Survivors**	**p**		
n	27	78			
Gestational age at diagnosis (weeks)	24.1±2.5	27.6±3.2	0.000		
Gestational age at delivery (weeks)	26.6±1.5	30.8±2.5	0.000		
Birth weight (g)	483±155	1109±371	0.000		
Abnormal uterine artery Doppler	24 (88.9)	61 (78.2)	0.225		
Umbilical artery [a]PI	2.9±0.9	1.6±0.8	0.000		
Middle cerebral artery PI	1.2±0.4	1.5±0.4	0.002		
Ductus venosus [a]PI	1.3±0.4	0.7±0.3	0.000		
Umbilical artery end diastolic velocity				OR[b] (95% CI)	
Absent	14 (51.8)	23 (29.4)	0.037	2.57 (1.05±6.32)	0.039
Reversed	11 (40.7)	7 (8.9)	0.000	6.97 (2.34±20.77)	0.000
Ductus Venosus					
Elevated [a]PI	15 (55.6)	16 (20.5)	0.002	4.51 (1.71±11.92)	0.002
Absent/reversed atrial systolic velocity	7 (25.9)	3 (3.8)	0.001	8.75 (2.07±36.92)	0.003

Data are expressed as mean±standard deviation or n (%) where appropriate
[a:] PI; Pulsatility index, [b:] OR; Un-adjusted Odds Ratio

weight of 665 g (sensitivity 88.8%, specificity 92%, AUC 0.970, p<0.001) provided the best prediction of survival. Mean UA and DV PI were significantly higher and MCA PI was significantly lower in pregnancies that survived than those that had perinatal mortality (p<0.01). ROC curve for the detection of perinatal mortality by UA, DV and MCA PI is illustrated in Figure 2 and the areas under the curve equal to 0.875, 0.867 and 0.729 (p<0.001) for UA, DV, and MCA PI respectively were determined. Incidences of absent/reversed EDF in UA and absent/reversed DV a-wave were significantly higher in pregnancies with perinatal mortality than survival (p<0.01). Logistic regression analysis of UA absent/reversed EDF, abnormal DV Doppler, and absent/reversed DV a-wave revealed ORs of 2.57, 6.97, 4.51 and 8.75, respectively for perinatal mortality in EO-FGR group (Table 3).

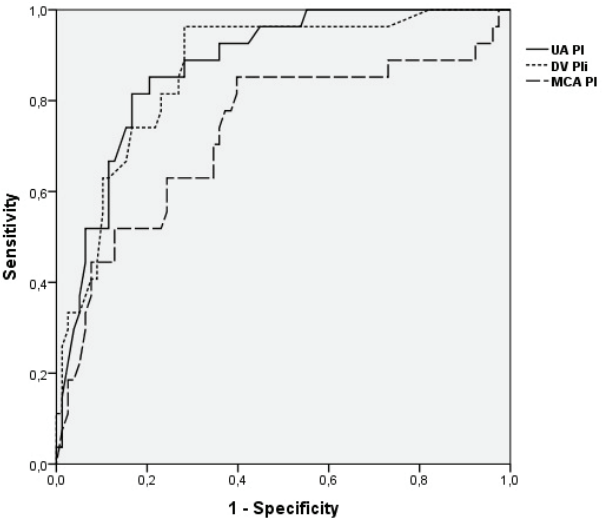

Figure 2: Receiver operating characteristics (ROC) curve analysis of umbilical artery, ductus venosus and middle cerebral artery for the detection of perinatal mortality in the early-onset fetal growth restriction group

In the LO-FGR group, there was no perinatal mortality and 11 (20%) composite adverse outcomes. Of the pregnancies with LO-FGR, 11 had spontaneous vaginal delivery, 9 had CS due to previous CS or breech presentation and 29 had induction of labor, of which 8 was emergency CS performed due to fetal distress. The obstetric characteristics and Doppler features of LO-FGR pregnancies with composite adverse and normal perinatal outcomes are shown in Table 4. The mean gestational age at diagnosis and delivery were not significantly different (p>0.05), whereas mean birth weight was significantly lower in LO-FGR pregnancies with composite adverse outcome than the normal outcome (p<0.001). The incidence of CPR below the 5th percentile was significantly higher in LO-FGR pregnancies with the composite adverse outcome than the normal outcome (p<0.001).

DISCUSSION

The present study, comparable with previous studies, demonstrates that EO- and LO-FGR have completely different perinatal and obstetric outcomes with higher incidences of perinatal mortality, preeclampsia and Doppler abnormalities in EO-FGR pregnancies (3, 15, 16). Defective placentation reflected by abnormal UtA and UA Doppler velocimetry findings is the main cause of EO-FGR, in which pregnancy is not accepted to continue beyond 34 weeks with such improper placenta formation (4, 17). Whereas in LO-FGR, although the placenta is formed properly, villous immaturity seems to be the main cause of mild placental insufficiency with mostly normal UA and UtA Doppler velocimetry (2, 5). We have observed high incidence of UtA Doppler abnormality (81%) and absent/reversed EDF in UA (52%) in EO-FGR pregnancies; however, all of the LO-FGR fetuses had UA PI within normal limits.

In our study group, all of the perinatal mortalities were observed in the EO-FGR pregnancies. Survival rates according to gestational age at delivery were similar to those

Table 4: The obstetric characteristics and Doppler features of late-onset fetal growth restriction pregnancies that had composite adverse outcome and normal outcome

	Normal outcome	Composite adverse outcome	p
n	44	11	
Gestational age at diagnosis (weeks)	35.2±1.5	34.7±2.1	0.380
Gestational age at delivery (weeks)	37.9±1.1	37.3±1.2	0.119
Birth weight (g)	2450±352	1985±282	0.000
Abnormal uterine artery Doppler	4 (9.1)	1 (9.1)	1.000
CPR[a] below the 5th	5 (11.3)	7 (63.6)	0.000

[a]: CPR; Cerebroplacental ratio
Data are expressed as mean±standard deviation or n (%) where appropriate

reported by multicentric TRUFFLE study (18). Gestational age at delivery and birth weight were the strongest predictors of perinatal mortality in the EO-FGR group, these findings were in accordance with the literature (18, 19). Gestational age greater than 27 weeks and birth weight of 665 g provided the best prediction of survival, which also were similar to previous studies (20, 21). Doppler evaluation of fetal vessels has become the primary method of fetal surveillance and management of EO-FGR pregnancies. UA Doppler reflects placental dysfunction, whereas MCA and DV Doppler reflect a brain sparing effect and myocardial dysfunction (4, 6). In our EO-FGR group, UA and DV PI were more effective than MCA PI in predicting perinatal mortality. In accordance with previous studies, our data also supports that MCA PI is unlikely to be helpful for targeting the best time of delivery in EO-FGR (22). Meta-analysis evaluating Doppler indices in EO-FGR fetuses has demonstrated that UA and/or DV absent/reversed EDFs are at a substantially increased risk for perinatal mortality (23). We have observed higher incidences of UA absent/reversed EDF and elevated PI/absent a wave in DV in pregnancies with perinatal mortality than survivors. In the regression model including Doppler parameters of UA absent/reversed EDF, increased DV PI and absent/reversed a wave in DV revealed ORs of 2.57, 6.97, 4.51 and 8.75 respectively for perinatal mortality in our EO-FGR group. Cardiotocography, Doppler examination and biophysical profiles are fetal surveillance methods used in the management of EO-FGR. TRUFFLE trial showed a better outcome by the integrated use of both DV and computerized cardiotocography short-term variation (cCTG-STV) in the management of EO-FGR (18). The authors of the TRUFFLE study emphasized that before 32 weeks of gestation, delaying delivery until there is an absent DV a-wave, abnormalities in cCTG-STV or recurrent decelerations in fetal heart rate is likely to be safe and possibly associated with a more favorable 2-year outcome in EO-FGR (6). As cCTG was not available, the role of this method was not addressed; however, loss of variability and recurrent decelerations in CTG were indications for delivery in our study group. Optimal delivery timing is a challenge in the management of pregnancies with EO-FGR. Gestational age, maternal conditions, CTG and Doppler results should be taken together in the decision making. Our data confirm that UA reversed EDF and absent a wave in DV are highly associated with perinatal mortality.

No perinatal mortality was observed in our LO-FGR group; however, a composite adverse outcome was found in 20% of pregnancies. Adverse neonatal outcomes have been reported in pregnancies with LO-FGR (5, 24). Many recent studies have demonstrated that low CPR is related with a higher rate of cesarean delivery, low Apgar score, neonatal unit admission, and neonatal complications (25, 26). Low CPR reflects a brain sparing affect as a result of cerebrovascular dilation due to hypoxia and such LO-FGR fetuses are more suspectable to delivery complications. We have also observed higher incidence of low CPR in fetuses with the composite adverse outcome than normal outcome. Although the number of LO-FGR pregnancies are limited in our study group, our data supports the role of CPR in the management of LO-FGR pregnancies.

CONCLUSION

EO- and LO-FGR groups pose different perinatal and obstetric outcomes. Optimal timing of delivery is still the main challenge in management of early severe FGR. Birth weight and gestational age at delivery are the most important variables for perinatal outcome. UA reversed EDF and absent DV a wave are highly associated with perinatal mortality. In LO-FGR, CPR <5th percentile is related with a higher risk of delivery complications and may play a role in the management of such pregnancies.

Ethics Committee Approval: This study was approved by the Institutional Ethical Review Board of Istanbul University-Cerrahpasa Cerrahpasa Faculty of Medicine (Date: 15.12.2020, No: 160381)

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Author Contributions: Conception/Design of Study- R.M., V.A., D.K.; Data Acquisition- R.M., V.A., İ.B.O.; Data Analysis/Interpretation- R.M., V.A., D.K.; Drafting Manuscript- R.M., V.A., İ.B.O.; Critical Revision of Manuscript- R.M., V.A., D.K., İ.B.O.; Approval and Accountability- R.M., V.A., D.K., İ.B.O.

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PRETERM LABOR CONDITIONS OF WOMEN WITH HIGH-RISK PREGNANCY AND RELATED FACTORS: A RETROSPECTIVE COHORT STUDY

YÜKSEK RİSKLİ GEBELİKLERDE ERKEN DOĞUM VE İLGİLİ FAKTÖRLER: RETROSPEKTİF KOHORT ÇALIŞMASI

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ABSTRACT

Objective: High-risk pregnancy is a situation associated with pregnancy involving a real or potential risk for the health and well-being of the mother and the fetus. Our study aimed to examine the preterm labor conditions of pregnant women followed up at a high-risk pregnancy outpatient clinic and related factors.

Material and Methods: Designed as a retrospective cohort study, the study was carried out with the participation of 293 pregnant women who presented to a high-risk pregnancy outpatient clinic in the Batman province of Turkiye and were followed up between March 2017 and January 2019.

Results: The top three high-risk conditions determined in the pregnant women were pregnancy over the age of 35 (n=83, 28.3%), pregnancy in a shorter interval than two years (n=71, 24.2%) and consanguineous marriage (n=60, 20.5%), respectively. Twenty-point-one percent (n=59) of the pregnant women had preterm labor. It was determined that the rates of caesarean section births and births at a private hospital among the pregnant women who had preterm labor were significantly higher in comparison to the pregnant women who gave birth at term (p=0.001, p=0.037, respectively). It was determined that the risk of preterm labor increased OR=5.6 (1.2-25.6) times in the pregnant women with anemia, OR=12.5 (1.3-122.7) times in those with intrauterine fertilization and OR=32.6 (3.2-332.5) times in those with multiple pregnancies.

Conclusion: Anemia, multiple pregnancy and intrauterine fertilization increase the risk of preterm labor. Quality prenatal care services are important in terms of protecting the health of the mother and the newborn.

Keywords: Preterm birth, risk factors, anemia, multiple pregnancy, intrauterine fertilization

ÖZET

Amaç: Riskli gebelik annenin ve fetüsün sağlığı veya iyiliği için gerçek veya potansiyel bir tehlikeye sahip hamilelikle ilişkili bir durumdur. Çalışmamızda riskli gebelik polikliniğinde takibi yapılan gebelerin preterm eylem durumu ve ilişkili faktörlerin incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Retrospektif kohort olarak tasarlanan bu çalışma Batman ilinde (Türkiye) riskli gebe polikliniğine başvuran Mart 2017- Ocak 2019 tarihleri arasında takibi yapılan 293 gebe ile gerçekleştirilmiştir.

Bulgular: Gebelerde tespit edilen ilk üç risk durumu sırasıyla 35 yaş üzeri gebelik (n=83, %28,3), iki yıldan kısa aralıklı gebelik (n=71, %24,2), akraba evliliği (n=60, %20,5) şeklindedir. Riskli gebelerin %20,1'inde (n=59) preterm eylem gerçekleşmiştir. Preterm eylem gerçekleşen gebelerin miadında doğum yapan gebelere göre sezaryen doğum ve özel hastanede doğum yapma durumu istatistiksel olarak fazla olduğu tespit edilmiştir (sırasıyla p=0,001, p=0,037). Preterm eylem riski değerlendirildiğinde, anemisi olan gebelerde OR=5,6 (1,2-25,6) kat, intrauterin fertilizasyon olan gebelerde OR=12,5 (1,3-122,7) kat, çoğul gebelerde OR=32,6 (3,2-332,5) kat arttığı tespit edilmiştir.

Sonuç: Anemi, multiparite ve intrauterin fertilizasyon preterm eylem riskini artırmaktadır. Kaliteli doğum öncesi bakım hizmetleri gebe ve bebek sağlığını korumada önemlidir..

Anahtar Kelimeler: Preterm doğum, risk faktörleri, anemi, çoğul gebelik, intrauterin fertilizasyon

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INTRODUCTION

Pregnancy is a process in which women experience changes brought about by new biological, physiological and sociological conditions (1). The World Health Organization (WHO) reported that in 2017, 295,000 women (approximately 810 women per day) lost their lives due to preventable causes associated with pregnancy and birth, and 94% of these deaths occurred in countries with lower and lower-middle income levels (2). In the High-Risk Pregnancy Management Guide prepared by the Turkish Ministry of Health, risk assessment is made under the headings of medical history (e.g., cardiovascular diseases, gynecological diseases, endocrine diseases), obstetric history (e.g., previous uterine surgery, pelvic mass, recurrent miscarriage, eclampsia-preeclampsia) and evaluation of current pregnancy (e.g., younger than 18 years old, older than 35 years old, Rh/rh incompatibility, multiple pregnancy, pregnancy in a period of shorter than two years after previous pregnancy). In the case of the presence of these conditions determined in this context in the pregnant woman, she is regarded as carrying a high risk and is referred to a hospital where an obstetrician is available (3). An important condition that may occur in high-risk pregnancies and affect the fetus is preterm labor. Approximately 11% of the births (around 15 million babies) in the world per year are early-term births (4). Many countries have reported an increase in the rate of early term births in the last 20 years, and this general trend has been verified by global research conducted by the WHO (5, 6). The prevalence of preterm births in Turkiye was determined to be around 12% (7). Compared to the ones born at term, premature babies are at a higher risk during their lifetime in terms of various disorders, including neurodevelopmental disorders, gastrointestinal complications, cerebral palsy, sensory deficiencies, learning difficulties, and respiratory diseases (8).

In this study, it was aimed to determine the causes of preterm labor in high-risk pregnant women. This study had two main hypotheses. The first hypothesis was that the presence of anemia in pregnant women increases the risk of preterm labor. The second was that the risk of preterm labor is higher in pregnancies induced by assistive reproductive techniques.

MATERIALS AND METHODS

This is a retrospective cohort study. The Turkish Ministry of Health has determined risk factors for pregnant women and created a high-risk pregnancy identification form. The population of this study consisted of pregnant women who presented to a high-risk pregnancy outpatient clinic located in the Batman province in Turkiye between March 2017 and January 2019. It was determined that 298 pregnant women presented to the clinic between the specified dates. The inclusion criterion was determined as meeting at least one of the three criteria determined by the Turkish Ministry of Health (medical history, obstetric history, and evaluation of current pregnancy) (Table 1). Pregnant women who were not followed-up (moving to another city, failure to attend clinic follow-up) and those whose pregnancy resulted in a miscarriage were excluded from the study. To elaborate, three of the pregnant women who presented to the high-risk pregnancy outpatient clinic were out of the scope of the study due to moving, one was not attending her follow-up, and one had a miscarriage on the 81st day of her gestation. The study protocol was approved by the Batman State Hospital Ethics Committee (Date: 06.10.2019, No: 200)

The minimum required sample size for this study, which was planned to assess the risk of preterm labor in high-risk pregnant women, was calculated using the G*Power program. A logistic regression analysis, which is a method in the Z-test family, was conducted for the calculation. Based on the result of the study by Rahman et al., with an alpha (α) error margin or 0.05 and an estimated power of 80%, the minimum required sample size was found as 148 participants (9).

Variables

All data for the study were obtained from the records of the high-risk pregnancy outpatient clinic. The risk assessment of the pregnant women was made under three categories determined by the Ministry of Health (medical history, obstetric history, and evaluation of current pregnancy). Seventy-two risk factors in total were inquired about, 19 of which were in the medical history, 17 in the obstetric history and 36 in the evaluation of the current pregnancy. Additionally, information regarding the age of the pregnant women, their parity, gravidity and miscarriage status, vitamin D and iron supplementation status, Td vaccination status and the place and method of birth (vaginal, C/S) was collected. The number of pregnancy follow-ups were calculated over the individuals' visits to the outpatient clinic. The period of pregnancy at labor was calculated according to the time difference between the pregnant woman's last date of menstruation and the date of labor. The pregnant women whose calculated period was <37 weeks were evaluated as preterm labor.

Statistical analysis

The statistical analyses of the study were performed with the Statistical Package for the Social Sciences software, version 25.0 (SPSS). Frequencies and percentages were used for descriptive information on the categorical variables, and means and standard deviations were used for the numerical data that displayed a normal distribution, while the numerical data that did not display a normal distribution is represented by medians (25th percentile-75th percentile). The normality of the distribution

Table 1: High-risk pregnancy definition criteria for pregnant women of the Ministry of Health

Medical history	**Obstetric history**	**Current pregnancy**	
Cardiovascular disease	Uterine surgery	Under 18 years old	Trauma during pregnancy
Gynecological disease	Pelvic mass, myoma, uterine malformation	Over 35 years old	Severe infection anemia
Diabetes mellitus	Recurrent miscarriage	Rh incompatibility	Commandment
Endocrine disease	Low birth weight	Multiple pregnancies	Body mass index>30kg/m^2
Epilepsy	Macrosomic baby history	Pregnancy more than two years	Body mass index<18kg/m^2
Cerebrovascular and neurological disease	Stillbirth, newborn death	Smoking and alcohol use	Abnormal pregnancy after infertility
Psychiatric disease	Preeclampsia or eclampsia	Grand multiparity	Pap smear (+)
Chronic hypertension	Preterm labor	Gestational diabetes mellitus	Cystitis
Respiratory system disease	Postterm birth	Placenta previa	Intrauterine growth restriction
Renal disease	Baby with anomaly	Venous thromboembolism	Inconsistency of uterus size with 80th gestational week
Hematological disease	Gestational diabetes mellitus	Heir	Phenylketonuria
Infectious disease	Venous thromboembolism	Polyhydramnios-oligohydramnios	Intrauterine device and pregnancy
Rheumatological disease	Ectopic pregnancy	Fetus with abnormality	Pregnancy following tube ligation
Venous thromboembolism	Rh incompatibility	Cervical insufficiency	Threat of miscarriage
Neoplasms	Antepartum and postpartum bleeding history	Vaginal bleeding	Gestational hypertension
Orthopedic disorder	Difficult and intrusive birth	Preeclampsia or eclampsia	Height less than 150 cm
Consanguineous marriage	Placenta previa, abruptio placentae	Surgical intervention during pregnancy	Pelvic mass, myoma, or uterine malformation
Drug use and addiction		Hyperemesis gravidarum requiring hospitalization	
Low socioeconomic status		Preterm labor	

of the continuous variables were tested by using visual (histogram and probability charts) and analytical (Kolmogorov-Smirnov Test) methods. In the comparison of the categorical variables in independent groups, Pearson's chi-squared test or Fisher's exact test was used. In the comparison of two independent groups, Student's t-test was employed for the variables with normal distribution, while Mann-Whitney U Test was used for those without normal distribution. The variables that were found to be statistically significant ($p<0.05$) or $p<0.200$) were included in the logistic regression model. In the logistic regression model, the Enter and Backward methods were used. In the model, the correction was made according to age. The results were evaluated in a 95% confidence interval by accepting an alpha error of 0.05.

RESULTS

The mean age of the 293 pregnant women who were included in the study was 29.6±6.4 years, and their ages ranged between 16 and 52 years. The descriptive characteristics of the pregnant women are presented in Table 2. Seven (2.4%) out of the 293 pregnancies resulting in live births were twin pregnancies. Ninety-seven-point-six percent (n=286) of the pregnant women were taking iron supplements, and 79.5% (n=233) were taking vitamin D

Table 2: Distribution of the characteristics of the pregnant women

	Number (%)
Birth	
At term	234 (79.9)
Preterm	59 (20.1)
Birth method	
Vaginal Birth	259 (88.4)
C/S	34 (11.6)
Sex of the baby born (n=303)	
Girl	145 (47.9)
Boy	158 (52.1)
Place of birth	
State hospital	174 (59.4)
Private hospital	119 (40.6)
Total number of pregnancies	
Mean±SD	4.0±2.5
Median (Min-Max)	4.0 (1-15)
Live birth	
Mean±SD	3.2±2.1
Median (Min-Max)	3.0 (0-13)
Miscarriage history	
Yes	126 (43)
No	167 (57)
Pregnancy follow-up number	
<4 follow-ups	56 (19.1)
≥4 follow-ups	237 (80.9)

supplements. It was observed that 9.9% (n=29) of the pregnant women had never been vaccinated against tetanus.

According to the results of the analysis on the medical histories of the pregnant women, 8.9% (n=26) had endocrine diseases, 2.0% (n=6) had chronic respiratory diseases, 1.7% (n=5) had diabetes, 1.4% (n=4) had cardiovascular diseases, 1.4% (n=4) had rheumatic diseases, 1.0% (n=5) had hypertension, and 0.3% (n=1) had epilepsy. Twenty-point-five percent (n=60) of the pregnant women reported that they had a consanguineous marriage. It was also determined that 4.4% (n=13) of the pregnant women had a low socioeconomic level.

According to the results of the analysis on the obstetric histories of the pregnant women, none of them had a history of uterine surgery, low birth weight, fetal macrosomia, post-term birth, antepartum and postpartum birth, venous thromboembolism, difficult and interventional birth, placenta previa, or abruptio placentae. It was observed that 0.3% (n=1) had a pelvic mass, myoma, uterine malformation, 0.3% (n=1) had a baby with a history of anomaly, 0.7% (n=2) had a history of gestational diabetes, 0.7% (n=2) had eclampsia-preeclampsia, 1% (n=3) had a history of preterm labor, 4.8% (n=14) had a history of still birth or newborn morbidity, 6.8% (n=20) had a history of recurrent miscarriage, and 13.7% (n=40) had a history of ectopic pregnancy.

In the analysis on the current pregnancies of the participants, it was determined that there was no case of fetal anomaly, gestational diabetes, cervical failure, eclampsia or preeclampsia, surgical intervention during pregnancy, severe infection, early membrane rupture, abnormal pap smear, intrauterine growth restriction (IUGR), phenylketonuria, intrauterine device (IUD) pregnancy, pregnancy following tube ligation, height less than 150 cm, pelvic mass, myoma, or uterine malformation. Zero-point-three percent (n=1) of the pregnant women had a body mass index of <18 kg/m^2, 0.3% (n=1) had experienced a trauma during pregnancy, 0.7% (n=2) had polyhydramnios-oligohydramnios, 0.7% (n=2) had vaginal hemorrhage, 0.7% (n=2) had hyperemesis gravidarum that required hospitalization, and 0.7% (n=2) had a risk of miscarriage. Moreover, 1.7% (n=5) had placenta previa, 1.7% (n=5) had post-infertility pregnancy, 2.0% (n=6) had preterm labor, 2.4% (n=7) had pregnancy before 18 years of age, 2.4% (n=7) had a multiple pregnancy, 2.7% (n=8) had anemia, 5.5% (n=16) had varicose veins, 9.6% (n=28) had the habit of smoking, 19.8% (n=58) had grand multiparity, 24.2% (n=71) had a pregnancy interval of shorter than two years, and 28.3% (n=83) had pregnancy over the age of 35 years.

It was determined that the frequency of use of the C/S birth method was significantly higher in the pregnant women who had preterm labor in comparison to those who gave birth at term (p=0.001). Besides, it was found that the rates of giving birth at a private hospital in the pregnant women who had preterm labor were significantly higher in comparison to the pregnant women who gave birth at term (p=0.037) (Table 3).

Table 3: The relationship of preterm labor condition with the birth method of the pregnant women and the place of birth

	At term (n=234)	Preterm (n=59)	P
Birth method			
Vaginal	214 (82.6)	45 (17.4)	0.001
C/S	20 (58.8)	14 (41.2)	
Place of birth			
State hospital	146 (83.9)	28 (16.1)	0.037
Private hospital	88 (73.9)	31 (26.1)	

It was determined that the risk of preterm labor was 5.6 times (1.2-25.6) higher in the pregnant women with anemia, 12.5 times (1.3-122.7) higher in those with intrauterine fertilization and 32.6 times (3.2-332.5) higher in those with multiple pregnancy (Table 4).

Table 4: Factors affecting preterm labor condition of the pregnant women

			Univariate		Multiple Logistic Regression/Model 1		Multiple Logistic Regression/Model 2	
Maternal characteristics	**At term (n=234)**	**Preterm (n=59)**	**P**	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**	**OR (95% CI)**
Age (Mean±SD)	30.0±6.8	29.6±6.3	0.663		0.929	1 (0.9-1.1)		
Parity median (25P-75P)	3 (2-5)	4 (2-6)	0.503					
Miscarriage median (25P-75P)	0 (0-1)	0 (0-1)	0.460					
Birth median (25P-75P)	3 (1-4)	3 (2-4)	0.472					
Smoking								
Yes	25 (89.3)	3 (10.7)	0.191	0.5 (0.1-1.5)	0.178	0.4 (0.1-1.5)		
No (ref)	209 (78.9)	56 (21.1)						
Low socio-economic status								
Yes	12 (92.3)	1 (7.7)	0.477					
No	222 (79.3)	58 (20.7)						
Low body mass index ($<18 kg/m^2$)								
Yes	1 (100)	0 (0)	1.000					
No	233 (79.8)	59 (20.2)						
Obstetric history								
Preterm birth								
Yes	2 (66.7)	1 (33.3)	0.492					
No	232 (80)	58 (20)						
In-vitro fertilization								
Yes	1 (20)	4 (80)	0.006	16.9 (154.6)	0.036	11.7 (1.2-116.8)	0.030	12.5 (1.3-122.7)
No (ref)	233 (80.9)	55 (19.1)						
Multiple pregnancy								
Yes	1 (14.3)	6 (85.7)	<0.001	26.4 (3.1-223.7)	0.004	31.2 (3.0-320.8)	0.003	32.6 (3.2-332.5)
No (ref)	233 (81.5)	53 (18.5)						
Anemia								
Yes	3 (37.5)	5 (62.5)	0.010	7.1 (1.7-30.8)	0.027	5.8 (1.2-27.4)	0.027	5.6 (1.2-25.6)
No (ref)	234 (79.9)	54 (20.1)						

Model 1 R^2=0.183, Model 2 R^2=0.173

DISCUSSION

In this study, the prevalence of preterm labor among the pregnant women who were in the risky category determined by the Turkish Ministry of Health was found to be as high as 20.1%. In studies conducted at various centers across Turkiye, it has been reported that the incidence of preterm birth ranged between 10% and 19.1% (10-12). The incidence of preterm birth across Turkiye bears similarity to the incidence of preterm birth in the entire world (11.1%). This incidence ranges from 5% to 11% in European countries (6.7% in Spain, 5.9% in Sweden, 6.6% in France, 5.5% in Finland), while it is around 18% in some African countries (18.3% in Kenya, 14.2% in Tanzania, 12.2% in Nigeria). Among some Asian countries, this rate is 13.0% in India, 7.1% in China and 14.0% in Bangladesh. More than 60% of preterm babies were born in South Asia and Sub-Saharan Africa (6, 7, 13). Preterm birth is also a problem for high-income countries such as the United States (US). According to the Centers for Disease Control and Prevention, one out of every 10 births occurred as preterm birth in 2019 (14). The reason why the prevalence of preterm birth was found to be high in our study may have stemmed from the fact that all pregnant women included in the study were in the risky category, while in the studies mentioned above, pregnant women were included without considering their risk categories. In another study conducted in California, it was determined that women with risk scores ≥3.0 in pre-pregnancy and first trimester training and testing samples had a preterm

labor rate of about 40% (15). In a study conducted in New Zealand, it was determined that women with two or three risk factors (OR=2.87) were at a greater risk of preterm labor (16). Comprehensive studies have defined many risk factors for preterm labor (17, 18). In addition to maternal demographic characteristics such as ethnic origin, age and socioeconomic status, these include pregnancy characteristics such as multiple pregnancy, shortened cervix and urogenital system infections (19). The risk of preterm labor in pregnant women included in our study was found to be higher in those with anemia, those with intrauterine fertilization and those with multiple pregnancy (p=0.027, p=0.030 and p=0.030 respectively) compared to other pregnant women.

Today, the use of assisted reproductive technologies (ART) is quite widespread. One of the results of the popularity of ART is to make three or more embryo transfers to obtain a higher probability of pregnancy and the consequent progressive increase in the incidence of twin, triplet and multiple pregnancies. High rates of multiple births accompanied by complications of prematurity are well-documented (20, 21). According to the National Vital Statistics' report, in the US, preterm birth rates were found as 9.9% for single births, 57.4% for twin births and 92.7% for births involving triplets or more babies (22). In a meta-analysis covering different countries, it was determined that in multiple pregnancies that occurred as a result of in vitro fertilization and intracytoplasmic injection, the highest reported prevalence of preterm birth was observed in Singapore [80.2% (95% CI: 75.3–84.3)], followed by Italy [75.8% (95% CI: 66.0–83.5)], Australia (57.1% (95% CI: 47.2–66.5)], Germany [56.0% (95% CI: 44.0–67.3)] and China [52.8% (95% CI: 48.6–57.1)] (23). In the study conducted by Luke et al. that included a broad population of pregnant women from 14 different cities, the pregnant women who had received IVF treatment had a 1.48 times (95% CI 1.37-1.61) higher chance of having a very early preterm birth (22-27 weeks), a 1.52 times (95% CI 1.45–1.59) higher chance of having an early preterm birth (22-32 weeks) and a 1.48 times (95% CI 1.45–1.15) higher chance of having a preterm birth (22-36 weeks) in comparison to other pregnant women (24). In their study carried out in Massachusetts, Stern et al. determined that ART-treated pregnant women had a 1.40 times (adjusted odds ratio [AOR] 1.40, 95% CI 1.25-1.50) higher change of preterm labor than other pregnant women (25). Another study identified the rate of preterm births (<32 weeks) in infants born as a result of in vitro fertilization as 2 times higher (AOR, 2.13; 95% CI 1.80-2.52) (26). A meta-analysis study reported that in women who got pregnant by receiving IVF or intracytoplasmic sperm injection (ICSI) treatment (singleton pregnancies), the rates of spontaneous preterm labor before 37 weeks and spontaneous preterm labor before 34 weeks increased (respectively, OR 1.75; 95% CI, 1.50-2.03; I^2=39% and OR 1.78; 95% CI, 1.03-3.08; I^2=6%) (27).

Despite the success of mother and child health programs, anemia is still the leading cause of maternal deaths and adverse pregnancy outcomes (e.g., preterm birth, low birth weight) in developing countries. In our study, it was determined that the pregnant women with anemia had a higher risk of preterm labor. Haider et al. determined that the risk of preterm labor increased in pregnant women with anemia by 1.21 times (1.13 to 1.30). Likewise, in another study conducted by Rahman et al., this risk was found to increase by 1.63 times (95% CI: 1.33, 2.01), while Rahmati et al. reported an increase in this risk by 1.56 times (95% CI: 1.25-1.95) (9, 28, 29). A retrospective cohort study in California observed that the children of pregnant women with anemia were born preterm at higher rates (8.9% versus 6.5% adjusted for maternal characteristics and obstetric complications RR 1.3, 95% CI: 1.3–1.4) (30). A study in Taiwan revealed that the preterm labor rates of pregnant women with anemia (Hemoglobin level<10.8 g/dl) increased (adjusted OR: 2.16, 95% CI: 1.54-3.03) (31). A randomized-controlled study carried out in South India reported that the preterm labor risk of women with iron deficiency anemia was more than 3 times higher than other pregnant women (RR: 3.46 (1.81–6.61); p=0.0002) (32).

In a study conducted in Turkiye in 2020, the preterm labor rates were found to be significantly higher in pregnant women with anemia compared to pregnant women without anemia (p<0.05) (33). Another study in Turkiye (provinces of Rize and Istanbul) found higher rates of preterm labor in pregnant women with lower hemoglobin levels (OR, 2.42; 95% CI: 1.07–5.49) (34).

In our study, another factor that increased the risk of preterm labor was identified as multiparity. Muniro et al. determined that grand multiparity increased preterm birth risk (AOR 1.28; CI: 1.05-1.56). Similarly, Koullali et al. reported that preterm birth risk increased in pregnant women who were having their fifth pregnancy (OR 1.26; 95% CI: 1.13-1.41) compared to pregnant women having their second pregnancy (35, 36). However, it has not yet been fully understood how parity affects the incidence of preterm births in terms of its biological mechanism, and studies carried out on the relationship between high parity and adverse pregnancy outcomes have pointed to conflicting results (37). A systematic review performed in 2010 demonstrated that grand multiparity and great grand multiparity were not associated with increased preterm birth risk (OR 0.96, 95% CI 0.77, 1.19 and OR 1.32, 95% CI: 0.61-2.83) (38). A study in China determined that multiparity reduced the risk of preterm labor in comparison to nulliparity (ARR 0.91, 95% CI: 0.89-0.93) (39). Chen et al. showed that nulliparity increased the risk of all stages of preterm labor 1.55 times in comparison to multiparity, whereas Dahman similarly stated that it increased this risk 2.08 (respectively, OR 1.55, 95% CI: 1.52-1.59 and OR 2.08, p<0.002) (40, 41).

Limitations

There were some limitations in our study. As our study was record-based and relied on recorded data, there may have been errors in data entry related to the pregnant women. In our retrospective study, we could not obtain data on social factors that might have affected preterm labor such as education and employment status. We also did not have data on exposure duration and severity of some risk factors that were determined (anemia, smoking), so, this may have prevented us from obtaining definitive results regarding the causes of preterm labor.

CONCLUSION

The prevalence of preterm labor, which is a risk factor, in the pregnant women who were included in this study was found to be higher than the prevalence of preterm birth determined across Turkiye. In our study, in compliance with our hypotheses, it was observed that the risk of preterm labor increased in cases of anemia in pregnant women and pregnancies induced by assistive reproductive techniques. In future studies, investigating the prevalence of preterm labor based on different types of anemia and different types of assistive reproductive techniques will help us understand the etiology of preterm labor in more detail and prevent it.

Ethics Committee Approval: This study was approved by the Ethics Committee of Batman Regional State Hospital (Date: 10.06.2019, No: 200).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- S.P.Y., C.B.; Data Acquisition- C.B.; Data Analysis/Interpretation- C.B.; Drafting Manuscript- S.P.Y., C.B.; Critical Revision of Manuscript- S.P.Y., C.B.; Approval and Accountability- S.P.Y., C.B.

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TRANSFER OF BRACHIALIS MUSCLE TO TRICEPS FOR RESTORATION OF ELBOW EXTENSION IN PATIENTS WITH OBSTETRIC PALSY SEQUELAE

DOĞUMSAL BRAKİYAL PLEKSUS PARALİZİSİNDE BRAKİYALİS TRİSEPS TENDON TRANSFERİNİN SONUÇLARI

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ABSTRACT

Objective: Elbow extension deficit following brachial plexus injuries can sometimes be overlooked. Elbow flexion deficit is more prominent in the early stages of obstetric paralysis and is used as an indication for early nerve surgery. However, in the future extension deficits may become more numerous.

Residual disabilities following obstetric paralyses can be dynamically addressed by tendon transfers. In patients with obstetric paralysis, canonical donor muscles such as the deltoid or biceps may be insufficient for restoration of elbow extension. The brachialis muscle, because of its deep and secluded position, may be considered as one of the more recent options for selection as a donor in these patient groups.

In this study, the efficiency of brachialis to triceps transfer both in elbow extension and in shoulder abduction was assessed.

Materials and Methods: Seven obstetrical palsy patients with varying degrees of sequelae around the shoulder and elbow underwent a brachialis to triceps transfer procedure. All patients had previously undergone a modified Hoffer procedure. Ranges of motion in shoulder and elbow joints were recorded before and after the transfer. A minimum of M3+ in elbow flexion was set as a prerequisite for the transfer.

ÖZET

Amaç: Brakiyal pleksus yaralanmaları sonrası dirsek ekstansiyonu sorunları genellikle ön planda tutulmaz. Obstetrik paralizinin erken evrelerinde dirsek fleksiyon kusurları ön plandadır ve erken dönem sinir cerrahisi endikasyonu için klinik bir belirteç olarak kullanılır. Yıllar geçtikçe fleksiyon işlevinin geri kazanımıyla birlikte dirsekte ekstansiyon kayıpları ön plana çıkabilir.

Tendon transferleri obstetrik paralizi sekellerinin geç dönem dinamik rekonstrüktif tedavi seçenekleri arasında yer almaktadır. Obstetrik paralizili hastalarda deltoid ve biseps gibi klasik donörlerin güçleri dirsek ekstansiyonunu sağlamaya yetmeyebilir.

Brakiyalis kası, kolda derin yerleşimiyle gizli bir alternatif olabilir.

Bu çalışmada dirsek ekstansiyon kusuru sekeli bulunan obstetrik paralizi hastalarında brakiyalis triseps tendon transferinin dirsek ekstansiyonu ve omuz ekstansiyonu üzerine etkileri incelenmiştir.

Gereç ve Yöntem: Çalışmaya dirsek ve omuz eklemleri çevresinde sekelleri bulunan ve bu amaçla brakiyalis triseps tendon transferi uygulanmış yedi obstetrik paralizi hastası dâhil edilmiştir. Tüm hastalara daha öncesinde modifiye Hoffer prosedürü uygulanmıştı. Hastalarda omuz ve dirsek aktif ve pasif eklem hareket açıklıkları gonyometrik olarak analiz edildi. Brakiyalis transferi, minimum dirsek fleksiyon gücü M3+ olan hastalara uygulandı.

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Results: The elbow extension was improved from a median of -70° (interquertile range, IQR: 20°) to a median of -10° (IQR: 35°) in the follow-up ($p<0.05$). Shoulder abduction was improved from a median of 140° (IQR: 5°) to a median of 170° (IQR: 15°) ($p<0.05$). Elbow flexion power was found to be diminished from median M4 (Q1: M3+, Q3: M5) to M3 (Q1: M3, Q3: M3+) ($p<0.05$).

Conclusions: Brachialis to triceps transfer was found to be a suitable alternative in palliative surgery of obstetric palsy patients in terms of elbow extension. Loss of elbow flexion power was within acceptable range.

Keywords: Tendon transfer, obstetric paralysis, elbow joint, shoulder joint

Bulgular: Dirsek ekstansiyonu ameliyat öncesi ortanca -70° (interkuartil açıklık, IQR: 10°)'den ameliyat sonrası izlemde ortanca -10° (IQR: 35°)'ye çıkmıştır ($p<0,05$). Omuz abdüksiyonu ise ameliyat öncesi ortanca 140° (IQR: 5°)'den ameliyat sonrası izlemde ortanca 170° (IQR: 15°)'ye çıkmıştır ($p<0,05$). Dirsek fleksiyonu ise ameliyat öncesi ortanca M4 (IQR: M3+, M5)'den, ameliyat sonrası ortanca M3 (IQR: M3, M3+)'e gerilemiştir ($p<0,05$).

Sonuç: Brakiyalis triseps transferi obstetrik paralizi sekeli bulunan olgularda dirsek ekstansiyonunu sağlamak için geçerli bir seçenek olarak ortaya konmuştur. Bununla birlikte dirsek fleksiyonunda istatistiki olarak anlamlı bir kayıp gözlenmektedir. Bu kayıp klinik olarak kabul edilebilir düzeylerdedir.

Anahtar Kelimeler: Tendon transferi, obstetrik paralizi, dirsek eklemi, omuz eklemi

INTRODUCTION

Functional recovery of the affected limb is the main goal in the treatment of neonatal or obstetric brachial plexus palsy. The term obstetric palsy refers to a clinical condition proposed to result from an antenatal injury to immature brachial plexus including nerve roots, and/or their branches. Although rare (0.06 to 0.26%), the disease is known to cause significant morbidity in the affected population. At least 10 to 30 percent of cases do not achieve complete recovery (1-3, 4).

Various options for treatment of obstetric plexus lesions are available, ranging from hand therapy, direct and indirect neural repair, and surgical interventions addressing the sequelae of the primary lesion which include permanent loss of muscle strength, joint, ligamentous, or bone abnormalities (5).

In the natural course of the disease, many infants show excellent recovery with a full active range of motion in all joints. Some end up with little to moderate disability, predominantly in the upper roots. In rare examples, a devastating flaccid limb with marked disability can be the outcome (4, 6).

The wide range of severity level in disease presentation is further complicated by the diversity of affected muscle groups and age of the patient at presentation. However, some patterns are more prominent in older age groups - such as lack of shoulder abduction (6). The age and problem-based clinical approach to an obstetrical brachial plexus injury patient in our institution is schematized in detail in Figure 1.

The importance of elbow extension following traumatic or obstetric injuries of the brachial plexus is at times understated and has been studied less than elbow flexion (7). The most probable explanation for this may be the contribution of gravity to passive extension of the elbows in the standing position. However, when the shoulder is abducted, raising the hand over the head requires a certain extent of elbow extensionto assure joint stabilization (8).

As most patients with paralytic plexus injury sequelae present with weakness in elbow flexion, a flexion contracture may be seen as a paradoxical outcome (9). However, restoration of elbow extension is an integral part of upper extremity surgical reconstructions. Improvements in completing daily activities such as lowering an object from above, handwriting, steering a wheelchair, driving a car and swimming depend on restoration of elbow extension function (10).

An elbow flexion contracture can be addressed by z-lengthening of the biceps tendon, release of lacertus fibrosus, and brachialis, as well as joint capsule (9). Some reconstructive options previously put forth for elbow extension have stood the effects of time. Examples are transfer of the posterior head of deltoid muscle to triceps (Moberg procedure), and transfer of the biceps to triceps (Friedenberg procedure) (11, 12). However, if those motors are also paralyzed (as is the case with most obstetric paralysis patients), alternative methods of elbow extension restoration become more important (8). However, literature is scarce on this subject (13). Some newer approaches, like the transpositional transfer of brachioradialis to triceps (14), and more recently, transfer of lower trapezius for elbow extension have been suggested (13).

Transfers of the brachialis muscle, with its fairly consistent anatomic location deep in the arm may have some advantages, such as sparing other muscles for further transfers with less morbidity (13, 15).

In their cross-sectional study of patients with various elbow problems, Chuang et al. included 10 patients who had been treated with a brachialis to triceps tendon transfer, and four more who had been treated with trans-

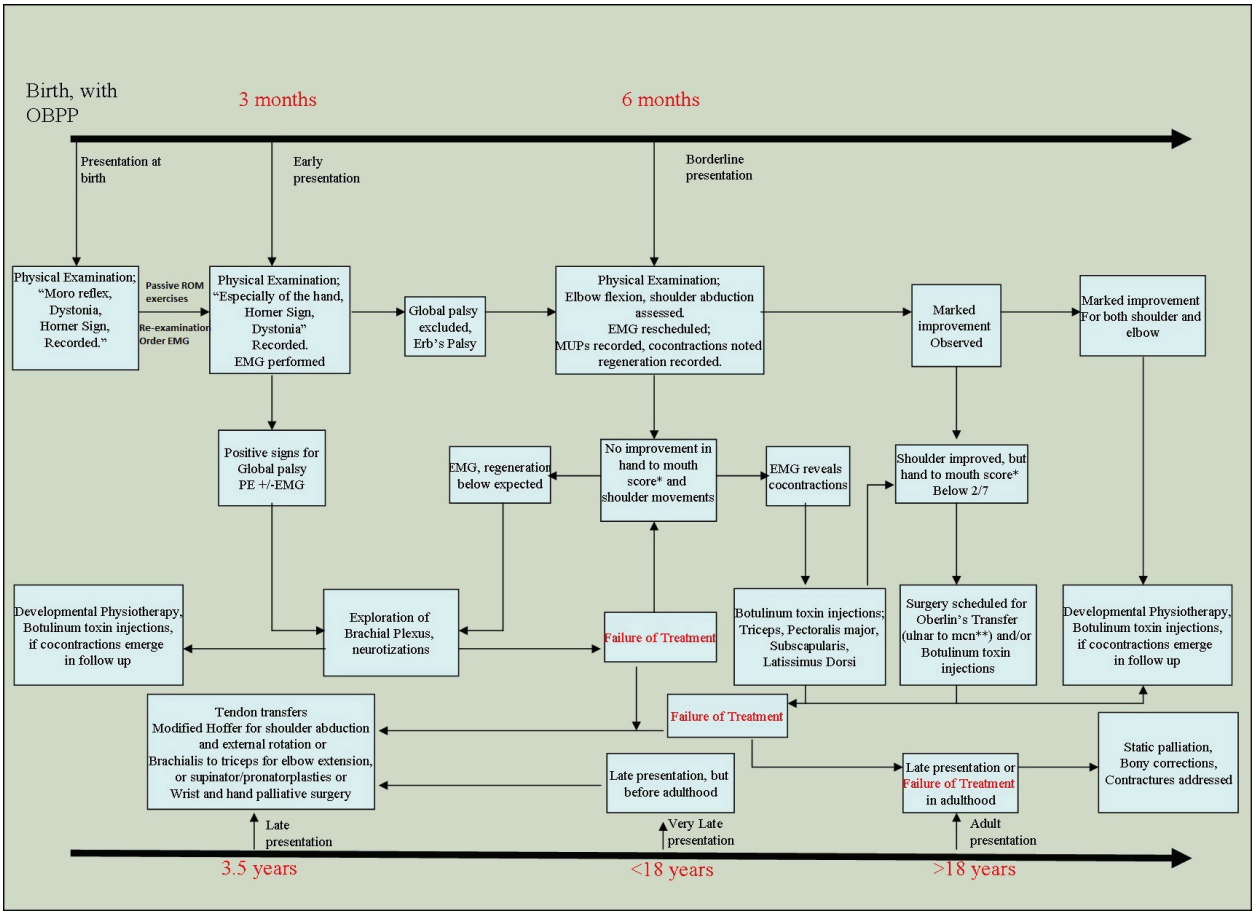

Figure 1: Clinical decision-making progress regarding obstetric brachial plexus palsy patients in our institution
*: A score of four less in the muscle grading system of the Hospital for Sick Children. Lack of elbow flexion against gravity.
**: Partial ulnar nerve transfer to musculocutanous nerve. "mcn", musculocutanous nerve.

fer of both biceps and brachialis (16). However, half of those patients (in both approaches) needed a secondary free vascularized muscle transfer for restoration of elbow flexion (16).

In this study, to restore elbow extension, a brachialis to triceps tendon transfer was performed in seven cases with obstetrical palsy sequelae that had abduction and external rotation deficit in their shoulders and extension deficit in their elbows. All of the patients had previously undergone a latissimus dorsi and teres major transfer to address the deficit in shoulder abduction and external rotation.

MATERIALS AND METHODS

This retrospective case study was approved by Istanbul University, Istanbul Medical Faculty, Ethical Committee for Clinical Research, filed with date and number: 30.11.2021-613417.

This study was conducted in a retrospective fashion. The authors declare that the operative techniques used are scientifically proven and just. The authors also declare that they have read the World Medical Association's Declaration of Helsinki (1964) including its 6th revision (2008) and that this study follows the declaration.

Seven global obstetrical palsy patients with varying degrees of sequelae around shoulder and elbow underwent a brachialis to triceps transfer procedure in our clinic over a two-year period were included. The median age at the time of operation was 6 years, IQR: 10 (min/max, 5/16). All the patients had previously undergone a latissimus dorsi and teres major to greater tubercle of the humerus transfer to improve the shoulder abduction and external rotation with a lapse of two years. Lack of elbow extension against gravity was the indication for the transfer.

Preoperative and postoperative assessments

Active ranges of motion in shoulder and elbow joints, as well as passive ranges of motion in the affected joints (to omit established contractures) were recorded with goniometric analyses by the physiotherapy team in the presence

of the surgical team before the brachialis transfer (after the latissimus dorsi/teres major transfer). The motor capacity of the brachialis muscle was graded according to the British Medical Council (BMC) muscle grading system with modifications made by Terzis et al. (17). A minimum of M3+ in elbow flexion was set as a prerequisite for the transfer.

Active ranges of motion in the joints were assessed with goniometric analyses by the physiotherapeutic team in the presence of the surgical team. Elbow flexion capacity was assessed according to the BMC grading system to note the extent of loss after the transfer.

Surgical technique

The brachialis muscle was dissected through an anterior elbow crease incision preserving the biceps tendon. A careful dissection should be carried out from there on; as medial to the tendon lies the median nerve and brachial artery and lateral to the tendon lies the radial nerve. The distal 1/3 of the brachialis muscle was dissected free after detachment from its insertion at the ulnar bone and transferred posteriorly and laterally to the olecranon area through a subcutaneous tunnel. Another incision was made dorsally at the olecranon area to dissect the distal portion of the triceps tendon. The transferred brachialis muscle tendon was interwoven to the triceps tendon in an end-to-side fashion.

After closure, cast immobilization was applied while the elbow was fully extended. After the operation and a four week period of cast immobilization, physical therapy was initiated with an isolated passive range of motion exercises and splinting until the sixth week. Re-education exercises and electrical stimulation were started at the sixth postoperative week, gentle resistive exercises were initiated between the 8th and 12th weeks and resistive exercises were begun at the 12th week (Figures 2, 3).

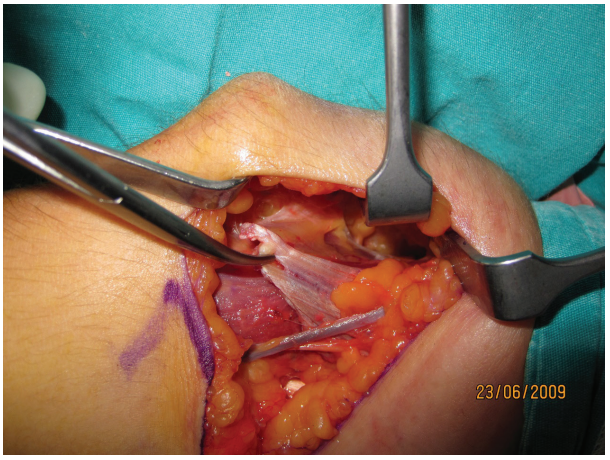

Figure 2: A sufficient excursion of the brachialis muscle is assured before transfer of the muscle laterally and posteriorly to the olecranon region

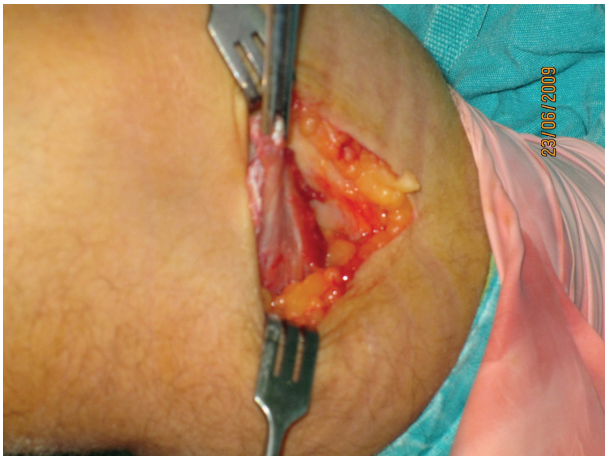

Figure 3: A posterior incision is made above olecranon to visualize the insertion of the triceps tendon. Brachialis tendon is delivered to this incision through a subcutaneous tunnel

Statistical analyses

Statistical analyses were performed using SPSS v. 18.0 (IBM Corp., Armonk, NY, USA). The quantitative assessment of the acquired data was made via descriptive statistics. The changes in elbow extension, shoulder abduction or elbow flexion with surgical intervention were tested by Wilcoxon signed-rank test with a p-value adjusted at 0.05.

RESULTS

Median follow-up was 12 months, IQR: 17 (min/max, 6/23 months). The elbow extension improved from a median of -70°, IQR: 20° (min/max, -90°/-40°; here 0° means full elbow extension while minus degrees dictate extension deficit) to a median of -10° IQR: 35° (min/max, -40°/0°). The improvement in elbow extension was found to be statistically significant ($p<0.05$). Shoulder abduction was improved significantly ($p<0.05$) from a median of 140° IQR: 5° (min/max, 135°/150°) to a median of 170° IQR: 15° (min/max, 165°/180°) (Figure 4).

Elbow flexion power was found to be diminished from a median M4 (interquartile range, M3+ to M5, BMC grading) to a median M3 (interquartile range, M3 to M3+). This downgrading was found to be statistically significant ($p<0.05$). Hand to mouth activity was preserved. The results are summarized in Table 1.

DISCUSSION

Since elbow extension is mostly performed passively in daily life and is of secondary importance when compared to elbow flexion, muscle transfer to triceps is not commonly mentioned in the obstetrical palsy literature. The methods to achieve improved elbow extension in

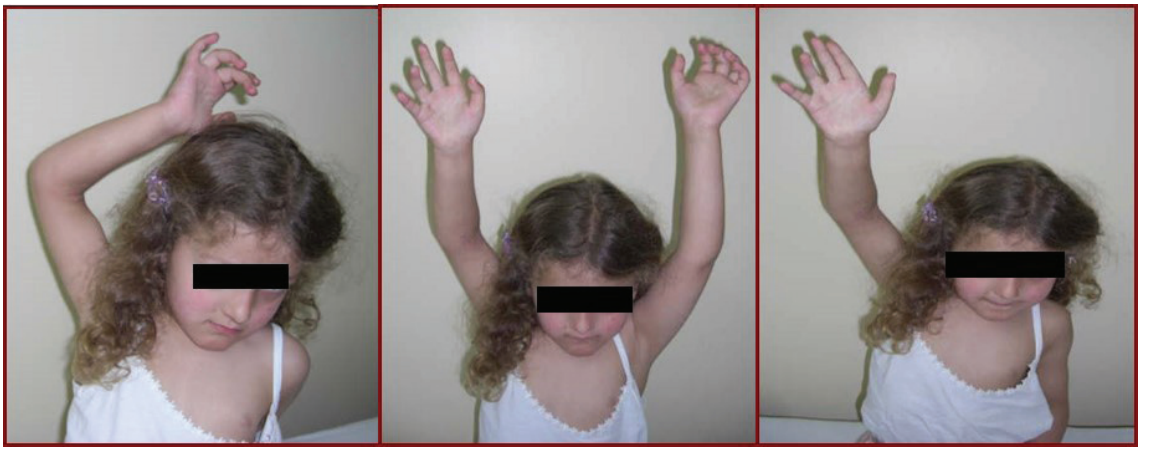

Figure 4: After a brachialis to triceps transfer this patient could achieve an adequate elbow extension and shoulder abduction.

Table 1: Preoperative and postoperative evaluation of the patients that underwent a brachialis to triceps procedure are presented

Patients	Age	Previous surgery	Follow-up, months	Preop active elbow extension (°)	Postop active elbow extension (°)	Preop active shoulder abduction	Postop active shoulder abduction	Preop active elbow flexion	Postop active elbow flexion
1	6	Yes*	12	-80	-40	140	170	M4	**M3+**
2	5	Yes**	12	-70	-10	135	160	M5	**M4**
3	6	Yes*	23	-90	0	145	185	M3+	**M3**
4	5	Yes*	23	-60	-10	140	175	M3	**M3-**
5	16	Yes*	6	-70	-35	150	180	M4	**M3**
6	6	Yes*	23	-40	0	140	170	M5	**M3**
7	15	Yes*	6	-70	-8	140	165	M4	**M3+**
Median	**8.4**		**15**	**-70**	**-10**	**140**	**170**	**M4**	**M3**
P* **				**0.018**		**0.017**		**0.016**	

*: A modified Hoffer procedure to improve shoulder abduction, **: A modified Hoffer procedure to improve shoulder abduction followed by two Botulinum toxin A injections to subscapularis muscle to improve shoulder external rotation, ***: Wilcoxon Signed Rank Test was used to test the significance of postoperative changes of median values.

patients with obstetric palsy include traditional tendon transfers such as biceps, latissimus dorsi, and posterior deltoid to triceps transfers, free gracilis muscle transfer, biceps/brachialis transfer and lower trapezius transfer (12, 13, 16, 18, 19). Brachioradialis muscle transfer was also popularized by our team a while ago, but we prefer to preserve this muscle for future pronatoplasty operations (14).

Utilization of brachialis muscle for transfers around the elbow was first suggested by Chuang, however it was Bertelli who introduced this transfer for elbow extension in a more organized manner (16, 20). The rationale behind using brachialis over biceps was the risk of losing elbow flexion power. This particular risk is also present with a brachialis transfer. In our group of patients, there was minimal donor site morbidity both from a functional and a cosmetic point of view. Elbow flexion power diminished significantly (a downgrade from a median M4 to a median M3) but since biceps tendon was preserved, hand to mouth function was not grossly affected. This may occur as a surprising outcome as the brachialis muscle has a slightly greater physiological cross-sectional area (PCSA) than biceps brachii (5.4 cm^2 and 5.1 cm^2, respectively). However, isometric moment-generating capacity, which is a function of not only PCSA, but also of average

moment arm and pennation angle is greater for biceps brachii than that for brachialis muscle, excluding the extremes of elbow flexion angles (21). Additionally, an anatomic study conducted by Leonello et al. ensured that brachialis muscle constantly has two separate muscle bellies that may enable a partial transfer (15). By doing so, the risk of losing elbow flexion power may be minimized. Another option for restoration of elbow extension may be distant transfers such as a free gracilis transfer (18). A rather lengthy operative time and adding a considerable technical difficulty, these transfers are mainly suitable for recently paralyzed muscles such as traumatic brachial plexus injuries or early obstetric paralyses. However, some patients (as was the case for patients in this study) are late referrals with obstetric paralyses. With their considerable denervation time, a distant transfer may not be suitable without extraplexal neurotisations, which in turn may result in the loss of precious time before physiotherapy, especially if multiple transfers have been made.

Restoration of shoulder abduction with transfer of latissimus dorsi and teres major has proven successful in our clinic (22). Nevertheless, the lack of elbow extension becomes more obvious after this transfer although shoulder abduction and extension are improved dramatically. Realizing that when an "elbow extension splint" is applied, an improvement in shoulder abduction can be achieved, we decided to perform a tendon transfer to mimic the effects of the splint. Anatomically and physiologically, elbow extension did not augment shoulder abduction. However, it was observed that when a splint extended the elbow, the shoulder abduction was higher. This might be due to better stabilization of the elbow joint. The child may have the opportunity to concentrate solely on the shoulder during abduction (This statement was made by Michael Tonkin during the Asia Pacific Hand Society Meeting at Taiwan 2009) (Figure 5).

Utilization of brachialis muscle for various purposes in the upper extremity is a new concept. Not surprisingly, the first attempts to use this rather secluded and powerful muscle, were made to restore elbow extension (16, 20). Chuang et al. reported their results with the transfer of the muscle instead of just releasing it to counteract the powerful elbow flexors in favor of extension, which is not emphasized enough (16). Bertelli, however, carried the concept onwards, describing the technique in detail (20). Our clinic took the next step in utilizing this muscle for various purposes in upper extremity palsies with promising results.

Apart from having been designed in a retrospective fashion, this study has certain limitations, for example,it lacks preoperative and postoperative qualitative measures such as quality of life scores, and functional scores such as the Hospital for Sick Children Scale. Another shortcoming may be the lack of electromyographic studies and having a rather small group of patients. Additionally, to obtain solid evidence on the advantage of this technique compared to the alternatives, a comparative study should be conducted to include alternative methods for elbow extension restoration. Statistical differences among these methods in respect to gains in elbow extension as well as loss in elbow flexion or other donor morbidities should be tested.

Figure 5: Shoulder abduction and elbow extension were both improved markedly after the triceps transfer. Note that no additional interventions were made for shoulder abduction.

CONCLUSION

In selected cases of obstetrical brachial plexus palsy, brachialis tendon transfer to triceps tendon is a promising alternative for restoration of elbow extension, if not, providing elbow stability, which in turn facilitates shoulder abduction.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 30.11.2021, No: 613417)

Peer Review: Externally peer-reviewed.

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THE EFFECT OF SHORT-TERM VIBRATION ON SOMATOSENSORY TEMPORAL DISCRIMINATION THRESHOLD

KISA SÜRELİ VİBRASYON UYGULAMASININ SOMATOSENSORİYEL TEMPORAL DİSKRİMİNASYON EŞİĞİ ÜZERİNE ETKİSİ

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ABSTRACT

Objective: This study will evaluate the changes in the somatosensory temporal discrimination threshold (STDT) after focal muscle vibration. The hypothesis was that the STDT, which is related to the functions of basal ganglia and somatosensory cortex, would deteriorate during application of peripheral muscle vibration if it had indirect central effects.

Materials and Methods: A total of fifteen healthy subjects (mean age 24.3±5.6;18-60) years) were prospectively included in the study. The researchers performed recordings of sensory threshold and the STDT on the second finger before, during, and after vibration in all subjects. A 100 Hz vibration was applied on the forearm flexor muscles for two minutes. The recordings were repeated four times: during, immediately after, one minute after, and three minutes after vibration.

Results: The mean STDT was 95.0±30.0 ms in recordings before vibration. During vibration, the STDT was significantly longer (146.9±52.6 ms) as compared to previbration recordings. However, the STDT value reduced immediately after the vibration and returned to previbration levels at one minute recordings (p=0.001, Friedman test).

Conclusion: The STDT value was longer during vibration. The longer STDT values during vibration suggest that the central effects of vibration can occur either directly or indirectly.

Keywords: Somatosensory temporal discrimination threshold, vibration, central effects

ÖZET

Amaç: Çalışmamızın amacı, fokal kas vibrasyonu sonrası somatosensoriyel temporal diskriminasyon eşiğindeki (STDT) değişiklikleri değerlendirmektir. Hipotezimiz, vibrasyonun dolaylı santral etkileri olması durumunda bazal ganglionlar ve somatosensoriyel korteks fonksiyonları ile ilgili olan STDT'nin, periferik kas vibrasyonu uygulaması sırasında STDT'de değişiklikler oluşabileceğiydi.

Gereç ve Yöntem: Çalışmaya prospektif olarak toplam on beş sağlıklı birey (ortalama yaş 24,3±5,6 years; 18-60 years) dahil edildi. Tüm deneklerde vibrasyon öncesinde, sırasında ve hemen sonrasında işaret parmağında önce duyusal eşik ve sonrasında STDT ölçümleri gerçekleştirildi. Önkol fleksör kaslarına 100 Hz'den iki dakika süreyle vibrasyon uygulandı. Kayıtlar, vibrasyon sırasında, hemen sonrasında, bir dakika sonra ve üç dakika sonra olmak üzere dört kez tekrarlandı.

Bulgular: Ortalama STDT değeri vibrasyon uygulanmadan önce 95,0±30,0 ms idi. Vibrasyon sırasında, STDT önceki kayıtlara kıyasla anlamlı olarak daha uzun olarak bulundu (146,9±52,6 ms). Ancak, vibrasyondan hemen sonra STDT değeri önemli ölçüde azaldı ve bir dakikalık kayıtta vibrasyon uygulanmadan önceki seviyelerine düştüğü gözlemlendi (p=0,001, Friedman testi).

Sonuç: Çalışmamızda, STDT değeri vibrasyon sırasında daha uzun olarak bulunmuştur. Daha uzun süre olan STDT değeri, vibrasyon etkisi altında, doğrudan veya dolaylı olarak titreşim duyusunun merkezi etkisi olabileceğini düşündürmüştür.

Anahtar Kelimeler: Somatosensoriyel temporal diskriminasyon eşiği, vibrasyon, merkezi etkileri

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INTRODUCTION

Vibration is best known to activate group Ia afferent fibers. Direct vibration stimulation on a muscle or tendon enhances muscle spindle activity leading to an excitatory response being known as tonic vibration reflex (TVR) (1, 2). The effect of vibration is not only on sensory nerve endings, but also provides increased neuromuscular activity (3). During high-frequency vibration, numerous skin afferents and interneurons are occupied and inhibition or facilitation on motoneuron are hypothesized to occur indirectly (4).

The effects of vibration have been studied using different electrophysiological parameters and have shown to indirectly affect various levels in the nervous system. When applied on the same muscle, vibration has a strong suppressive effect on H reflex amplitude and excitability (5-7). Vibration caused minor shortening of the cutaneous silent period end latency and duration of second inhibitory phase (8). Vibration also inhibits short interval intracortical inhibition or increases the amplitude of motor evoked potentials (9) whereas no effect was shown on the magnitudes of somatosensory evoked potentials (10). The effects on short interval intracortical inhibition or motor evoked potentials were attributed to the central effects.

This study evaluated the changes in somatosensory temporal discrimination threshold (STDT) after focal muscle vibration. The hypothesis was that STDT, which is related to the functions of the basal ganglia and somatosensory cortex, would deteriorate during application of peripheral muscle vibration if it had indirect central effects.

MATERIALS AND METHODS

The prospective study included fifteen healthy subjects (mean age of the subjects 24.3±5.6 years; age range 18-60 years; 9 men, 6 women). None of the subjects had a history of any neurological or systemic diseases or any medication use. Individuals who have contraindications in terms of electrophysiological studies or diseases that may affect the results such as peripheral nervous system disorders, movement disorders, or use of medications were excluded from the study.

The STDT was recorded in a quiet, low-light laboratory, using a Nihon Kohden 5504 (Japan) device, and commercially available standard bipolar stimulating electrode while subjects were seated.

We first determined the sensory threshold increasing the amplitude from 1mA in steps of 0.5mA. The sensory threshold was defined for each subject by delivering a series of stimuli to the index finger as the minimal intensity perceived by the subject in 5 of 5 consecutive stimuli. In the second step, the STDT was studied by delivering paired stimuli to the index finger that started with an inter-stimulus interval (ISI) of 10 ms and dramatically increasing the ISIs (in 10 ms steps) over experimental procedures already used in previous research (11, 12). The subjects were asked if they could distinguish a single stimulus or two separate stimuli by saying "one" or "two" after each stimulation. The first-time interval in which the subject perceived consecutive stimuli to be two different stimuli was the STDT value. After that, the average STDT value was calculated by taking two more measurements and averaging three measurements.

A 100Hz vibration was applied on the forearm flexor muscles for two minutes. The STDT values were measured again during vibration. We used high-frequency (100 Hz) vibration because higher frequency vibration (~100 Hz) generates a strong illusion of movement with a velocity related to the frequency of vibration. The vibration, 100 Hz in frequency and 1 mm in amplitude, was applied using the Beurer Hand Held Massager (M70, Ulm, Germany).

The recordings of STDT were repeated immediately after, one minute after and three minutes after the application of vibration. The timing of recordings was adapted from previous studies (8, 10).

Data analyses were performed using the SPSS 20 software statistical package (SPSS Inc., Chicago, IL, USA). First, we identified the normality of distribution using the Shapiro-Wilk test. Because the data was non-normally distributed, the STDT values before, during, and after the focal muscle vibration application were compared with the Friedman test and the Wilcoxon test was used for Post-hoc analysis. A p value ≤0.05 was deemed significant.

This study was approved by the Ethical Committee of Istanbul University Cerrahpasa- Cerrahpasa Faculty of Medicine (Date:11.06.2019, No: 86733). All participants gave informed consent.

RESULTS

The mean sensory threshold was 2.3±0.3mA. The mean STDT was 95.0±30.0ms in recordings before vibration. During vibration, the STDT was significantly longer (146.9±52.6ms) compared to the previbration recordings (p=0.001, Friedman test, Table 1). However, the STDT value was reduced immediately after the termination of vibration, and it returned to the previbration levels at one minute and three minute recordings (Tables 1, 2). Figure 1 shows boxplots of the STDT values before, during, and after the vibration.

Table 1: The change of mean STDT values before, during, immediately after, 1 minute after and 3 minutes after the application of vibration (mean±SD)

Parameter	Before	During	Immediately after	1 minute after	3 minutes after	p
STDT (ms)	95.0±30.0	146.9±52.6*	103.1±28.4	95.6±29.6	95.0±27.8	0.001*

*: Friedman test, STDT: somatosensory temporal discrimination threshold, SD: Standard deviation, ms: millisecond

Table 2: The p values of Wilcoxon Signed Ranks test between before vibration and each condition

STDT	p
During	0.00096*
Immediately after	0.284
1 minutes after	0.284
3 minutes after	1

*: Wilcoxon Signed Ranks test, between before vibration and during vibration p<0.001, STDT: Somatosensory temporal discrimination threshold

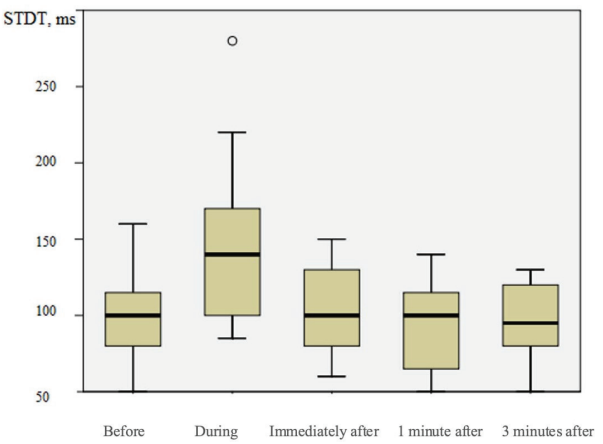

Figure 1: Boxplots of STDT values before, during, immediately after, and immediately, 1 minute after and 3 minutes after the application of vibration.

ms: millisecond

As seen in Figure 1, there was an outlier during vibration recordings. When we repeated the statistics excluding this subject, we obtained the same results.

DISCUSSION

The major finding in this study is that STDT is longer during vibration and the effect of vibration is lost immediately after vibration. Vibration is a powerful stimulator of group Ia afferent fibers. Nevertheless, the effect of the vibration is non-discriminatory and can activate mechanoreceptors other than primary spindle endings (13).

When the skin was vibrated around its resting level, the slowly adapting afferents displayed the same response characteristics as the rapidly adapting and Pacinian afferents. This suggests that the mechanisms underlying the responses of all three mechanoreceptors are similar (14).

The Group Ia afferent of the muscle spindles are activated by the implementation of high-frequency vibration on muscle and tendon directly, this implementation also affects in a smaller degree, the secondary afferents and Golgi tendon organ (15). Cortical areas of the brain receive and operate proprioceptive information when the high-frequency vibration is applied directly, which produces evoked cortical potentials (16). The amplitude of the auditory startle reflex was most likely reduced by the application of continuous high-frequency vibration on the dominant hand (17). But, the latter effect was attributed to the sensory filtering at the brainstem, prepulse inhibition. The activated areas in the brain after muscle vibration are associated with motor function and are responsible for voluntary motor command and sensorimotor integration such as the posterior parietal cortex (18, 19).

In temporal discrimination, there is a role of interaction between the cortical structures, cerebellum, and subcortical structures such as basal ganglia. The important cortical structures are assumed to be the primary somatosensory cortex and pre-supplementary motor area, which focuses attention during the task. Subcortical structures engaged in this task are putamen, superior colliculus, and substantia nigra (20). Keeping in mind that vibration has indirect central effects and the STDT is a function of central structures, this study suggests that vibration may induce longer STDT through its central suprasegmental effects.

However, there are other mechanisms that could have an impact on these results such as habituation, surround inhibition (SI) or collision. For example, the second response is reduced when the two closely timed stimuli are given in a rapid sequence (21). The SI occurs at more than one level of the somatosensory system. In healthy individuals, the sum of the two individual peripherals input is bigger than the size of a dual input. SI is the suppression of the excitability of the area surrounding the active neural network. Through the SI, the motor system facil-

itates the activation of the muscles responsible for the implementation of selected motor programs and inhibits the activation of muscle antagonists that are not targeted (22, 23). In physics, a collision is the abrupt and forceful coming together of two objects through direct contact. When two objects collide, the sum of their momentum before the impact is equal to the sum of their momentum after the impact. In electrophysiology, two distinct types of stimuli may collide, and the result will change the ultimate behavior (24).

There were certain limitations in this study. The STDT was recorded only on the vibrated extremity. The number of subjects included in the study was small. The study findings were not correlated with other electrophysiological measures such as somatosensory evoked potentials or high-frequency oscillations.

In conclusion, vibration might cause longer STDT values through its central suprasegmental effects.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Cerrahpaşa Faculty of Medicine Clinical Research Ethics Committee (Date: 12.06.2019, No: 86733).

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THE INFLUENCES OF LACTOBACILLUS CELL-FREE SUPERNATANTS ON GROWTH AND VIRULENCE PROPERTIES OF *CAMPYLOBACTER JEJUNI* IN HUMAN ADENOCARCINOMA (HT-29) CELL CULTURE

LAKTOBASİLLERDEN ELDE EDİLEN HÜCRESİZ SÜZÜNTÜLERİN İNSAN ADENOKARSİNOM (HT-29) HÜCRE KÜLTÜRÜNDE *CAMPYLOBACTER JEJUNİ*'NİN ÜREME VE VİRÜLANS ÖZELLİKLERİ ÜZERİNE ETKİLERİ

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ABSTRACT

Objective: Lactobacilli are the most commonly used probiotics. We examined the influence of cell-free supernatants (CFSs) of *Lactobacillus acidophilus* (La), *L. fermentum* (Lf), *L. plantarum* (Lp) and *L. rhamnosus* (Lr) on growth, adhesion and invasion of *Campylobacter jejuni* 81116 and RM1221 in human adenocarcinoma colon cells (HT-29). We also analyzed the influences of CFSs, *C. jejuni* and their combinations on HT-29 cell viability.

Materials and Methods: Growth and adhesive-invasive bacteria counts were determined using the spectrophotometric method and colony counting method, respectively. We used methyl thiazolyl diphenyl-tetrazolium bromide (MTT) assay for detection of HT-29 cell viability.

Results: During two and four hours of incubation, the growth of RM1221 was significantly decreased ($p<0.0001$) with the effects of the tested CFSs, while the decrease in growth of the 81116 strain was only significant ($p<0.05$) in the presence of La and Lp. All CFSs except La reduced the growth of both *C. jejuni* isolates at 24 hours of incubation. The adhesion of *C. jejuni* 81116 was significantly ($p<0.0001$) reduced in the presence of all CFSs. La and Lr statistically significantly ($p<0.05$ and $p<0.005$, respectively) reduced the adhesion of *C. jejuni* RM1221. Invasion of *C. jejuni* strains was shown not to be affected in presence of all CFSs. *C. jejuni* and each CFSs were found to influence the Human colon adenocarcinoma cells (HT-29) viability differently.

ÖZET

Amaç: Laktobasiller en yaygın kullanılan probiyotiklerdendir. Çalışmamızda, *Lactobacillus acidophilus* (La), *L. fermentum* (Lf), *L. plantarum* (Lp) ve *L. rhamnosus* (Lr)'nin hücresiz süzüntülerinin (CFS) *Campylobacter jejuni* suşlarının (81116 ve RM1221) üremesi, adezyonu ve invazyonu üzerine etkilerini inceledik. Aynı zamanda, CFS'lerin, *C. jejuni* suşlarının ve CFS+*C. jejuni* kombinasyonlarının HT-29 hücre canlılığındaki etkilerini araştırdık.

Gereç ve Yöntem: Üreme ile adezif ve invazif bakterilerin sayıları, sırasıyla, spektrofotometrik ve koloni sayma yöntemleri ile belirlenmiştir. Çalışmamızda, Metil tiazolil difeni-tetrazolium bromid (MTT) deneyini, hücrelerin (HT-29) canlılığını belirlemede kullandık.

Bulgular: İki ve dört saatlik inkübasyonlarda, tüm CFS'ler RM1221 suşunun, La ve Lp CFS'leri ise, 81116 suşunun üremesini anlamlı düzeyde azaltmıştır (sırasıyla, $p<0,0001$, $p<0,05$). Tüm CFS'ler (La hariç) 24 saatlik inkübasyonda her iki suşun da üremesini baskılamıştır. *C. jejuni* 81116'nin adezyonu tüm CFS'lerin varlığında istatistiksel olarak anlamlı düzeyde baskılanmıştır ($p<0,0001$). *C. jejuni* RM1221'nin adezyonu La ve Lr süzüntüleri varlığında istatistiksel olarak anlamlı düzeyde baskılanmıştır (sırasıyla, $p<0,05$ ve $p<0,005$). Suşların invazyon özellikleri süzüntülerin varlığında etkilenmemiştir. İnsan kolon adenokarsinom (HT-29) hücrelerinin canlılığı hem *C. jejuni*'nin ve hem de her bir CFS'nin varlığında farklı yönlerde etkilenmiştir.

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Conclusion: Our results suggest that CFSs have suppressive effects on the growth and adhesive properties of *C. jejuni* in a time-dependent manner. The viability of HT-29 depends on incubation time and which strain is tested.

Keywords: *C. jejuni*, lactobacilli, growth, adhesion, invasion, cell viability

Sonuç: Sonuçlarımız CFS'lerin *C. jejuni*'nin üreme ve adezyonunu temas süresine bağlı olarak baskıladıklarını göstermektedir. HT-29 hücrelerinin canlılığı inkübasyon süresi ve incelenen suşa bağlı olarak etkilenmiştir.

Anahtar Kelimeler: *C. jejuni*, laktobasiller, üreme, adezyon, invazyon, hücre canlılığı

INTRODUCTION

Probiotics are known as living microorganisms that provide advantages for the host's health by stimulating the immune system, competing with pathogens for receptor binding and production of acids, bacteriocins, bio-surfactants and hydrogen peroxide to inhibit pathogens (1–4). In recent years, many studies have reported the roles of probiotics not only as supportive therapeutics but also as an alternative treatment method for infectious diseases, gastrointestinal tract diseases and control of oral health. Lactobacilli are known as the most commonly used probiotic microorganisms (5-7). Although they are known to have inhibitory effects on pathogens, the effects of their products have not been investigated extensively on *Campylobacter jejuni*.

C. jejuni, a foodborne pathogen, causes gastroenteritis and can be responsible for post-infectious complications in humans (8, 9). Adhesion-invasion mechanisms of the bacterium are very important during infectious processes and are related to cell death and the distribution of mucosal barriers in the host (10, 11). The increase of antibiotic-resistant strains is an important problem that leads to public health concerns and an economic burden (8, 9). Therefore, alternative and supportive options need to be considered. There are studies that present promising results to cope with stages of infection using the antagonist relationship between probiotic microorganisms and pathogens (12-20).

This study aimed to investigate the influence of lactobacilli cell-free supernatants (CFSs) on the growth and virulence properties (adhesion and invasion abilities) of two *C. jejuni* strains (81116 and RM1221) in the human adenocarcinoma cells (HT-29), mimicking host conditions. The study also examined the effects of *C. jejuni* strains and CFSs of lactobacilli, together or separately, on the viability of HT-29 cells.

MATERIALS AND METHODS

Bacteria and preparing of cell-free supernatants

Two *Campylobacter* strains *(C. jejuni* 81116 and RM1221) were kindly provided by Dr. György Schneider, (University of Pécs, Hungary). *C. jejuni* strains were grown in Brucella broth (BB) (Besimik, Turkiye) under microaerophilic conditions at 37°C for 48 hours.

Lactobacilli (*Lactobacillus acidophilus* ATCC 314-La, *L. fermentum* ATCC 9338-Lf, *L. plantarum* ATCC 14917-Lp and *L. rhamnosus* ATCC 53103-Lr), which are commonly sold in pharmacies and markets, were examined (21, 22).

De-Man Rogosa-Sharpe (MRS) broth (Conda, Spain) was used on growth of Lactobacilli under anaerobic conditions at 37°C for 24 hours. Following overnight cultivation of *Lactobacillus* strains, supernatants were collected via centrifugation at 4000 rpm for 30 minutes at 4°C, then filtered with 0.2 µm pore size filters (12, 23).

Cell culture

Human colon adenocarcinoma cells (HT-29) were used in our experiments and specific cell culture conditions as previously defined (13, 15).

HT-29 cells were seeded in 96-well microplates for bacterial growth and cell viability assay, seeded in 24-well plates for invasion and adhesion experiments. To provide a confluent monolayer cell culture, density was adjusted as approximately 5×10^4 cells for 24-well and 1×10^4 cells for 96-well plates. Plates were incubated at 37°C, under 5% CO_2 conditions for 24 hours.

Infection of HT-29 cells with *C. jejuni*

The overnight cultures of *Campylobacter* isolates were prepared in Brucella broth at 37°C. For infection of HT-29 cells, a suspension of approximately 10^9 CFU/mL of each strain was used.

Before inoculation of *C. jejuni*, Dulbecco's Modified Eagle Medium (DMEM) containing antibiotics was replaced with antimicrobial solution-free DMEM and CFSs were added into each well (20 µL in each 96-well plates and 50 µL in each 24-well plates). The plates were incubated for one hour at 37°C.

HT-29 cells were inoculated with *C. jejuni* and microaerophilic conditions were provided for incubation (as seen below). All assays were performed three times.

Bacterial growth

Bacteria were incubated for two, four and 24 hours to investigate the alterations of growth in the presence/absence of CFSs. The influence of each CFSs was detected by measuring the changes in absorbance at 600 nm. Bac-

terial growth in cell culture with CFSs was compared to cell culture without CFSs (as negative control).

Bacterial adhesion and invasion
HT-29 cells were incubated with *C. jejuni* for three hours at 37°C under microaerophilic conditions. The effect of each CFS on bacterial adhesion and invasion was determined by comparing colony counts (as CFU/mL) from cell lysates of HT-29 grown in the presence/absence of CFSs. We determined colony counts of adhesive and invasive bacteria as described previously (24, 25).

Bacterial adhesion
Phosphate buffer saline (PBS) was used to wash the wells three times to remove unbound bacteria after incubation for three hours. Then, the cells were lysed using 500 µl 1% Triton X-100 for 10 minutes at 37°C under 5% CO_2 conditions. Following the homogenization and inoculation of cell lysates on Mueller–Hinton agar (MHA) (Spesera, Turkiye) supplemented with sheep blood (5% defibrinated), media was incubated at 37°C for 48 hours under microaerophilic conditions.

Bacterial invasion
After bacterial inoculation and three hours incubation, the wells were washed with PBS three times. Then, a medium supplemented with 300 ng/ml gentamicin was added to each well for killing non-invasive (extracellular) bacteria. Microaerophilic conditions were provided for incubation of the plates and they were incubated for two hours at 37°C. The lyses of HT-29 cells were provided using Triton X-100 as mentioned above. For detection of invasive bacteria, the homogenized cell lysates were inoculated on MHA (Spesera, Turkiye) supplemented with sheep blood and incubated for 48 hours under microaerophilic conditions at 37°C.

Viability of Human adenocarcinoma colon cells (HT-29)
A methyl thiazolyl diphenyl-tetrazolium bromide (MTT) assay was used for detecting cell viability. The effects of CFSs and *C. jejuni*, together and separately, on the viability of HT-29 were investigated. Experimental conditions were prepared as mentioned above and HT-29 cells were incubated for 24 and 48 hours under microaerophilic conditions.

Following incubation, the wells were washed with PBS three times to remove residue. Then, a fresh culture medium was added. According to Mosmann, MTT was prepared (12 mM, Neofrox 3580 MTT) and added into each well. The HT-29 cells were incubated at 37°C for four hours under microaerophilic conditions (26).

After incubation, the media were aspirated to remove the content from the wells. Then, Dimethyl sulfoxide (DMSO) was added into each well. The plates were incubated at room temperature for 10 minutes to dissolve the formazan crystals into a colored solution. Lastly, absorbance values were measured at 540 nm via the spectrophotometric method.

The cell viability of HT-29 was investigated by comparing the absorbance values of dissolved formazan crystals produced by HT-29 cells in the presence/absence of CFSs or *C. jejuni*, separately and together.

Statistical analysis
The significant differences between experimental conditions and control conditions were calculated. Results were analyzed with two-way ANOVA followed by Dunnett's multiple comparisons test for growth alterations. One-way ANOVA followed by Dunnett's multiple comparisons test was performed for adhesion and invasion results. Alterations of cell viability were detected by two-way ANOVA followed by Dunnett's and Sidak's multiple comparisons test. The significant differences between experimental conditions and control conditions were evaluated. All results were presented as mean±SD. Differences with p values less than 0.05 were accepted as indicative of statistically significant differences.

RESULTS

Bacterial growth
After incubation for two and four hours, CFSs of La and Lp significantly reduced ($p=0.046$ and $p=0.039$, respectively for two hours and $p=0.043$ and $p=0.029$, respectively, for four hours) the growth of *C. jejuni* 81116 strain; all CFSs decreased significantly ($p<0.0001$) the growth of *C. jejuni* RM1221 (Figure 1a, Figure 1b).

According to the 24 hour incubation results, the growth *C. jejuni* 81116 strain was statistically significantly decreased ($p=0.0034$ for Lf, $p<0.0001$ for Lp and $p=0.0025$ for Lr) in the presence of all CFSs except for La. It was found that all CFSs were shown to decrease ($p<0.0001$) the growth of *C. jejuni* RM1221 statistically significantly. Furthermore, the most effective inhibition was seen for *C. jejuni* RM1221 in the presence of each CFSs at 24 hours incubation (Figure 1b).

Bacterial adhesion
The adhesion of *C. jejuni* 81116 was found to be significantly reduced ($p<0.0001$) in the presence of all CFSs. La and Lr CFSs' were found to significantly reduce ($p=0.0068$, and $p=0.026$, respectively) the adhesion of *C. jejuni* RM1221 (Figure 2).

Bacterial invasion
The effects of all CFSs on bacterial invasion were found statistically insignificant ($p>0.05$) for both *C. jejuni* strains (Figure 3).

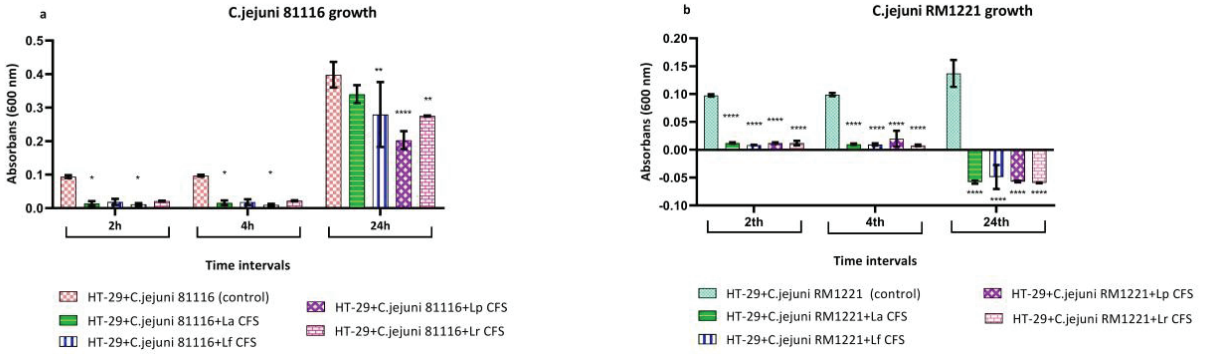

Figure 1: The influences of CFSs on the growth of *C. jejuni*

The growth of *C. jejuni* grown in HT-29 with and without CFSs were examined using two way ANOVA followed by Dunnett's multiple comparisons test.

*, **, ****: Significance levels were as p<0.05, p<0.005 and p<0.0001, respectively.

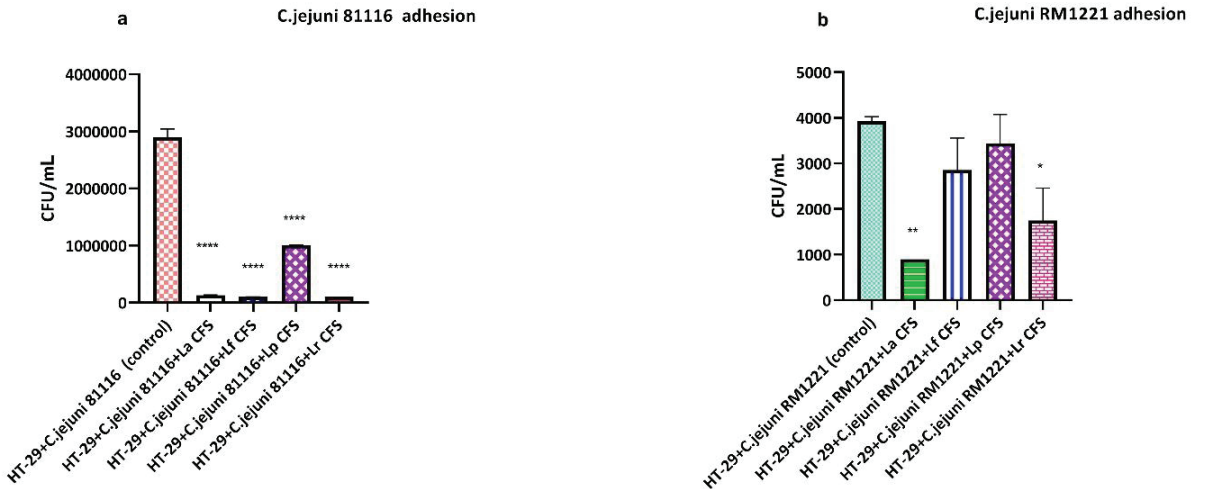

Figure 2: The influences of CFSs on adhesion of *C. jejuni*

The adhesion of *C. jejuni* in HT-29 with/without CFSs was analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test.

*, **, ****: Significance levels were as p<0.05, p=0.0068 and p<0.0001, respectively.

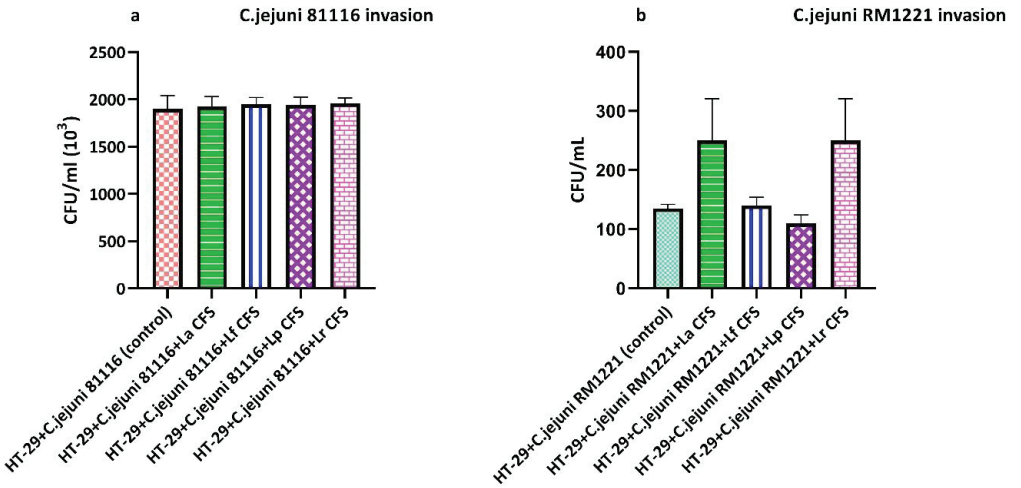

Figure 3: The influences of CFSs on the invasion of *C. jejuni*

The invasion of *C. jejuni* in HT-29 with/without CFSs was analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test.

Viability of HT-29 cells

According to the 24 hour incubation results, the viability of HT-29 cells infected with *C. jejuni* RM1221 increased ($p<0.0001$). The HT-29 cell viability was shown to be decreased significantly ($p<0.0001$) by each CFSs (Figure 4).

After 48 hours, the HT-29 cell viability was significantly reduced in the presence of CFSs (Lf- $p=0.001$, Lp- $p<0.0001$ and Lr- $p=0.0005$) and *C. jejuni* RM1221 ($p=0.0097$), separately (Figure 4).

The influence of *C. jejuni* 81116 on HT-29 cell viability was found to be insignificant ($p>0.05$) on both at 24 and 48 hours (Figure 4).

According to the 24 hour incubation results, we found that La, Lp and Lr CFSs statistically significantly decreased the viability of *C. jejuni* 81116 infected HT-29 cells ($p=0.01$, $p=0.001$ and $p=0.0036$, respectively). According to the results of the 48 hour incubation, the viability of HT-29 cells infected with *C. jejuni* 81116 was found to be significantly decreased ($p=0.008$, $p=0.001$, respectively) in the presence of La and Lf CFSs; however, Lr was found to significantly ($p<0.0001$) increase the viability of infected HT-29 cells (Figure 5a).

According to the results of the 24 hour incubation, it was found that all CFSs statistically significantly ($p<0.0001$) reduced the viability of *C. jejuni* RM1221 infected HT-29 cells. After 48 hours of incubation, we found that CFSs of La, Lf ($p<0.0001$) and Lp decreased (p: 0.0004) the viability of *C. jejuni* RM1221 infected HT-29 cells (Figure 5b).

DISCUSSION

Our study showed inhibitory effects of CFSs, obtained from the *Lactobacillus* species on *C. jejuni* isolate in human adenocarcinoma (HT-29) cell culture, mimicking host conditions. Numerous studies have reported that *L. acidophilus, L. gasseri, L. fermentum, L. johnsonii, L. reuteri, L. crispatus, L. paracasei, L. plantarum,* and *L.salivarius* or their cell-free supernatants repress the growth of the *Campylobacter* species (15, 27-32). Consistent with previous results, our findings showed that at the first four hours of incubation, the growth of *C. jejuni* RM1221 was reduced by all lactobacilli CFSs, while *C. jejuni* 81116 was only significantly reduced by La and Lp CFSs in HT-29 cell cultures. After 24 hours, all CFSs reduced the growth of both *C. jejuni* RM1221 and 81116 strains, except CFSs of La, which did not alter the growth of *C. jejuni* 81116.

It is well known that adhesion is one of the most important stage for the colonization of colon cells by microbes. Previous studies have found that *L. acidophilus, L. casei, L. rhamnosus* and *L. plantarum* decreased the adhesion of *C. jejuni* strains while *L. rhamnosus* and *L. salivarius* did not prevent the adhesion of *C. jejuni* (15, 29, 33-36). In our study, we found that all CFSs decreased the adhesion of *C. jejuni* 81116 to HT-29 cells while the adhesion of *C. jejuni* RM1221 strain was found to be decreased in the presence of only CFSs of La and Lr. Although anti-adhesive properties of CFSs are shown to be strain-specific, we may conclude that lactobacillus strains have an inhibitory effect on the adhesion of *C. jejuni* strains in general.

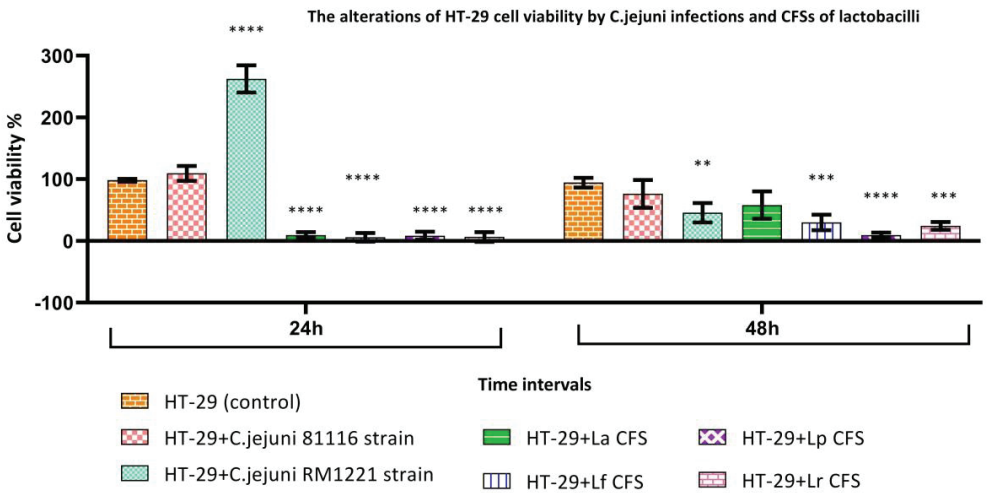

Figure 4: The influences of CFSs and *C. jejuni* infections, separately on HT-29 cell viabilities

The cell viabilities with/without CFSs were analyzed using two-way ANOVA followed by Dunnett's multiple comparisons test
,*,****: Significant at $p=0.0097$, $p\leq0.001$ and $p<0.0001$ levels, respectively.

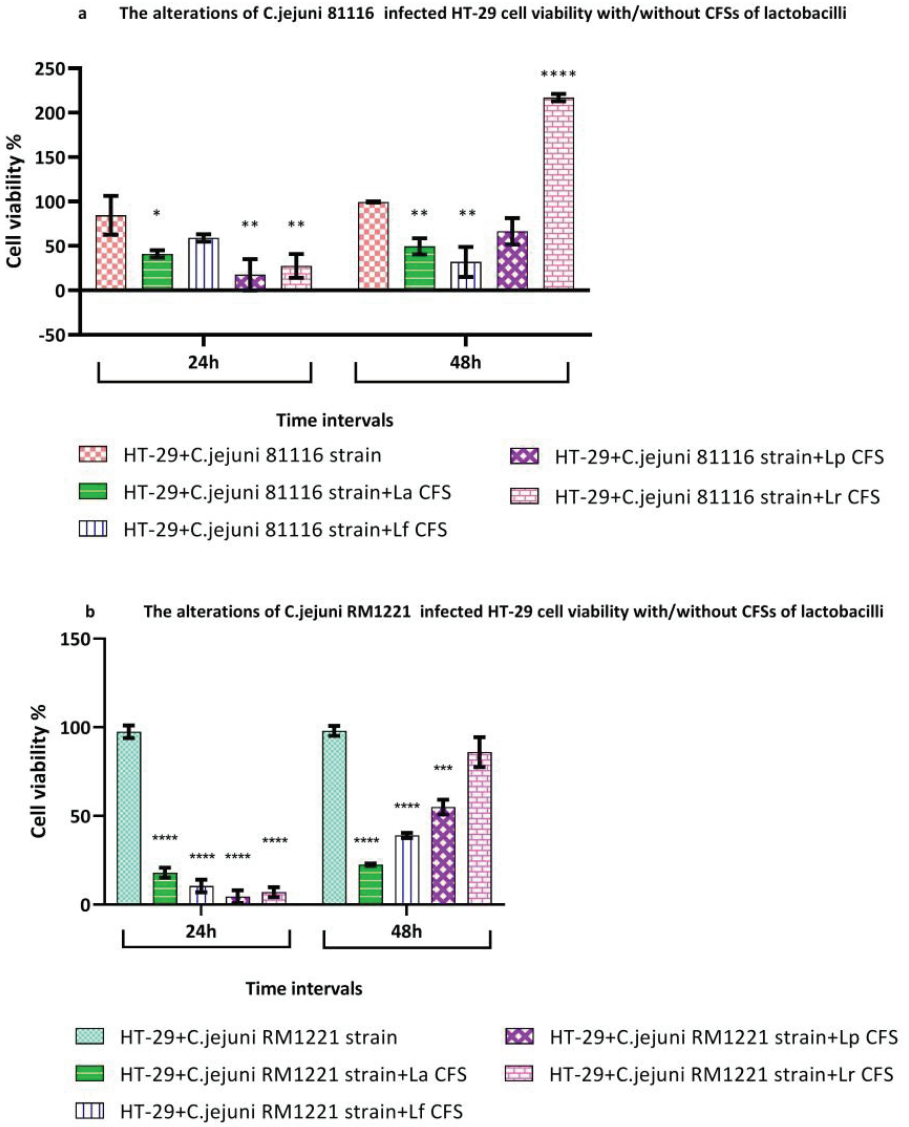

Figure 5: The influences of CFSs and *C.jejuni* infection co-presence on HT-29 cell viabilities

The cell viabilities with/without CFSs were analyzed using two-way ANOVA followed by Sidak's and Dunnett's multiple comparisons tests, respectively

*, **, ***, ****: Significant at p=0.01, p≤0.008, p<0.0005 and p<0.0001 levels, respectively.

Furthermore, invasion is another important stage during the infectious process. Although there are a limited number of studies investigating the effects of lactobacilli and their products on the invasion of *C. jejuni*, it appears that their effects are commonly defined as repressive. According to previous findings, *L. helveticus, L. acidophilus, L. paracasei, L. rhamnosus, L. lactis, L. gasseri* and *L. salivarius*, decrease the invasion of *C. jejuni,* but *L. rhamnosus* does not exhibit the same effect (10, 15, 37). Consistent with Wine et al., we showed that CFSs did not alter the invasion of two *C. jejuni* strains tested (37).

In our study we did not analyze which mechanisms were responsible for the inhibition of adhesion and invasion processes. However, previous studies have proposed that probiotics could exclude and/or displace the pathogens in a competitive way (15, 37).

The influence of CFSs, *C. jejuni* and their combinations on HT-29 cell viability were also analyzed in our study. Many studies have reported that lactobacilli and their products affect the host cell viabilities (PSI cl.1, B1OXI, CLAB, Caco-2, HOB, HT-29, HeLa, AGS, MCF-7 and CF cell lines) (13, 34, 39-41). While Pogačar et al. showed that

L. plantarum and *L. rhamnosus* strains did not have any cytotoxic effects on pig and chicken epithelial cells at 24 hours of incubation, Kalaycı-Yüksek et al. reported that CFSs of La, Lf and Lp decreased the viability of HOB cells for three hours of incubation (13, 34). Consistent with earlier research the viabilities of AGS, MCF-7, HT-29 and HeLa cells were found to be gradually reduced depending on incubation and concentration of *L. acidophilus* CFS (13, 41). We found in our study that all CFSs, except La, decreased the viability of HT-29 cells at both incubation periods. Presumably, the effects of lactobacilli and/or their products on cell viability may be related to their acidic pH.

It has been shown that *C. jejuni* causes a cytotoxic effect on pig and chicken epithelial cells (34). However, Bouwman et al. have shown that different *C. jejuni* strains did not induce any cytotoxicity on macrophages. Interestingly, our results have shown that the effect of *C. jejuni* infection on the viability of HT-29 cells is strain-dependent. While *C. jejuni* 81116 did not affect the viability of HT-29 cells at both incubation periods, *C. jejuni* RM1221 increased at 24 hours. However, HT-29 cell viabilities were found to be decreased if the exposure was prolonged to 48 hours. We assume that cell viability is associated with exposure time, types of infected cell lines, and biological properties of *C. jejuni* strains tested.

Moreover, we investigated the effects of CFSs in combination with *C. jejuni* infection on host cell viability. *L. plantarum* and *L. rhamnosus* were shown to have protective effects on the viabilities of *C. jejuni* infected pig and chicken epithelial cells at 24 and 48 hours of incubation. It has also been shown that different lactobacilli combinations decreased the cell viabilities which were infected with *C. jejuni* at both 24 and 48 hours of incubation (34). Consistent with these findings, in our study, CFSs of La, Lp and Lr decreased the viability of HT-29 cells infected with *C. jejuni* 81116 at 24 hours of incubation. However, at 48 hours of incubation, the viability was increased in the presence of Lr CFSs. Furthermore, CFSs of Lf acted as a suppressive on the viability of *C. jejuni* 81116 infected-HT-29 cells. Similar results were observed on the HT-29 cell viabilities infected with *C. jejuni* RM1221 at 24 hours incubation. All CFSs decreased HT-29 cell viability, but suppressive effects of Lr disappeared when incubation was prolonged to 48 hours. It is clear that the influence of CFSs on the viability of infected HT-29 cells is strain-dependent.

In conclusion, the present study demonstrated that CFSs obtained from the *Lactobacillus* species showed inhibitory effects on *C. jejuni* growth and adhesive properties in cell culture. Furthermore, in this study, we found that CFSs have a suppressive effect on the viability of infected/non-infected HT-29 cells which may be related to the acidic properties of CFSs. Although our results showed that the inhibitory effects of CFSs vary depending on exposure time and strains, it is possible to suggest that their inhibitory effects on the biology of *Campylobacter* infections may be taken into consideration.

However there were some limitations in our study. Further clarity is needed as to which inhibitory products of lactobacilli have the most effective roles on pathogens. Also, molecular aspects could identify the mechanisms which are affected during these interactions. In this frame, our findings provide preliminary insights for *in vivo* future studies to focus on the identification of these inhibitory roles of lactobacilli.

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ANTI-TUBERCULOSIS TREATMENT IN PRESUMED OCULAR TUBERCULOSIS PATIENTS WITH GRANULOMATOSIS OCULAR PATHOLOGIES: SINGLE CENTER EXPERIENCE FROM TURKIYE

GRANÜLOMATOZ GÖZ PATOLOJİSİ SAPTANARAK GÖZ TÜBERKÜLOZU KABUL EDİLEN HASTALARDA ANTİTÜBERKÜLOZ TEDAVİ: TÜRKİYE TEK MERKEZ DENEYİMİ

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ABSTRACT

Objective: Ocular tuberculosis is still considered to be a controversial issue in terms of both terminology and diagnostic criteria and treatment options. There is no common diagnostic/treatment algorithm even though new approaches exist.In this study, the results of the treatment of "presumed ocular tuberculosis patients" are evaluated.

Materials and Methods: Anti-tuberculosis treatment was given to cases who had latent tuberculosis infection, had no other diagnosis and who did not respond to steroid treatment. These cases were considered to have presumed ocular tuberculosis.

Results: Sixty four cases who were considered to have "presumed ocular tuberculosis" received anti-tuberculosis treatment for a minimum of three months and were followed up regularly. The 64 cases were made up of 32 women and 32 men, with a mean age of 42.1±12.09 years. Mean antituberculosis treatment duration was 8.18±2.17 months (3-15 month). After the treatment, 47 (73.4%) of 64 patients reported improvement in vision. Only 36 of these 64 cases (31 bilateral, 5 unilateral, 67 eyes in total) were evaluated with objective vision test before and after the treatment. Disease involvement was uveitis (anterior, intermediate, posterior, panuveitis) in 61 eyes (91%), and retinal vasculitis in 6 eyes (9%). According to the vision analysis among 67 eyes, 42 (62.7%), visual acuities were detected to be improved

ÖZET

Amaç: Göz tüberkülozu hem terminoloji, hem tanı kriterleri hem de tedavi seçenekleri açısından halen tartışmalı bir konu olarak kabul edilmektedir. Yeni yaklaşımlar olmasına rağmen ortak bir tanı/tedavi algoritması bulunmamaktadır. Bu çalışmada "Göz tüberkülozu hastalarının" tedavi sonuçları değerlendirilmiştir.

Gereç ve Yöntem: Latent tüberküloz enfeksiyonu olan, başka tanısı olmayan, steroid tedavisine yanıt vermeyen ve göz tüberkülozu düşünülen olgulara antitüberküloz tedavisi verildi.

Bulgular: Göz tüberkülozu düşünülen ve en az üç ay süreyle antitüberküloz tedavisi gören ve düzenli takipleri yapılan 64 olgu (32 kadın, 32 erkek, ortalama 42,1±12,09 yıl) değerlendirildi. Ortalama antitüberküloz tedavi süresi 8,18±2,17 ay (3-15 ay) idi. Tedaviden sonra 64 hastanın toplam 47'si (%73,4) görmesinde iyileşme bildirdi. Bu hastaların sadece 36'sında (31 bilateral, 5 tek taraflı, toplam 67 göz) tedavi öncesi ve sonrası objektif görme değerlendirmesi yapılabildi. Bu hastalarda hastalık tutulumu 61 gözde (%91) üveit (ön, orta, arka, panüveit), 6 gözde (%9) retinal vaskülitti. Altmış yedi gözde yapılan görme analizine göre, 42 (%62,7)'sinin görme keskinliklerinde değişken düzeylerde iyileşme tespit edildi. Ortalama sağ göz görme keskinliği tedavi öncesi 0,56±0,049, tedavi sonrası 0,71±0,047 idi (p<0,001). Ortalama sol göz görme keskinliği tedavi öncesi 0,60±0,51, tedavi sonrası 0,73±0,043 idi (p<0,004).

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at variable levels. Mean right eye visual acuity was 0.56±0.049 before treatment, and 0.71±0.047 after treatment (p<0.001). Mean left eye visual acuity was 0.60±0.51 before treatment, and 0.73±0.043 after treatment (p<0.004).

Conclusion: In the presence of latent tuberculosis infection, successful results can be obtained with antituberculosis treatment for cases diagnosed as presumed ocular tuberculosis.

Keywords: Extra-pulmonary tuberculosis, granulomatosis ocular pathologies, ocular tuberculosis

Sonuç: Latent tüberküloz enfeksiyonu varlığında göz tüberkülozu düşünülen olgularda antitüberküloz tedavisi ile başarılı sonuçlar alınabilir.

Anahtar Kelimeler: Ekstrapulmoner tüberküloz, granülomatöz göz patolojileri, göz tüberkülozu

INTRODUCTION

Tuberculosis is one of the top 10 causes of death worldwide. In 2020, 9.9 million people had tuberculosis, and 1,3 million died from the disease. Globally calculated tuberculosis incidence is 127 per 100.000. According to WHO's data, in 2020, 15% of 6.624.523 tuberculosis cases reported worldwide had extra-pulmonary tuberculosis (1). Turkiye is among the countries with medium tuberculosis incidence. According to the data of the Turkish Ministry of Health, tuberculosis incidence was 10.6 per 100.000 in 2020 in Turkiye. Of these patients, 34.3% were reported to have extra-pulmonary tuberculosis (2). Among the patients with tuberculosis, there are studies reporting eye involvement ranging from 1.4% to 18% (3-5). The reason of these differences is that there is no standardized diagnostic criteria. Furthermore, tuberculosis incidence of the country or the region is important for the cross-sectional studies searching the tuberculosis frequency among all uveitis cases. The ratio varies between 1-4% in the countries with low tuberculosis incidence, while it is between 10-26% in the countries with high tuberculosis incidence (6). In a study conducted in our country, this rate was 1.3% (7).

Clinically, intraocular tuberculosis may be due to direct infection or indirect immune-mediated hypersensitivity response to mycobacterial antigens when there is no defined active systemic lesion elsewhere or the lesion is thought to be inactive. Choroidal tubercles and tuberculomas are reported to be the most common intraocular manifestations of tuberculosis (8). However, tuberculous uveitis is a hardly diagnosed disease since its symptoms imitate uveitis of different etiology, and since it is hard to obtain materials for microbiologic and pathologic examination. Tuberculous uveitis is a vision-threatening disease that inevitably leads to blindness if not properly diagnosed and treated (9).

On the other hand, ocular tuberculosis is still considered to be a controversial issue in terms of both terminology and diagnostic criteria and treatment options (8, 10). There is no common diagnostic/treatment algorithm even though new approaches exist (11). In this study, the clinical approach that we used for defining presumed ocular tuberculosis and the treatment results of the cases with ocular tuberculosis were evaluated.

MATERIALS AND METHODS

All patients with suspicion of ocular tuberculosis who were referred from the Istanbul Medical Faculty Ophthalmology Department of Istanbul University to the Pulmonology Department of Istanbul University between 2008-2016 were evaluated. All the subjects voluntarily signed their informed consent. The study was carried out according to the principles of the Helsinki Declaration. It was approved by Istanbul Medical Faculty Ethical Committee of Istanbul University (Date: 11.06.2018, No: 738).

All patients were asked whether they previously had active tuberculosis or not, and had close contact history with a tuberculosis patient. Tuberculin skin test (TST) and/or Quantiferon test, chest X-ray and if needed computerized thorax tomography were performed in all patients. Non - tuberculosis infections (HIV, fungal infections etc., sarcoidosis, connective tissue diseases, and vasculitis were investigated, and such patients were excluded. Surgical biopsy, bronchoscopic transbronchial lung biopsy and/or transtracheal/transbronchial lymph node aspiration were performed in patients in which sarcoidosis and tuberculosis cannot be differentiated.

Patients with sarcoidosis, active tuberculosis of any organ other than the eye or patients who did not have tuberculosis infection were excluded. Patients with latent tuberculosis infection who did not have any other pathology explaining the ocular involvement and who did not respond to previously given prednisolone and/or other immunosuppressive therapies were given 9-month anti-tuberculosis treatment (ATT). Of these patients, the ones who received treatment for at least three months were evaluated.

The objective response to the treatment of tuberculosis was evaluated by testing visual acuity before and after the ATT. The visual acuity was compared separately for both eyes. Patients who underwent treatment and had follow-up data of pre-treatment and post-treatment were analyzed.

Statistical analysis

Statistical evaluations were performed using SPSS (Statistical Package Social Science 21.0 package program). Descriptive values were given as mean, standard deviation, median and minimum-maximum. Categorical variables were expressed as number of cases and percentage value. Whether continuous variables were appropriate to normal distribution or not were analyzed using Shapiro–Wilktests. Wilcoxon test was used to compare the changes in right and left visual acuity before and after the ATT. A p value <0.05 was considered as statistically significant.

RESULTS

We evaluated 104 patients who were referred to our Outpatient Clinic of Pulmonary Diseases with the diagnosis of presumed ocular tuberculosis. Of the 104 patients evaluated, 40 were excluded because of sarcoidosis, presence of active tuberculosis in lung or extrapulmonary involvement than the eye. The remaining 64 (61.5%) cases were given ATT with the clinical diagnosis of presumed ocular tuberculosis.

Clinical parameters and treatment response of these patients who received ATT and underwent regular follow-up were evaluated. Demographic and clinical characteristics of 64 cases were given in Table 1. Of those 64 patients, 32 (50%) were female and 32 (50%) were male, and the mean age was 42.1±12.09 years (18-69 years). The mean disease duration was 8.64±6.99 months (1-31 months). Nonspecific minimal sequel changes in chest X-ray and/or thoracic computed tomography (CT) were present in most of the cases (60.9%, n=39). There were no radiological clinical signs of active tuberculosis.

Table 1: Demographics and clinical findings of the patients

	n=64
Age (year) Mean±SD	42.19±12.09
Gender (Female/male) (n)	32/32
Disease duration (month) Mean±SD	8.64±6.99
TST (mm) Mean±SD	19.28±4.58
ATT duration (month) Mean±SD	8.18±2.17
Quantiferon positivity (n, %)	64 (100%)
Bilateral disease/unilateral (n, %)	33/31 (51%/48.4%)
Sequel lesion in chest X-ray and/or Thoracic CT (n, %)	39 (60.9%)
Tuberculosis contact (n, %)	18 (28.1%)
Tuberculosis history (n, %)	7 (10.9%)
Immunosuppressive treatment history (n, %)	62 (96%)
Hepatotoxicity (n, %)	2 (3.1%)
Patient subjective treatment response (n, %)	47 (73.4%)

TST: tuberculin skin test, ATT: anti-tuberculosis treatment, CT: Computed tomography

Approximately one third (28%, n=18) had contact with active tuberculosis, and 7 (10.9%) had a history of tuberculosis. TST values of all patients were ≥10 mm, and the mean TST values were 19.28±4.58 mm (10-32mm). Quantiferon TB test was performed in 64 (100%) cases, and all were positive. In all of the nine cases in which interferon-gamma release assay (IGRA) test could not be performed, TST was ≥10mm, and in 7 of them it was ≥15mm (77%).

Sixty two cases (96.8%) were previously treated with corticosteroids (local and/or systemic) and other immunosuppressive therapies (azathioprine, methotrexate, etc.) before the treatment of tuberculosis, and there was no response to these treatments. Two patients received concomitant steroid and ATT. Treatment decision was based on the presence of typical findings of tuberculosis such as choroidal tubercles, exclusion of other diseases and the presence of latent tuberculosis infection. Duration of ATT was 8.18±2.17 month (3-15 month). Treatment was stopped in 3 and 5 months due to the hepatotoxicity in 2 cases (3.1%). ATT was given at least for 6 months in other cases.

After treatment, 47 of 64 patients (73.4%) reported improvement in vision. Objective visual evaluation was performed in 36 of 64 patients (31 bilateral, 5 unilateral, a total of 67 eyes) before and after treatment.

Eye involvement type of 36 patients (31 bilateral, 5 unilateral total 67 eyes) who had complete visual acuity evaluation before and after treatment was uveitis (anterior, intermediate, posterior, panuveitis) in 61 eyes (91%), and retinal vasculitis in 6 eyes (9%) (Table 2). Mean visual acuity of the right eyes was 0.56±0.049 before the treatment, and 0.71±0.047 after the treatment (p<0.001). Mean visual acuity of the left eyes was 0.60±0.51 before treatment, and 0.73±0.043 after treatment (p=0.004) (Table 3). Visual acuity was improved in 42 (62.7%) of 67 eyes at any level. There was no significant difference between the types of ocular involvement in terms of treatment response.

Table 2: Ophthalmologic findings of the eyes with possible ocular tuberculosis

Ophthalmologic findings of 67 eyes	n, (%)
Anterior uveitis	5 (7.5%)
Intermediate uveitis	5 (7.5%)
Posterior uveitis	23 (34%)
Panuveitis	28 (42%)
Vasculitis	6 (9%)

Table 3: Pre-treatment and post-treatment visual acuities

	Right eye (n=33) Mean±SD; Median (Min-Max)	Left eye (n=34) Mean±SD; Median (Min-Max)
Pre-treatment	0.54±0.34; 0.50 (0.05-1.0)	0.60±0.35; 0.75 (0.05-1.0)
Post-treatment	0.67±0.34; 0.80 (0.05-1.0)	0.70±0.31; 0.85 (0.10-1.0)
p value	0.002	0.02

DISCUSSION

This study reveals that anti-tuberculosis treatment may be successful in presumed ocular tuberculosis cases where active clinical tuberculosis is not detected and the presence of latent tuberculosis infection is shown.

Because of epidemiological differences and the differences in definition, the rate of detection of ocular tuberculosis within tuberculosis cases and in cases with uveitis shows great differences among countries. Ocular tuberculosis was reported to be between 1% and 85%, and tuberculosis uveitis was between 1% and 26% in cases with tuberculosis (10, 12). In different endemic regions in Europe, eye involvement in case of tuberculosis changes from 10% to 85%, and this data indicates the absence of standardization in the definition (13).

For the diagnosis of uveitis, the identification of clinical signs such as broad-based synechiae, retinal vasculitis, multifocal choroiditis, and serpiginoid choroiditis may be suggested in some endemic regions (8, 14, 15). Retinal perivasculitis and multifocal serpiginoid choroiditis, in particular, have been reported to have positive predictive values of ≥90% (8, 14). Nevertheless, in non-tuberculosis-endemic areas, broad or extensive posterior synechiae occur much more frequently in eyes with HLA-B27 or sarcoid associated uveitis than intraocular tuberculosis, and so this particular sign may be less predictive in such settings (16, 17). In our study, most cases were defined as granulomatous uveitis, but no significant difference was found between the groups in terms of treatment response.

In the study of Lou et al., which was performed in United States and Europe, it was found that the diagnosis and treatment of tuberculosis uveitis were not found to be compatible (18, 19). Although most clinicians are questioned in countries with a low incidence of tuberculosis, most clinicians routinely perform TST, IGRA, chest X-ray and chest CT similar to our approach in countries with a high incidence of tuberculosis.

Neither TST nor IGRA tests can differentiate latent infection from active tuberculosis. On the other hand, there is a study reporting a TST sensitivity of 59% in cases of histopathologically proven eye tuberculosis (13). In recent years, IGRA tests have been used more frequently, but they cost a lot or low and medium income developing countries, and have a high risk of false positive results in countries with low incidence of tuberculosis (20). However, IGRA is preferred in high-risk cases, especially those who use anti-TNF-alpha drugs (21). The combined use of TST and IGRA tests is one of the recommendations. Anget al. reported a positive predictive value of 84.6%, and a negative predictive value of 78.9% in a group of patients with both a positive TST and Quantiferon test, analyzed for their response to ATT (21). This approach is adopted in our study. In 86% of our cases, TST and IGRA were positive. In all of the 9 cases in which IGRA test could not be performed, TST was ≥10mm and ≥ 15mm in 7 (77%). Although PCR-based diagnostic studies have high specificity in eye tuberculosis cases, sensitivity values have been reported to be low (22). However, in recent years, high positive predictive value rates of around 80% have been demonstrated in multitargeted PCR tests, and this method has been introduced in new diagnostic algorithms (23, 24). Gupta A. et al. suggested to classify tuberculosis uveitis patients as ''confirmed'', ''probable'', or ''presumed'' in their study performed in India which is a high incidence country for tuberculosis (24). Confirmed case was defined when detection of Mycobacterium tuberculosis in ocular fluid or tissue as well as clinical findings. Probable case was defined as the presence of tuberculosis in lung or extrapulmonary tuberculosis and TST or IGRA test positivity although there is no microbiological evidence of *Mycobacterium tuberculosis*. The presumed case was defined as TST/IGRA positivity with clinical eye tuberculosis findings in cases where other possibilities are excluded.

The main problem in clinical practice is that the cases as the ones in our study are defined as presumed. It is not certain whether ATT treatment should be started in these cases, whether treatment should be done with steroids or how long the duration of the treatment should be (10). Several studies have shown that tuberculosis treatment with systemic steroids leads to better response in tuberculosis cases (25). Moreover, it was reported that steroid treatment alone may cause complications in these cases. Many studies have reported that inflammation is controlled by the addition of tuberculosis therapy in patients with recurrent disease on steroid treatment (26-28). Even 60% success has been reported with tuberculosis treatment alone (28). In our study, objective improvement was

detected in 62.7% of the patients who were defined as presumed ocular tuberculosis and received ATT. In the literature, similar results to our study were reported between 60%-76.6% (5, 29, 30). In this regard, such improvement with anti-tuberculosis treatment alone is remarkable in our cases that had ongoing disease despite the long-term steroid treatment (mean 8.6 months, range: 1-31 months). However, in these cases with presumed tuberculosis uveitis, the use of corticosteroids with anti-tuberculosis treatment from the beginning may be considered to be a more appropriate option for shortening the duration of disease and preventing permanent disorders.

Although there is no generally accepted consensus, a standard treatment regimen of six months is recommended for most patients. However, there is no consensus among experts on this issue; there are nine months or longer treatment recommendations (15). Similarly, in the study conducted among eye specialists, it was found that approximately 50% of the experts recommended treatment for 9 months or longer, and in developed countries, it was seen that cases were referred to infection or tuberculosis specialists for the decision of ATT (18). Due to the possibility of eye toxicity and lack of treatment experience in these cases, ethambutol was not used, and the ATT duration was nine months in our study.

Another question is to evaluate the results of the treatment. Visual acuity is difficult to measure because it is affected by many factors. The intraocular cell count seems to be a more reliable outcome measure although it may be influenced by inter-observer variability and inaccuracy in reporting (5). On the other hand, it should be kept in mind that standard treatment may be unresponsive in delayed complicated cases (31).

A significant limitation of our study is that only 56% (36/64) of the presumed ocular tuberculosis cases suggested ATT could be evaluated objectively. The other patients were evaluated according to their self-reports about the improvement in vision.

It is important to note that in this study and many of other studies, the definition of clinical and treatment outcomes was inconsistent largely due again to the lack of an exact definition of ocular tuberculosis, variation in duration and type of ATT, and the variable use of concomitant corticosteroid therapy. Therefore, a consensus is required to guide clinicians for the treatment duration of ocular tuberculosis to avoid unnecessary treatment, over exposure to side effects, and to reduce the risk of introducing drug resistance (10).

In conclusion, this study suggests that anti-tuberculosis treatment can be successful in cases of presumed ocular tuberculosis cases where other diseases are excluded and the presence of silent tuberculosis infection is shown.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 11.06.2018, No: 738).

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EVALUATION OF HELICOBACTER PYLORI FREQUENCY AND GASTRIC ANTRUM PATHOLOGY FINDINGS IN PATIENTS WITH PORTAL HYPERTENSION

PORTAL HİPERTANSİYONU OLAN HASTALARDA HELİCOBACTER PYLORİ SIKLIĞININ VE MİDE ANTRUM PATOLOJİ BULGULARININ DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: In our study, we aimed to evaluate the prevalence of Helicobacter pylori (H. Pylori) and the characteristics of antrum pathologies in patients with portal hypertensive gastropathy.

Materials and Methods: Antrum pathologies and the presence of H. Pylori evaluated with Giemsa stain in gastroscopies of 203 patients with portal hypertensive gastropathy.

Results: A total of 203 patients, 119 male, 84 female, with portal hypertensive gastropathy was included in our study. In patients with portal hypertension, the rate of H. Pylori positivity was 15.3%. This rate was determined as 18.5% under 65 years old and 8.8% over 65 years old. On the basis of gender, H. Pylori positivity rates were 21.8% in male patients and 6% in female patients. The most common antrum pathologies in the patients, in order of frequency, were reactive gastropathy with a rate of 41.4%, active gastritis with a rate of 23.6%, and edema with a rate of 22.2%. The rate of intestinal metaplasia was found to be 9.9%.

Conclusion: In the patients with portal hypertension included in our study, the H. Pylori positivity rate was found to be 15.3%. This rate was found to be below the H. Pylori positivity rate in our country. The most common antrum pathology in the patients was reactive gastropathy with a rate of 41.4%, while the rate of intestinal metaplasia was found to be 9.9%.

Keywords: Portal hypertensive gastropathy, H. pylori, gastroscopy, prevelance

ÖZET

Amaç: Çalışmamızda portal hipertansif gastropatisi (PHG) olan hastalarda Helicobacter pylori sıklığını ve antrum patolojilerinin özelliklerini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Portal hipertansiyonu olan 203 hastanın endoskopi ünitemizde yapılmış olan gastroskopilerinde portal hipertansif gastropati saptanan hastaların antrum patolojileri ve Giemsa boyası ile değerlendirilen H. Pylori varlığı retrospektif olarak araştırılmıştır.

Bulgular: Çalışmaya, portal hipertansif gastropatisi olan, 119'u erkek, 84'ü kadın, toplam 203 hasta dahil edilmiştir. Portal hipertansif gastropatisi olan hastalarda, H. Pylori pozitiflik oranı %15,3 olarak saptandı. Bu oran 65 yaş altında %18,5, 65 yaş üzerinde ise %8,8 olarak saptanmıştır. Cinsiyet bazında, H. Pylori pozitiflik oranları erkek hastalarda %21,8, kadın hastalarda %6 olarak tespit edildi. Hastalarda en sık görülen antrum patolojileri sıklık sırasına göre: %41,4 oranı ile reaktif gastropati, %23,6 aktif gastrit ve %22,2'lik oran ile ödem olarak saptandı. İntestinal metaplazi oranı ise %9,9 olarak saptandı.

Sonuç: Çalışmamıza dahil ettiğimiz PHG'i olan hastalarda, H. Pylori pozitiflik oranı %15,3 olarak saptandı. Bu oranın ülkemiz genelindeki H. Pylori pozitiflik oranının altında olduğu görülmüştür. Hastalarda en sık görülen antrum patolojisi %41,4 oranı ile reaktif gastropati iken, intestinal metaplazi oranı %9,9 olarak saptandı.

Anahtar Kelimeler: Portal Hipertansif Gastropati (PHG), H. Pylori, gastroskopi, prevelans

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INTRODUCTION

Portal hypertension (PHT), defined as an increase in blood pressure in the portal system, causes varicose veins in the gastrointestinal system as well as portal hypertensive gastropathy (PHG), enteropathy, and colopathy, which is characterized by characteristic mucosal pictures, primarily in the stomach (1, 2).

PHG is identified in patients with cirrhotic PHT and/or non-cirrhotic PHT by the presence of endoscopic findings in the stomach mucosa that appear as a mosaic pattern of erythematous, tiny, polygonal regions bordered by a reticular whitish border. Although cirrhosis is the most common cause of PHT, PHG can also arise in non-cirrhotic PHT instances. The pathophysiology of PHG is not well understood (3, 4). It is thought that the hemodynamic changes that develop on the basis of PHT are effective in the development of PHG by causing hyperdynamic congestion, which also leads to changes in gastric mucosal blood flow. Furthermore, alterations in this gastric mucosal microcirculation cause the release of several cytokines, hormones, and growth factors, resulting in a decrease in mucin secretion, making the stomach mucosa more sensitive and vulnerable to harmful elements (5, 6).

H. pylori, which is one of the most damaging factors in the gastric mucosa, is also accepted as an oncogenic bacterium. It has been proposed that bacterial virulence, host, and environmental factors all have a role in making H. pylori illness a complex process (7, 8). It advances and colonizes from the epithelial layer of the stomach mucosa to the basal layer by being influenced by numerous bacterial virulence factors such as flagella, as well as host gastric mucosal variables such as acidity and mucosal structure (8, 9). While PHG causes some hemodynamic and hormonal changes that may make the gastric mucosa vulnerable, there are conflicting results in the literature regarding how the relationship between this gastropathic stomach and H. pylori progresses and how the frequency of H. pylori changes in this patient group (3, 9). In this study, we aimed to investigate the frequency of H. pylori in patients with PHG in our patient group.

MATERIALS AND METHODS

A total of 203 patients with PHG were included in the study. The presence of H. pylori was explored retrospectively in patients who were reported to have esophageal varices and/or fundus varices in gastroscopic examinations performed in the endoscopy unit of Istanbul University, Istanbul Faculty of Medicine during 2014-2015 and whose antrum was biopsied. Patients whose biopsy specimens were unsuitable for histological analysis and/or H. pylori was not detected in the biopsy preparations, as well as those on proton pump inhibitors, were excluded from the study. The diagnosis of PHG was made based on the presence of typical endoscopic findings in patients with cirrhosis or non-cirrhotic PHT. Endoscopically, the typical findings of PHG were classified based on the presence of four main lesions which are mosaic-like pattern, presence of red spot lesions, presence of cherry-red spots, and presence of black-brown spots. Endoscopic evaluation was performed with Fujinon EG-450 WR5 flexible fiberoptic endoscopies under white light. The statistical significance level (a) was taken as 5% in the calculations and the SPSS (IBM SPSS for Windows, ver.22) statistical package program was used for the calculations. Power was obtained by taking at least 0.80 and Type-1 Error (a) 0.05 for each variable when computing the sample size for our investigation. Descriptive statistics for continuous (quantitative) variables are expressed as median, mean, standard deviation, minimum and maximum. Pearson correlation analysis was used to investigate the link between antrum diseases and H. pylori. The study was carried out in accordance with the Principles of the Declaration of Helsinki. Approval for the study was obtained from the ethics committee of Istanbul University, Istanbul Faculty of Medicine (Date: 25.06.2020, No: 104060).

RESULTS

A total of 203 patients with PHG, 119 male and 84 female, were included in our study. There were 135 patients under the age of 65 and 68 patients above the age of 65. In patients with PHG, the rate of H. pylori positivity was 15.3% (Table 1).

Table 1: H. pylori frequency

Groups	Frequency	Percentage
Negative	172	84.7%
Positive	31	15.3%

This rate was calculated to be 18.5% for those under the age of 65, and 8.8% for those over the age of 65. On the basis of gender, H. pylori positivity rates were 21.8% in male patients and 6% in female patients. In the patients' histological examinations, the most prevalent antrum diseases in order of frequency are: reactive gastropathy (41.4%), active gastritis (23.6%), and edema (22.2%) (Table 2).

The rate of intestinal metaplasia was found to be 9.9%. When the relationship between antrum pathologies and H. pylori was considered, it was discovered that the relationships between vascularectasis-vascular congestion, edema, reactive gastropathy, active gastritis, lymphoid-hyperplasia variables, and H. pylori were statistically significant because the sigma value was greater than 0.05. When the correlation coefficients are examined, it is seen that the highest correlation is between active gastritis and H. pylori (r=0.666). As a result, there is a strong and positive relationship between active gastritis and H. py-

Table 2: Evaluation of antrum pathologies frequency distribution

	Groups	Frequency	Percentage
Vascular ectasia-Vascular congestion	Negative	178	87.7%
	Positive	25	12.3%
Edema	Negative	158	77.8%
	Positive	45	22.2%
Reactive gastropathy	Negative	119	58.6%
	Positive	84	41.4%
Intestinal metaplasia	Negative	183	90.1%
	Positive	20	9.9%
Foveolar hyperplasia	Negative	167	82.3%
	Positive	36	17.7%
Active gastritis	Negative	155	76.4%
	Positive	48	23.6%
Inactive gastritis	Negative	181	89.2%
	Positive	22	10.8%
Lymphoid hyperplasia	Negative	193	95.1%
	Positive	10	4.9%

lori. Following that, the strongest link is shown between reactive gastropathy and H. pylori, with the correlation coefficient being negative, indicating that H. pylori drops while gastropathy increases, and H. pylori increases while gastropathy declines (Table 3).

Other antrum diseases (intestinal metaplasia, foveolar hyperplasia, inactive gastritis) and H. pylori were not statistically significant because their sigma values were greater than 0.05.

DISCUSSION

The link between PHG and H. pylori infection has been fascinating, both in terms of prevalence and the consequences of these two diseases on the development and the severity of each other's disorders. Various research studies have been reported in this regard, evaluating both the influence of H. pylori on PHG and cirrhosis, as well as the presence and effect of H. pylori on the backdrop of PHG (8-10).

In a study conducted by Ozaydın N. et al., who investigated the prevalence of H. pylori in 2186 individuals in our country, they discovered an 82.5% prevalence of H. pylori positivity. Korkmaz M. et al. discovered a 49.5% positivity rate for H. pylori in healthy persons in another investigation (11, 12). In the study of Karadag M et al., one of the studies evaluating the frequency of H. pylori in cirrhotic patients, the prevalence of H. pylori was found to be 47%. Again, in the same study, the prevalence of H. pylori was found to be 27% in cirrhotic patients with portal gastropathy, and H. pylori in cirrhotic patients without portal hypertensive gastropathy, its prevalence was found to be 60% (13). In this research article, the prevalence of H. pylori in cirrhotic patients was similar to those found in a previous study analyzing H. pylori prevalence rates in the general population, while the incidence of H. pylori positivity was much lower in cirrhotic and PHG patients. These findings are similar to the low prevalence of H. pylori that we found in patients with PHG in our study. In our study, we found the frequency of H. pylori to be 15% in patients with portal hypertensive gastropathy. Again, in one of the few studies in the literature analyzing the prevalence of H. pylori in PHG, Mc Cormack et al. discovered that the frequency of H. pylori was 26% in cirrhotic patients and 38% in the control group. They also discovered that H. pylori positive reduced as the severity of the PHG rose (14). One reason for the reduced H. pylori positive in PHG is the lack of a favorable milieu for H. pylori colonization in the congestive gastric mucosa (15).

In contrast to these studies, which found that the frequency of H. pylori was reduced in PHG, Sathar SA, et al. examined a total of 140 cirrhotic patients, 70 with PHG and 70 without PHG, and discovered that the overall H. pylori positivity rate was 35.7%, while the H. pylori positivity rate in cirrhotic patients with PHG was 44.3 percent. whereas 27.1% did not have PHG (16). Similarly, Puri S. et al. investigated the prevalence of H. pylori in 60 cirrhotic patients and discovered a rate of H. pylori positivity

Table 3: Correlation analysis results between antrum pathologies and H. pylori

		H. pylori	H. pylori (Male)	H. pylori (Female)	H. pylori (Under age 65)	H. pylori (65 and above)
Vascular ectasia Vascular congestion	Pearson Correlation	**-0.159(*)**	-0.169	-0.113	**-0.175(*)**	-0.121
	Sig. (2-tailed)	0.023	0.067	0.308	0.043	0.324
	n	203	119	84	135	68
Edema	Pearson Correlation	**-0.227(*)**	**-0.280(*)**	-0.136	**-0.260(*)**	-0.158
	Sig. (2-tailed)	0.001	0.002	0.217	0.002	0.197
	n	203	119	84	135	68
Reactive gastropathy	Pearson Correlation	**-0.357(*)**	**-0.412(*)**	**-0.234(*)**	**-0.395(*)**	**-0.268(*)**
	Sig. (2-tailed)	0.000	0.000	0.032	0.000	0.027
	n	203	119	84	135	68
Intestinal metaplasia	Pearson Correlation	-0.002	0.003	0.051	-0.120	**0.264(*)**
	Sig. (2-tailed)	0.972	0.978	0.642	0.167	0.030
	n	203	119	84	135	68
Foveolar hyperplasia	Pearson Correlation	-0.054	-0.042	-0.016	-0.047	-0.040
	Sig. (2-tailed)	0.447	0.654	0.887	0.590	0.743
	n	203	119	84	135	68
Active gastritis	Pearson Correlation	**0.666(**)**	**0.746(**)**	**0.428(*)**	**0.690(**)**	**0.611(**)**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000
	n	203	119	84	135	68
Inactive gastritis	Pearson Correlation	-0.104	-0.169	0.051	-0.091	-0.121
	Sig. (2-tailed)	0.140	0.067	0.642	0.294	0.324
	n	203	119	84	135	68
Lymphoid hyperplasia	Pearson Correlation	**0.283(*)**	**0.264(*)**	**0.291(*)**	**0.365(*)**	-0.054
	Sig. (2-tailed)	0.000	0.004	0.007	0.000	0.661
	n	203	119	84	135	68

*: According to the Pearson correlation coefficient (r), there is a weak degree of statistically significant correlation at the sig. 0.005 significance level.
**: According to the Pearson correlation coefficient (r), there is a moderate/high degree of statistically significant correlation at the 0.005 significance level.

of 55%. While the positive rate was 67% in patients with PHG, it was found to be 33% in patients without PHG (17). The reason for the higher prevalence of H. pylori in patients with PHG is increased expression of Inducible Nitric Oxide Synthase resulting in high reactive oxygen species, congestion, increased secretion of cytokines such as TNF alpha, IL-8, etc., and it increases the virulence of H. pylori while creating a synergistic effect between H. pylori and PHG (18). Our study differs from other studies examining the prevalence of H. pylori in PHG in terms of both number and examination method, because it has the maximum number of cases and H. pylori is identified by staining with Giemsa in samples taken from stomach biopsies in all patients. We think that this is the reason for the lower rate of H. pylori positivity prevalence we found compared to other studies in the literature.

The most common pathological finding in the antral biopsies we acquired from PHG patients was reactive gastropathy, followed by active gastritis, edema, and vascular ectatic alterations. According to Chandanwale SS. et al., who examined the gastrointestinal pathologies of 30 patients with PHG, the most prevalent pathological result was dilated congestive capillaries and edema (19). PHG is mostly characterized by mucosal changes in the proximal stomach, and therefore, in our study evaluating antral biopsies, unlike Chandanwale SS et al., it was thought that one of the reasons we saw edema and vascular conges-

tion in the third frequency might be related to biopsy localizations (19). In our investigation, we discovered a negative connection between H. pylori and gastropathy, similar to the findings of Mc Cormack et al. There was a positive correlation between active gastritis and H. pylori.

The positive features of our study are that we analyzed more patients than previous studies on the frequency of H. pylori in patients with PHG, and that H. pylori was tested by biopsy as a standard in all patients. The limitations of our study are the fact that it is a retrospective study, and the absence of cirrhotic differentiation in the etiology of PHG can be considered.

In conclusion, PHG is a diagnosis that necessitates both clinical and endoscopic assessment. Despite the fact that H. pylori is associated with active gastritis in this patient population, the prevalence of H. pylori in gastropathy is decreasing.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 25.06.2020, No: 104060).

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GENE MUTATION PATTERNS OF RIFAMPICIN IN MULTIDRUG-RESISTANT *MYCOBACTERIUM TUBERCULOSIS* COMPLEX STRAINS

ÇOĞUL İLACA DİRENÇLİ *MYCOBACTERİUM TUBERCULOSİS* KOMPLEKS SUŞLARINDA RİFAMPİSİNİN GEN MUTASYON PATERNLERİ

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ABSTRACT

Objective: The aim of this study was to evaluate the GenoType®MTBDR*plus* test for detection of rifampicin (RIF) resistance in MDR *Mycobacterium tuberculosis* complex strains.

Materials and Methods: Twenty-five multidrug-resistant (MDR) *Mycobacterium tuberculosis* clinical strains were used, gene mutations causing RIF resistance were investigated by GenoType®MTBDR*plus* and the results were compared with the results of the BACTEC 460 TB system. The strain was sequenced if there was no mutation and absence Wild Type (WT). *Mycobacterium tuberculosis* ATCC 35838 was used as a quality control (QC) strain.

Results: There was 96% compliance between the GenoType®MTBDR*plus* and BACTEC 460 TB system for the finding of RIF resistance. The most frequent mutation zone in MDR strains was *rpoB* S531L promotor zone (13 strains, 52%). The other *rpoB* gene mutations were H526Y (three strains, 12%) and H526D (three strains, 12%), while five strains (20%) had Δ2-Δ5 mutations in the wild type probes. There was no mutation in only one strain (4%) by GenoType®MTBDR*plus* but it was found to be as resistant to rifampicin by the BACTEC 460 TB system. This strain was sequenced and detected to have triple mutations. Mutations were found on codons 489, 493, and 503.

Conclusion: GenoType®MTBDR*plus* showed good compatibility with the BACTEC 460 TB system in detecting rifampicin resistance, and it was thought that GenoType®MTBDR*plus* could be an effective and reliable test for RIF susceptibility testing in MDR-TB patients, providing a significant advantage in technical time.

Keywords: Tuberculosis, rifampicin, resistance, GenotypeMTBDR*plus*, *Mycobacterium*, *rpoB*

ÖZET

Amaç: Bu çalışmada, çoğul ilaca dirençli (ÇİD) *Mycobacterium tuberculosis* kompleks suşlarında rifampisin (RIF) direncinin saptanmasında GenoType®MTBDR*plus* testinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Yirmi beş ÇİD *Mycobacterium tuberculosis* klinik suşu kullanılmış, RIF direncine neden olan gen mutasyonları GenoType®MTBDR*plus* ile araştırılmış ve sonuçlar BACTEC 460 TB sistemi sonuçları ile karşılaştırılmıştır. Eğer mutasyon veya Vahşi Tip (VT) bandı yoksa suş sekanslanmıştır. Kalite kontrol (QC) suşu olarak *Mycobacterium tuberculosis* ATCC 35838 kullanılmıştır.

Bulgular: Rifampisin direncinin saptanmasında GenoType®MTBDR*plus* ve BACTEC 460 TB sistemi arasında %96 uyum bulunmuştur. ÇİD suşlarda mutasyonun en sık *rpoB* S531L promotor bölgede (13 suş, %52) olduğu görülmüştür. Diğer *rpoB* gen mutasyonları H526Y (üç suş, %12) ve H526D (üç suş, %12) bölgesinde iken, beş suşta (%20) vahşi tip problarında Δ2-Δ5 mutasyonları saptanmıştır. Bir suşta (%4) GenoType®MTBDR*plus* ile mutasyon bulunamamış, ancak BACTEC 460 TB sistemi ile rifampisine dirençli olduğu belirlenmiştir. Bu suş sekanslanmış, 489, 493 ve 503 kodonlarında üçlü mutasyona sahip olduğu tespit edilmiştir.

Sonuç: Rifampisin direncinin saptanmasında GenoType®MTBDR*plus*, BACTEC 460 TB sistemi ile iyi uyum göstermiştir ve ÇİD-TB hastalarında RIF duyarlılık testi için GenoType®MTBDR*plus*'ın etkili ve güvenilir bir test olabileceği ve bunun teknik zaman açısından da önemli bir avantaj sağlayacağı düşünülmüştür.

Anahtar Kelimeler: Tüberküloz, rifampisin, direnç, GenotypeMTBDR*plus*, *Mycobacterium, rpoB*

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INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* complex (MTBC), is one of the leading causes of disease in the world. *M. tuberculosis* is responsible for 97.9% of the disease. The incidence of the disease has increased and decreased over the past thousands of years, but this disease has remained a permanent threat to public health (1-3).

According to the 2020 data of the World Health Organization (WHO), 10 million new cases of tuberculosis were determined and approximately 1.4 million people died from tuberculosis in 2019. Global cases were reported 44% from Southeast Asia, 25% from Africa, 18% from the West Pacific, 8.2% from the Eastern Mediterranean, 2.5% from Europe and 2% from the Americas. India, Indonesia, China, Nigeria, Pakistan, Bangladesh and South Africa have been reported as countries with the highest number of cases (4).

According to the TB dispensary in Turkiye 2020 report, 11,786 TB patients were identified in 2018. Of the patients, 6,778 (57.5%) were male and 5,008 (42.5%) were female. The case rate was 16.5/100 000 in men and 12.3/100 000 in women. The total case rate was determined as 14.4/100 000 in 2018. The most common drug resistance in new tuberculosis cases whose drug susceptibility tests were studied was isoniazid with 11.6%. The total resistance rate was 19.2% in the cases in which DST was studied, while the MDR-TB rate was 2.6% in new cases and 9.9% in previously treated cases (5).

In many developed countries, a rapid rise has been detected in tuberculosis with the increase in Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) cases. In addition to HIV/AIDS infections, cases of multidrug-resistant tuberculosis (MDR-TB; least resistant to isoniazid (INH) and rifampicin (RIF) from first-line anti-TB drugs) also played an important role in the re-increase of tuberculosis. In addition to MDR strains, the emergence of extensively drug-resistant TB (XDR-TB; resistant to isoniazid, rifampicin, any quinolone and one of kanamycin, capreomycin or amikacin) strains, non-compliance with treatment, patient management mistakes, unsupervised treatment, limited or intermittent drug supply, use of substandard drugs and bad tuberculosis control programs have also been among the factors causing the increase in tuberculosis (4, 6, 7).

In the last 20 years, new molecular methods have been developed for diagnosis of tuberculosis. These methods allow resistance to various antituberculosis drugs, especially RIF, to be detected in a very short time and to start treatment in a short time. At the same time, epidemiological data are obtained by investigating which gene mutations are dominant in many countries through these methods (8, 9).

In this study, it was aimed to evaluate the GenoType®MTBDR*plus* test for detection of rifampicin resistance in MDR *Mycobacterium tuberculosis* complex strains.

MATERIALS AND METHODS

Bacterial strains

Bacterial strains were isolated from clinical specimens, which came to the Istanbul University, Istanbul Faculty of Medicine, Department of Medical Microbiology, Mycobacteriology Laboratory using the BACTEC 460 TB system. Sensitivity to antituberculosis drugs (INH, RIF, ethambutol (EMB), streptomycin (SM)) was investigated using this system, and was determined to be resistant to rifampicin and isoniazid.

Ethical statement

The approval of the Istanbul University Faculty of Medicine Ethics Committee was obtained for this study (Date: 30.11.2011, No: 2118).

Identification of rifampicin resistance by molecular assay

Gene mutations causing RIF resistance were investigated by GenoType® MTBDR*plus* assay version 2.0 (HainLife Sciences, Nehran, Germany) (10).

DNA isolation

One milliliter of culture was taken from the liquid medium and transferred to a sterile screw-capped Eppendorf tube and incubated for 20 minutes at 95°C in a heat block. DNA was obtained by incubating in an ultrasonic water bath for 15 minutes.

DNA amplification

The total reaction volume for each strain was set to be 50 µl; 35 µl of the primer nucleotide mixture in the kit, 5 µl of 10x PCR buffer, 2 µl of $MgCl_2$, 3 µl of ultra pure water, 0.2 µl of TaqGold polymerase and 5 µl of the sample were added in sterile PCR tube. The PCR mix was subjected to the PCR steps listed in Table 1 (10, 11).

Table 1: PCR conditions

	Pre-PCR	PCR	PCR	Extension
Temperature/time	95°C→15 min	95°C→30 sec 65°C→2 min	95°C→25 sec 50°C→40 sec 70°C→40 sec	70°C→8 min
Cycle number	**1**	**10**	**20**	**1**

Hybridization

Hybridization and detection were performed according to the manufacturer's instructions. Twenty microliters of denaturation solution were added to 20 µl of amplified sample and mixed. The solution was incubated at 21°C for 5 min. One milliliter of hybridization buffer was added and shaken. After, the membrane strips were placed in the hybridization solution and incubated for 30 min at 45 °C. Each strip was washed twice. One milliliter of diluted substrate was added and colorimetric detection of the hybridized amplicons was obtained (12).

The Genotype MTBDR*plus* strip includes 27 probes. It contains hybridization (CC) and amplification (AC) controls to confirm the test protocols. *M. tuberculosis* is identified by the use of the *M.tuberculosis* complex-specific (TUB) probe. The *rpoB*, *katG*, and *inhA* control probes detect *rpoB*, *katG*, and *inhA* regions, respectively. The eight *rpoB* wild-type probes (WT1 to WT8), two *inhA* wild-type probes (WT1 and WT2), and one *katG* wild-type probe are used to determine the mutations that lead to RIF and INH resistance. The ten mutant probes were specifically designed to hybridize sequences of the frequently detected four *rpo*B, four *inh*A and two *kat*G mutations: *rpoB* MUT1 D516V, *rpoB* MUT2A H526Y, *rpoB* MUT2B H526D and *rpoB* MUT3 S531L, *inhA* MUT1 C15T, *inhA* MUT2 A16G, *inhA* MUT3A T8C and *inhA* MUT3B T8A, *katG* MUT S315T1 and *katG* MUT S315T2 (13).

If a band occurs at the *rpo*B mutation site, the strain is resistant to RIF. In addition, the strain is accepted as resistant to RIF if there is no band in the mutation region but there was a faint band in the WT region (10).

DNA sequencing

The strain was sequenced in the absence of mutation or absence of the WT band. A 411bp fragment of the *rpoB* gene was amplified using *rpoB* primers (Forward primer: 5'- TACGGTCGGCGAGCTGATCC-3', Reverse primer: 5'-TACGGCGTTTCGATGA ACC-3') (14). Product size (411 bp) was verified by agarose (2%) gel electrophoresis. PCR product was cleaned up using ExoSAP-IT™ PCR Product Cleanup kit (Thermo Fisher Scientific, USA). Cycle sequencing was performed with BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA). After that, sephadex purification was done and the product was loaded in the ABI 3130 XI 16 Capillary Genetic Analyser (Hitachi, Japan). Clustal Omega online tool (https://www.ebi.ac.uk/Tools/msa/clustalo/) was used for DNA sequence comparisons (15).

Quality control

Mycobacterium tuberculosis ATCC 35838 was used as a quality control (QC) strain for all methods. This strain is resistant to rifampicin.

RESULTS

Both male 15 (60%) and female 10 (40%) TB patients' samples were included in the study. Twenty-five strains were isolated from 24 sputum and one abscess samples.

Drug susceptibility testing of 25 strains was determined using the BACTEC 460 TB method. Resistance was determined to be INH+RIF in six strains (24%), INH+RIF+EMB in eight strains (32%), INH+RIF+SM in four strains (16%) and INH+RIF+SM+EMB in seven strains (28%) (Figure 1).

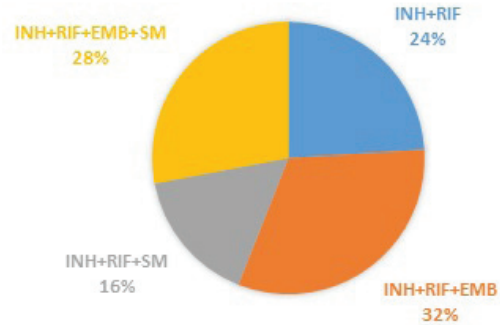

Figure 1: Sensitivity test results by the BACTEC 460 TB method

Gene mutations causing RIF resistance were investigated by GenoType® MTBDR*plus*. The most frequent mutation zone in MDR strains was the *rpoB* S531L promotor zone (13 strains, 52%). The other *rpoB* gene mutations were H526Y (four strains, 16%), H526D (one strain, 4%) and D516V (one strain, 4%) while five strains had Δ2-Δ5 mutations (20%) in the WT probes. There was no mutation in only one strain (4%) by GenoType®MTBDR*plus* but it was found to be as resistant to rifampicin by the BACTEC 460 TB system (Table 2).

In seventeen of the twenty-five isolates, WT1/WT4/WT7 (one; 4%), WT2/WT3/WT4 (one; 4%), WT3/WT4 (two; 8%), WT6/WT7/WT8 (one; 4%), WT8 (12; 48%) faint bands were present. On the other hand, WT6/WT7/WT8 (one; 4%), WT3/WT4 (one; 4%), WT3/WT7 (one; 4%), WT6/WT8 (one; 4%), and WT7 (four; 16%) absence bands were available in eight isolates (Table 2).

The strain that was found susceptible by GenoType® MTBDR*plus* was sequenced and triple mutations were detected. Mutations were found on codons 489, 493 and 503 (Table 3).

DISCUSSION

Molecular methods provide faster diagnosis than traditional methods. However, it is recommended that these tests be applied in conjunction with gold standard culture methods. The sensitivity and specificity of studies in

Table 2: Gene mutation pattern detected by GenoType MTBDR*plus*

Gene	Number of isolates	Wild type band faint	Wild type band absence	Mutation band	Mutation
	1	WT2/3/4	WT6/7/8	-	-
	2	WT3/4	-	-	-
	1	-	WT3/4	*rpo*B MUT1	D516V
	1	-	WT3/7	-	-
	1	WT6/7/8	-	*rpo*B MUT2A	H526Y
rpoB	1	WT1/4/7	WT6/8	-	-
	3	-	WT7	*rpo*B MUT2A	H526Y
	1	-	WT7	*rpo*B MUT2B	H526D
	12	WT8	-	*rpo*B MUT3	S531L
	1	-	-	*rpo*B MUT3	S531L
	1	-	-	-	-

Table 3: Sequence result of the susceptible strain

Codon	Amino acid change	Nucleotide change
489	Gln → Hist	CAG → CAC
493	Asn → Isoleucin	AAC → ATC
503	Lys → Isoleucin	AAG → ATC

which RIF resistance is detected by molecular methods vary between 92-100% and 85-100%, respectively (16-22).

In our study, the performance of the GenoTypeMTBDR*plus* assay for the detection of RIF-resistant strains of *M. tuberculosis* was evaluated on 25 MDR *M. tuberculosis* isolates. Twenty-four (96%) of the 25 isolates, which were determined to be rifampicin-resistant with the BACTEC 460 TB method, were found to be rifampicin-resistant with GenoType MTBDR*plus*. Our result was similar to other reports from India, France, Germany and Ethiopia (16, 23-25). One isolate (4%) was susceptible to rifampicin. It was observed that the GenoType MTBDR*plus* showed good accordance with the BACTEC 460 TB system. The resistance in the mutation undetectable strain is thought to be related to the gene region not detected by the GenoType MTBDR*plus* kit. Our sequence study confirmed this idea. We found triple mutation on codons 489, 493 and 503. These codons are outside the gene region range included in the GenoType MTBDR*plus* kit. Cavusoglu et al. detected rifampin resistance in 22 (53.7%) of 41 isolates with Genotype MTBDR assay. But two RIF-resistant isolates (4.8%) were identified as RIF sensitive. One strain had an Gln-490-His mutation and the other isolate had a CGG insertion between codons 514 and 515. These mutation regions were outside the 81-bp hotspot region and not detected by Genotype MTBDR assay (26). In our study, the result is identical with these study results.

Molecular methods provide information about the mutation regions that occur in the genes that cause resistance, as well as providing faster results. Studies have shown that mutations in different codons of the relevant *rpoB* gene are effective at different rates in the emergence of RIF resistance, and it has been reported that the percentage of mutations in the codon S531L of the *rpoB* gene is higher than the other codons (27, 28).

In our study, RIF-resistance-specific mutations were determined on *rpoB* MUT probes in 19 (76%) of the 25 MDR isolates by the GenoType MTBDR*plus* assay. This frequency rate was found to be similar with other studies (29-31).

In 2018, in the study by Kamiri et al., among the 35 specimens identified as RIF-resistant using the GenoTypeMTBDR*plus* assay, 23 (65%) carried the S531L, eight isolates (22%) showed mutation in codon H526Y and four isolates (11%) in D516V codon (32). In 2013, in the study by Maurya et al., the frequency of rpoB mutation was 28 in S531L (62.3%), eight in D516V (17.7%), six in H526Y (11.1%), one in the H526D (2.2%) region and nine (20%) unknown mutations (absence of one or more wild-type) among a total of 45 MDR-TB strains (23). In 2017, in the study by Abanda et al., among the 48 isolates determined as RIF-resistant using the GenoTypeMTBDRplus assay, 41 (85%) had the S531L mutation and three isolates (6%) showed mutation in codon H526Y. The frequency of codon 531 mutation in this study was 85%, which is higher than generally reported (19). In 2013, in the study by Yadav et al., among all 66 RIF-resistant strains, 51 (72%) had a mutation in *rpoB* S531L. Four strains (6%) had a mutation in *rpoB* H526D (4/66), two in (3%) *rpoB* D516V (2/66) and two (3%) in *rpoB* H526Y (33).

The result of this study showed that of the 19 specific mutations of the *rpoB* gene, 13 mutations (52%) were at

codon S531L. The remaining 16% (4/25) had mutations at codon H526Y, 4% (1/25) at codon H526D and 4% (1/25) at codon D516V. Mutations at codon S531L and H526Y are known to be the most prevalent RIF resistance. Our results displayed a similar profile with the previous study (30, 34, 35). But our frequency rate is lower in codon S531L. This may be due to the variability of strains between countries. Mutation percents in the D516V and H526Y codon were found to be lower in our study compared with percents reported from Europe (36). We think that smaller sample sizes cause the difference. This idea is supported by the work by Farooqi et al. (37). On the other hand, five of our MDR isolates have only WT band absence and faint band but don't have a corresponding MUT band. This result is associated with mutations that cause drug resistance. In addition to that, silent mutation that doesn't result in any amino acid difference may cause this pattern, or the presence of less common mutations in the *rpoB* gene that cannot be detected by the GenoType MTBDR*plus* test may lead to this result (11, 16).

The present study showed that TB is more common among males (60%) and affects all age groups. This finding was in alignment with several reports (38-40). Males generally are known to be more susceptible to TB than females because of their relatively large social network, which increases their risk of infection, in addition to the higher prevalence of smoking, which has a confirmed association with TB (41, 42).

Consistent results were detected in this study, when compared with the results obtained worldwide. However the frequency rate of mutations was found to be low. We think that the use of a small number of isolates led to this result, because when previous studies are reviewed it appears that more isolates are used. The GenoType MTBDR*plus* assay has high sensitivity and specificity. However it identifies limited mutation regions.

CONCLUSION

In conclusion, GenoType® MTBDR*plus* showed good compliance with the BACTEC 460 TB system in the detection of rifampicin resistance and it was thought that GenoType® MTBDR*plus* might be an effective and reliable test for the susceptibility testing of rifampicin in MDR-TB patients. Additionally, this technique also provided a significant advantage in terms of time.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 30.11.2011, No: 2118).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.Y.S., M.U.; Data Acquisition- E.Y.S., M.U.; Data Analysis/Interpretation- E.Y.S., M.U.; Drafting Manuscript- E.Y.S., M.U.; Critical Revision of Manuscript- E.Y.S., M.U.; Approval and Accountability- E.Y.S., M.U.

Conflict of Interest: Authors declared no conflict of interest

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COMPARISON OF SINGLE DOSE AND MULTI-DOSE hCG STIMULATION TESTS

TEK DOZ VE ÇOĞUL DOZ hCG UYARI TESTLERİNİN KARŞILAŞTIRILMASI

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ABSTRACT

Objective: The human chorionic gonadotropin (hCG) stimulation test is widely used to assess testicular steroidogenesis. This test evaluates Leydig cell function and helps to evaluate testosterone synthesis in testicular enzyme defects, hypogonadism, 46,XY sexual development disorders (DSD), and micropenis. This study will compare testosterone responses to a single dose of 5000 IU/m^2 and three consecutive days of 1500 IU/m^2 hCG regimens.

Materials and Methods: The study evaluated 18 patients who were admitted with micropenis, hypospadias, or undescended testis (Group 1) and 18 patients with a molecular diagnosis of androgen receptor insensitivity (56%) or 5α reductase deficiency (44%) (Group 2). The median (interquartile range; IQR) age of the patients were 1.4 (0.8-4.2) years for Group 1 and 0.7 (0.3-0.8) years for Group 2. Baseline testosterone levels were checked and after a single dose of hCG 5000 IU/m^2 injection into the first group, testosterone levels were observed on day one and day four. In the second group, after the injection of hCG 1500 IU/m^2 for three days, testosterone levels were checked on the fourth day.

Results: There was no significant difference in height, weight, BMI SDS, and baseline testosterone levels between groups. After hCG, stimulation testosterone responses did not vary among the groups. Testosterone levels were increased 40-fold and 40.1-fold on day four compared to the baseline in Group 1 and Group 2. In Group 1, testosterone response was significantly higher on day four compared to day one (p=0.001).

Conclusions: The hCG tests performed with a single dose or multidoses showed that there was no difference between testosterone responses and these two tests were applicable. Since

ÖZET

Amaç: hCG uyarı testi, testis steroidogenezini değerlendirmek için yaygın olarak kullanılmaktadır. Bu test hipogonadizm, 46,XY cinsel gelişim bozukluğu veya mikropenisi olan hastalarda Leydig hücre fonksiyonunu, testise bağlı enzim kusurlarını değerlendirmektedir. Bu çalışmada tek doz 5000 IU/m^2 ve 3 gün 1500 IU/m^2 hCG testlerine testosteron yanıtlarının karşılaştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya mikropenis, hipospadyas veya inmemiş testisi (Grup 1) olan 18 hasta ile moleküler olarak tanılı androjen reseptör kusuru (%56) ve 5α redüktaz eksikliği (%44) olan 18 hasta (Grup 2) dahil edildi. Hastaların median (çeyrekler aralığı) yaşı Grup 1 için 1,4 (0,8-4,2) yıl, Grup 2 için 0.7 (0,3-0,8) yıl idi. Bazal testosteron düzeyi ölçüldükten sonra ilk gruba tek doz 5000 IU/m^2 hCG uygulanarak testosteron düzeyleri 1. ve 4. gün ölçüldü. İkinci gruba ise 3 gün 1500 IU/m^2 hCG uygulanarak testosteron düzeyleri 4. gün ölçüldü.

Bulgular: Grup 1 ve Grup 2 arasında boy, kilo, vücut kitle indeksi SDS ve bazal testosteron seviyeleri arasında anlamlı bir fark yoktu. hCG uyarısı sonrasında testosteron yanıtlarında da anlamlı fark saptanmadı. Testosteron seviyeleri bazale göre kıyaslandığında 4. günde Grup 1'de 40 kat ve Grup 2'de 40.1 kat artış gösterdi. Grup 1 hastalarında testosteron yanıtı 1. gün ile karşılaştırıldığında 4. günde anlamlı olarak daha yüksek idi (p=0,001).

Sonuç: Tek doz ve çoğul doz ile gerçekleştirilen hCG uyarı testlerinin sonucunda testosteron yanıtları benzer çıkmış olup bu sonuç her iki testin de uygulanabilir olduğunu göstermiştir. 5000 IU/m^2 testi daha az enjeksiyon gerektirdiği ve uygulaması kolay olduğu için Leydig hücre fonksiyonunu değerlendirmede bu test tercih edilebilir. Yeterli testosteron yanıtı için tek doz 5000 IU/m^2

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the 5000 IU/m^2 hCG test requires fewer injections and is easy to administer, this test may be preferred to an evaluation of the Leydig cell function. For an adequate testosterone response, the researchers suggest measuring testosterone levels on the fourth day after the single dose of 5000 IU/m^2.

Keywords: Disorders of sex development, hCG stimulation test, testosterone, 46,XY

hCG uygulandıktan sonra 4. gün testosteron düzeylerini ölçmeyi önermekteyiz.

Anahtar Kelimeler: Cinsiyet gelişim bozukluğu, hCG uyarı testi, testosteron, 46,XY

INTRODUCTION

Testosterone is the main product of steroidogenesis, and it is produced by Leydig cells. At birth, following the perinatal decline, the hypothalamic-pituitary-gonadal (HPG) axis is active until 3-6 months of extrauterine life and then lies dormant until the onset of puberty (1). The HPG axis reactivates after the pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus. This reactivation stimulates the secretion of luteinizing hormones (LH) which leads to testicular testosterone production at the onset of puberty (1). Testosterone and its metabolites drive pubertal development of sexual characteristics and sex differentiation of male patients. Therefore, the evaluation of testosterone concentration and its precursors is helpful to identify the disorders of testicular function and development (1).

The human chorionic gonadotropin (hCG) is a glycoprotein which is produced by the human placenta. hCG is usually obtained from the urine of pregnant women (uhCG), however, a recombinant hCG preparation (rhCG) is also produced (2, 3). hCG activates the LH/hCG receptor on Leydig cells and stimulates sex steroid secretion (2). The hCG stimulation test has been used frequently for the evaluation of testicular steroidogenesis. This test determines Leydig cell function and is used for the evaluation of testicular enzyme deficiencies in hypogonadism, bilateral nonpalpable gonads, micropenis, infertility, 46,XY disorders of sex development, and delayed puberty (4-6). Nevertheless, many different hCG test protocols are reported particularly in terms of the number of injections, application doses, and blood drawing time. Therefore, this study wanted to investigate hCG test protocols with three days 1500 IU/m^2 and single dose 5000IU/m^2, and to define the criteria for a normal response and improvement of the procedure.

MATERIALS AND METHODS

In this study, 36 prepubertal boys were enrolled (Tanner stage I), aged between 0.02-10.4 years old. Eighteen patients were presented with hypospadias, cryptorchidism, or micropenis but a diagnosis had not been obtained yet (Group 1). In Group 1 the hCG stimulation test was performed with one intramuscular injection of hCG 5000 IU/m^2 given early in the morning. Testosterone levels were measured immediately before the injection and then 24 and 96 hours after hCG injection (Group 1).

Another 18 boys had a molecularly verified diagnosis of 5α reductase deficiency (n=8) or androgen insensitivity syndrome (n=10) and received 3 daily 1500 IU/m^2 hCG injection. Blood samples for testosterone were taken just before the hCG injection and then 24 hours after the last injection (Group 2).

Anthropometric measurements of the patients for height and weight were taken by the same auxologist. Body mass index (BMI) was calculated with the formula: BMI = weight (kg)/height (m^2). Standard deviation scores (SDS) of these measurements were calculated using national data (7). Haycock formula was used to calculate the body surface area (BSA).

All blood samples were measured at the same endocrine laboratory using the same methods. Plasma T levels were measured by electrochelumiscence (ECLIA) (Cobas Roche Diagnostics, Mannheim, Germany).

The study protocol was approved by the local Ethics Committee (Date: 17.07.2020, No: 985). Written informed consent was obtained from all parents.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) for Windows 21.0 was used for statistical analysis. The distribution between variables was evaluated with the Shapiro–Wilk test. Comparisons were made between single versus multiple doses, and in Group 1 testosterone 24 hours versus 96 hours after injection. Nonparametric tests were used to compare the study variables. The Mann-Whitney U test was used to compare differences between the two independent groups. The Wilcoxon rank-sum test was performed to compare paired repeated testoterone levels in the groups. The Friedman's two way analysis of variance (ANOVA) by ranks test was used to compare the baseline, first day and fourth day testosterone levels within the same groups. Data presented as median [interquartile range (IQR)]. p value <0.05 was accepted as statistically significant.

RESULTS

The median age of patients were 1.4 (0.8-4.2) years for Group 1 and 0.7 (0.3-0.8) years for Group 2. The ages of the patients in Group 1 were higher than Group 2. Accordingly, patients in Group 1 had a higher BSA than Group 1. The characteristics of the patients are presented in Table 1. Baseline testosterone levels were prepubertal in four in Group 2 were significantly higher than testosterone levels of day one in Group 1 (p<0.001). None of the patients developed side effects related to the hCG test. Comparisons of hCG tests are given in Table 2 and Table 3. Figure 1 shows the median testosterone levels of both groups.

Table 1: Characteristics of patients in Group 1 and in Group 2. Data presented as median (interquartile ranges).

	Group 1 (5000IU/m², single dose) n=18	Group 2 (1500 IU, 3 days) n=18	p
Age (years)	1.4 (0.8-4.2)	0.7 (0.3-0.8)	**0.002**
Weight SDS	-0.6 (-1.4-0.4)	0.02 (-1.1-0.7)	0.613
Height SDS	-1.1 (-2.1 and -0.4)	-0.3 (-1.5-0.2)	0.273
BMI SDS	0.1 (-0.7-1.1)	-0.2 (-0.9-0.5)	0.273
BSA	0.5 (0.4-0.6)	0.4 (0.3-0.5)	**0.01**
Diagnosis n (%)			
Androgen insensitivity syndrome	NA	55.6	
5α reductase deficiency		44.4	

SDS: standard deviation score, BMI: body mass index, BSA: body surface area, NA: not available

Table 2: Comparison of hCG tests. Data presented as median (interquartile ranges)

	Group 1 (n=18) 5000 IU/m², single	Group 2 (n=18) 1500 IU/m², 3 days	p*
Testosterone (ng/mL)			
Baseline	0.03 (0.03-0.03)	0.2 (0.03-0.8)	**0.025**
Day 1	0.4 (0.2-1.7)	-	-
Day 4	3.3 (1.9-5.3)	4.9 (2.8-6.1)	0.152
Increase (fold)			
Baseline/Day 4	40 (41.6-179.3)	40.1 (3.4-171.8)	0.143
Baseline/Day 1	14 (5.6-57.7)	-	-

*: Mann–Whitney U test

all patients. In Group 1, after the injection of 5000 IU/m², testosterone levels increased to 0.4 (0.2-1.7) ng/mL and 3.3 (1.9-5.3) ng/mL on day one and day four, respectively. Testosterone level on day one was significantly higher than baseline testosterone levels (p=0.005). In addition, testosterone levels on day four were significantly higher than the baseline (p<0.001). In Group 2 the stimulated testosterone level was 4.9 (2.8-6.1) ng/mL and was significantly higher according to the baseline levels (p<0.001). Peak testosterone levels of Group 1 and Group 2 did not differ (p=0.152) however, both were significantly higher than their baseline values. Testosterone levels increased 40-fold in Group 1 and 40.1-fold in Group 2 compared to the baseline (p=0.142). The testosterone levels of day

Table 3: Comparison of hCG tests in the groups

	Group 1 p	Group 2 p
Testosterone (ng/mL)* Baseline-Day 1-Day 4	<0.001	-
Testosterone (ng/mL) Baseline-Day 1**	0.005	-
Testosterone (ng/mL) Baseline-Day 4**	<0.001	<0.001
Increase testosterone Day 1-Day 4**	0.005	-

*: Friedman's two-way analysis of variance by ranks, **: Wilcoxon signed-rank test

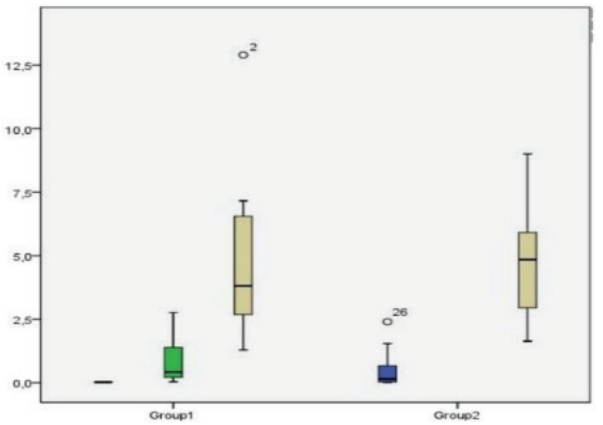

Figure 1: Median testosterone levels of Group 1 and Group 2

DISCUSSION

This study compared a single dose of 5000 IU/m^2 and three consecutive days of 1500 IU/m^2 hCG regimens. Peak testosterone levels in both tests were found to be similar. In Group 1, the testosterone level was significantly higher on day four than day one. As a result, a 5000 IU/m^2 hCG injection and blood drawn on day four was found more pratical than the other options.

The hCG stimulation test is the gold standard test to determine Leydig cell function. In recent years, assays like Insl3, anti-mullerian hormone (AMH), and inhibin B were identified for the evaluation of male reproductive system (8). However, the hCG stimulation test remains a key step in identifying the testicular hormone secretion. Testosterone responses of hCG stimulation tests help to differentiate bilateral nonpalpable gonads, hypogonadotropic hypogonadism, and constitutional delay of puberty from anorchia and some enzymatic defects of testicular steroidogenesis (9).

Nevertheless, poor standardization of hCG test protocols, lack of normative values, and different biochemical methods for steroid hormone measurements are some of the restrictions of hCG stimulation tests (2). hCG is a polypeptide hormone and has similar structure and action with LH because it interacts with the same receptor. In clinical practice, hCG is used when LH activity is needed. hCG has a long plasma half-life and stimulates Leydig cell steroidogenesis through the activation of LH/hCG receptors (10, 11).

In recent years, a recombinant hCG (rhCG) has been defined (250 µg/ampule; ~6500 IU). When compared with uhCG (5000 IU, single dose) the testosterone responses did not differ. Peak testosterone values were reached 72 hours after injections, and the increase over baseline was significantly high in both groups (12). In another study, a single dose application of 6500 IU rhCG was given to unilateral and bilateral cryptorchid boys and testosterone levels were increased from 10 ng/dl to 247.8±135.8 ng/dL and 253.6±128.0 ng/dL: respectively, 7 days after the injection. These studies suggest that rhCG is a favourable test to replace the urinary hCG test for the evaluation of Leydig cell steroidogenesis (3). However, it is more expensive, but rhCG can be used in countries in which uhCG is unavailable (3).

There are a few hCG stimulation tests reported in the literature. Different forms of hCG tests were performed and discrepancies of serum testosterone responses were reported. Previously, 5000 IU/1.7 m^2 single dose hCG was administered to prepubertal boys and their testosterone levels were measured before the injection and thereafter at 0.5, 1, 2, 3, 6, 12, 24, and 36 hours, and then daily for 6 days. The early response (at approximately 2-4h) of serum testosterone was absent in all boys, whereas the late response was constant at 2-5 days (13). Ishii et al. performed the hCG test with a dose of 3000 IU/m^2 for three consecutive days and gave a cut-off testosterone level of 1.1 ng/mL for patients who needed further evaluation and hormone replacement treatment was required (14). In another experiment, the peak testosterone level was reached on the third day and the maximal response was maintained through the fifth day, in normal adult males injected with 10.000 IU of hCG (15). In another study, hCG was applied three consecutive days and the daily dose was adjusted according to the age (1 year old, 500 units; 1-10 years, 1000 units; >10 years, 1500 units) and testosterone levels were measured 24 hours after the final injection. The study found the positive predictive value of the hCG test to be 89% and the negative predictive value of the hCG test to be 100% (16). Kolon et al. investigated 77 prepubertal boys and the hCG dose was applied according to weight (100 IU/kg) or BSA (5000 IU/1.7 m^2). Serum testosterone levels were measured at 72 and 96 hours after the injection. The researchers recommended that a single weight or BSA based hCG dose with testosterone measurement after three or four days was cost effective, practical, and reliable in testicular evaluation (17). Great variability in dosing schedules of hCG tests exist and Table 4 lists some of the previously recommended hCG protocols from the literature (13-20).

The main limitations of this study were the absence of a healthy control group and a small number of patients because this was a single center study for a rare disease. Secondly, a genetic analyses could not be obtained from all the patients so Group 1 patients did not have a certain diagnosis. However, most studies of hCG stimulation tests did not have a molecularly diagnosed patient cohort.

CONCLUSION

This study investigated the testosterone response after one and three daily doses of hCG protocols in prepubertal boys. Testosterone response in both groups was significantly high according to baseline values. Therefore, the hCG test is a reliable method for the evaluation of testosterone biosynthesis. Peak testosterone levels of both groups did not vary significantly. When a comparison of the easy applicability, practicality, and economy of both tests was done, it is suggested that performing a 5000 IU/m^2 rather than the 1500 IU/m^2 three day protocol would be the best method.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 17.07.2020, No: 120372)

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Author Contributions: Conception/Design of Study- A.D.K., Ş.P.; Data Acquisition- A.D.K., L.A., E.K.Ö.; Data Analysis/Interpretation- A.D.K., A.P.Ö., M.Y.; Drafting Manuscript- A.D.K., F.B., M.Y.; Critical Revision of Manuscript- F.D., Ş.P.; Approval and Accountability- A.D.K., E.K.Ö., L.A., A.P.Ö., M.Y., Ş.P., F.B., F.D.

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N-TERMINAL PROHORMONE OF BRAIN NATRIURETIC PEPTIDE IN THE DIAGNOSIS AND MANAGEMENT OF PERSISTENT PULMONARY HYPERTENSION IN NEWBORNS

YENİDOĞANLARDA PERSİSTAN PULMONER HİPERTANSİYON TANISI VE YÖNETİMİNDE N-TERMİNAL PROHORMON BRAİN NATRİÜRETİK PEPTİD

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ABSTRACT

Objective: This study aimed to investigate the levels and utility of amino/N-terminal prohormone of brain natriuretic peptide (NT-proBNP) in the diagnosis of neonatal persistent pulmonary hypertension (PPHT).

Materials and Methods: Infants born at ≥34 weeks of gestation were included in this retrospective cross-sectional study. In total, 33 newborns diagnosed with PPHT were included in the patient group and 50 healthy newborns were included in the control group. Patient and control groups were compared in terms of plasma NT-proBNP levels measured in the umbilical cord (UC) and at 72 hours of life.

Results: The NT-proBNP levels in UC and at 72 hours were significantly higher in neonates with PPHT compared to controls ($p<0.01$). For the diagnosis of PPHT, UC NT-proBNP cut-off value was >2760.5 pg/ml, sensitivity was 90.7%, and specificity was 96.6% ($p<0.01$). For NT-proBNP at 72 hours, the cut-off value was >1414 pg/ml, sensitivity was 95.9%, and specificity was 91.2% ($p<0.01$). The mean UC NT-proBNP levels were 1094.7±603 pg/ml and mean NT-proBNP levels at 72 hours were 875.7±423 pg/ml in the control group.

Conclusion: NT-proBNP levels are high during the initial days of life. They are useful in the diagnosis and follow-up of PPHT in newborns with hypoxemic respiratory failure and high fraction of inspired oxygen (FiO_2) requirement, especially in cases where transthoracic echocardiography (TTE) cannot be performed.

Keywords: NT-proBNP, persistent pulmonary hypertension, newborn, umbilical cord, echocardiography

ÖZET

Amaç: Bu çalışmada, beyin natriüretik peptidinin (NT-proBNP) amino/N-terminal prohormonunun duzeylerinin ve yenidoğan kalıcı pulmoner hipertansiyon (PPHT) tanısında kullanımının araştırılması amaçlandı.

Gereç ve Yöntem: Bu retrospektif kesitsel çalışmaya ≥34 gebelik haftasında doğan bebekler dahil edildi. Hasta grubuna PPHT tanısı konan 33 yenidoğan, kontrol grubuna ise 50 sağlıklı yenidoğan alındı. Hasta ve kontrol grupları, göbek kordonunda (UC) ve 72 saatlik yaşamda ölçülen plazma NT-proBNP seviyeleri açısından karşılaştırıldı.

Bulgular: UC'da ve 72. saatte NT-proBNP seviyeleri, kontrollere kıyasla PPHT'lu yenidoğanlarda anlamlı derecede yüksekti ($p<0,01$). PPHT tanısı için UC NT-proBNP eşik değeri >2760,5 pg/ml, duyarlılık %90,7 ve özgüllük %96,6 ($p<0,01$) idi. Yetmişikinci saatte NT-proBNP için eşik değeri >1414 pg/ml, duyarlılık %95,9 ve özgüllük %91,2 idi ($p<0,01$). Kontrol grubunda ortalama UC NT-proBNP seviyeleri 1094,7±603 pg/ml ve 72 saatte ortalama NT-proBNP seviyeleri 875,7±423 pg/ml idi.

Sonuç: NT-proBNP seviyeleri yaşamın ilk günlerinde yüksektir. Özellikle transtorasik ekokardiyografinin (TTE) yapılamadığı durumlarda, hipoksemik solunum yetmezliği ve inspire edilen oksijen fraksiyonu (FiO_2) gereksinimi yüksek olan yenidoğanlarda PPHT tanı ve takibinde faydalıdır.

Anahtar Kelimeler: NT-proBNP, persistan pulmoner hipertansiyon, yenidoğan, göbek kordonu, ekokardiyografi

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INTRODUCTION

Persistent pulmonary hypertension in newborns is a common critical complication of the neonatal lungs. The incidence of persistent pulmonary hypertension (PPHT) ranges between 0.4 and 6.8 per thousand liveborn term infants with an associated neurodevelopmental morbidity ranging from 30% to 35% and mortality ranging from 3% to 39% (1-5). PPHT occurs when pulmonary vascular resistance does not decrease after birth. When respiratory distress is complicated by oxygenation issues and low saturation, PPHT should be suspected. Because most diseases in newborns start with respiratory problems, PPHT is often difficult to diagnose (6, 7). PPHT is caused by a variety of factors, such as perinatal asphyxia, pneumonia, transient tachypnea of the newborn (TTN), respiratory distress syndrome (RDS), pulmonary vasospasm, congenital diaphragmatic hernia (CDH), aspiration of amniotic and fluid meconium, sepsis, drugs, idiopathic, and maternal urinary tract infection during pregnancy, which is mainly manifested as pulmonary arteriolar remodeling, hyperplasia, and vasospasm (8, 9).

N-terminal prohormone of brain natriuretic peptide (NT-proBNP), the amino-terminal portion of the preprohormone, is secreted into the peripheral blood in equimolar portions to brain natriuretic peptide (BNP). BNP has a short half-life of 20 minutes and is unstable at room temperature. In contrast, NT-proBNP has a longer half-life of 60–120 minutes and is stable under a range of storage conditions (10). BNP is an endogenous natriuretic peptide hormone secreted from the cardiac ventricular myocytes in response to an increase in ventricular wall stretch volume and ventricular pressure loading. Hypoxia also affects the secretion of NT-proBNP in human cardiac myocytes. The physiologic activities of BNP include the inhibition of sympathetic activity, induction of natriuresis, diuresis, vasodilatation, and inhibition of the renin–angiotensin–aldosterone system. The net effect is a reduction of intravascular volume and ventricular preload and afterload. NT-proBNP levels are elevated soon after birth, peaking at 24 h of life, decreasing thereafter for up to four months, and remaining unaltered until the age of 15 years. NT-proBNP levels are elevated in PPHT, but their measurement is not sensitive enough to contribute to routine diagnosis (11-13).

In this study, umbilical cord (UC) NT-proBNP and postnatal 72-hour NT-proBNP levels were measured in patients with PPHT and echocardiographic examination was performed. The results were compared with the healthy control group. The usefulness of NT-proBNP in the diagnosis and follow-up of PPHT was evaluated. NT-proBNP levels were also analyzed in subgroups of patients with PPHT.

MATERIALS AND METHODS

This work is designed as a prospective study. Parental consent was obtained for all patients. Ethics committee approval was obtained retrospectively from the ethics committee of Tekirdağ Namık Kemal University Faculty of Medicine (Date: 18.06.2020, No: 46048792-050.01.04-E.).

A total of 38 neonates that were born between July 2018 and July 2020 at >34 weeks of gestation and hospitalized at the neonatal intensive care unit with PPHT were included in the study. Patients with multiple congenital anomalies (1 patient), acute renal failure (1 patient), and other structural congenital heart diseases (3 patients), except small atrial septal defect, were excluded from the study. A total of 33 patients were finally included in the patient group. Fifty newborns born at >34 weeks of gestation who were followed-up for reasons such as jaundice and/or nutritional problems and did not have neonatal sepsis or respiratory problems were included in the control group by the sequential random-access method. Data were obtained from the electronic registry system and patient files. Parameters such as gestational week, gender, birth weight, 1st and 5th minute Apgar scores, pH in umbilical cord blood gas, base excess, NT-proBNP levels in the UC and at 72 hours, transthoracic echocardiography (TTE) at postnatal six hours, vasoactive inotropic score (inotrope and pressor score), oxygenation index (OI), Downes score (DS, for respiratory failure) at postnatal 1 hour, nasal continuous positive airway pressure (CPAP), intubated mechanical ventilation (MV) time, and length of hospital stay were recorded.

NT-proBNP levels were analyzed by electrochemiluminescence immunoassay using the Elecsys-cobas e602 analyzer (Roche Diagnostics, Germany). The NT-proBNP test was studied from the umbilical cord in the first 30 minutes after birth and from the baby's venous blood (0.5 ml) at the postnatal 72nd hour (measuring range is 5-35.000 pg/ml).

The total vasoactive inotropic score was obtained by summing daily inotrope score (dopamine [μg/kg/min]×1)+(dobutamine [μg/kg/min]×1)+(milrinone [μg/kg/min]×10) and pressor score (norepinephrine [μg/kg/min]×100)+(vasopressin [U/kg/min]×10,000)+(epinephrine [μg/kg/min]×100).

Severity of respiratory failure was evaluated using the DS within the first hour after birth. A score of 0–3 points was evaluated as mild, 4–6 points as moderate, and 7–10 points as severe respiratory failure (14). Oxygenation, in arterial blood gas analysis, the OI was calculated as mean airway pressure (cm H_2O)×FiO_2×100/PaO_2 in mmHg.

The first TTE was performed at postnatal 6 (±1) hours in all infants. Echocardiography was performed by a pediatric cardiologist in all enrolled patients using the Acuson SC 2000 ultrasound system transducer (Siemens, Germany) at a frequency bandwidth of 2.5–8 Mhz. Two-dimensional views were used to detect cardiac septal defects. For

accurate measurement of these defects, shunt direction in color flow mapping was determined. Doppler examinations were performed to assess pulmonary blood flow. PPHT was defined as hypoxemia with echocardiographic findings of elevated pulmonary artery systolic pressure (>35 mmHg), position of the intraventricular septal wall configuration (shape, size, and position to the right and left ventricles), and right-to-left shunting through a patent foramen ovale (intracardiac shunt) or patent ductus arteriosus (extracardiac shunt) or both. Pulmonary artery systolic pressure (PASP) of the newborn was measured using the tricuspid regurgitation pressure gap (TRP) method (PASP=TRP+10 mmHg). Left ventricular ejection fraction (LVEF) was calculated using the following formula: (end diastolic diameter−end systolic diameter)/(end diastolic diameter)×100. The severity of PPHT was graded as mild (pulmonary arterial systolic pressure <⅔ systemic systolic pressure), moderate (⅔-to systemic pressure), or severe (suprasystemic pressure) (15).

In the etiology of PPHT, various risk factors have been identified, including pneumonia, neonatal sepsis, pneumothorax, meconium aspiration syndrome (MAS), neonatal RDS, perinatal asphyxia, TTN, CDH, late prematurity (≥34 to <37 weeks of gestation), small for gestational age (SGA; birth weight <10% percentile for gestational week), nondiabetic large for gestational age (LGA; birth weight >90% percentile for gestational week), infant of a diabetic mother (IDM), oligohydramnios, and maternal drug use.

Statistical analysis

Pasw statistics software version 18 was used for data analysis. Data were presented as mean±standard deviation. Student's t test for quantitative independent variables was used for analyzing the differences between the two groups. Comparison between multiple subgroups was performed using one way analysis of variance. The correlation between quantitative variables was investigated using Pearson's chi-squared test. A p-value of <0.05 was considered statistically significant. Receiver operating characteristic (ROC) curve was drawn according to the relationship between NT-proBNP levels and pulmonary artery systolic pressure in the UC and at the postnatal 72nd hour.

RESULTS

There was no difference between the patient and control groups in terms of gender, gestational week, birth weight, mode of delivery, 1st minute Apgar score, maternal age, and number of pregnancies (p>0.05). PASP was higher, 5th minute Apgar score, pH, base excess and LVEF were lower in the patient group compared to the control group (p<0.05). The mean NT-proBNP levels of both measurements were found to be higher in the patient group than in the control group (p<0.01) (Table 1). The mean PASP was 28±2.3 mmHg in the control group. In the control group, mean UC NT-proBNP was 1094.7±603 pg/ml, 5th percentile was 298.5 (95% CI 248.5–547.6), 50th percentile was 986.7 (95% CI 878–1114), and 95th percentile was 2549.7 (95% CI 1712.1–3352); mean NT-proBNP at postnatal 72 hours was 875.7±423 pg/ml, 5th percentile was 346.6 (95% CI 299.7–452), 50th percentile was 789.3 (95% CI 678–939), and 95th percentile was 1589.3 (95% CI 1328.4–2521).

Table 1: Demographic features of the patient and control groups

Features	Patient group (n=33)	Control group (n=50)	p-value
Gender (female/male)	14/19	25/25	0.32
Gestational week (mean)	37.5±1.9	38.0±1.6	0.20
Birth weight (gr)	3079.2±714.6	3141.7±511.2	0.64
Normal vaginal delivery (%)	8 (24%)	17(34%)	0.24
Maternal age (years)	29.4±6.4	28.6±4.6	0.51
Number of pregnancies	2.3±1.2	2.4±1.4	0.64
1st minute Apgar score	6.8±2.01	7.4±1.2	0.15
5th minute Apgar score	8.0±1.2	8.6±0.7	**0.03**
UC pH	7.27±0.13	7.35±0.06	**<0.01**
UC base excess (mmol/L)	-5.79±4.24	-3.60±2.57	**0.01**
UC NT-proBNP (pg/ml) (mean)	16204.0±13459.9	1094.7±603.0	**<0.01**
NT-proBNP at 72 hours (pg/ml)(mean)	3559.5±3400.3	875.7±423	**<0.01**
PASP (mmHg)	54.6±12.5	28±2.3	**<0.01**
Ejection fraction (%)	65.2±2.5	67.84±2.8	**<0.01**

UC: umbilical cord, PASP: pulmonary artery systolic pressure, NT-proBNP: N-terminal prohormone of brain natriuretic peptide

The etiology of PPHT included neonatal pneumonia (36.4%), neonatal sepsis (30.3%), late preterm birth (30%), MAS (24.2%), IDM (18%), perinatal asphyxia 5 (15%), RDS (15%), SGA (15%), pneumothorax (12%), TTN (12%), nondiabetic LGA (9%), oligohydramnios (9%), and idiopathic factors (9%). Almost all patients had more than one risk factor (Table 2).

There was no statistical difference among the mild, moderate, and severe PPHT groups in terms of gender, gestational week, birth weight, mode of delivery, and LVEF. In the severe PPHT group, NT-proBNP in UC and at 72 hours, need for inotropic support, oxygenation index, respiratory failure score, and PASP values were higher than the other two groups ($p<0.05$). In addition, nasal CPAP/intubated MV time and lengths of hospital stays were longer in the severe PPHT group. Moreover, 1st and 5th minute Apgar scores, UC pH, and base excess were lower in the severe PPHT group, albeit not significantly ($p>0.05$). One patient in the severe PPHT group died (Table 3).

ROC analysis of UC NT-proBNP with PASP revealed the following: Area Under the Curve (AUC) 0.938, cut-off >2760.5 pg/ml, sensitivity 90.7%, and specificity 96.6% ($p<0.01$). ROC analysis of postnatal 72 hour NT-proBNP with PASP revealed the following: AUC 0.968, cut-off >1414 pg/ml, sensitivity 95.9%, and specificity 91.2% ($p<0.01$) (Table 4, Figure 1). The Pearson's correlation analysis revealed a strong positive correlation of UC NT-proBNP with PASP and oxygenation index and a moderate positive correlation with vasoactive inotropic score and Downes score. There was a moderately significant negative correlation between UC NT-proBNP and pH, 1st minute Apgar score, and base excess ($p<0.05$). NT-proBNP at postnatal 72 hours had a strong positive correlation with vasoactive inotropic score and a moderate positive correlation with PASP and oxygenation index ($p<0.05$). In addition, there was a moderate negative correlation between postnatal 72nd hour NT-proBNP and 5th minute Apgar score, UC pH and base excess ($p<0.05$) (Table 5). For PPHT, the stepwise regression model was applied in the univariate regression analysis. A significant correlation was observed between UC NT-proBNP and 1st minute Apgar score, OI, DS, VIS, PASP, and intubated MV time ($p<0.05$, explanatoriness 93.9%). In addition, a significant correlation was found between NT-proBNP at 72 hours and VIS ($p=0.01$) (Table 6).

DISCUSSION

In this study, plasma NT-proBNP levels in the patient and control groups were evaluated at two different time points. It was observed that NT-proBNP levels at both timepoints were higher in the patient group compared to the control group. Further, these levels increased as the clinical presentation of the disease became more severe.

Plasma NT-proBNP levels are very high during the first few days of life in healthy neonates, presumably owing to the fetal-to-neonatal transition of circulation. After this period, NT-proBNP levels decrease rapidly until the end of the 1st week of life and then decreases slowly until the end of the neonatal period (16). In our study, NT-proBNP levels of the included neonates were found to be higher than those of children and adults (17). It was observed that mean UC NT-proBNP levels decreased on the 3rd postnatal day in all groups, which was consistent with the results of a study by Zhu et al. (18). Schwachtgen et al. reported mean UC NT-proBNP levels of 818 (281–2595) pg/ml in 62 healthy infants. These results were also close to the UC NT-proBNP levels observed in our control group (19).

Table 2: Etiologies of persistent pulmonary hypertension

Causes	Mild (n=14)	Moderate (n=11)	Severe (n=8)	Total (n=33) (%)
Neonatal pneumonia	4	6	2	12 (36.4%)
Neonatal sepsis	4	3	3	10 (30.3%)
Late preterm birth (>34 week of gestation)	4	3	3	10 (30%)
Meconium aspiration syndrome	1	2	5	8 (24.2%)
Infant of a diabetic mother	3	1	2	6 (18%)
Respiratory distress syndrome	2	2	1	5 (15%)
Perinatal asphyxia	1	1	3	5 (15%)
Small for gestational age	3	1	1	5 (15%)
Transient tachypnea of the newborn	3	1	0	4 (12%)
Pneumothorax	2	1	1	4 (12%)
Nondiabetic large for gestational age	0	2	1	3 (9%)
Oligohydramnios-pulmonary hypoplasia	0	1	2	3 (9%)
Idiopathic	1	1	1	3 (9%)

Table 3: Features of patients with persistent pulmonary hypertension

	Mild (n=14) Mean±SD	Moderate (n=11) Mean±SD	Serious (n=8) Mean±SD	p value
Gender (female) (%)	7 (50%)	5 (45%)	2 (25%)	0.50
Gestational week	37.9±2.2	37.36±2	37.1±1.7	0.63
Birth weight (gm) (mean)	2983.9±631.4	3127.8±599.6	3180.0±1023.5	0.80
Vaginal delivery (%)	3 (21.4%)	3 (27.2%)	2 (25%)	0.8
Umbilical cord NT-proBNP (pg/ml)	4931.7±3412.2	21587.3±14004.9	28528.6±7288.5	**<0.01**
NT-proBNP at 72 hours (pg/ml)	2645.2±1117.6	2877.2±1817.2	6097.9±6004.4	**0.04**
Vasoactive inotropic score	21.0±13.9	47.3±23.1	124.6±86.7	**<0.01**
Oxygenation index (mean)	20.1±3.5	29.8±3.2	37.50±1.5	**<0.01**
Downes score (mean)	5.2±2.1	7.5±1.6	9.4±1.1	**<0.01**
Intubated MV time (hours)	38.4±21.5	39.3±41.4	52.1±39.8	0.73
Nasal CPAP (hours)	31.1±31.1	47.1±30.8	55.5±13.4	0.18
1st minute Apgar score	7.15±2.3	7.13±1.8	6.36±1.8	0.59
5th minute Apgar score	8.25±1.6	8.08±1.3	7.91±1.0	0.85
UC pH	7.33±0.11	7.24±0.13	7.21±0.12	0.10
UC base excess (mmol/L)	-4.8±3.3	-5.6±5.1	-7.9±4.1	0.24
PASP (mmHg)	44.4±2.3	53.4±4.4	73.7±6.9	**<0.01**
Ejection fraction	65.4±2.7	65.1±2.6	65.0±2.1	0.95
Hospital stay (days)	12.4±4.4	14.6±8.2	17.1±8.1	0.30

SD: standard deviation, CPAP: continuous positive airway pressure, MV: mechanical ventilation, UC: umbilical cord, PASP: pulmonary artery systolic pressure, NT-proBNP: N-terminal prohormone of brain natriuretic peptide

Table 4: ROC curve analysis of NT-proBNP levels measured in UC and at 72 postnatal hours with PASP in patients vs. controls

	AUC	Cutoff	p value	95% CI	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
UC NT-pro BNP	0.938	>2760.5	<0.01	0.877–0.999	90.7	96.6	96.5	90.7
Control NT-pro BNP	0.968	>1414	<0.01	0.933–1	95.9	91.2	91.2	94
PASP (mmHg)	1	>36.5	<0.01	1–1	100	100	100	100

ROC: receiver operating characteristic, NT-proBNP: N-terminal prohormone of brain natriuretic peptide, UC: umbilical cord, PASP: pulmonary artery systolic pressure, AUC: area under the curve, CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value

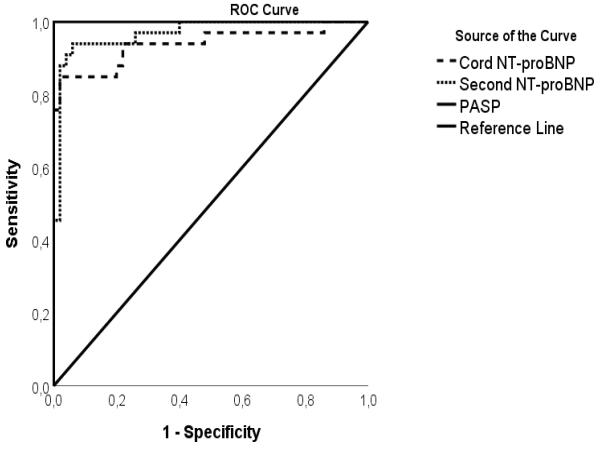

Figure 1: Receiver operating characteristic curve analysis according to the relationship between NT-proBNP levels in the umbilical cord and at postnatal 72 hours and pulmonary artery systolic pressure.

There is no apparent lung pathology involved in idiopathic PPHT. Secondary causes may be detected in three-quarters of the cases. In our study, the most common etiology of PPHT was infection (sepsis and/or pneumonia), late preterm birth, MAS, IDM, perinatal asphyxia, RDS, and SGA. There were three patients (9%) in the idiopathic group. The identified risk factors were similar to those of a study by Steurer and Bhattacharya (20, 21).

In the present study, gender, gestational week, maternal age, number of pregnancies, and mode of delivery had no effect on NT-proBNP levels (p>0.05). Similarly, Mannarino et al. reported that gender, maternal age, gestational week, and mode of delivery did not affect BNP levels in 36 preterm and 34 full-term infants (22). Seong et al. reported UC NT-proBNP levels of 766.2±536.9 pg/ml in 84 healthy newborns and demonstrated that low Apgar scores and pH were associated with increased NT-proBNP levels (23). In our study, the mean UC NT-proBNP levels in the

Table 5: Pearson's correlation analysis of NT-proBNP levels in the umbilical cord and at postnatal 72 hours

	UC NT-proBNP		NT-proBNP at postnatal 72 hours	
	r	p	r	p
Pulmonary artery systolic pressure	0.75	**<0.01**	0.54	**<0.01**
Vasoactive inotropic score	0.58	**<0.01**	0.84	**<0.01**
Oxygenation index	0.75	**<0.01**	0.40	**0.02**
Downes score	0.54	**<0.01**	0.33	**0.05**
1st minute Apgar score	-0.36	**0.04**	-0.29	**0.10**
5th minute Apgar score	-0.25	**0.16**	-0.35	**0.04**
Cord pH	-0.52	**<0.01**	-0.37	**0.03**
Cord base excess	-0.41	**<0.01**	-0.37	**0.03**

Table 6: Stepwise regression model analysis in the PPHT group

NT-proBNP	Model	Unstandardized beta	p-value	95% confidence interval
Umbilical cord	Vasoactive inotropic score	98.962	**0.02**	14.1–183.8
	1st minute Apgar score	-3753.574	**<0.01**	-4768.1– -2738.9
	Oxygenation index	616.517	**0.01**	176.7–1056.3
	Pulmonary artery systolic pressure	443.168	**0.01**	105–781.3
	Intubated mechanical ventilation time (hours)	59.37	**0.03**	111.8–6.86
Control	Vasoactive inotropic score	21.042	**0.01**	5.56–37.61

control group were slightly higher than those reported by Seong et al. This may be attributed to the presence of late preterm infants in our study group, individual differences in postnatal adaptation, and/or study methodology.

In the present study, increased UC NT-proBNP levels were negatively correlated with 1st minute Apgar scores and pH. Further, increased NT-proBNP levels at 72 hours were negatively correlated with 5th minute Apgar scores and pH. These findings indicated difficulties in respiratory adaptation in the perinatal period. In cases with severe MAS and severe asphyxia, a 5th minute Apgar score of <5 has been shown to be six times more associated with mortality (14). In a study by Neves, NT-proBNP was found to be associated with MV time, length of stay in the intensive care unit, need for inotropic support, and low cardiac output syndrome (24). In our study, infants with high PASP and UC NT-proBNP levels had higher inotrope scores, higher oxygen requirement, longer periods of intubated MV time, and lower 1st minute Apgar scores. Further, NT-proBNP levels at 72 hours were higher in patients requiring inotropic support.

NT-proBNP levels are closely correlated with cardiac shunt volume, increasing with decreasing LVEF, and are positively correlated with increasing PASP (25). In our study, patients with the highest levels of pulmonary artery pressure had worse clinical presentation and higher NT-proBNP levels. LVEF was lower in the patient group compared to the control group, but no significant correlation was found between NT-proBNP levels and LVEF. Increased pressure in blood vessels and/or myocardial hypoxia induce the synthesis and secretion of BNP. A previous study on 28 newborns with respiratory distress showed that NT-proBNP was much higher in the severe respiratory failure subgroup (26). In the present study, NT-proBNP levels, respiratory failure scores, and inotropic support and oxygen requirements were higher in the severe PPHT group. In addition, MV time and length of hospital stay were longer in the severe PPHT group. There were no statistical differences between the mild PPHT and moderate PPHT groups. It has been reported that PPHT accompanied by pulmonary hypoplasia results in a worse treatment response, with a mortality rate of 16.4% (4). In our study, inhaled nitric oxide (INO) therapy was used in two patients with severe PPHT. One of our three patients with oligohydramnios/pulmonary hypoplasia did not respond to INO and/or other treatments and died.

Downes score is used to evaluate respiratory failure in preterm/term infants. Rusmawati et al. showed that the sensitivity and specificity of DS were high in 88 newborns

with hypoxemic respiratory failure (14). In the present study, the clinical presentation was more severe and oxygen and inotropic support requirements were higher in patients with high DS and NT-proBNP levels.

Oxygenation index is used to determine INO and extracorporeal membrane oxygenation (ECMO) requirements in newborns with hypoxemic respiratory failure such as persistent pulmonary hypertension (9). Heindel et al. found a correlation between the severity of left ventricular dysfunction and pulmonary hypertension and elevated NT-proBNP levels at postnatal 48 hours and the need for ECMO in 44 patients with CDH. They also reported a significant correlation between elevated NT-proBNP levels at postnatal 6 hours and inotrope score (27). It has been shown that a repeated increase in NT-proBNP levels during the reduction of INO dose for PPHT treatment is associated with an increase in pulmonary vasoconstriction (7). Since INO could be used in only two patients in our study, the relationship between NT-proBNP and INO treatment response was not evaluated.

It has been shown that the rate of early deaths or ECMO requirement was higher when the NT-proBNP levels at postnatal 6 hours were ≥4682.5 pg/ml in patients with CDH-induced PPHT (27). In the present study, the threshold values for NT-proBNP levels were found to be lower due to the etiological differences in our patient group and the different blood test time. In 46 patients with perinatal asphyxia and myocardial injury, the cutoff for NT-proBNP levels was 3612.5 pg/ml (AUC 0.80), with sensitivity of 83.3% and specificity of 80.5% (25).

The strength of the study was that the diagnostic value of NT-proBNP measurement at two different time points and its relationship with the clinical follow-up of the disease were investigated in newborns with PPHT and healthy newborns.

The limitations of the study were that the number of patients was small, it was single-center, the best time to measure NT-proBNP levels was not known, different echocardiographic evaluation methods were not used, and there were no long-term morbidity results.

CONCLUSION

NT-proBNP levels are quite high in the first days of life. NT-proBNP can be a useful biomarker in the diagnosis and follow-up of PPHT when used with other parameters such as respiratory failure and high FiO_2 requirement.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Tekirdag Namik Kemal University, Non-Invasive Clinical Research Ethics Committee (Date: 18.06.2020, No: 46048792-050.01.04-E.).

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COMPREHENSIVE GERIATRIC ASSESSMENT IN PRACTICE: WHAT DO PATIENTS SAY?

KLİNİK PRATİKTE KAPSAMLI GERİATRİK DEĞERLENDİRME: HASTALAR NE DÜŞÜNÜYOR?

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ABSTRACT

Objective: Comprehensive Geriatric Assessment (CGA) is one of the cornerstones of geriatric medicine. In this study, we aimed to find out how satisfied patients aged 60 and over were with the application of CGA and whether they would express these complaints if geriatric-syndromes were not questioned.

Materials and Methods: Patients who applied to the geriatric outpatient-clinic were included. An 18-question survey was applied by the geriatric nurse. The satisfaction and benefit expectations of the patients regarding both the application of CGA and specific sub-areas of CGA (urinary- fecal incontinence, falls, sleep, Mini-Nutritional-Assessment, Mini-Mental-State-Examination) were evaluated. They were asked whether they would express their complaints if these questions were not asked.

Results: One-hundred-fifty patients were included in the study. The mean-age was 73.7±7 years. One-hundred-forty-eight patients were satisfied with the CGA and 139 of them thought it was beneficial. Seventy-three (49%) patients had urinary-incontinence, and 29 (19%) patients said that they would not report urinary-incontinence if this question had not been asked. Seventeen patients (11%) had fecal-incontinence and 16% of all patients said that they would not report fecal-incontinence if this question had not been asked. Twenty-nine of 85 patients stated that although they had a history of falling within the last year, they would not have stated this situation if this question was not asked. Ninety-three percent of the patients stated that they were satisfied with the Mini-Mental-State-Examination and that they thought this test would be beneficial for them.

ÖZET

Amaç: Kapsamlı-Geriatrik-Değerlendirme (KGD) geriatrinin temel taşlarındandır. Bu çalışmada 60 yaş ve üzeri hastaların KGD'nin uygulanmasından ne kadar memnun olduklarını ve geriatrik sendromlar sorgulanmasaydı; bu şikayetleri ifade edip etmeyeceklerini öğrenmeyi amaçladık.

Gereç ve Yöntem: Geriatri polikliniğine başvuran hastalar dahil edildi. Geriatri hemşiresi tarafından 18 soruluk memnuniyet anketi uygulandı. Hastaların, hem KGD uygulanmasıyla ilgili hem de KGD'nin bazı alt-alanlarından özgün olarak (idrar-dışkı inkontinansı, düşme, uyku, Mini-Nütrisyonel-Değerlendirme) memnuniyet ve faydalanım beklentileri değerlendirildi. Kendilerine bu sorular sorulmasaydı şikayetlerini ifade edip etmeyecekleri soruldu.

Bulgular: Çalışmaya 150 hasta dahil edildi. Ortalama yaş 73,7±7 yıl idi. Yüzelli hastanın 148'i KGD'nin yapılmasından memnun olduğunu, 139'u kendileri için faydalı olduğunu düşündüklerini belirtti. Yüz elli hastadan 73'ünün (%49) üriner inkontinansı vardı ve 150 hastanın 29'u (%19) bu soru kendilerine sorulmamış olsaydı idrar inkontinansı olsa dahi belirtmeyeceklerini söylediler. On yedi hastanın (%11) fekal-inkontinansı mevcuttu ve tüm hastaların %16'sı bu soru kendilerine sorulmasaydı fekal-inkontinansı olsa dahi belirtmeyeceklerini söylediler. Düşme hikayesi olan 85 hastanın 29'u son bir yıl içinde düşme hikayesi olmasına rağmen bu soru kendilerine sorulmamış olsa bu durumu belirtmeyeceklerini belirtti. Hastaların %93'ü Mini-Mental-Testin yapılmasından memnun olduklarını ve bu testin kendilerine faydası olacağını düşündüklerini belirttiler.

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Conclusion: Although it takes time and is tiring, CGA is satisfactory for patients and enables the recognition of geriatric syndromes that may remain hidden.

Keywords: Comprehensive geriatric assessment, satisfaction, geriatric syndrome, benefit

Sonuç: Vakit alması ve yorucu olmasına rağmen KGD hastalar için memnuniyet vericidir ve gizli kalabilecek geriatrik sendromların tanınmasını sağlamaktadır.

Anahtar Kelimeler: Kapsamlı geriatrik değerlendirme, memnuniyet, geriatrik sendrom, faydalanım

INTRODUCTION

The elderly population aged 65 and over has been increasing all over the world and in our country. According to the data of the Turkish Statistical Institute for 2019, while the ratio of the elderly population in the total population was 8.0% in 2014, it increased to 9.1% in 2019 (1).

The encounter with the elderly population in health services is increasing with ageing of the population. Evaluation of older adults should be done with a "comprehensive geriatric assessment" (CGA), which includes physical, functional, environmental, social, and psychological evaluation. CGA is an interdisciplinary evaluation in which multiple problems of elderly individuals are revealed, these problems are defined and explained, the reserves and stability of individuals are classified, their capacities and long-term needs are determined, a coordinated treatment plan is developed, and multifaceted medical, functional, psychosocial, and environmental evaluations are made (2).

The aim of CGA is to protect the health of older adults with preventive medicine and early diagnosis of diseases; to increase the functional capacity and quality of life of older adults; to optimize medical care; screening for geriatric syndromes such as depression, dementia, delirium, falls, pressure injuries, osteoporosis, polypharmacy, vision and hearing problems, malnutrition, sarcopenia and planning their treatment and follow-up; to take appropriate care of the patient by providing optimum environmental and social support; improve care costs and results; to prevent repeated and unnecessary hospital admissions and unnecessary tests (3). It is also used to identify the frail older adults, who are the most at risk for geriatric problems.

CGA is an examination that requires attention and knowledge and takes quite a long time. It is known that some patients may not want to have the tests done because it is a time-consuming process for both the patient and the physician, and some physicians may be hesitant to make all the evaluations.

Patient satisfaction is an important measure of patient-reported experience and can also be viewed as an outcome in itself and as an integral part of the outcomes of any healthcare organization. It has been identified as very important for the assessment of healthcare quality.

In addition, patient satisfaction can be defined as the measurement of needs and desires and affects patient compliance and continuity of care. For these reasons, the evaluation of patient satisfaction in the procedures performed is of particular importance

In this study, we aimed to investigate whether patients aged 60 and over are satisfied with the application of CGA, their expectation of benefit, and whether they would spontaneously express these syndromes if CGA was not performed.

MATERIALS AND METHODS

Study population

Patients who were aged 60 and over and applied to the outpatient-clinic of Istanbul University, Istanbul Medical Faculty were included in the study. All patients who applied to the geriatric outpatient clinic between February 2015 and April 2015, approved to participate in the survey, and underwent CGA were included in the study. Patients who could not come to the outpatient clinic because they were bedridden and were followed only by their relatives, patients who could not cooperate, and patients who could not understand and answer questions were not included in the study. Among 161 patients who applied to the Geriatrics outpatient clinic, 11 were excluded from the study because they did not meet the inclusion criteria. All the remaining 150 patients agreed to participate in the study. The study was designed as descriptive. Ethical approval was obtained from the ethics committee of Istanbul University, Istanbul Faculty of Medicine (Date: 28.04.2021, No: 190217). Informed consent was obtained from all participants.

Comprehensive geriatric assessment

Standardized Mini-Mental State Examination (SMMSE) was administered to evaluate cognitive and mental status. SMMSE is a test consisting of eleven items and evaluated over a total of 30 points, gathered under five main headings as orientation, recording memory, attention, and calculation, recall and language. The cut-off point for the total score was determined as 23-24 points (4-6).

Yesavage Geriatric Depression Scale was used for mood assessment. This test consists of 30 self-reported, easy-to-answer questions for the older adults. It is a scale that does not include symptoms that may occur due to non-depressive causes, especially somatic symptoms such as sleep

disorders, sexual dysfunction, aches and pains in the body, and the answers are only "yes" and "no". A total score of 0-10 indicates normal, 11-13 probable depression, and 14 and above indicates definite depression (7, 8).

Nutritional assessment was performed with the Mini Nutritional Assessment-Short Form (MNA-SF). This test is scored between 0-14. A score of 0-7 is considered "malnutrition", a score of 8-11 is considered "at risk of malnutrition", and a score of 12 and above is considered "normal" (9, 10).

Presence of urinary incontinence was questioned, and if present, the type (stress, urge, mixed, overflow, functional) was recorded. In addition, fecal incontinence was questioned. Falls in the past year were questioned.

While assessing sleep problems, it was questioned whether the patient had difficulty in falling or staying asleep, whether there were involuntary early awakenings, whether he found his sleep sufficient, frequency of waking up at night, and whether he used sleeping pills. In addition, the presence of REM sleep behavior disorder, obstructive sleep apnea syndrome and restless legs syndrome were questioned. In case of any of these, it was considered that the patient has a "sleeping problem".

Satisfaction survey

An 18-question satisfaction survey (Table 1) was administered to the patients by the geriatric nurse in a separate room immediately after the physicians performed the CGA, with a face-to-face interview. Patients were asked to answer questions as yes, no, or maybe. It was especially emphasized that the answers of the patients in this questionnaire would certainly not affect their treatment. In the questionnaire, the satisfaction and utility expectations of the patients regarding the application of CGA, both general and specific to some sub-domains of CGA (urinary incontinence, fecal incontinence questioning, falling questioning, sleep questioning, Mini Nutritional Assessment, and Mini-Mental-State-Examination), were evaluated. They were also asked whether they would have expressed these complaints if they had not been asked these questions.

Statistical analysis

Statistical analyzes were performed using the SPSS 21.0 program. After normal distribution analysis was per-

Table 1: Survey questions and answers

	Yes (%)	No (%)	Maybe (%)
Overall, were you satisfied with these tests?	148 (98.7%)	1 (0.7%)	1 (0.7%)
Do you think these tests will be useful to you?	139 (92.7%)	4 (2.7%)	7 (4.7%)
Were you satisfied with the implementation of the SMMSE?	145 (96.6%)	3 (2%)	2 (1.4%)
Do you think performing the SMMSE was beneficial for you?	140 (93.3%)	7 (4.7%)	3 (2%)
Were you satisfied with the implementation of the Geriatric Depression Scale?	146 (97.3%)	4 (2.7%)	-
Do you think performing the Geriatric Depression Scale was beneficial for you?	139 (92.6%)	7 (4.7%)	4 (2.7%)
Were you satisfied with the questioning about fall?	147 (98%)	1 (0.7%)	2 (1.3%)
If this question was not asked, would you state that you had falls?	114 (76%)	35 (23.3%)	1 (0.7%)
Were you satisfied with the sleep questioning?	149 (99.3%)	-	1 (0.7%)
If this question was not asked, would you state that you have a sleep problem?	138 (92%)	10 (6.7%)	2 (1.3%)
Were you satisfied with the nutrition test application?	97	1.5	1.5
Do you think applying a nutrition test was beneficial for you?	146 (97.3%)	2 (1.3%)	2 (1.3%)
MNA test A (decreased appetite); If this question was not asked, would you state that you have a decrease in appetite?	127 (84.7%)	21 (14%)	2 (1.3%)
MNA test B (weight loss); If this question was not asked, would you express weight loss?	129 (86%)	18 (12%)	3 (2%)
Were you satisfied with the urinary incontinence questioning?	149 (99.3%)	-	1 (0.7%)
If this question was not asked, would you state that you have urinary incontinence?	120 (80%)	30 (20%)	-
Were you satisfied with the fecal incontinence questioning?	148 (98.7%)	2 (1.3%)	-
If this question was not asked, would you state that you have fecal incontinence?	124 (82.6%)	24 (16%)	2 (1.3%)

SMMSE: Standardized Mini Mental State Examination, MNA: Mini Nutritional Assessment

formed, numerical data were given as mean±standard deviation. Categorical data were given as percentages.

RESULTS

The mean age was 73.7±7 years (min 60, max 92 years). Thirty-nine (26%) were male, 111 (74%) were female. Seventy-three (48.9%) patients had urinary incontinence, 17 (11%) patients had fecal incontinence, 85 (56.7%) patients had a history of falling in the past year, and 63 (42%) patients had sleep problems. According to the loss of appetite and weight loss questionnaires included in the MNA-SF, 32 (21.3%) patients had loss of appetite and 41 (27.3%) patients had weight loss. Of the 150 patients who participated in the study, 148 (98.7%) stated that they were satisfied with the CGA, and 139 (92.7%) of these patients stated that they thought it was beneficial for them. The detailed results of the 18 questions in the survey were summarized in Table 1.

Twenty-two (14.7%) of 73 (48.7%) patients with urinary incontinence stateted that they would not have stated this if this question had not been asked. Seven (4.7%) of 17 (11.3%) patients with fecal incontinence stated that although they had fecal incontinence, they would not have stated it if this question had not been asked. Twenty-nine (19.4%) of the 85 (56.7%) patients with a history of falling stated that although they had a history of falling within the last year, they would not have stated this situation if this question had not been asked to them. Seven (4.7%) of 63 (42%) patients with sleep problems stated that although they had sleep problems, they would not have stated this if this question had not been asked. Only 3 (2%) patients out of 41 (27.3%) patients with weight loss stated that they would not have stated this if they had not been asked this question despite their weight loss. Of the 32 (21.3%) patients with loss of appetite, only 1 (0.7%) patient stated that if this question had not been asked, he would not have stated this situation although he had loss of appetite. The detailed results were shown in Table 2.

DISCUSSION

In this study, it has been shown that the CGA is pleasing to patients and is useful in detecting geriatric syndromes that may remain hidden if not asked. It has been observed that especially urinary incontinence, fecal incontinence, and falls can remain hidden more than other geriatric syndromes.

In the international literature, studies on CGA and patient satisfaction are limited. In the study of four-item patient satisfaction surveys conducted by Ekerstad et al. in the emergency unit, it was shown that acute care in a CGA unit with direct admission was associated with higher levels of patient satisfaction compared to traditional acute care (11). Renoux et al., in their study to evaluate patient satisfaction in primary care after geriatric evaluation, found that 72% of the participants (n=89) were completely satisfied with the evaluation. In our study, 99% of the patients were satisfied with these tests; 93% of the patients reported that they thought these tests would be beneficial for them (12). Daure et al. evaluated geriatric evaluation in primary care from the physician's side this time and evaluated the satisfaction of 26 general practitioners (GPs) in performing CGA. 92% of the GPs surveyed expressed an 'extremely positive' or 'positive' opinion about the detection of previously unidentified health problems and the improvement of patient care after assessment. In addition, 73% of GPs stated that they had better knowledge about frailty syndrome and cognitive impairment after evaluation (13).

Geriatric syndromes refer to clinical conditions that are frequently seen in older adults, which can impair quality of life and increase morbidity and mortality. Common geriatric syndromes are malnutrition, immobilization, depression, dementia, delirium, falls, incontinence, pain, osteoporosis, pressure ulcers, sleep problems, and polypharmacy. Geriatric syndromes often remain undetected, hidden, and therefore cannot be treated unless questioned by a healthcare professional. In the study

Table 2: The results of the questionnaire

	n (%)	Those who would not report a problem despite having it if a question about it had not been asked: n(%)	Those who have complaints but say "I wouldn't have stated it if the question wasn't asked n(%)	Those who have no complaints and say "I wouldn't have stated it if the question had not been asked n(%)
Falls	85 (56.7%)	35 (23.3%)	29 (19.4%)	3 (2%)
Sleep problem	63 (42%)	10 (6.7%)	7 (4.7%)	3 (2%)
Decrease in appetite	32 (21.3%)	21 (14%)	1 (0.7%)	15 (10%)
Weight loss	41 (27.3%)	18 (12%)	3 (2%)	10 (6.7%)
Urinary incontinence	73 (48.7%)	30 (20%)	22 (14.7%)	7 (4.7%)
Fecal incontinence	17 (11.3%)	24 (16%)	7 (4.7%)	16 (10.7%)

of Iliffe et al., it was determined that the needs of the older adults in the areas of senses (vision and hearing), physical competence (mobility and falls), incontinence, cognition and emotional stress (depression and anxiety) were not met in primary care (14). In the geriatric screening study of Piccoliori et al. involving 894 patients, 7.8 of 32 potential problems were identified per patient, and 1.4 of these problems were unknown to the general practitioner (15). Rjin et al. observed that many geriatric syndromes were defined with CGA, but only a few of them were considered as problems by the patient. In the study of Rjin et al., the median of geriatric syndromes determined per participant was eight, while the median of geriatric syndromes recognized by the participant was one (16). In our study, some of the participants stated that they would not state this as a complaint unless they were asked about the presence of geriatric syndromes. Urinary incontinence is a geriatric syndrome that has profound effects on the quality of life of geriatric patients and their caregivers (17). Despite this, in our study, 22 (30%) of 73 patients with urinary incontinence stated that they would not have stated this situation if this question had not been asked to them. Seven (41%) of 17 patients with fecal incontinence stated that they would not have stated that they had fecal incontinence if this question had not been asked to them. As a matter of fact, fecal incontinence is a syndrome that has a great psychological impact and significantly impairs the quality of life of geriatric patients and caregivers. Moreover, the economic cost of the resources used in its treatment becomes a major problem for the social health system (18). For this reason, it is important to question fecal incontinence as a part of CGA for its early diagnosis and necessary interventions. Falls, another geriatric syndrome, are the main cause of morbidity and disability in older adults. More than one-third of people aged 65 and over fall each year, and in half of such cases, falls recur. The risk doubles or triples if there is a cognitive impairment or a previous history of falls. In our study, 29 (34%) of 85 patients with a history of fall in the last year reported that they would not report this situation to the doctor unless asked. In other words, if we did not do this questioning for the elderly patients who had falls in our study, approximately one third of them would not report their falls as a complaint.

Almost 40% of the older adults cannot be fed enough to meet their daily energy needs, and two out of three older adults skip a meal (19). In the study in which the nutritional status of older adults who applied to geriatric outpatient clinics in Turkiye were screened by MNA, the rate of malnutrition was found to be 6.9%, and the rate of those at risk of malnutrition was 26.7% (20). Anorexia prevalence was reported 21.5% in community dwelling older adults and independently associated with decreased muscle mass and strength in Turkiye (21, 22). Malnutrition is associated with prolonged hospitalization, increased risk of falls, decreased physical function, poor quality of life, increased risk of life-threatening complications, and increased mortality. It has also been shown to be associated with higher healthcare costs (23, 24). We have seen in our study that a geriatric syndrome, whose prevalence, and results are so important for the elderly, cannot be detected in some patients and the necessary interventions cannot be made unless asked by the doctor.

Limitations of the study

There are some limitations of our study. The study was descriptive and performed in a three-month period, therefore the number of patients was low. Although the patients were informed that it would not affect their treatment during the filling out of the questionnaire, a falsely high satisfaction rate may have been detected due to face-to-face interviews. On the other hand, they may have answered the question "Would you have told me if we hadn't asked about the existence of geriatric syndromes?" as "I would have said yes" because of the hesitation from the face-to-face interview. In summary, the conditions for filling out the survey were not ideal, but we do not think this situation greatly affected the results. In our study, only quantitative methods were used to evaluate the satisfaction of individuals. However, the use of qualitative research methods as well as quantitative methods will provide a more in-depth examination of the subject by obtaining information about people's motivations, thoughts and attitudes. In particular, it would be helpful to add a qualitative assessment to gain insight into the underlying reasons why people ``wouldn't have said their complaints if they hadn't been asked". On the other hand, the CGA satisfaction survey was conducted for the first time in our country and the satisfaction of specific CGA subheadings was evaluated for the first time in our country. However, the fact that some specific areas, not all subdomains of CGA, were evaluated in the satisfaction survey can be considered as a limitation.

CONCLUSION

The patients found the CGA application very satisfactory and stated that it was beneficial. In addition, CGA provides recognition of geriatric syndromes that may remain hidden. Although geriatric syndromes are common clinical conditions in older adults, which are known to cause deterioration in quality of life, decrease in functionality, increase in morbidity and even mortality, we see that some geriatric syndromes are not reported to the doctor unless asked in approximately one third of the patients. This demonstrates the importance of screening for geriatric syndromes that are a substantial part of CGA.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Ethics Committee of Istanbul University, Istanbul Faculty of Medicine (Date: 28.04.2021, No: 190217).

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NATURAL HISTORY OF CONGENITAL ISOLATED MILD AORTIC VALVE AND MILD PULMONARY VALVE STENOSIS: A SINGLE-CENTER FOLLOW-UP STUDY

KONJENİTAL İZOLE HAFİF AORT KAPAK VE HAFİF PULMONER KAPAK DARLIĞININ DOĞAL SEYRİ: TEK MERKEZLİ BİR TAKİP ÇALIŞMASI

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ABSTRACT

Objective: Most of the available information on the natural history of aortic stenosis (AS) and pulmonary stenosis (PS) in children is based on studies carried out over the past 35-40 years using cardiac catheterization. This study aimed to reveal the natural history of congenital isolated mild valvular AS and PS in children using serial Doppler echocardiographic examinations.

Materials and Methods: A total of 125 children, 50 with mild AS and 75 with mild PS, who underwent Doppler echocardiography were included in this study. The prognoses of mild AS and PS were compared according to age, gender, and valvular gradient at the time of initial diagnosis.

Results: The mean age of patients was 26.1±37.6 months at diagnosis. The mean follow-up duration was 27.65±21.60 (1-120) months. There was a significant decrease in the final gradient of the PS group compared to the baseline (23.58±6.97 vs. 19.88±11.21 mmHg, p=0.001). In the AS group, there was an increase in the final gradient, which was more pronounced in patients ≤1-year-old (22.42±6.12 vs. 27.74±14.12 mmHg, p=0.002). Four percent of patients in the PS group and 12% of patients in the AS group progressed to moderate to severe stenosis. All patients who progressed in the PS group were ≤1-year-old and male.

Conclusion: The results showed that mild PS had a better prognosis than mild AS and that the risk of progression in AS was higher. Careful follow-up should be performed in mild PS cases ≤1-year-old, especially in boys, since progression may be detected, even if infrequently. Mild AS should also be followed closely, as the disease may show progressive characteristics in all age groups.

Keywords: Congenital, aortic stenosis, pulmonary stenosis, natural history, pediatrics, echocardiography

ÖZET

Amaç: Hafif aort darlığı (AD) ve hafif pulmoner darlığın (PD) doğal seyri hakkındaki bilgilerin birçoğu geçmiş 35-40 yıllık kardiak kateterizasyon kullanılarak yapılmış olan çalışmalara dayandırılmaktadır. Bu çalışmanın amacı, çocuk hastalarda izole hafif valvüler AD ve izole hafif valvüler PD'nin doğal seyrini, seri Doppler ekokardiyografik ölçümler ile ortaya koymaktı.

Gereç ve Yöntem: Bu çalışmaya hafif AD tespit edilen 50 ve hafif PD tespit edilen 75, toplam 125 çocuk dahil edildi. Hafif AD ve PD'nin klinik seyirleri cinsiyet, ilk tanı anındaki yaş ve valvüler gradiyente göre karşılaştırıldı.

Bulgular: Tanı yaşı ortalaması 26,1±37,6 ay (1 gün-13 yaş) idi. Çocukların ortalama izlem süresi 27,65±21,60 (1-120) aydı. PD grubunun final gradiyentlerinde başlangıca göre belirgin azalma saptandı (19,88±11,21'e karşı 23,58±6,97 mmHg, p=0,001). AD grubunda bir yaş altı hastalarda daha belirgin olmak üzere tüm hastaların final gradiyentlerinde artış saptandı (27,74±14,12'ye karşı 22,42±6,12 mmHg, p=0,002). AD grubunda final gradiyentin ortalaması, PD grubuna göre daha yüksekti (27,74±14,12'ye karşı 19,88±11,21 mmHg, p=0,001). PD grubunda toplamda hepsi erkek ve ≤1 yaş olan %4 hastada orta-ileri darlığa progresyon görüldü. AD grubunun %12'sinde orta-ileri darlığa progresyon görüldü. Hafif AD olanlardan iki hastaya, hafif PD olanlardan ise bir hastaya girişim yapıldı.

Sonuç: Çalışmamızda hafif PD'nin hafif AD'ye göre seyrinin daha iyi olduğu ve AD'nin progresyon gösterme riskinin daha yüksek olduğu gösterildi. Bir yaş ve altındaki, özellikle erkek hafif PD olgularında sık olmasa da progresyon saptanabileceğinden dikkatli takip yapılmalıdır. Hafif AD ise, hastalık her yaş grubunda progresif özellik gösterebileceğinden yakın takip edilmelidir.

Anahtar Kelimeler: Konjenital, aort darlığı pulmoner darlığı, doğal seyir, pediatri, ekokardiyografi

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INTRODUCTION

Valvular aortic stenosis (AS) is defined as left ventricular outflow tract obstruction at the aortic valve level and is the fifth most common congenital heart lesion. Its prevalence is between 4-7%. Valvular pulmonary stenosis (PS) is defined as obstruction at the pulmonary valve level in the right ventricular outflow tract and is the fourth most common congenital heart lesion. Its prevalence varies between 8-10% (1, 2). Most of the existing information on the natural history of mild AS and mild PS is based on serial studies using cardiac catheterization carried out over the past 15-20 years (1, 3, 4). Doppler echocardiography has been used frequently in cases of mild to moderate AS and PS, as it is a non-invasive diagnostic method, facilitates diagnosis and treatment, and can be used with patients of all ages (5). Cardiac catheterization is a highly invasive diagnostic method for these patients. Furthermore, Doppler echocardiography provides more information regarding valve thickness and stenosis than catheterization procedures (5, 6). This study aimed to demonstrate the natural history of congenital isolated mild valvular AS and congenital isolated mild valvular PS in children by comparing them according to age and gender using serial Doppler echocardiographic measurements.

MATERIAL AND METHODS

The patients (n=125) were those with congenital isolated valvular AS (n=50) and congenital isolated valvular PS (n=75), who underwent Doppler echocardiography in the Department of Pediatric Cardiology.

The patients were divided into two subgroups according to their age at the time of diagnosis as ≤1-year-old and >1-year-old. All patients had mild stenosis at initial diagnosis (pressure gradient less than 41 mmHg measured via Doppler echocardiography). Echocardiographic measurements were obtained retrospectively from medical records.

The exclusion criteria were as follows: i) patients who had only a single echocardiographic examination); ii) patients with additional cardiac anomalies except for small patent ductus arteriosus and patent foramen ovale; iii) patients with abnormal ventricular function; and iv) patients with systemic diseases or genetic disorders.

The study was approved by the Istanbul Kanuni Sultan Süleyman Training and Research Hospital Ethics Committee (Date: 25.06.2009, No: 279). The study conforms to the principles outlined in the Declaration of Helsinki.

Transthoracic Doppler echocardiography

The study was performed using the Acuson 128/XP 10 echocardiography system and an age-appropriate transducer. Pulmonary and aortic valve morphologies and the status of the ventricles were evaluated by two-dimensional echocardiography. A quantitative assessment of the severity of valve stenosis was estimated using the aortic and pulmonic valve pressure gradient. The transvalvular pressure gradient (P) calculated from the peak instantaneous continuous-wave Doppler echocardiographic velocity measurements obtained using the Bernoulli equation was used to assess the severity of stenosis, as follows: $P=4x(\text{maximum velocity})^2$ (7).

Statistical analysis

Statistical analysis was performed with SPSS 19.0 for Windows (IBM Corp. since v. 19.0, Chicago, IL, USA). Continuous data was expressed as the mean±SD, categorical data was expressed as number and percentages. Kruskal–Wallis test was used for comparison among non-parametric variables between groups. Mann Whitney U test was used for comparisons of parameters between two groups. A Chi-square test was used to assess the differences in categorical variables between the groups. Intraclass correlation analysis was used to examine the relationships between parameters. A p-value <0.05 was considered statistically significant.

RESULTS

The children in the study population (n=125) were girls (n=51, 41%) and boys (n=74, 59%). The ages of the children at initial diagnosis ranged from 1 day-13 years, with an average of 26.1±37.6 months. The duration of follow-up ranged from 1 month-10 years, with an average of 27.7±21.6 months from initial diagnosis. The children were divided into the following two groups: mild PS group (n=75) and, mild AS group (n=50). While the age of 63% (n=79) of the children at initial diagnosis was ≤1-year-old, 37% (n=46) of the children were >1-year-old. There were no statistically significant differences between the duration of follow-up for the children in the AS and PS groups (p>0.05). In the mild PS group, the number of children ≤1-year-old was higher at initial diagnosis than in the mild AS group. (n=60, 80% vs. n=19, 38%, p=0.001). The ratio of boys to girls in the AS group was statistically significantly higher than that of the PS group (n=37, 74% vs. n=37, 49%, p=0.006) (Table 1).

There was no statistically significant difference between the initial gradients of the AS and PS groups (22.42±6.12 vs. 23.58±6.97 mmHg, p=0.380). However, the final gradient was statistically significantly higher in the AS group than in the PS group (27.74±14.12 vs. 19.88±11.21 mmHg, p=0.001). In children with PS, the decrease in the final gradient compared to the initial gradient was statistically significant (23.58±6.97 vs. 19.88±11.21 mmHg, p=0.001). In children with AS, the increase in the final gradient compared to the initial gradient was statistically significant (22.42±6.12 vs. 27.74±14.12 mmHg, p=0.002) (Figure 1).

Table 1: Comparison of age, gender, and follow-up duration of the groups

	PS group (n=75)	AS group (n=50)	p-value
Age at initial diagnosis			
≤1 year, n (%)	60 (80%)	19 (38%)	0.001
>1 year, n (%)	15 (20%)	31 (62%)	
Gender			
Girl, n (%)	38 (51%)	13 (26%)	0.006
Boy, n (%)	37 (49%)	37 (74%)	
Follow-up duration (months)	26.6±21.1 (20)	29.3±22.6 (23)	0.386

There was a significant difference between the levels of change in the final gradients between the AS and PS groups compared to the initial gradients (p=0.001). Children with AS had a higher rate of increase in terms of the final gradient relative to the initial gradient as compared to children with PS (60% vs. 24%). In contrast, children with PS had a higher rate of decrease in terms of the final gradient relative to the initial gradient compared to children with AS (65% vs. 26%).

In the children with PS, all 31 girls aged ≤1 year at diagnosis had a mild final gradient. Of the 29 boys aged ≤1 year at diagnosis, the final gradient for 26 of them was mild, for two was moderate, and for one was significant. The final gradient of all children with PS aged >1 at diagnosis was mild. In the children with AS, five girls aged ≤1 year at diagnosis had a mild final gradient, and one had a moderate final gradient. Ten boys aged ≤1 year at diagnosis had a mild final gradient while one had a moderate final gradient, and two had a severe final gradient. Of the seven girls aged >1 year at diagnosis, six had a mild final gradient while one had a moderate final gradient. Of the 24 boys aged >1 year at diagnosis, 23 had a mild final gradient while one of them had a moderate final gradient (Table 2).

In the PS group, the initial gradients of the children aged ≤1-year-old were statistically significantly higher than the initial gradients of those aged >1-year-old (24.63±6.79 vs. 19.40±6.25 mmHg, p=0.013). There was no statistically significant difference between the final gradients of the children according to age at diagnosis (20.83±11.75 vs. 16.07±7.94 mmHg, p=0.160). In children ≤1-year-old, the decrease in the final gradient compared to the initial gradient was statistically significant (24.63±6.79 vs. 20.83±11.75 mmHg, p=0.002). In children >1-year-old, there was no statistically significant change in the final gradient compared to the initial gradient (19.40±6.25 vs. 16.07±7.94 mmHg, p=0.136) (Figure 2A).

In the AS group, in children aged ≤1-year-old, the increase in the final gradient compared to the initial gradient was

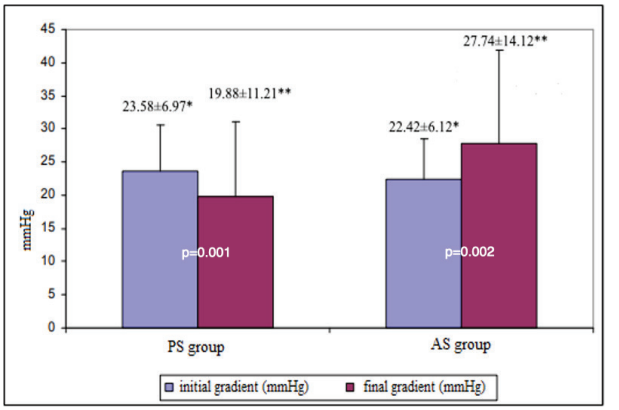

Figure 1: Comparison of the initial and final gradients of the children with PS and AS

*: p=0.380, **: p=0.001

statistically significant (21.84±7.08 vs. 31.95±19.28 mmHg, p=0.002). In children aged >1-year-old, there was no statistically significant change in the final gradient compared to the initial gradient (22.77±5.55 vs. 25.16±9.18 mmHg, p=0.121). There were no statistically significant differences between the initial and final gradients of the children according to age at diagnosis (Figure 2B).

When comparing children with PS in terms of gender, the decrease in the final gradient compared to the initial gradient for the girls was statistically significant (22.76±5.90 vs. 17.71±7.53 mmHg, p=0.001). However, for boys, there was no statistically significant change in the final gradient compared to the initial gradient (24.43±7.92 vs. 22.11±13.78 mmHg, p=0.133) (Figure 3A).

In girls with AS, the increase in the final gradient compared to the initial gradient was statistically significant (20.23±5.38 vs. 26.54±13.02 mmHg, p=0.010). Again, for the boys, the increase in the final gradient compared to the initial gradient was statistically significant (23.19±6.24 vs. 28.16±14.63 mmHg, p=0.030) (Figure 3B).

Bicuspid aortic valves were detected in 18 of the 50 children with mild AS, and no statistical difference was found between those with and without bicuspid valves in terms of age at initial diagnosis (p=0.777), final gradients (p=0.730), and follow-up durations (p=0.412).

There was a statistically significant difference in the changes in the final gradients compared to the initial gradients between children with PS and AS at ≤1 year of age. A decrease was seen in the final gradients from the baseline in children with mild PS, while an increase was seen in children with mild AS (-3.80±10.37 vs. 10.10±17.03 mmHg, p=0.001).

There was a statistically significant difference in the changes in final gradients compared to initial gradients between children with PS and AS at >1 year of age. A decrease was

Table 2: Distribution of final gradients and follow-up durations by age and gender in children with mild aortic and pulmonary stenosis

	Age at initial diagnosis	Gender	Final gradient	Follow-up duration (months)
Aortic stenosis	≤1 year	Girls (n=6)	Mild: 5	17.8±7.4
			Moderate: 1	19
			Severe: 0	-
		Boys (n=13)	Mild: 10	17.0±10.9
			Moderate: 1	14
			Severe: 2	16.7±10.4
	>1 year	Girls (n=7)	Mild: 6	38.8±40.6
			Moderate: 1	18
			Severe: 0	-
		Boys (n=24)	Mild: 23	36.8±21.0
			Moderate: 1	17
			Severe: 0	-
Pulmonary stenosis	≤1 year	Girls (n=31)	Mild: 31	26.3±25.3
			Moderate: 0	-
			Severe: 0	-
		Boys (n=29)	Mild: 26	24.8±18.4
			Moderate: 2	44.0±7.1
			Severe: 1	2
	>1 year	Girls (n=7)	Mild: 7	19.9±13.0
			Moderate: 0	-
			Severe: 0	-
		Boys (n=8)	Mild: 8	38.1±14.2
			Moderate: 0	-
			Severe: 0	-

Table 3: Comparison of changes in final gradients from initial gradients in PS and AS groups according to age at diagnosis

Age		Change in final gradient from baseline (mmHg)	p-value
≤1 year	PS group	-3.80±10.37	0.001
	AS group	10.10±17.03	
>1 year	PS group	-3.33±10.42	0.024
	AS group	2.39±7.22	

seen in the final gradients from the baseline in children with mild PS, while an increase was seen in children with mild AS (-3.33±10.42 vs. 2.39±7.22 mmHg, p=0.024) (Table 3).

DISCUSSION

Children with congenital mild valvular PS and mild valvular AS with congenital intact septum without any additional cardiac pathology were included in our study. We aimed to reveal the natural history of children with PS and AS whose initial diagnosis ages were ≤1 and >1 and to provide accurate data on their prognoses for their families. Our study was conducted with a total of 125 children, 51 (41%) girls and 74 (59%) boys. Age at diagnosis ranged from one day to 13 years, and the children were examined

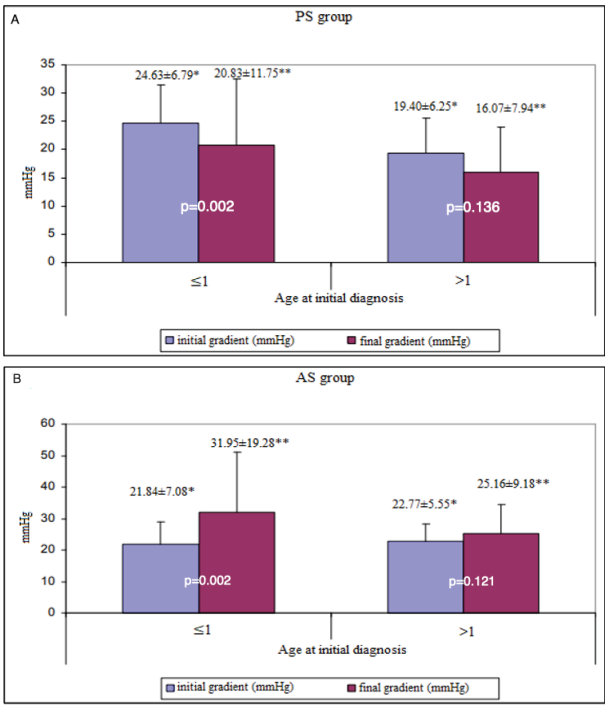

Figure 2: A) Comparison of the initial and final gradients of the children with PS according to the age at initial diagnosis
*: p=0.013, **: p=0.160

B) Comparison of the initial and final gradients of the children with AS according to the age at initial diagnosis
*: p=0.365, **: p=0.508

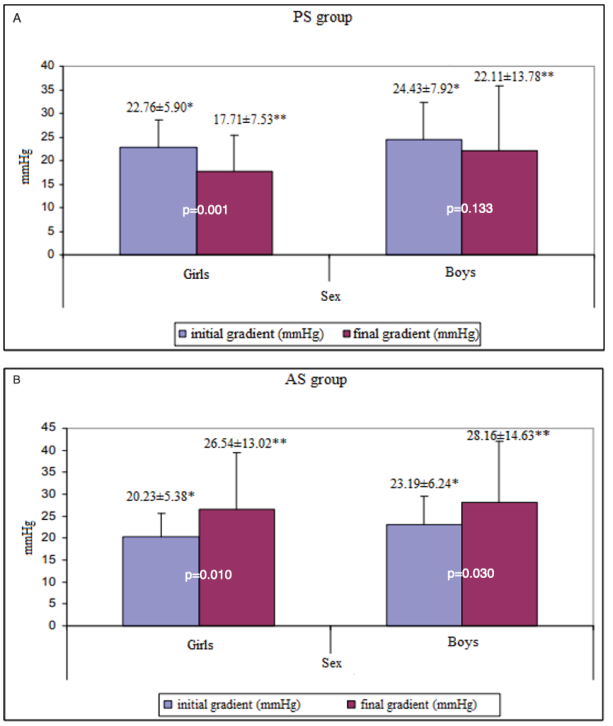

Figure 3: A) Comparison of the initial and final gradients of the children with PS according to the gender
*: p=0.362, **: p=0.298

B) Comparison of the initial and final gradients of the children with AS according to the gender
*: p=0.141, **: p=0.731

in two groups: PS (n=75) and AS (n=50). The most important results of our study were as follows: i) when the two homogeneous groups with no statistical differences in baseline gradients and mean follow-up times were compared, we found that the natural histories of patients with mild PS were better than those with mild AS; ii) while all of the children with progressive PS were boys, two-thirds of the children with progressive AS were also boys; iii) in children with PS at ≤1-year-old, progression was significantly more pronounced than in children >1-year-old; iv) in children with AS, the progression at ≤1-year-old was found to be significant compared to those >1-year-old; and iv) it was determined that the rate of progression in children with mild PS, whether ≤1 or >1-year-old, was more often benign than in children with mild AS who had similar follow-up periods.

The age at diagnosis of 63% of the children was ≤1 year. The reason for such early diagnosis was that the study was conducted in a maternity hospital where every newborn is examined early. The age at diagnosis of children with PS was earlier than those with AS. The age at first diagnosis was ≤1 year in 80% of the children with PS while it was >1 year in 62% of the children with AS.

Studies have shown that PS is equally common in boys and girls and that AS is four times more common in boys (8-10). In our study, while the boy/girl ratio of children with PS was equal, it was 3:1 in children with AS, which is consistent with the literature.

Considering the distribution of the final gradients in children with mild AS, it was observed that 8% had moderate AS and 4% had severe AS. That is, the progression of mild AS was observed in 12% of our children. Progression was higher in children with mild AS at ≤1 year old than in children aged >1 year. Of the 19 children with mild AS at ≤1 year of age, progression was detected in 21% of cases, while it was only detected in 6% of the 31 children aged >1 year. In the AS group 66% of children who progressed were boys.

In a study that included 142 children with mild AS, there was clinical progression within ten years of initial diagnoses in only 12% of the children (10). However, in our study, progression was detected in 12% of the children over a much shorter time, just 29.3±22.6 months.

Kitchiner et al. studied 187 children who presented with mild congenital AS; 63% were boys. Additional cardiac

lesions were present in 51 children. The median age at presentation was two years (range, 0-15), and the median duration of follow-up was ten years (range, 1-28). Thirty two patients (17%) progressed to require intervention (28 surgical, five balloon valvuloplasty) at a median age of 10.5 years. As a result of their study, they recommended follow-up into adulthood (11). In contrast, children with additional cardiac lesions were excluded from our study. Intervention was required in two of our children (4%) with mild AS who presented with progression at follow-up. The shorter median follow-up period and the exclusion of patients with additional cardiac anomalies in our study can be explained as the reason for the lower rate of intervention.

According to the literature, the course of valvular PS is benign in children with a systolic gradient below 40 mmHg in the first year of life. In the study "Natural History Study of Congenital Heart Defects", 261 children were evaluated; the researchers concluded that the risk of progression was higher in children under four years of age who had high initial gradients. Only 22 children under the age of two were included in that study (12). In the second "Natural History Study of Congenital Heart Defects" study, patients with a gradient of 25 mmHg and below were followed, and no increase was detected (13). Both studies concluded that mild PS is a static lesion and rarely progresses. Mody et al. enrolled 68 patients with isolated valvular PS diagnosed by cardiac catheterization. They concluded that mild stenosis in infants <1-year-old could become severe later in life, while mild stenosis at >1-year-old was unlikely to become severe. The study showed that moderate to severe stenosis was progressive in all age groups (14).

However, studies in the neonatal period suggested that PS is a progressive lesion. A study concluded that mild PS was a non-static pathology that should be followed carefully, especially in infants. This study observed that 25% of infants with mild PS during the neonatal period developed significant stenosis in the later period, and half of these underwent surgery (15). Anand et al. followed 51 infants with asymptomatic PS in 1997 and observed rapid progression that required surgery in 6 (15%) of them (8). Finally, Rowland et al. examined 56 infants with mild PS with a diagnosis age of <1 month, and progression was detected in 16 patients (1).

In the present study, a significant decrease was found in the final gradients of children aged ≤1 year and >1 year with mild PS. All children with progressive mild PS were ≤1-year-old and boys. Of 60 the children ≤1-year-old with mild PS, two (3%) progressed to moderate stenosis and one (1.5%) to severe stenosis. Balloon valvuloplasty was required in one patient who progressed to severe stenosis.

Our study is one of few aiming to examine the natural history of children with isolated aortic and isolated pulmonary stenosis. The literature contains studies from the previous 40-50 years; aside from the present study, no similar current studies yet exist.

Our study has limitations as well as strengths. First of all the sample size was relatively small because the data was derived from a single center. The follow-up period was short and the adult age echocardiographic measurements of the patients were not available.

CONCLUSION

Based on the results of this single-center follow-up study, congenital mild valvular PS was shown to have a benign course in cases detected after one year of age. However, children diagnosed before the age of one year, especially boys, should be followed up more carefully because progression may be detected, including significant progression, although it is not common. Cases of congenital mild valvular AS should be followed closely, as the disease shows progressive characteristics in all age groups, especially those under one year old.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Ethical Committee of the Kanuni Sultan Suleiman Training and Research Hospital (Date: 25.06.2009, No:279).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- K.Ö.; Data Acquisition- S.B., K.Ö.; Data Analysis/Interpretation- K.Ö., P.K.Ö.; Drafting Manuscript- P.K.Ö.; Critical Revision of Manuscript- K.Ö.,S.B., P.K.Ö.; Approval and Accountability- S.B., P.K.Ö., K.Ö.

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RESEARCH / ARAŞTIRMA
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A BIBLIOMETRIC ANALYSIS OF THE RELATIONSHIP BETWEEN DIABETES AND ARTIFICIAL INTELLIGENCE*

DİYABET VE YAPAY ZEKÂ İLİŞKİSİ ÜZERİNE BİR BİBLİYOMETRİK ANALİZ

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ABSTRACT

Objective: The applications of artificial intelligence in the field of medicine are progressing at a remarkable speed. Within the scope of this study, it is aimed to make a detailed examination of the studies published on diabetes and artificial intelligence, to determine any trends in these studies over time, to examine which subjects have been researched more and to explain the global interest in the subject.

Material and Methods: In this study, 2534 studies published between 1985 and 2020 in the field of diabetes and artificial intelligence were analyzed using the R programming language through bibliometric data obtained from the Scopus database. Correlation analysis, ANOVA and regression analysis were performed to determine the relationship between the number of articles and years.

Results: According to the analysis results, the number of publications between 1985 and 2015 was 604 and over the last 5 years, the number of publications have tripled to 1930. It was found that the country with the highest number of publications with 358 publications and 10426 citations was the United States of America (USA). Moreover, in the analyzed studies, the most used keywords and the use of these words together was also examined and the top 10 source platforms where the studies were published most have been presented in the study. According to regression analysis, it can be predicted that the number of articles to be published for 2021 is 242.

Conclusion: As a result of the analysis in this study, it was determined that artificial intelligence and diabetes applications have

ÖZET

Amaç: Yapay zekânın sağlık alanındaki uygulamaları dikkat çekici hızla ilerlemektedir. Bu çalışma kapsamında diyabet ve yapay zekâ konusunda yayınlanan çalışmaların detaylı bir incelemesinin yapılması, çalışmaların zaman içindeki eğiliminin belirlenmesi, hangi konularda daha fazla araştırma yapıldığının incelenmesi ve konuyla ilgili küresel ilginin açığa çıkarılması amaçlanmıştır.

Gereç ve Yöntem: Bu çalışmada 1985-2020 yılları arasında diyabet ve yapay zekâ alanında yayınlanan 2534 çalışma Scopus veri tabanından elde edilen bibliyometrik verilerle R programlama dili kullanılarak analiz edilmiştir. Yıllara göre makale sayısı arasındaki ilişkiyi açığa çıkarmak için, korelasyon analizi, varyans analizi (ANOVA) ve regresyon analizi gerçekleştirilmiştir.

Bulgular: Yapılan analiz sonuçlarına göre, 1985-2015 yılları arasında üretilen yayın sayısının 604 olduğu ve son 5 yıldaki yayın sayısının bu sayıyı üçe katlayarak 1930'e çıkardığı tespit edilmiştir. Bununla birlikte, 358 yayınla en çok yayın yapan ve 10426 atıfla en çok atıf alan ülkenin, Amerika Birleşik Devletleri olduğu saptanmıştır. Ayrıca, incelenen araştırmalarda en çok kullanılan anahtar kelimeler ve bu kelimelerin bir arada kullanımı ve çalışmaların en çok yayınlandığı ilk 10 kaynak platform da araştırmada sunulmuştur. Gerçekleştirilen regresyon analizine göre 2021 yılı için yayınlanabilecek makale sayısının 242 olarak tahmin edilmiştir.

Sonuç: Sonuç olarak, yapay zekânın altın çağını yaşadığı günümüzde, yapay zekâ ve diyabet uygulamalarının, küresel bazda önemli araştırma konularından biri hâline geldiği saptanmış olup, diyabeti önlemek veya erken dönemde teşhis etmek için

* This study was presented orally at the 7th International Management Information Systems Conference.

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become one of the important global research topics in today's golden age of artificial intelligence, and it was also determined that there is an urgent need for artificial intelligence supported scientific studies to prevent diabetes or diagnose diabetes in the early period.

Keywords: Diabetes mellitus, artificial intelligence, bibliometric analysis

yapay zekâ destekli bilimsel çalışmalara yoğun bir ihtiyacın bulunduğu tespit edilmiştir.

Anahtar Kelimeler: Diyabet, yapay zekâ, bibliyometrik analiz

INTRODUCTION

The incidence of diabetes is increasing day by day with the spread of obesity and sedentary lifestyles and it has become a global health problem. In fact, it could be said that diabetes is the health crisis of the century (1). According to the International Diabetes Federation's 9th Diabetes Atlas data in 2019, since its first report in 2000, the estimated number of patients with diabetes (type 1 and type 2 combined, both diagnosed and undiagnosed) was 151 million people aged between 20 to 79 years (4.6% of the global population) and increased to 463 million people (9.3% of the global population). The report predicts that this number, which was estimated to be 463 million in 2019, will reach 578 million in 2030, and if sufficient action plans are not implemented for this pandemic, this number will reach 700 million (10.9%) in 2045. In addition, the number of deaths from diabetes and its complications was estimated to be 4.2 million in 2019. In the same report, it was stated that 10 percent of global health expenses are spent on diabetes, while 3 out of every 4 people with diabetes (79%) live in low-middle income countries (2). The prevalence of diabetes is also increasing in Turkiye, parallel to its global increase. The first of the studies conducted to determine the prevalence of diabetes in Turkiye is TURDEP I (Turkiye Diabetes, Hypertension, Obesity and Endocrinology Diseases Prevalence Study-I) study (3). According to the results of the study, the diabetes prevalence was found to be 7.2% (24788 subjects, age≥20, mean 40.88±14.86). Moreover, according to the results of the TEKHARF 2013 (Turkish Adult Risk Factor survey 2013) study, which was conducted to explain the change in the prevalence of diabetes across the country in the last 12 years (768 subjects, age range ~40-80), it was found that the overall prevalence of diabetes increased by 80%, which corresponds to an annual increase of 5% (4). According to the Turkiye Chronic Kidney Disease Prevalence Research (the Chronic Renal Disease in Turkiye - CREDIT) results (10872 participants, mean age 40.5±16.3 years) diabetes prevalence was 14.3% in women, and 10.9% in men and it is overall reported as 12.7% (5). According to the TURDEP-II study, the prevalence of diabetes in adults 20 years and older was determined as 16.5% and it was stated that according to the data of 2010, there were 6.5 million diabetic people in Turkiye (6).

Long-term complications of diabetes include retinopathy, potentially leading to impaired vision; nephropathy, leading to kidney failure; peripheral neuropathy with risk of foot ulcer, Charcot joints; amputation and autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular diseases. However, if proper diabetes management is provided, these serious complications can be delayed or avoided altogether (2). Research results demonstrate that, with the increasing incidence of diabetes, early detection and prevention of both diabetes and diabetes complications are critical.

Artificial intelligence (AI) has become one of the most important fields of study worldwide. AI is defined as the ability of a computer or a computer-controlled machine to perform tasks related to higher mental processes such as reasoning, inferring and learning from past experiences, which are generally assumed to be human-specific qualities (7). AI applications have become prominent especially in the field of medicine. It is one of the important research areas that will benefit humanity the most and research is progressing at a remarkable pace (8). To illustrate this, with the aging population in the world, chronic diseases such as diabetes, cancer, and dementia have tended to increase and physicians, nurses and all employees who are in direct contact with the patient, working in the health sector, make a serious effort to adapt to the patient's condition when they come across such patients. It is obvious that in such cases, AI will be very useful as a decision supporting system (9). Digital medicine and related research have the potential to continuously monitor patients' symptoms, physiological data, behavior, and lifestyle remotely through AI, wearable devices, sensors and smartphone technologies. Thus, it has revolutionary effects in the prevention of diabetes, from the creation of personal nutrition plans to the regulation of the necessary diabetes treatment (10).

It has been stated that AI can help reduce the global prevalence of diabetes of 8.8% by changing the way diabetes is prevented, detected, and managed. Furthermore, it was stated that it would positively affect the decision-making process and thus cause a paradigm change in the management of diabetes based on data (11). AI technologies with machine learning algorithms have started to be used for the prediction and management of diabetes and related syndromes from the diagnosis of

diabetes, diabetic retinopathy and diabetic nephropathy to blood glucose control, and studies in this area are increasing day by day (12-16).

Studies on the use of AI in the diagnosis and management of diabetes have become increasingly common in the literature (17). The development and progress of this increasing number of studies, the level of interest around the world, and especially the subjects of these research studies, have been an object of curiosity. Regardless of the field, before conducting a new scientific study, it is necessary to determine which new trends and gaps are in the field, which methods and models are used, the aspects of the study to be conducted that differ from other studies and what innovations it can bring to the literature. Literature studies are carried out before the application and it is stated that it is possible to carry out these studies more technically using bibliometric data (18).

Within the scope of this study, it is aimed to make a detailed examination of the studies published on diabetes and AI, to determine the trend of the studies over time, as stated above, to examine which subjects have been researched more and to explain the global interest in the subject. Thus, it is aimed to present a preliminary literature that will guide researchers who want to conduct similar studies in the literature. Below, the data and the method used in the study, the findings obtained, and the inferences about the past, present and future of diabetes studies related to AI based on the findings obtained are shared respectively.

MATERIALS AND METHODS

In line with the purpose of the study, the bibliometric analysis technique was used. Bibliometry is defined as being a part of research evaluation methodology and the numerical analysis of the publications produced by individuals or institutions in a specific area in a specific region and the relations between these publications (19). This type of analysis is based on the identification of publications in a particular subject area (20). Within the scope of bibliometric analysis, processes such as quantitative evaluation of publications and citation data in a field of science, measuring the progress in the related field, determining conceptual relations and related network maps, and interpreting trends are carried out. In more general terms, bibliometric analysis can be examined under two main headings - performance analysis and mapping. Performance analysis is based on numerical calculations such as the number of publications and citations by country and author, and determination of the most frequently used words. Mapping aims to reveal the relationship between the mentioned variables. Various measures such as the number of publications and citations per year, citation distribution, number of citations per publication, number of authors per publication, collaboration index, and h-index are used for performance analysis. Citation analysis, co-citation analysis, bibliographic coupling, co-word analysis, and co-authorship analysis are used for mapping (21).

In order to perform bibliometric analysis, bibliometric data must first be obtained. There are many databases such as Web of Science, Scopus, PubMed, Google Scholar, EMbase, and SpringerLink, all of which can be used to obtain data. Scopus is a database of studies in high-quality scientific journals, conference proceedings and international books. It ensures that only the highest quality data is indexed through rigorous content selection and re-evaluation by an independent Content Selection and Advisory Board. Following the same rigorous standards, it also creates a comprehensive author and institution profile with the support of profiling algorithms (22). For these reasons, the Scopus database was chosen to obtain the necessary data for this study.

Within the scope of the study, the bibliometric data of academic studies in accordance with the following constraints were obtained from this database.

- Including "diabetes-artificial intelligence" or "diabetes-machine learning" word groups in the title, summary, or keywords
- Published in the form of article, book series, books and conference proceedings
- Published/accepted for publication between 1985-2020
- Published in English

Regarding 2534 studies in total:

- Quote information
  - Author (s) name (s), author (s) ID (s), study title, year, reference title, volume-issue-page information, citation number, document type, stage in the publication process, access type, DOI number.
- Bibliographic information
  - Institution, ISSN, PubMed ID, publisher, editor, original language, responsible author address information, abbreviated source title.
- Summary and keyword information
  - Summary, keywords, indexed keywords.
- Supporting information
  - Number, acronym, sponsor, financing text.
- Other information
  - Trade name and manufacturers, accession numbers, conference name, attached references.

Although the same database was searched with different word groups, some studies were repeated as machine learning is a subfield of AI. A total of 668 duplicate studies (determined according to DOI numbers), were excluded from the data set used in the analysis. The study carried

out was limited to the database used and the preferred word groups.

The term AI includes many sub-branches such as machine learning, data mining, evolutionary algorithm, expert systems, etc. Therefore, while obtaining bibliometric data on the articles, different combinations of the word groups related to these sub-branches could be used together with AI. In order to obtain data in this study, the researchers limited the search word groups to AI-diabetes and diabetes-machine learning because all the branches mentioned are sub-branches of AI as stated. AI was chosen in this study since it is a very inclusive concept. While the sections in which the word groups to be searched in the articles were limited to only the title or keywords, the abstract section is considered as the article section where the word groups are searched in order to obtain the data of the studies in the sub-branches, and the search space was expanded. At this point, it was thought that the concept of AI would be included at least in the summary section for studies on expert systems, data mining, etc. Another situation is minimizing the repetition of the articles the data of which will be extracted. The data of the articles listed in the search with each word group is a subgroup dataset. The data set, which is the result of combining all these sub-datasets, is used for the analysis. Therefore, if each sub-branch word group is used to obtain data, some articles will repeat in the data collected for each word group, since the aforementioned fields are related to each other. This repetition is minimized with the selected inclusive word groups. Machine learning is also among the mentioned sub-fields, but the diabetes-machine learning word group was also used in this study. At this point, the researchers used this word group especially in order to see which algorithms would come to the fore in the articles on the development of the prediction model.

Codes were written in R programming language by using RStudio editor for bibliometric data analysis (23,24). With the R programming language, code packages prepared for bibliometric analysis studies, as well as for many different analysis types, have been developed and various functions are included in these packages. The packages and functions used in this study are given below:

Package:

- bibliometrix (25): The bibliometrix package is a tool that contains many functions that can be used to perform quantitative research in bibliometrics and scientometrics. The functions in this package generally provide solutions for three purposes. Making bibliometric data suitable for analysis with R programming language, performing bibliometric analysis of a publication data set, creating matrices needed for co-citation, coupling, collaboration and common word analysis.
- tidyverse (26): The tidyverse package is an opinionated collection of R packages designed for data science. All packages share an underlying design philosophy, grammar, and data structures.

Functions used from bibliyometrix package:

- readFiles(): This function is used to assign the exported SCOPUS files into a large character object.
- convert2df(): This function is used to import and convert bibliographic files and API objects.
- biblioAnalysis(): This function is used to perform bibliometric analysis.
- biblioNetwork(): This function has been used to create bibliographic networks.
- networkPlot(): This function was used to plot bibliographic networks.

Functions used from the tidyverse package:

- duplicated(): The function was used to identify repetitive studies in the data set.

In addition, correlation analysis, one-way analysis of variance (ANOVA) and regression analysis were carried out in order to determine the relationship between the number of articles by years. All the statistical analyses were performed using SPSS 21.0 software. A value with a p-value of ≤0.05 were considered statistically significant.

RESULTS

The findings obtained as a result of the bibliometric analysis study performed within the steps specified in the method section have been shared under this title. 1500 of the 2534 publications included in the analysis are articles, 978 are conference papers, 52 are book chapters, and 4 are books. The percentage distribution of the publications is given in Figure 1.

The frequency distribution of the publications included in the analysis by years was also obtained. While the num-

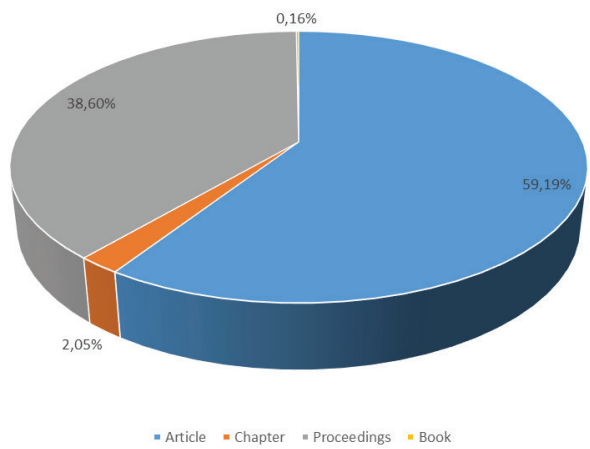

Figure 1: Percentage distribution by type of publications

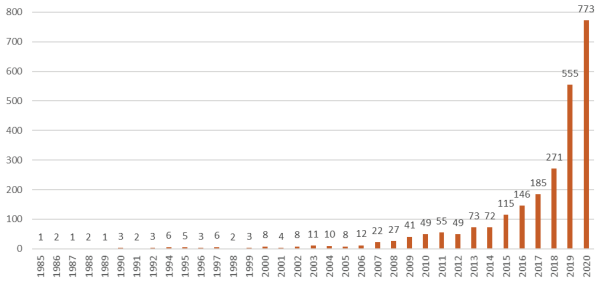

Figure 2: Counts depending on the years
*SCP: Single Country Publication, MCP: Multi Country Publication

to the total number of multi-author articles (27, 28). The cooperation index, calculated accordingly, is 4.21.

Findings about the relationship between the country and the number of publications are given in Table 2 and Figure 3. Findings on the top 10 producing countries are also shared in the table. Accordingly, the country producing the most publications with 358 publications was the United States of America (USA), while the other countries in the top 10 were China, India, United Kingdom, Korea, Spain, Italy, Australia, Germany, and France. When the multi-country collaborative publication rates (MCP_Ratio)

Table 1: Author-publication number relationship

Total number of authors	Number of publications with a single author	Number of publications per author	Number of authors per publication	Collaboration index
10,274	109	0.247	4.05	4.21

Table 2: Country-publication relationship

Country	Number of publications	SCP	MCP	MCP_Rate
USA	358	277	81	0.226
China	207	157	50	0.242
India	191	169	22	0.115
United Kingdom	92	60	32	0.348
Korea	66	44	22	0.333
Spain	65	45	20	0.308
Italy	51	46	5	0.098
Australia	37	25	12	0.324
Germany	33	20	13	0.394
France	28	20	8	0.286

SCP: Single country publication, MCP: Multi country publication

ber of publications between 1985 and 2015 was 604, the number of publications in the last 5 years has tripled to 1930. Publication counts by years can be seen in Figure 2. Although the annual average increase rate was calculated as 20.93%, when Figure 2 is analyzed, it is seen that the number of publications has increased especially in the last 5 years and reached the highest level with the publication of a total of 773 studies in 2020.

Findings regarding the relationship between author and publication number are given in Table 1. Accordingly, the total number of authors was 10,274, while the number of publications with a single author was 109. Although the majority of the studies had multiple authors, the number of authors per article was 4.05. The Collaboration Index (CI) is the ratio of the total authors of multi-author articles

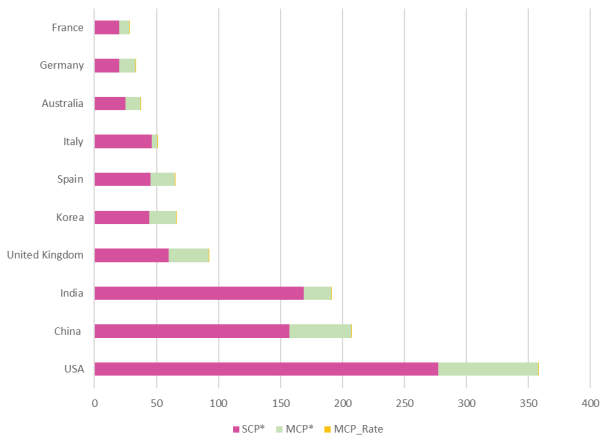

Figure 3: Country-publication relationship chart

Table 3: Number of citations within the scope of the country-publication relationship

Country	Total number of citations	Number of citations per publication
USA	10426	29.12
China	2669	12.89
India	2330	12.20
United Kingdom	1639	17.82
Turkiye	1352	54.08
Australia	1346	36.38
Singapore	1137	54.14
Korea	1118	16.94
Israel	1077	59.83
Italy	1002	19.65

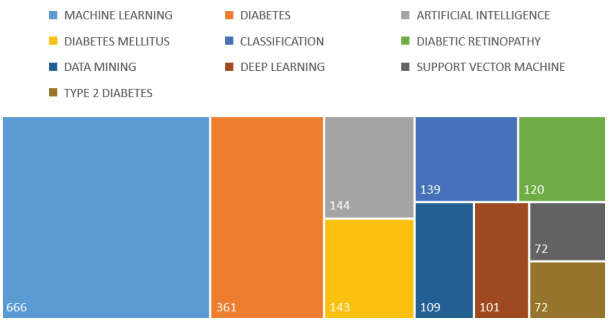

Figure 4: Frequency distribution of keywords

among all the publications of the countries was examined, Germany, United Kingdom, Korea, Australia, and Spain were in the top 5.

The findings regarding the number of citations obtained depending on the country-publication relationship are given in Table 3. Accordingly, the USA was the country with the highest number of citations with 10426 citations. When the table is examined, the top 10 countries with the most citations were the USA, China, India, United Kingdom, Turkiye, Australia, Singapore, Korea, Israel, and Italy. Although the USA publications had the most citations, when the number of citations per publication was examined, Israel came first with a rate of 59.83%.

In the analyzed studies, the most used keywords and the use of these words together were also examined. Accord-

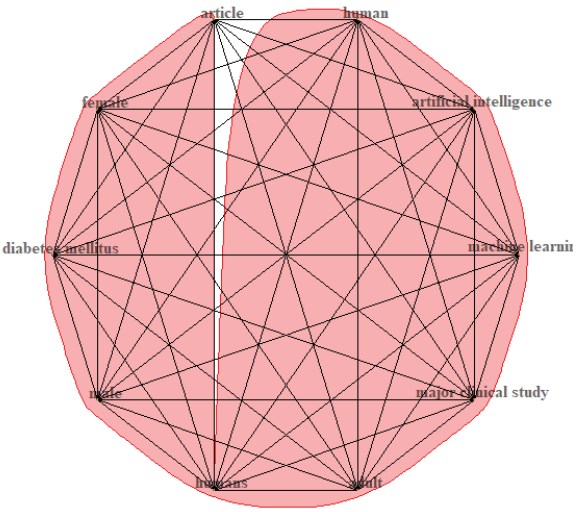

Figure 5: Relationship chart between keywords

Table 4: Top 10 sources for publications

Platform	Number of publications
Lecture Notes in Computer Science (Including Subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)	116
Journal of Diabetes Science and Technology	55
Studies in Health Technology and Informatics	46
Advances in Intelligent Systems and Computing	45
Artificial Intelligence in Medicine	43
Communications in Computer and Information Science	36
ACM International Conference Proceeding Series	34
Plos One	33
Journal of Biomedical Informatics	31
Procedia Computer Science	27

Table 5: Summary of the created regression model

Model	R	R^2	Adjusted R^2	Std. Error of the estimate	Change statistics			
					R^2 change	F change	df1	df2
Model 1	0.613[a]	0.376	0.357	127.917	0.376	20.474	1	34

a: Dependent variable: articles, df: degrees of freedom

Table 6: ANOVA[a] results

Model		Sum of squares	df	Mean square	F	p
Model 1	Regression	335019.287	1	335019.287	20.474	0.000[b]
	Residual	556335.269	34	16362.802		
	Total	891354.556	35			

a: Dependent variable: articles, b: Predictors: (constant), years, df: degrees of freedom

Table 7: Results regarding the prediction of the number of articles by years

Model		Unstandardized coefficients		Standardized coefficients	t	p	95.0% Confidence interval for B	
		B	Std. error	Beta			Lower bound	Upper bound
Model 1	(Constant)	-101.406	43.543		-2.329	0.026	-189.897	-12.916
	Years	9.286	2.052	0.613	4.525	0.000	5.116	13.457

t: test value, p: probability value, B: beta coefficient

ingly, the most used keywords were "Machine Learning", "Diabetes", "Artificial Intelligence", "Diabetes Mellitus", "Classification", "Diabetic Retinopathy", "Data Mining", "Deep Leaning", "Support Vector Machine", and "Type 2 Diabetes". The frequency distribution of keywords is given in Figure 4, and the situation of using words together is given in Figure 5.

The top 10 source platforms where the studies were published most are given in Table 4.

The results of the regression model created according to the number of articles by years are given in Table 5. The correlation between the number of articles by years was positive, linear and significant ($r=0.613$, $p\leq0.01$).

The output of the ANOVA, which tests the significance of the regression model, is given in Table 6. When the ANOVA results regarding the prediction of the number of articles by years were evaluated, there was significance ($F(1.34)=20.474$, $p\leq0.01$). The regression model established for the prediction of the number of articles by years was significant.

The results of the prediction of the number of articles for the year 2021 are given in Table 7.

When the coefficients of the model were evaluated, 37.6% of the total variance was explained and the years had a significant effect on the number of articles ($\beta=9.286$; $\beta std=0.613$; $t(34)=4.525$; $p:0.000\leq0.001$).

The regression equation obtained for the model was:

$Y=-101.406+9.286X$.

The calculation to predict the number of articles for the year of 2021 (the 37th year) was:

$Y=-101.406+9.286(37)=-101.406+343.582\approx242$.

Accordingly, it can be predicted that the number of articles to be published for 2021 is 242.

CONCLUSION

AI technologies continue to transform our habits and the health sector is taking its share from this transformation. The importance of digital transformation is increasing with the integration of AI technologies in the prevention, monitoring and management of diabetes, which is one of the most important public health problems of today. With the effect of many factors such as obesity, unhealthy diet and sedentary lifestyle, diabetes has become a pandemic and has become a global public health problem. Therefore, it can be said that there is an urgent need for AI supported scientific studies to prevent diabetes or diagnose it at an early stage.

In this study, a bibliometric analysis study was carried out with the data of 1029 studies, which included "diabetes-artificial intelligence" or "diabetes-machine learning" word groups in the title, a summary or keywords, and which were published or accepted for publication between 1985-2020 in the form of articles, book chapters and conference papers. As a result of the analysis, quite remarkable findings were obtained. It was determined that the number of studies conducted since 2015 was triple the number of studies published between 1985 and 2015. These findings are not surprising given that AI has had its second golden age since 2010 (29). Moreover, when the number of studies carried out up until the end of 2020 is examined, it can be stated that this increased interest will continue in the 2021 according to regression analysis.

When the most used keywords in the studies and the situations of using these words together were examined, it was seen that the term "machine learning" was used more frequently than AI. Accordingly, it can be said that instead of smart applications such as wearable technologies and remote monitoring systems, the studies were mainly based on predictive analysis based on the data set (in other words, machine learning from experience). Likewise, the concept of classification was seen in keywords. This also supports the idea that the studies were prediction oriented. Although there are three basic methods in data mining - classification, clustering and association analysis - the classification method was at the forefront of the study results. In this respect, it can be thought that studies primarily focus on the benefit of prevention rather than monitoring of the disease. A more technical result is the keyword obtained especially for the algorithms used in machine learning. These are artificial neural network based deep learning algorithms and they support vector machines. Although there are many algorithms used for classification, the prominence of deep learning and support vector machines algorithms was interpreted as these two algorithms are used extensively in studies in this field. Although deep learning is the algorithm that has recently gained interest and is being used widely, it is surprising that support vector machines are among the keywords. It can be said that this algorithm is frequently preferred due to the nature of the data set used in studies in the relevant field and the desired results.

When the number of publications was examined, it was seen that the country that produced the most publications in this field with 358 publications and the most cited country with 10426 citations was the USA. The fact that the USA is one of the most preferred countries in scientific publication production, projects and collaborations and attaches importance to multidisciplinary work shows that this result is not surprising. However, when the number of citations per publication was examined, it was determined that the USA lags behind Israel (29.12 vs 59.83 respectively). Additionally, Singapore and Turkiye have a high number of citations per publication. However, if we consider that there are few publications in these countries, it may have been highly cited by other authors because of the lack of options.

It was determined that the published publications were predominantly multi-author and the average number of people per publication was above four. From this point of view, it was seen that teamwork especially stands out in this area. Considering the subject in general, it can be stated that the partnership of teams working in the field of health and informatics has gained importance in such research.

According to the study, Turkiye is unfortunately not among the top 10 countries producing the most publications in this field. In Turkiye, whose success in the field of health has been proven worldwide (30), the importance of health tourism is highlighted and where there is a desire to make a breakthrough with various investments (31), it needs studies to be carried out in this field for Turkiye to take its place among the leading countries. Despite the shortage of publications, it seems that Turkiye is among the top 10 countries in terms of the number of citations received. There could be many reasons why publications are cited more frequently. These reasons should be investigated in further studies separately.

When it comes to the limitations of this study, the study was carried out adhering to the data set obtained from a single database. For this reason, different databases may be considered in future studies. While listing the publications, a search was made for diabetes, machine learning and AI word groups. In future studies, these words could be diversified, customized or different combinations could be tried and the work could be expanded by accessing the data of different publications.

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A SCALE DEVELOPMENT AND VALIDATION STUDY: COMMUNICABLE DISEASES RISK AWARENESS AND PROTECTION SCALE

BULAŞICI HASTALIKLAR RİSK FARKINDALIĞI VE KORUNMA ÖLÇEĞİ GELİŞTİRME

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ABSTRACT

Objective: It is important to measure the risk awareness and protection levels of individuals to be protected from infectious diseases that maintain their importance for society by showing a dynamic course. This study was aimed to develop the "Communicable Diseases Risk Awareness and Protection Scale" as a measurement tool for adult individuals.

Materials and Methods: This is a methodological research, and the purposeful sampling method was used. The item pool prepared by the researchers has been provided with scope validity with expert opinions. After the pilot study, the 60-item draft scale was applied to the research group, consisting of 740 individuals. After an item analysis and an exploratory factor analysis, a confirmatory factor analysis was performed on the draft scale.

Results: As a result of the principal components analysis and the Varimax rotation method, a six-factor structure was formed with the explained total variance of 45.21%. Since the Chi-square/df:2.78, RMSEA:0.049, CFI:0.97, GFI:0.97, AGFI:0.97, NFI:0.96, and RFI:0.96 met the fit criteria, the construct validity of the scale was confirmed. In reliability analysis: Cronbach α value of the scale was 0.91; sub-dimensions were between 0.60-0.78. The Spearman-Brown coefficient was 0.86, and the test-retest correlation value was 0.95.

Conclusion: It has been determined that the 'Communicable diseases risk awareness and protection scale,' consisting of 6 sub-dimensions and 36 items, was determined to be valid and reliable. The increase in the total score indicates a high level of risk awareness and protection from communicable diseases.

Keywords: Communicable diseases scale, scale development, validity, reliability

ÖZET

Amaç: Dinamik bir seyir göstererek toplum açısından önemini koruyan bulaşıcı hastalıklardan korunmak için bireylerin risk farkındalığı ve korunma düzeylerinin ölçülebilmesi önemlidir. Bu çalışmada yetişkin bireyler için bir ölçme aracı olarak 'Bulaşıcı Hastalıklar Risk Farkındalığı ve Korunma Ölçeği'nin geliştirilmesi amaçlanmıştır.

Gereç ve Yöntem: Metodolojik tipte bir araştırmadır ve amaçlı örnekleme yöntemi kullanılmıştır. Araştırmacılar tarafından hazırlanan madde havuzunun kapsam geçerliliği uzman görüşleri ile sağlanmıştır. Pilot çalışma sonrasında 60 maddelik taslak ölçek, 740 kişiden oluşan araştırma grubuna uygulanmıştır. Madde analizi ve açımlayıcı faktör analizi sonrasında, taslak ölçek için doğrulayıcı faktör analizi yapılmıştır.

Bulgular: Temel bileşenler analizi ve Varimax döndürme yöntemi sonucunda toplam varyansı %45,21 olarak açıklanan altı faktörlü yapı oluşturulmuştur. Ki-kare / sd:2,78, RMSEA:0,049, CFI:0,97, GFI:0,97, AGFI:0,97, NFI:0,96, RFI:0,96 uyum ölçütlerini karşıladığından ölçeğin yapı geçerliliği doğrulanmıştır. Güvenirlik analizinde: Ölçeğin Cronbach α değeri 0,91; alt boyutları 0,60-0,78 arasındadır. Spearman-Brown katsayısı 0,86 ve test-tekrar test korelasyon değeri 0,95'ti.

Sonuç: Altı alt boyut ve 36 maddeden oluşan 'Bulaşıcı hastalıklar risk farkındalığı ve korunma ölçeği' geçerli ve güvenilir olarak belirlenmiştir. Toplam puan artışı, bulaşıcı hastalıklara risk farkındalığı ve korunma düzeyinin arttığını göstermektedir.

Anahtar Kelimeler: Bulaşıcı hastalıklar ölçeği, ölçek geliştirme, geçerlik, güvenilirlik

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INTRODUCTION

Communicable diseases where microorganisms play a role in aetiology can lead to social problems such as panic, anxiety, absenteeism, economic damage, and density in health institutions by causing death, disability, and epidemics (1). Microorganism-related factors, the environment, and individual and social risk factors play a role in transmissions, such as air, droplets, contact, water, food, and sexual or hospital-borne transmissions (2). It can spread to a large part of society, between countries and even continents, in a very short period by infecting sensitive and healthy persons (1). The spread of infectious agents to every region of the world has become easier due to globalization, rapid urbanization, collective travelling, climate change, and global warming (2). Although progress has been made in the control of communicable diseases, it does not lose its importance in terms of morbidity and mortality due to its dynamic structure and continues to be one of the leading public health problems of society (1).

As for the control of communicable diseases, the knowledge, attitudes, and beliefs of the individuals and society are as important as health systems, services and technologies. The levels of risk awareness, correct attitudes and behaviors of individuals provide high benefits to the environment and society in preventing infection and spread (2). The fact that individuals with wrong attitudes and behaviors are factors in the spread of the disease in society creates the need for measuring and evaluating the attitudes and behaviors. Knowing the ways of transmission of infectious diseases will lead individuals to be more careful about protection. In this regard, mistakes known to be true or truths known to be false will make individuals and society susceptible to infectious diseases. For example, there may not be a sufficient level of knowledge for protection from infectious diseases in society on issues such as handwashing, ventilation, handshaking, eating and drinking or personal care environments, crowded areas, vaccinations, sexual transmission, water, and food. This situation reveals the necessity of measuring the attitudes and behaviors of individuals towards infectious diseases. Therefore, developing an objective measurement tool that measures the level of risk awareness and protection levels of communicable diseases will help determine the risk awareness level of both the individual and society.

In the literature reviews, it was determined that there was no scale available in Turkish or English that measures the communicable diseases risk awareness and protection levels of society. Some studies measure the level of knowledge about specific communicable diseases, mostly for special groups. No study measures the levels of awareness, attitudes, and behaviors of individuals related to protection from communicable disease risks in daily and general life. For this purpose, it is necessary to develop a scale with proven validity and reliability.

The aim of this study was to develop a qualified, valid and reliable scale, and all steps of scale development studies were applied in every stage. It was aimed to develop the "Communicable disease risk awareness and protection scale (CDRAPS)," which will enable the measurement of the general risk awareness and protection levels of communicable diseases in adult individuals.

MATERIALS AND METHODS

This study, which was carried out to develop a scale, is methodological research. It was planned that the scale was intended for society and adult individuals that constitute the target group. The population of the study consisted of individuals aged 18 and above who applied to family health centres (FHC) in Kayseri province and its districts. The research was conducted between February 2019 and February 2020. Data collection was carried out at Family Health Centers (FHCs) located in Kayseri city centre and Yeşilhisar, Yahyalı, and İncesu districts between May-November 2019. The study was conducted with a sample group, a pilot study group, and a post-test group.

A criterion was used to calculate the sample size, which is applied in all scale development studies. The number of individuals corresponding to 10-20 times the item pool was taken into account for the sample (3, 4). For this purpose, 740 individuals, corresponding to approximately 12 times the item pool (60 items), were included in the sample. To give a quality to the scale, it was aimed to select a sample group that would reflect the differences (heterogeneity) of society. The "maximum diversity sampling method," which is a non-randomized and purposive sampling method, was used as the sampling method. It was aimed to select inclusive and heterogeneous participants according to each characteristic that was intended to be measured. To fulfil this goal, Kayseri is divided into two areas, urban and rural. The urban areas, Kocasinan, Melikgazi, and Talas, are in the centre. The rural area, Akkışla, Bünyan, Develi, Hacılar, İncesu, Pınarbaşı, Sarıoğlan, Sarız, Tomarza, Yahyalı, Özvatan, Felahiye, and Yeşilhisar, are outside the center. The total population of Kayseri's districts in 2019 and their distribution by gender are given in Table 1.

Kocasinan, Melikgazi, and Talas were chosen as urban areas. Yeşilhisar, Yahyalı, and İncesu districts were determined as rural areas by lot. After Kayseri was divided into urban and rural areas, each region was divided into socioeconomic levels. During the study, similar distributions were attempted in terms of gender, age, and educational level. Periodic descriptive statistical analyses of the data, collected from urban and rural areas, were made to try to equalize their distribution in terms of age group, gender, education level, marital status, and place of residence (Table 2).

The descriptive characteristics of the research group are given in Table 2.

Table 1: Total population of Kayseri districts in 2019 and distribution by gender

County/Town	Total population	Male population	Female population	Male %	Female %
Melikgazi	571.166	285.154	286.012	49.9	50.1
Kocasinan	396.912	197.248	199.664	49.7	50.3
Talas	163.773	81.790	81.983	49.9	50.1
Develi	65.745	33.044	32.701	50.3	49.7
Yahyalı	36.208	18.272	17.936	50.5	49.5
Bünyan	30.603	17.166	13.437	56.1	43.9
İncesu	27.969	14.232	13.737	50.9	49.1
Pınarbaşı	24.080	12.546	11.534	52.1	47.9
Tomarza	22.166	11.296	10.870	50.9	49.1
Yeşilhisar	16.098	8.086	8.012	50.1	49.9
Sarıoğlan	14.552	7.318	7.234	50.3	49.7
Hacılar	12.414	6.263	6.151	50.5	49.5
Sarız	9.583	4.902	4.681	51.2	48.8
Akkışla	6.247	3.166	3.081	50.7	49.3
Felahiye	5.861	2.980	2.881	50.8	49.2
Özvatan	4.164	2.098	2.066	50.4	49.6

Table 2: Sociodemographic characteristics of the research group

Features		Number	%
Gender	Male	361	48.8
	Female	379	51.2
Age group	18-29	180	24.3
	30-39	196	26.6
	40-49	182	24.6
	50+	182	24.6
Educational status	Secondary school graduate and below	227	30.7
	High school graduate	260	35.0
	University graduate and above	253	34.3
Location of longest residence	Urban (city centre)	431	58.3
	Rural (county, town and village)	309	41.7
Marital status	Never married	174	23.6
	Married	516	69.7
	Deceased/separated	50	6.7
Total		**740**	**100.0**

A heterogeneous, comprehensive, and wide variance distribution was formed in the research group.

No sampling method and sample size calculations were made for the pilot application and test-retest analysis, and individuals who agreed to participate were included.

In this study, the concept of infectious diseases was evaluated according to the risks and protection behaviors of all transmission routes. An item pool, consisting of approximately 95 items, was prepared by the researchers from the literature. General risk factors covering all infectious diseases in society and ways of prevention

have been researched in the literature and have prepared the items related to attitudes and behaviors that can be applied in daily life. The item pool should be three or four times more than the number of items considered in the final scale (5, 6). The answer choices were designed to be in a five-point Likert type. Response choices for items measuring awareness/attitude are "Strongly Disagree" to "Strongly Agree;" for the items measuring behavior, the response choices range from "Never" to "Always."

Scope, content, and appearance validity were ensured by taking expert opinions for the item pool (7). The expert panel consisted of six faculty members from Erciyes University Faculty of Medicine, Department of Public Health. The item pool consisting of 95 items prepared by the researchers was discussed one by one in a face-to-face panel consisting of six people. After the items were added and removed during the panel, a new pool of 75 items was formed. These items were sent to the committee of experts via e-mail. The expert committee consisted of public health, infectious diseases, and assessment-evaluation specialists throughout Turkiye. 21 experts who gave back feedback scored each question according to its suitability, and as a result, a draft scale consisting of 60 items was created. This draft scale was evaluated in terms of Turkish spelling and grammar rules by two experts from the field of Turkish Language and Literature.

The draft scale, which was shaped after expert opinion, was tested by interviewing 25 persons suitable for the target group, and necessary corrections were made by the researchers.

The data was collected by the researchers through the method of self-reporting since privacy regarding communicable diseases may affect the correct response rates of individuals. Missing data, extreme values, parallelism, and singularity problems in variables were evaluated (8). Item total scores provided the assumption of the normal distribution with the Kolmogorov-Smirnov test. It was observed that multivariate normality was not provided by the Mardia's multivariate normality test ($p<0.001$) (9).

Statistical analysis

The mean, standard deviation, minimum-maximum values, kurtosis, and skewness coefficients of the items were shown with the descriptive statistical analysis of the items. In item analyses, item-total correlation coefficients were calculated with the Pearson correlation analysis, and an independent sample t-test was applied to 27% lower and upper groups.

Factor Analyses were made for the construct validity of the scale. In the exploratory factor analysis (EFA), the Kaiser-Mayer-Olkin (KMO) coefficient, which reveals the sampling adequacy, was examined. The Barlett Sphericity Test, which tests the conformity of the data to factor analysis based on normality assumption, was conducted. A Principal Component Analysis (PCA) was performed as a factor analytical method since the data did not provide multivariate normality. Kaiser criterion, scree-plot graph, and exploratory factor analysis were used to determine the number of factors. The rotated components matrix was formed with the Varimax rotation method, which maximizes the sum of the variances of the quadratic factor loads in each factor (4).

After item analysis and EFA, the scale became 37 items. A confirmatory factor analysis (CFA) was conducted to verify the created sub-dimensions. The unweighted least squares method was used as the parameter estimation method. Fit indices ($\chi 2$, SRMR, RMR, CFI, NFI, RFI, IFI) were used to test the model fit (10).

Internal consistency analysis, scale-size Pearson correlation analysis, and the test-retest method were used to determine the reliability of the scale. Spearman-Brown two equivalent half-reliability coefficients and Cronbach α coefficients were calculated for the overall factor and sub-dimensions for internal consistency. The test-retest correlation coefficient was the stability coefficient of the scale as a result of the test-retest method, and the intraclass correlation coefficient was calculated as the generalization coefficient.

Statistical analysis TURCOSA Cloud (Turcosa Analytics Co. Ltd., Turkiye) was made with software and LISREL 8.72 statistical package program (11,12). For statistical significance, a p-value of <0.05 at a 95% confidence level was considered significant.

To conduct the study, the study was approved by the Erciyes University Faculty of Medicine Clinical and laboratory Research Ethics Committee (Date:20.03.2019, No:96681246). Administrative permission was obtained from the Turkish Ministry of Health and the Kayseri Provincial Health Directorate with the number 49654233-604.02 dated 10.05.2019. Project support was received from the Scientific Research Projects unit of Erciyes University (Project ID: TTU-2019-9209). Verbal consent was obtained from the participants.

RESULTS

The arithmetic mean values of the items in the item pool ranged from 2.37±1.04 to 4.78±0.54. When the kurtosis and skewness values were examined, item 10 (-2.39 and 6.70), item 52 (-2.65 and 8.15), and item 56 (-3.03 and 10.56) were removed from the scale. It was determined that the items were sufficient to provide item discrimination power with a 27% lower-upper group analysis ($p<0.001$). It was determined that the corrected item-total correlation coefficients were positive and varied between 0.09 and

0.53. Items with a correlation value of less than 0.30 were removed starting from the lowest value. In this way, 20 items that did not correlate completely with the scale were removed from the draft scale.

Exploratory factor analysis (EFA) findings

Kaiser-Meyer-Olkin Value (KMO) is 0.922, and Bartlett's Test of Sphericity Chi-Square value is 7734.78 (sd:666, p<0.001). The calculated KMO fit measure is above 0.50, which is accepted as the critical value. In the Barlett's test, p-value <0.001 indicates that the data structure is sufficient for factor analysis. As a result of Principal Component Analysis (PCA), the common factor variance values of the items vary between 0.332 and 0.671. Since the sample size is over 200, factor loads above 0.3 are considered significant.

The eigenvalue is the sum of the squares of the factor loadings of each factor. It is a coefficient used in calculating the ratio of variance explained by each factor and in deciding the number of important factors. In general, factors with an eigenvalue of 1 and above are taken as significant factors (3). Considering these criteria regarding the eigenvalues of the factors, the total variance explained, and the scree plot graph findings,it was decided to accept the scale as having six factors. The variance rate explained by the six-factor structure is 45.21%. In social sciences, the total variance explained should be over 40%.

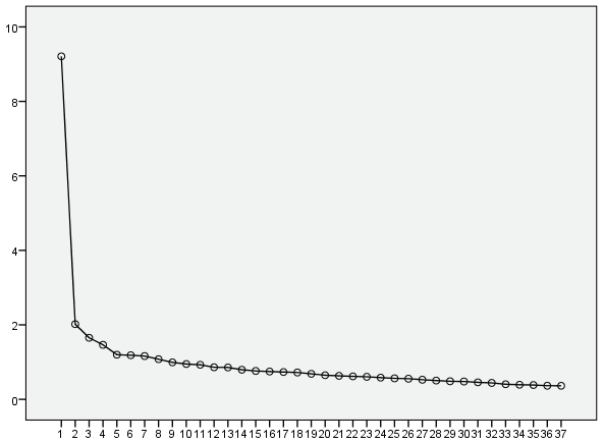

Figure 1: Scree plot graph of items

A Scree plot showing the eigenvalue and factor number of the PCA result is shown in Figure 1.

With the Kaiser Normalization and Varimax vertical rotation technique, it was examined whether the items met the acceptance level of the factor load criteria. It was determined that the factor loads of the 37 items are varied between 0.313 and 0.736.

Eigenvalue, variance explained, and cumulative variance of each factor in the draft scale before and after Varimax rotation is shown in Table 3.

Naming was made by the relevant dimensions under the concepts contained in the items collected under the factors. Factor 1 is named "Common Life Risk Awareness," Factor 2 "Self-Protection Awareness," Factor 3 "Protection Behaviors," Factor 4 "Handwashing Behaviors," Factor 5 "Social Protection Awareness," and Factor 6 is named as "Personal Contagion Awareness."

Confirmatory factor analysis results

A measurement model, which includes the six factors obtained as a result of EFA and the Items that make up these factors, was formed. Standardized coefficients, t-values, error variances, and explanatory rates of the model are shown in Table 4.

The standardized path coefficients of the items in the model are between 0.49-0.81; It has moderate to high potency levels. The t-values of the items in the model are statistically significant at a 95% confidence level. The error variances of the items in the model range between 0.35-0.76 and their explanatory values vary between 24% and 65%. In the model, Item 28, whose standard regression coefficient is below 0.5, was removed and the model was rebuilt. Standard regression coefficients and error variances of the new model are shown in Figure 2.

Table 3: Eigenvalue and explained variance ratios of factors before and after varimax rotation

	Initial values			Sum of squares of loads after rotation		
Factor	**Initial eigenvalue**	**Variance explained %**	**Cumulative variance %**	**Initial eigenvalue**	**Variance explained %**	**Cumulative variance %**
1	9.21	24.89	24.89	3.79	10.24	10.24
2	2.02	5.45	30.34	3.03	8.18	18.43
3	1.65	4.47	34.81	2.85	7.69	26.12
4	1.46	3.95	38.77	2.47	6.67	32.79
5	1.20	3.24	42.01	2.35	6.35	39.14
6	1.18	3.20	45.21	2.25	6.07	45.21

Table 4: Standardized coefficients, t-values, error variances and explanatory ratios of the model

	Items	Standardized coefficient	t-value	p	Error variance	R2
FACTOR 1	Item 18	0.49	12.05	0.041	0.76	0.24
	Item 16	0.57	14.79	0.039	0.67	0.33
	Item 36	0.52	13.75	0.037	0.73	0.27
	Item 15	0.53	14.15	0.037	0.72	0.28
	Item 41	0.72	23.30	0.031	0.48	0.52
	Item 23	0.53	13.72	0.038	0.72	0.28
	Item 35	0.58	15.15	0.038	0.66	0.34
	Item 45	0.62	18.47	0.034	0.61	0.39
	Item 33	0.58	16.41	0.035	0.66	0.34
FACTOR 2	Item 39	0.69	21.72	0.032	0.52	0.48
	Item 67	0.69	21.02	0.033	0.53	0.47
	Item 28	0.48	11.45	0.042	0.77	0.23
	Item 25	0.64	18.96	0.034	0.59	0.41
	Item 40	0.65	20.60	0.032	0.58	0.42
	Item 42	0.53	14.48	0.036	0.72	0.28
	Item 37	0.56	14.47	0.039	0.68	0.32
	Item 57	0.66	22.87	0.029	0.56	0.44
	Item 24	0.67	21.28	0.031	0.55	0.45
FACTOR 3	Item 62	0.62	18.86	0.033	0.62	0.38
	Item 53	0.52	14.10	0.037	0.72	0.28
	Item 58	0.66	19.93	0.033	0.57	0.43
	Item 65	0.61	17.92	0.034	0.63	0.37
	Item 63	0.51	12.14	0.042	0.74	0.26
	Item 43	0.60	18.90	0.032	0.64	0.36
	Item 50	0.54	15.25	0.036	0.71	0.29
	Item 49	0.61	19.92	0.031	0.63	0.37
FCT 4	Item 48	0.81	26.05	0.031	0.35	0.65
	Item 56	0.69	19.51	0.036	0.52	0.48
	Item 54	0.77	26.94	0.029	0.40	0.60
FCT5	Item 3	0.50	11.27	0.045	0.75	0.25
	Item 26	0.62	15.15	0.041	0.62	0.38
	Item 19	0.49	10.22	0.048	0.76	0.24
	Item 8	0.68	16.23	0.042	0.54	0.46
FCT6	Item 46	0.64	16.95	0.038	0.59	0.41
	Item 44	0.52	11.61	0.045	0.73	0.27
	Item 47	0.69	19.41	0.035	0.53	0.47
	Item 14	0.59	13.58	0.043	0.65	0.35

The model is statistically significant. Table 5 shows the fit criteria of the model as a result of confirmatory factor analysis.

In our study, the Standardized Root Mean Square Residual (SRMR) was acceptable for the model of the scale in the CFA result; x^2/sd, Root Mean Square Error

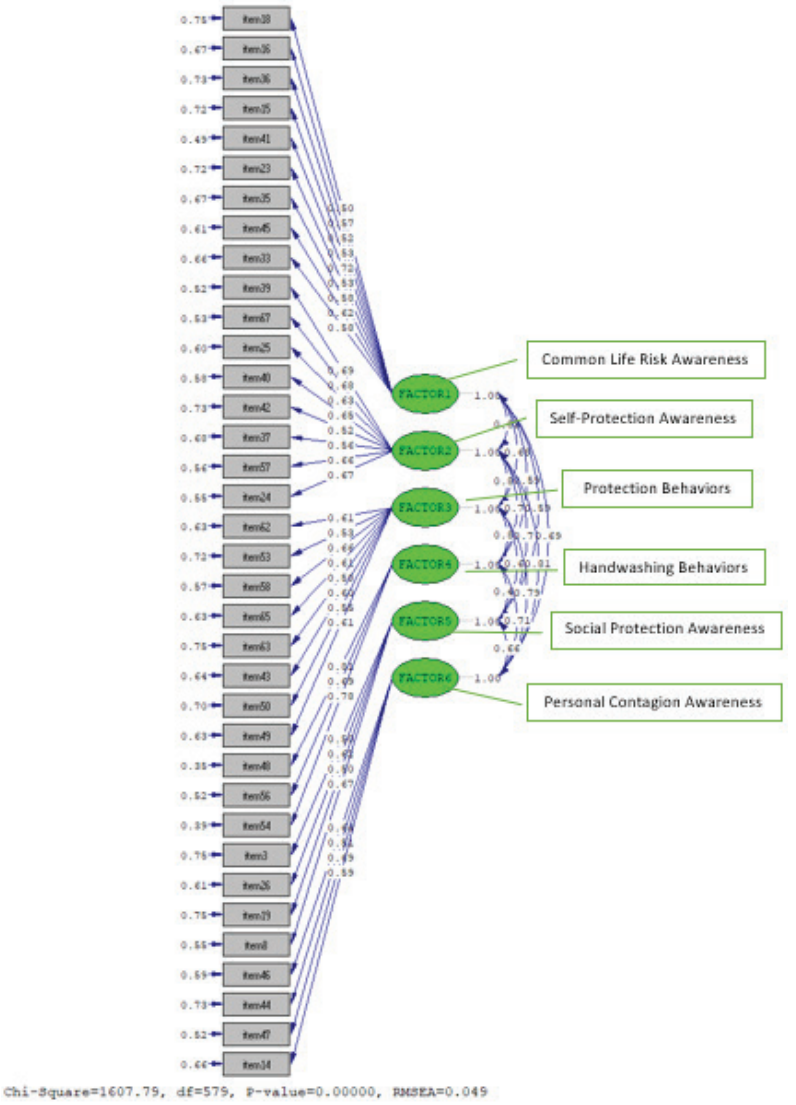

Figure 2: Confirmatory factor analysis result scale model

of Approximation (RMSEA), Comparative Fit İndex (CFI), Goodness of Fit İndex (GFI), Adjusted Goodness of Fit Index (AGFI), Normed Fit Index (NFI), Non-Normed Fit Index (NNFI), Incremental Fit Index (IFI), and Relative Fit Index (RFI) are a good fit.

Internal consistency reliability analysis results of the scale are shown in Table 6.

The correlations between sub-dimensions of the scale are between 0.27 and 0.64 and are statistically significant. The correlations between the sub-dimensions, and the scale total score are between 0.59 and 0.87 and are statistically significant.

The overall internal consistency coefficient of the scale is 0.91, and the two equivalent half reliability coefficient is 0.86. The internal consistency coefficients of the dimensions are between 0.60 and 0.78. The two equivalent half reliability coefficients of the dimensions are between 0.57 and 0.79. Four weeks after the application of the scale to 72 people from the study group, the same scale was applied again, and the stability coefficients were calculated.

The test-retest correlation coefficient is 0.95, and the in-class correlation coefficient is 0.97. These results show that the scale has test-retest reliability.

Items of the scale and factors titles are shown in Table 7.

DISCUSSION

The gradual increase in communicable diseases makes them not an individual health problem but turns them

Tablo 5: Confirmatory factor analysis result fit criteria of the model

	Fit values of the model	Good fit criteria*	Acceptable fit criteria*	Fit degree
x^2/sd	2.78	2-3	3-5	Good
RMSEA	0.049	<0.05	<0.08	Good
90% CI	0.046-0.052	<0.05	<0.08	
SRMR	0.058	<0.05	<0.08	Acceptable
CFI	0.97	0.95-1.00	0.90-0.95	Good
GFI	0.97	0.95-1.00	0.90-0.95	Good
AGFI	0.97	0.95-1.00	0.90-0.95	Good
NFI	0.96	0.95-1.00	0.90-0.95	Good
NNFI	0.97	0.95-1.00	0.90-0.95	Good
RFI	0.96	0.95-1.00	0.90-0.95	Good
IFI	0.98	0.95-1.00	0.90-0.95	Good

*Reference:10, RMSEA: Root Mean Square Error of Approximation, SRMR: Standardized Root Mean Square Residual, CFI: Comparative Fit Index, GFI: Goodness of Fit Index, AGFI: Adjusted Goodness of Fit Index, NFI: Normed Fit Index, NNFI: Non-Normed Fit Index, RFI: Relative Fit Index, IFI: Incremental Fit Index

Table 6: Internal consistency reliability analysis results of the scale

	Number of items	Cronbach α*	Spearman-Brown coefficient*
CDRAPS	**36**	**0.91**	**0.86**
Factor 1	9	0.78	0.75
Factor 2	8	0.78	0.79
Factor 3	8	0.76	0.77
Factor 4	3	0.70	0.68
Factor 5	6	0.60	0.57
Factor 6	6	0.63	0.64

*: Below 0.60 is not acceptable; Between 0.60 and 0.70 is acceptable as a minimum; Significantly between 0.70 and 0.80; It is very good between 0.80 and 0.90 (10).

into a social health problem and shows that they are a sociological problem. In this study, a measurement tool has been developed that will facilitate the measurement and evaluation of communicable diseases risk awareness and protection levels of individuals in society.

When the literature is examined, many studies on infectious diseases and transmission routes have been found, and it has been seen that they are directed to certain groups and specific transmission routes. However, studies evaluating the general risk awareness of the general population and infectious diseases are limited (13-118). Existing scales used in the literature are important information tests, but they were developed for a single specific disease (STD Information Test, AIDS information scale) (13, 14).

Studies investigating the level of knowledge of sexually transmitted diseases were mostly conducted on university students and less frequently on high school students but were conducted on substance addicts, brothel workers, marriage applicants, and those with some psychiatric diseases (antisocial personality disorder, schizophrenia, bipolar patients). In studies conducted with high school students and different universities and faculties in Turkiye, it has been determined that young people do not have enough knowledge about STDs and ways to prevent them (15-21); similarly, the level of knowledge is low also in substance addicts and some psychiatric patients (22-24). It has been determined that women working in brothels, defined as a risk group in terms of STD, have a high knowledge gap; most of them are not informed, and they do not even consider themselves in the risk group (25,26). In a study in which a young adult age group of soldiers participated, it was determined that the level of knowledge about STDs was lower among male individuals with a lower education level and those who came from the eastern regions (27). In studies conducted with married

Table 7: Factors titles and ıtems of the scale

FACTOR 1: Common life risk awareness
When I attend crowded organizations such as weddings and festivities, the possibility of infection make me nervous.
When I enter closed and crowded environments (such as cinemas, shopping malls, mosques, wedding halls), I get anxious because of the possibility of disease transmission.
I do not want to eat foods such as bagels and dried nuts sold openly in the street or bazaar due to the possibility of infectious diseases.
I do not want to go to places such as hot springs, baths, and swimming pools due to the possibility of disease transmission.
When touching places such as doorknobs, stair handles and bus handles in public areas, the possibility of disease transmission makes me nervous.
I believe that diseases can be transmitted in places such as restaurants and cafeterias.
I believe that diseases can be transmitted through treatments such as manicure, pedicure, and shaving at hairdressers.
I avoid consuming products such as yoghurt, cheese, and eggs that are sold outdoors in the market for fear of infectious diseases.
I believe that plastic toys in shopping malls create an infectious disease risk for children.
FACTOR 2: Self-protection awareness
I pay attention to whether the people around me cover their mouths when coughing, sneezing.
I behave with hesitation when using public restrooms.
I believe that I can be protected from some infectious diseases such as flu and cold by ventilating my environment.
I believe that communicable diseases can be transmitted by mosquitoes, houseflies, and some insects.
I believe that I can be protected from some infectious diseases such as flu and cold by eating right.
Entering the house with shoes makes me nervous as it can lead to disease transmission
When I go to health institutions, I avoid touching the surroundings due to the possibility of disease transmission.
When I touch money, I think I have to wash my hands because of the possibility of disease transmission.
FACTOR 3: Protection behaviors
I stay away from people around me when I have the flu or cold.
I take care of my diet to avoid infectious diseases.
I avoid shaking hands with people who have infectious diseases such as flu and cold.
I pay attention that the meat is well cooked due to the possibility of disease transmission.
I check the expiry dates of food while shopping.
Information on infectious diseases catches my attention.
I avoid eating cheese and butter made from unboiled or unpasteurized milk.
I especially research the measures that can be taken to prevent infectious diseases.
FACTOR 4: Handwashing behaviors
I wash my hands with soap before eating.
When I enter the house from outside, I wash my hands especially with soap.
When I cover my mouth with my hand while coughing or sneezing, I wash my hands immediately.
FACTOR 5: Social protection awareness
I believe getting vaccinated protects me from infectious diseases.
The increase in people who do not get vaccinated in society worries me.
I believe the chlorination of water is essential to prevent infectious diseases.
I believe handwashing protects me from many infectious diseases.
FACTOR 6: Personal contagion awareness
I take care to separate the personal belongings of family members in case of infectious diseases.
If I have an infectious disease, I will tell people who can be infected.
I believe having more than one sexual partner increases the possibility of infection.
I avoid using other people's personal belongings for fear of infectious disease.

men aged between 21-71 years, it was observed that the majority of them had a low level of knowledge about STDs (28).

Studies investigating the level of knowledge of blood-borne diseases were mostly conducted on health workers, students, risky occupational groups such as hairdressers, barbers and beauty salons, and substance addicts. The risk of encountering blood-borne diseases, especially the Hepatitis B virus, increase with occupational risk groups and substance abusers (29). In the studies conducted on hairdressers, barbers, and beauty salons, the level of knowledge about blood-borne diseases is insufficient, hepatitis B vaccination rates are low, appropriate disinfection rates are low, handwashing rates are low (30), protective measures (wearing gloves, wearing masks, using different towels and covers for each customer etc.) is low, and they do not know the protective procedures at the desired level (31-33). In one study, HBV DNA positivity rate was found to be 6.6% in razor blades used in barbershops in Samsun (34). The fact that most of these risks are risks that can be eliminated with some basic precautions shows once again the importance of individuals' awareness of infectious diseases prevention and risk awareness. In a study conducted in a hospital in Istanbul, only 60% of the nurses stated that they see infectious diseases as a hazard related to occupational health and safety (35). Hepatitis B knowledge levels were found to be low in studies conducted with high school students in different regions of Turkiye (36-40).

It has been determined that studies investigating the knowledge level of zoonotic diseases are less than studies on other modes of transmission. The studies were carried out on people living in rural areas, those engaged in farming and animal husbandry, those studying at health-related schools, and veterinary students. It was determined that one-third of the participants did not know that diseases can be transmitted from unboiled milk, most of them consumed raw milk, and the rate of using personal protective equipment was low (41). In a study conducted with people living in a semi-urban area, it was determined that individuals' knowledge and awareness levels about brucella disease were low (42). In another study, the presence of Brucella abortus, a Brucella species, as suspicious in 17.3% of 202 cows' milk samples collected from 14 villages, also helped to show this risk, which is important for society (43). In a study conducted with nurses working in hospitals located in the city center of Kocaeli, it was stated that the level of knowledge about zoonotic diseases was insufficient and only 5% of them thought that they had sufficient knowledge about zoonotic diseases (44). Especially, many zoonotic diseases (such as Crimean-Congo hemorrhagic fever, anthrax, rabies and brucellosis) continue to be an significant public health problem in Turkiye (45).

The fact that the knowledge levels of infectious diseases are insufficient even in these groups, which are thought to be risky in terms of specific transmission routes, arouses curiosity about the awareness levels of individuals from different segments of society. However, the lack of a standard measurement tool that measures awareness, attitudes and behaviors towards general risks in infectious diseases limits studies on this subject. Application of existing knowledge tests to the general population in research can cause difficulties. While these tests were developed to measure the level of knowledge of individuals, BHRFC is intended to measure individuals' general risk awareness of infectious diseases and their behavioral levels. Using these knowledge tests together with the 'Infectious Diseases Risk Awareness and Prevention Scale' developed in our research in studies where these knowledge tests are used in risk groups will provide new findings in the interpretation of the results. The existence of an objective measurement tool that measures the risk awareness and protection levels of the society about infectious diseases will not only make a significant contribution to the literature but also the risk awareness levels determined as a result of its use in different researches will guide the training for the society after determining the risk awareness levels as a result of its use in different researches.

In the study, the implementation of both the expert panel and the expert committee stages, in getting expert opinion for the item pool, contributed to the strengthening of the scope, content, and appearance validity of the scale. The research group has included 740 individuals corresponding to approximately 12 times the items. Comrey and Lee stated that 50 samples were very poor, 100 were poor, 200 were moderate, 300 were good, 500 were very good, and 1000 or more samples were excellent (46). Regarding the opinions about the sample size, it can be said that the sample size for the research group is sufficient for the scale development study. To conduct scale development studies with a sufficient number of participants will cause incorrect factor structures and inferences.

The total variance explained by the six-factor structure created as a result of EFA is 45.21%. The high explained variance is interpreted as an indicator that the related concept or structure is measured so well (47). According to Scherer, Wiebe, Luther, and Adams, explained total variance rate in social sciences between 40% and 60% is considered sufficient (48). In this framework, it is seen that the contribution of CDRAPS sub-dimensions to the total variance is sufficient.

The Cronbach Alpha reliability coefficients of the scale are over 0.80 (9), and the Spearman-Brown two-equivalent semi-reliability coefficients are above 0.70. It

is an indication that the scale has a high level of reliability (49). In social science research, a reliability coefficient of 0.70 or higher has been determined as an "acceptable" reliability coefficient. In our study, it can be said that the scale is of high reliability for the general and medium for the sub-dimensions.

In our study, the RMSEA value of 0.49 in the CFA model, which was established to ensure the construct validity of the dimensions, indicates a good fit (50).

As a result, the existence of an objective measurement tool that measures the communicable diseases risk awareness and protection levels of the society will both contribute significantly to the literature. The risk awareness levels determined as a result of the use of the scale in different studies may guide education for society. Health training for society should be planned on subjects that are not included in the scale although they are in the item pool.

It is thought that the use of this scale by different researchers will provide the necessary feedback to society and health planners. Applying the relevant scale to different groups in the society in new studies will increase the reliability and validity of the scale.

Powerful sides of the research

It is the first scale development study that can evaluate the risk and protection awareness levels of adult individuals on communicable diseases in Turkiye. It was made for the community. Developing the scale in heterogeneous groups belonging to the sociodemographic variable will increase its applicability in society. After the exploratory factor analysis, confirmatory factor analysis, which is a stronger analysis method, contributed to the strengthening of the construct validity of the scale.

Limitations of the research

This research is not a multicenter study consisting of individuals from different regions of Turkiye.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Erciyes University Clinical Research Ethics Committee (Date: 20.03.2019, No: 194).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- D.E., F.C.; Data Acquisition- D.E.; Data Analysis/Interpretation- D.E., Y.S.; Drafting Manuscript- D.E.; Critical Revision of Manuscript- D.E., F.C.; Approval and Accountability- D.E., F.C.

Conflict of Interest: Authors declared no conflict of interest

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CAN BRAIN EDEMA BE PREDICTED WITH OPTIC NERVE SHEATH DIAMETER MEASUREMENT IN CASES WITH DIABETIC KETOACIDOSIS?: A PRELIMINARY STUDY

DİYABETİK KETOASİDOZLU OLGULARDA OPTİK SİNİR KILIF ÇAPI ÖLÇÜMÜ İLE BEYİN ÖDEMİ ÖNGÖRÜLEBİLİR Mİ?: BİR ÖNCÜL ÇALIŞMA

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ABSTRACT

Objective: The clinical signs and symptoms of brain edema resulting from diabetic ketoacidosis (DKA) may not always be obvious. When the intracranial pressure increases, the optic nerve sheath diameter (ONSD) simultaneously increases and can be imaged with ultrasonography. We aimed to discuss the determinative features of ONSD measurements in brain edema (BE) in DKA.

Materials and Methods: Patients who were classified as having mild, moderate and severe DKA were included in the study. Transorbital ultrasonography was performed during the first two hours of treatment while the patients remained in the supine neutral position with their eyes closed. The optic nerve sheath diameters, which appeared as a hypoechoic double-edged line 3 mm deep to the globe, were measured. The same measurements were repeated in outpatient clinic controls. The ONSD values and metabolic, neurological conditions of the patients were compared.

Results: Eight patients with a mean age of 8.8±3 (Standard Deviation (SD)) years were included in the study. Seven of them presented with moderate to severe DKA. Two patients suffering from headaches were found to have mild BE according to the brain computerized tomography (CT). The ONSD was 5.7±0.93

ÖZET

Amaç: Diyabetik ketoasidozda (DKA) beyin ödeminin klinik belirti ve semptomları her zaman açık olmayabilir. İntrakraniyal basınç arttığında, optik sinir kılıfı çapı (OSKÇ) aynı anda artar ve ultrasonografi ile görüntülenebilir. Bu çalışmada, DKA'da beyin ödemi (BÖ) varlığında OSKÇ değerlerinin belirleyici özelliklerinin tartışılması amaçlandı.

Gereç ve Yöntem: Çalışmaya hafif, orta ve şiddetli DKA tanılı hastalar dahil edildi. Tedavinin ilk iki saatinde hastanın gözleri kapalı, sırtüstü nötral pozisyonda yatarken transorbital ultrasonografi uygulandı. Globun 3 mm derinliğinde hipoekoik çift kenarlı bir çizgi olarak görünen optik sinir kılıf çapları ölçüldü. Poliklinik kontrollerinde de aynı ölçümler tekrarlandı. Hastaların OSKÇ değerleri ile metabolik ve nörolojik durumları karşılaştırıldı.

Bulgular: Yaş ortalaması 8,8±3 (SS) yıl olan sekiz hasta çalışmaya dahil edildi. Bunlardan yedisinde orta-şiddetli DKA vardı. Bilgisayarlı beyin tomografisinde (BT) baş ağrısı olan iki hastanın hafif BÖ olduğu görüldü. Orta-şiddetli DKA'lı hastalarda OSKÇ 5,7±0,93 mm (ortalama±SD (Standard Sapma)), hafif DKA'lı tek hastada ise 4 mm idi. BT'de BÖ'lü 2 hastanın OSKÇ'si 6,8 mm ve 5,9 mm idi. Şiddetli DKA'lı beş hastanın tedaviden bir hafta sonraki poliklinik kontrolünde ortalama OSKÇ 4,4±0,32 mm idi.

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mm (mean±SD) in the patients with moderate-severe DKA and 4 mm in the single patient with mild DKA. The ONSDs of the two patients with BE on the CT were 6.8 mm and 5.9 mm. The mean ONSD of the five patients with severe DKA was 4.4±0.32 mm in the outpatient clinic checks.

Conclusion: The measurement of ONSD by USG may be a supportive method for predicting BE in children with DKA.

Keywords: Diabetic ketoacidosis, optic nerve sheath diameter measurement, brain edema

Sonuç: USG ile OSKÇ ölçümü, DKA'lı çocuklarda BÖ'yü tahmin etmek için destekleyici bir yöntem olabilir.

Anahtar Kelimeler: Diyabetik ketoasidoz, optik sinir kılıf çapı ölçümü, beyin ödemi

INTRODUCTION

Diabetic ketoacidosis (DKA) is the most common complication of Type 1 Diabetes Mellitus (DM). It's clinical picture consists of ketonemia, ketonuria, hyperglycemia and acidosis (1-3). The first diagnosis of type 1 DM is with a DKA attack in 15-67% of the patients (4, 5).

Brain edema is the most severe complication of DKA attacks and is seen in less than 1% of patients but can be fatal. Clinical signs and symptoms may not always be obvious. The pathogenesis is still not clear and associations with dehydration, cerebral hypoperfusion or sudden changes in serum osmolality have been suggested (2).

Detecting the presence of a brain edema (BE) as early as possible and being able to predict the condition before obvious clinical symptoms develop are critical. An effective, non-invasive and method that can provide guidance and prevent secondary damage is not currently available.

The optic nerve extends from the intracranial subarachnoid space to the intraorbital area as an extension of the brain and is surrounded by layers of the meningeal membrane. When intracranial pressure (ICP) increases, the structure reflects this increased subarachnoid pressure from the intracranial area directly to the intraorbital area. Increased ICP is observed as a simultaneous increase in the optic nerve sheath diameter (ONSD). ONSD measurements can be easily performed with ultrasonography at the bedside. This method has been shown to be effective and reliable in the diagnosis of increased ICP (6-9). However, there are very limited studies on the practicality and power of this method in patients with DKA. Here, in this preliminary evaluation, we discuss the possibility of predicting the presence of brain edema during a DKA attack using ONSD measurements.

MATERIALS AND METHODS

We evaluated the data of the eight DKA patients, six of whom were newly diagnosed, seen at the Çukurova University School of Medicine's Pediatric Emergency Department and followed-up and treated in our unit. This study was approved by the Ethical Committee of the Çukurova University (Date: 09.08.2021, No: 113).

Patients diagnosed with DKA were included in the study. They had been treated according to our hospital DKA treatment protocols. Patients were included after providing written consent and after detailed information about the ultrasonographic ONSD measurement procedure was provided to the patient and family. Their ultrasonographic ONSD measurements were all conducted by the same pediatric emergency physician who was trained in ultrasonography and who had more than 2 years of experience. The procedure was performed within the first two hours of treatment. The ultrasonographic ONSD measurements were then repeated when the patient attended their routine out patient follow-up one week after the DKA treatment was completed.

Transorbital ultrasonography (USG) was performed on the horizontal and sagittal axes with a Sonosite Edge® USG device with a 6-15 MHz receiver using a method reported to have good reliability in previous studies (6, 7). The patient's eyes were kept closed while they were in the supine neutral position without sedation. Both globes and the optic nerve sheath diameters, which appeared as a hypoechoic double-edged line 3 mm deep at the optic nerve exit site, were imaged. The optic nerve sheath diameter at this point was measured in both the longitudinal and transverse sections in the frozen images. The mean of the four measurements was calculated and recorded.

The clinical and laboratory values at presentation and the characteristics of the treatment process were obtained from the patient records. Patients were classified as mild (venous pH 7.20-7.30, bicarbonate 10-15 mmol/L), moderate (venous pH 7.10-7.20, bicarbonate 5-10 mmol/L) and severe (venous pH <7.10, bicarbonate <5 mmol/L)) DKA according to their metabolic state at presentation (10).

Statistical analysis

Descriptive statistical analysis was performed. All analyses were performed using the IBM SPSS Statistics version 20.0 statistical software package. Normally distributed variables were expressed as mean±SD. The statistical level of significance for all tests was considered to be 0.05.

RESULTS

The eight patients, aged 5.5 to 13 years, consisted of four males and four females. They had presented at our

emergency department with symptoms of fatigue, nausea, and vomiting. One patient was diagnosed with mild DKA and all the others with moderate to severe DKA. The vital signs of the patients, aside from acidotic respiration, were consistent with age and all patients were hemodynamically stable. Ophthalmoscopic examination revealed no papillary stasis. Only two patients had headaches and their Glasgow Coma Scale (GCS) scores were 12 and 13. Their brain computed tomography (CT) investigations revealed mild brain edema. Demographic, clinical, and laboratory characteristics and the ONSD values are presented in Table 1 while ONSD examples are presented in Figures 1a and 1b.

Fluid and insulin treatment was started in accordance with the unit's DKA treatment protocol (10). Two patients were monitored in the pediatric intensive care unit and the others at the pediatric endocrinology department. All were discharged with full recovery and without a problem.

The initial mean (SD) value of ONSD of the seven patients with moderate-severe DKA was 5.7 (0.93) mm. The ONSD of the patient with mild DKA was 4 mm (Figure 1a, b). The ONSD values of the two patients showing brain edema on the CT were 6.8 mm and 5.9 mm, respectively. The

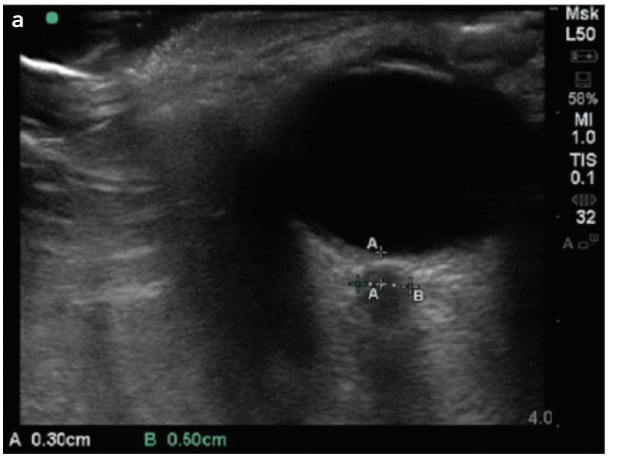

Figure 1a. A sample image from patients

mean (SD) ONSD of the five patients with moderate and severe DKA was 4.4(0.32) mm during the symptom-free follow-up one week after discharge and the difference was statistically significant (p: 0.008).

A high negative correlation was found between the ONSD and GCS values and the partial carbon dioxide ($PaCO_2$) levels in blood gases at admission (r: -0.859,

Table 1: Demographical, clinical, laboratory, and ONSD results of the patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Age (year)	12	13	6	6	7	13	8	6
Gender	M	F	F	F	F	M	M	M
Physical examination	Acidotic respiration	Acidotic respiration	Acidotic respiration	Acidotic respiration	Acidotic respiration	Acidotic respiration	Acidotic respiration	Acidotic respiration
GKS	15	15	12	13	13	15	13	14
Corrected Na	139	137	139	141	140	138	140	141
pH	7.0	7.09	6.8	7.08	7.1	7.2	7.1	7.17
HCO3	7	7.4	5	7.6	8.6	14	9	10
PaCO2	14	15	9	15	16	34	18	19
Blood sugar (mg/dl)	414	424	472	360	365	352	446	540
DM/year	6	6	New diagnosis	New diagnosis	New diagnosis	New diagnosis	New diagnosis	New diagnosis
Symptoms associated brain edema	Non	Non	Headache	Headache	Non	Non	Non	Non
DKA severity	Severe	Severe	Severe	Severe	Moderate	Mild	Moderate	Moderate
Braine edema at CT	Non applied	Non applied	+	+	Non applied	Non applied	Non applied	Non applied
Initial ONSD/mm	5.1	4.9	6.8	5.9	7.0	4	5.6	5.2
Control ONSD/mm	4.4	4	4.6	4.1	4.9	-	-	-

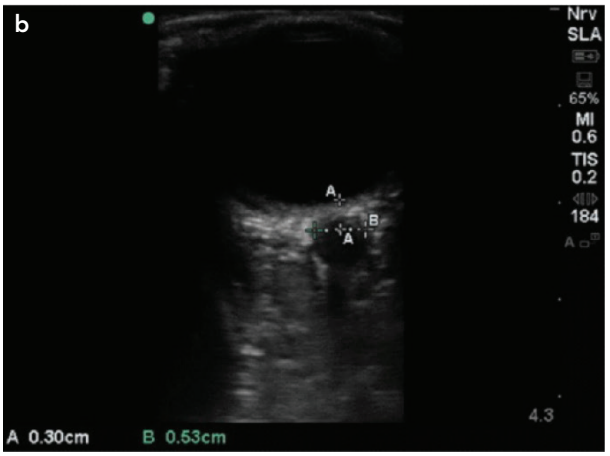

Figure 1b. A sample image from patients

p: 0.006 and r: -0.71, p: 0.04, respectively). A decrease in the GCS score from 15 was associated with an increase in ONSD measurements. This good level of correlation was statistically significant (r: 0.901 p: 0.02)

DISCUSSION

The results of this preliminary study suggest that the progress of clinical neurological findings and increased ONSD in children with moderate to severe DKA may be associated with the severity of the metabolic impairment and the presence of clinical and subclinical brain edema. Therefore, early diagnosis of BE is needed using a convenient method that is easy to administer at the bedside.

Detection of papillary stasis with fundoscopic examination is a late stage finding of brain edema and is therefore not reliable for early BE diagnosis. CT has risks such as radiation side effects and requires transport and sedation (8, 11, 12). Monitorization with invasive intracranial pressure measurement is the gold standard method for BE diagnosis but is not practical for emergencies (10).

The optic nerve sheath consists of three meningeal membranes. Fresh cadaver studies have shown that the cerebrospinal fluid circulates in the subarachnoid space and that the intracranial and intraorbital subarachnoid space pressure is the same (8, 12). It has been reported that the first marker in BE is an ONSD increase, and this is most prominently observed at a distance of 3 mm behind the globe (6-8). ONSD values measured with USG and Magnetic Resonance Imaging have been shown to be compatible in studies conducted in healthy children and adolescents (8, 12). Although a definite cut-off value has not been determined yet, an ONSD limit (cut-off) value with USG of 5.7-5.9 mm has been reported to have very high sensitivity (65-84%) and specificity (71-100%) when compared with the presence of brain edema on CT images (9, 13, 14).

The increase in ONSD as measured with USG has been shown to be useful in diagnosing BE early and monitoring the response to treatment in studies on patients with increased ICP due to traumatic or non-traumatic brain injury (6, 7, 9, 12-14).

A few studies have investigated the use of ultrasonographic ONSD measurements in the diagnosis and follow-up of BE in pediatric and adult hyperglycemia and in DKA patients. Bergman et al. found no statistically significant difference between the ONSD measurements in their study where they included patients aged 7-18 years with well-controlled DM, DKA and hyperglycemic non-DKA (15). Hensen et al. reported no significant difference between changes in ONSD during treatment in their pilot study on seven patients aged 4-17 years who were diagnosed with DKA without clinical suspicion of BE. They measured ONSD at the first hour, 8th hour and 24th hour of treatment (16). In our study the ONSDs of the seven patients with moderate and severe DKA were 4.9 to 7 mm (mean: 5.7±0.93 mm). The ONSD of our only patient with mild DKA was lower (4 mm). The ONSDs of two patients with severe DKA who underwent CT because they had headaches and low GCS scores (12 and 13) were 6.8 and 5.9 mm. These measurements were above the cut-off values that could indicate brain edema as reported in the literature, indicating that the DKA had become more severe. The ONSD increase also became significantly more pronounced (11, 13, 14).

The ONSD of these two patients measured at the symptom-free follow-up one week later was found to be 4.6 mm and 4.1 mm, respectively, and had decreased below the brain edema related cut-off values reported in the literature. A statistically significant difference was found between the mean ONSDs of the moderate to severe DKA patients measured at presentation and during the one-week follow-up. This change may indicate that the ONSD value is within normal limits when the children with DM are normoglycemic, but then increases compared to the basal values in the moderate to severe DKA state.

Another finding in our study, ONSD increase and/or brain edema in CT were present in patients with decreased GCS scores. There was a statistically significant negative correlation between the GCS values and the ONSD measurements of our patients. It can be recommended that evaluation of GCS at frequent intervals together with serial ONSD measurements in DKA patients with DKA may help in the early diagnosis of subclinical and clinical brain edema.

The results of this preliminary study suggest that the progress of clinical neurological findings and increased ONSD in children with moderate to severe DKA may be associated with the severity of the metabolic impairment and the presence of clinical and subclinical brain ede-

ma. However, prospective, and large-scale studies are required to determine the ONSD cut-off values that indicate the presence of clinically significant brain edema requiring intervention.

CONCLUSION

This study reveals that the measurement of ONSD using USG may be a supportive method for predicting BE in children with DKA. Prospective and large-scale studies are required.

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CASE REPORT / OLGU SUNUMU
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ELECTROPHYSIOLOGICAL CHARACTERISTICS OF AUTOSOMAL-RECESSIVE SPASTIC ATAXIA OF CHARLEVOIX-SAGUENAY IN A TURKISH FAMILY*

CHARLEVOİX-SAGUENAY'IN OTOZOMAL RESESİF SPASTİK ATAKSİ SENDROMU: BİR TÜRK AİLESİNDE ELEKTROFİZYOLOJİK ÖZELLİKLER

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ABSTRACT

The autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS), presenting with spinocerebellar ataxia, dysarthria, nystagmus, and spastic paraparesis, is a gradually progressive hereditary disease. Sensorimotor polyneuropathy may also accompany the symptoms. Herein, we present the electrophysiologic findings of a Turkish family with ARSACS in combination with clinical and genetic features to better describe the characteristics of the polyneuropathy in ARSACS. Regarding the electrophysiologic findings, however, the demyelinating characteristics were prominent and there were findings compatible with secondary axonal degeneration. Rare hereditary diseases such as ARSACS must be suspected in the presence of polyneuropathies with demyelinating characteristics accompanying pyramidal findings and ataxia.

Keywords: Charlevoix-Saguenay, ARSACS, electrodiagnosis, demyelinating polyneuropathy

ÖZET

Charlevoix-Saguenay'ın otozomal resesif spastik ataksi sendromu (ARSCAS), spinoserebellar ataksi, dizartri, nistagmus ve spastik paraparezi ile seyreden ilerleyici bir herediter hastalıktır. Sensörimotor polinöropati semptomlara eşlik edebilir. Bu vaka serisinde, ARSACS'a eşlik eden polinöropatinin niteliklerinin daha iyi anlaşılması amacıyla, ARSACS'lı bir ailede klinik ve genetik özellikler ile birlikte elektrofizyolojik bulgular sunulmuştur. Elektrofizyolojik bulgular, demiyelinizan özellikte bir polinöropati sendromu varlığı ile uyumlu olsa da, hastalarda ikincil aksonal dejenerasyonu işaret eden bulgularda mevcuttu. Demiyelinizan özellikli bir polinöropatiye piramidal bulgular ve ataksi eşlik ettiğinde ARSACS gibi nadir herediter hastalıktan şüphelenilmelidir.

Anahtar Kelimeler: Charlevoix-Saguenay, ARSACS, elektromiyografi, demiyelinizan polinöropati

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INTRODUCTION

The autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a progressive hereditary disease characterized by ataxia and spastic paraparesis. Sensorimotor polyneuropathy may also accompany the symptoms (1).

The sacsin gene *(SACS),* responsible for ARSACS, is located on chromosome 13q12.12, encoding the protein of the same name, and has been described by Engert JC et al. in 2000 (2). More than 200 mutations have been reported worldwide since then (1, 3, 4).

Moderate-to-severe axonal neuropathy with demyelinating characteristics were demonstrated in patients with ARSACS by electrophysiologic studies, and these findings were confirmed in biopsies (5, 6).

We present clinical, genetic, and electrophysiologic findings of a Turkish family with ARSACS to describe the pattern of polyneuropathy.

CASE REPORTS

Patient 1. A 41-year-old man was considered the index case, and his symptoms developed when he was 15. He became wheelchair dependent at the age of 40. An electrophysiologic test revealed demyelinating features affecting motor and sensory fibers, and accompanying secondary axonal involvement. Whole exome sequencing (WES) was performed for the index patient. WES revealed a novel homozygous truncating variant in the *SACS* gene (ENST00000382292.3:c.7720dupA, p.Arg2574LysfsTer4) leading to a frameshift. VarSome indicated the variant c.7720dupA as "pathogenic." American College Of Medical Genetics And Genomics (ACMG) scores for the variant are PVS1, PM2, and PP3, and the GERP score is 5.59. The variant was confirmed by Sanger sequencing in all available family members.

Patient 2. The elder brother of the index case was 55 years old, and he was bedbound due to similar symptoms that emerged when he was 15. Neurologic and electrophysiologic examinations could not be performed on this patient.

Patient 3. The 49-year-old sister of the index case developed poliomyelitis when she was four. She could hardly walk without support in the last ten years.

Patient 4. The niece of the index case was 18 years old; her symptoms developed at the age of 10. Her clinical process was moderate, compared with other family members. She managed to perform her daily activities independently.

Regarding the electrophysiologic characteristics, the median, sural, and peroneal superficial sensory nerve action potentials (SNAPs) of patients 1 and 3 could not be obtained. In patient 4, the amplitudes of median, sural, and peroneal superficial SNAPs were reduced and conduction velocities (CVs) of these nerves were slow. However, ulnar SNAP could not be obtained in patient 3. The amplitudes of ulnar SNAPs and CVs were reduced in patients 1 and 4. Tibial and peroneal compound muscle action potentials (CMAPs) could not be obtained in patient 1. The amplitudes of tibial and peroneal CMAPs were reduced, distal latencies were prolonged, and CVs were slow compatible with demyelination in patients 3 and 4. The amplitudes of ulnar CMAPs of all patients were normal, with reduced CVs.

The distal latencies of median CMAPs were prolonged and CVs were slow in all patients. The amplitude of median motor CMAP was reduced in patient 1, and the amplitudes of median CMAPs were normal in patients 3 and 4. In needle electromyography (EMG), denervation was not detected in muscles that were evaluated, and moderate neurogenic changes were detected on distally located muscles. The needle EMG of patient 3 revealed chronic neurogenic changes with significant asymmetric characteristics in lower extremities, which were interpreted as the sequela of poliomyelitis.

DISCUSSION

Charlevoix-Saguenay disease was first reported in 1978 in the Charlevoix-Saguenay-Lac-Saint-Jean region in Quebec, Canada among Canadians of French origin (7). The earliest finding is spasticity in the lower extremities, becoming apparent in early childhood, followed by ataxia (1, 8). Absent deep tendon reflex generally develops around the age of 25 due to progressive distal neuropathy (5).

Most of the mutations found in SACS gene were in exon 10, consisting of missense mutations, nonsense mutations, or frameshift variants (3). Besides the French-Canadian variants which were mainly truncating, several mutations were identified across Europe as well as in Turkiye (9-13). The clinical findings in these cases presented from Turkiye were consistent with the common clinical presentation of ARSACS, similar to our family. We reported a novel homozygous variant in the *SACS* gene leading a frameshift.

In our patients, the electrophysiologic pattern of polyneuropathy reveals a demyelinating type with secondary axonal degeneration. The type of peripheral nerve involvement is controversial in ARSACS. It was previously suggested that it was similar to axonopathy, myelinopathy, and intermediate forms of Charcot–Marie–Tooth disease (CMT) neuropathy (1, 5, 14). In patients 1 and 3, indiscernible motor and sensory responses were related to a more advanced stage of the disease

when compared to those in patient 4. Demyelinating features were present predominantly in motor nerve fibers. Secondary axonal degeneration was compatible with a length dependent pattern. None of the patients demonstrated equally homogenous deceleration of motor and sensory nerves CVs, which are characteristics for hereditary demyelination neuropathies. In addition, CMAPs showed an increased temporal dispersion as a sign of demyelination, particularly in lower extremity distal muscles.

As compared to our family, it was reported that neuropathy had both axonal and demyelinating characteristics in a Spanish family with ARSACS. Researchers suggested that this involvement, similar to intermediate forms of CMT neuropathy, was associated with both axon and myelin dysfunction in peripheral nerves (14). In another study consisting of five patients, the authors suggested that a myelin sheath defect had multifocal distribution; the process was associated with defect in myelin development, and the degeneration of peripheral axons accompanied the process (15). Previous cases reported from Turkiye also revealed both axonal, demyelinating or mixed types of polyneuropathy (9-13). These findings indicate that within autosomal recessive ataxias accompanied with axonal, demyelinating or mixed polyneuropathy, ARSACS disease should be considered in the diagnosis.

In conclusion, hereditary ataxias must be kept in mind when polyneuropathy accompanies in patients who present with symptoms of imbalance and family history. Although the involvement pattern of polyneuropathy is heterogeneous in electrophysiologic evaluation, rare hereditary diseases such as ARSACS should be suspected in the presence of polyneuropathy with demyelinating characteristics, accompanying pyramidal findings, and ataxia. This hereditary ataxia should be considered in the differential diagnosis of demyelinating neuropathies, some of which are immune mediated and responsive to immunosuppressant treatment.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 05.02.2021, No: 04).

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Author Contributions: Conception/Design of Study- L.M., M.B.B., E.K.O.; Data Acquisition- L.M., M.B.B., E.K.O., B.B., H.H., A.N.B., N.G.S.; Data Analysis/Interpretation- L.M., M.B.B., E.K.O., B.B., H.H., A.N.B., N.G.S.; Drafting Manuscript- L.M., M.B.B., E.K.O., B.B., H.H., A.N.B., N.G.S.; Critical Revision of Manuscript- L.M., M.B.B., E.K.O., B.B., H.H., A.N.B., N.G.S.; Approval and Accountability- L.M., M.B.B., E.K.O., B.B., H.H., A.N.B., N.G.S.

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A PARTIAL TRISOMY 9 CASE WITH DICENTRIC CHROMOSOME DUE TO THE ADJACENT-2 SEGREGATION OF MATERNAL RECIPROCAL TRANSLOCATION

MATERNAL RESİPROKAL TRANSLOKASYONUN ADJACENT-2 SEGREGASYONUNA BAĞLI OLUŞAN DİSENTRİK KISMİ TRİZOMİ 9 OLGUSU

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ABSTRACT

Duplication of the short arm (p) of chromosome (Chr.) 9 is a frequently seen abnormality while duplication of both p and long arm (q) is a rare chromosomal rearrangement derived mostly from parental translocations or inversions. The unbalanced products of the translocations are mostly derived from the 2:2 segregation of adjacent-1 division while the ones due the adjacent-2 patterns are rare. Here, a dysmorphic infant with a pure duplication of 9pter to 9q22.31 is reported due to the product of the adjacent-2 segregation of maternal reciprocal translocation between the 9q22.31 and 22p11.1. The affected infant had two normal and one derivative/dicentric Chr.9 (carrying the centromere regions of both Chr.9 and Chr.22) with one normal Chr.22. These results were confirmed by the fluorescence *in situ* hybridization technique. Array-comparative genomic hybridization confirmed the breakpoints precisely and revealed a 61.75 megabases duplication of Chr.9 consisting of many genes such as *BICD2, NTRK2, HNRNPK,* and *SMARCA2,* which are mostly related to developmental delay and growth retardation. Additionally, the infant had ear abnormalities, microcephaly, and extremity abnormalities, which were the other findings of trisomy 9. In sum, the case has presented as a rare example of adjacent-2 division of 2:2 segregation and a pure partial trisomy of 9pter to 9q22.31.

Keywords: Partial trisomy 9, reciprocal translocation, adjacent-2 segregation, dicentric chromosome, chromosomal rearrangement, growth retardation, fluorescence in situ hybridization, array-comparative genomic hybridization

ÖZET

Kromozom (Chr.) 9'un kısa koluna (p) ait duplikasyon sık olarak görülmekle birlikte hem kısa hem de uzun kolun (q) duplikasyonu daha çok ailesel translokasyonlar ve inversiyonlara bağlı oluşan ve nadir olarak görülen bir kromozomal yeniden düzenlenmedir. Ailesel translokasyonlara bağlı oluşan dengesiz gebelik ürünleri daha çok 2:2 segregasyonun adjacent-1 aktarımı ile oluşmakta iken, adjacent 2 aktarımı nadir bir durumdur. Bu çalışmada, annenin 9q22.31 ve 22p11.1 bölgeleri arasındaki resiprokal translokasyonuna bağlı 9pter ile 9q22.31 bölgeleri arasında duplikasyonu olan dismorfik bir çocuk sunulmaktadır. Etkilenmiş çocuk iki normal Chr.9, bir derivatif/disentrik Chr.9 (hem Chr.9, hem de Chr.22'nin sentromerini taşıyan), bir tane de normal Chr.22'ye sahiptir. Tüm bu sonuçlar floresan *insitu* hibridizasyon tekniği teyit etmiştir. Kırık bölgelerini doğru olarak belirleyen array-karşılaştırmalı genomik hibridizasyon tekniği *BICD2, NTRK2, HNRNPK* ve *SMARCA2* gibi büyüme gelişme geriliğine eşlik eden genleri de kapsayan kromozom 9'a ait 61,75 megabazlık duplikasyonu ortaya çıkarmıştır. Ek olarak mikrosefali ve ekstremite anomalileri gibi trizomi 9'a eşlik eden diğer bulgular da olguda bulunmaktadır. Özetle bu olgu, adjacent-2 tipi segregasyonun ve sadece 9pter-9q22.31 bölgelerini kapsayan kısmi trizomi 9'un nadir bir örneği olarak sunulmaktadır.

Anahtar Kelimeler: Kısmi trizomi 9, resiprokal translokasyon, adjacent-2 segregasyonu, disentrik kromozom, kromozomal yeniden düzenlenme, büyüme geriliği, floresan in situ hibridizasyon, array-karşılaştırmalı genomik hibridizasyon

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INTRODUCTION

Complete trisomy 9 is a quite rare but well-known syndrome with distinctive clinical features, with more than 150 cases reported in the database of the National Organization for Rare Disorders (NORD) up to today (1). The vast majority of the cases are mosaic due to the postzygotic error at any time in early development, or, rarely, due to the meiotic nondisjunction with subsequent loss of the trisomic cell line. The assumed complete trisomy 9 is a rare aneuploidy (which might represent a sampling error of variable tissue mosaicism) that is worthy of consideration (2). The partial trisomy of chromosome (Chr.) 9 (involving short; p and/or long; q arm) is mostly derived from a parental reciprocal translocation and is accompanied by a concurrent deletion or duplication of another chromosome due to the 2:2 or 3:1 segregation mechanisms (2, 3). The present partial trisomy 9 (pter to q22.31) case resulted from adjacent-2 segregation of the maternal reciprocal translocation between Chr.9 and Chr.22. Since the breakpoint was at the centromere of the Chr.22, the derivative chromosome 9 had two centromeres, and a dicentric chromosome [dic(9;22)] had been formed. This rare reciprocal translocation was inherited to the child with adjacent-2 segregation, which led to disomy 22 and partial trisomy 9 (pter to q22.31) and this unique case gave us the opportunity to delineate the accurate genotype-phenotype correlation of the duplication for this relevant segment of chromosome 9. Furthermore, we believe that this case will increase the familiarity of the geneticist with the unusual segregation pattern and also emphasize the importance of the usage of different techniques such as cytogenetic, fluorescence *in situ* hybridization (FISH) and array-comparative genomic hybridization (array-CGH) studies to determine the breakpoints of the chromosomal rearrangements.

CASE PRESENTATION

The 13-month-old girl patient was the first child born to a non-consanguineous 27-year-old mother and a 34-year-old father. At the 36th week of pregnancy, oligohydramnios was detected and an emergency C-section delivery was performed. At birth, her measurements were in the normal range for her birth week: 2160 g weight (10th centile), 44 cm length (10-25th centile), and 33 cm head circumference (75th centile). After the birth, the hypotonic baby was hospitalized in the neonatal intensive care unit for 53 days and was intubated for 23 days due to respiratory distress syndrome. One month after she was discharged from the hospital she started vomiting 6-7 times a day, and her weight gain stopped after the 7th month.

Developmental milestones were delayed: she was able to hold her head up at the age of 8 months. Physical examination at 13 months of age revealed that her measurements were small for her age (6200 g weight (3.47 SD), 68 cm length (-2.81 SD) and 40.5 cm head circumference (-4.81 SD). Brachydactyly was found (hand 8 cm and 3rd finger 3 cm, below the lowest percentile line). She had microcephaly and facial dysmorphic features such as hypertelorism, low set and cup-shaped ears, broad nasal root and bulbous nose, low hanging columella, short philtrum, thin and tented upper lip, and downturned corners of the mouth (Figure 1a, b). She also had a single transverse palmar crease in both hands and fifth finger clinodactyly. Both of her second toes were shorter than the others, and both of her feet were deviated laterally (Table 1). She could not sit without assistance, talk, or walk at time of physical examination.

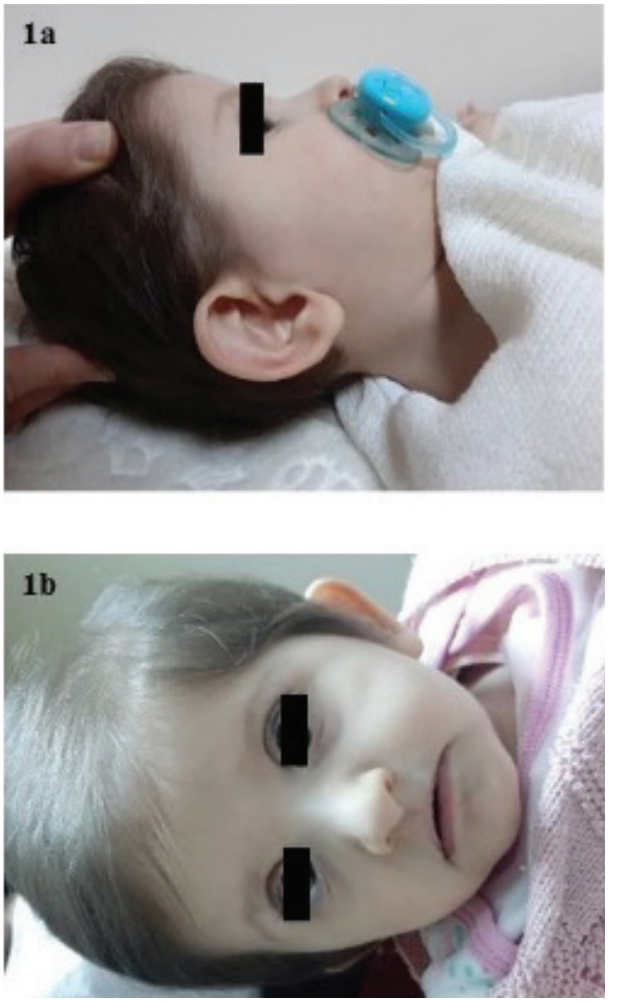

Figure 1: Dysmorphic signs of the patient (a,b): hypertelorism, bulbous nose, broad nasal root, low hanging columella, short philtrum, tented upper lip, downturned corners of the mouth, cup shaped ears

Abdominal ultrasonography (USG) at two months showed mild renal pelvis dilatation with renal pelvic AP with a diameter of 4-4.5 mm. Brain MRI findings were compatible with ventriculomegaly, which was evaluated as benign

Table 1: The comparison of the clinical findings, conventional cytogenetic and array studies in patients with partial trisomy 9 (pter to q22~q32)

	Karyotype	Chromosome 9 duplicated region	Other duplicated or deleted region	Array	Facial dysmorphism	Other clinical findings	Extremity abnormalities	Radiological findings	Age
Present case	46,XX,+9,dic(9;22)(9pter→9q22.31::22q11.1→22qter)mat	Trisomy 9pter to 9q22.31	No other chromosomal trisomy or monosomy	arr[hg19] 9p24.3p13.1(211,086-38,741,437)x3, 9q21.11q22.31(71,069,763-94,291,138)x3.	hypertelorism, esotropia, bulbous nose, broad nasal root, low hanging columella, short philtrum, tented upper lip, downturned corners of the mouth, cup shaped ears	hypotonia, developmental delay, growth retardation, sacral dimple	laterally deviated feet, joint laxity, brachydactyly, bilateral single transverse palmar creases, clinodactyly of fifth finger, relatively shorter second toes	Abdominal US: mild renal pelvis dilatation and renal stones ECHO: mitral valve prolapse, secundum atrial septal defect Brain MRI: ventriculomegaly	15 months old (living)
Dhangar S, et al. (2019)	46,XY,+der(9)t(9;14)(q22.1;q11.2)pat,-14	Trisomy 9pter to 9q22.1	Monosomy 14pter to 14q11.2	arr(GRCh38)9p24.3q22.1(203861-87860633)x3 pat	Mild macrocephaly, prominent forehead, low hair line, downward slanting of the eyes' epicanthic folds, hypertelorism, low set ears, large pinnae, long face, bulbous nose, thin upper lip, long philtrum, high arched palate	webbed neck, kyphoscoliosis, pilonidal sinus, delayed speech development and poor fine motor development, bilateral hearing loss	rocker bottom feet, short middle interphalangeal elevated foot, clinodactyly of fifth finger	MRI and CT scan of brain: generalized cerebral atrophy, dilated ventricles and arachnoid cyst 2D-ECHO: tiny patent ductus arteriosus US of abdomen: small size of both kidneys	5 years old (living)
von Kaisenberg CS, et al. (2000)	47,XX,+der(9)t(7;9)(q35;q22.2)mat	Trisomy 9pter to 9q22.2	Trisomy 7q35 to 7qter	N/A	micrognathia, hypertelorism, deep-set, posteriorly rotated ears, bulbous nose	postmortem examination: caudal hypoplasia and dysplasia of the cerebellar vermis, dilated foramen Magendie, enlarged cisterna magna micropolygyria female sex abnormalities	bilateral simian creases	US at 23 weeks of gestation: hypoplasia of the cerebellar vermis, marginal dilatation of the cisterna magna and the lateral ventricles X-ray: presence of only 11 ribs	23 weeks of gestation (termination of pregnancy)
Sutherland GR et al. (1976)-Case1	47,XX,+der(9),t(7;9)(p22;q32)mat	Trisomy 9pter to 9q32	Trisomy 7pter to 7p22	N/A	microcephaly, low hair line, low set ears, micrognathia, hypertelorism, microphthalmos, pinpoint pupils, large mouth, downturned corners of the mouth	widely open sagittal suture, palpable metopic suture, short, prominent sternum, sacral dimple Autopsy findings: persistent left superior vena cava, patent ductus arteriosus, bilobed right lung, small kidneys, hypoplastic cerebellar vermis, dilated fourth ventricle	flexion deformities of the arms and hands, bilateral simian creases, absence of the terminal phalanges of the thumb and index fingers, hyperconvex nails, fixed dislocation of the hips, unstable dislocated knees, talipes calcaneovalgus	bilateral hypoplasia of the pubic bones and an angulated ischia, dislocation of the head of the radius, hypoplasia of the distal humerus and fibulae, hypoplasia of the ala of the sacrum with poorly developed sacro-iliac joints	17 days (exitus)
Lopez-Felix J, et al.(2017)	47,XX,+der(9)t(8;9)(p21.3;q22.3)mat	Trisomy 9pter to 9q22.3	Trisomy 8pter to 8p21.3	N/A	Absent nasal bone		claw-like hands	US at 15.1 weeks of gestation: CRL (crown-rump length) of 77.6 mm (which accorded to 13.6 gestational weeks) US at 20 weeks of gestation: a fetus of 18 weeks, thickness and dilatation of right cardiac walls, echogenic focus in left ventricle	20 weeks of gestation (termination of pregnancy)
Metzke-Heidemann S, et al. (2004)-patient 2	47,XX,+der(9)t(7;9)(q35;q22.2)mat	Trisomy 9pter to 9q22.2	Trisomy 7q35 to 7qter	N/A	Unilateral cleft lip and cleft of both hard and soft palate	N/A	N/A	N/A	N/A

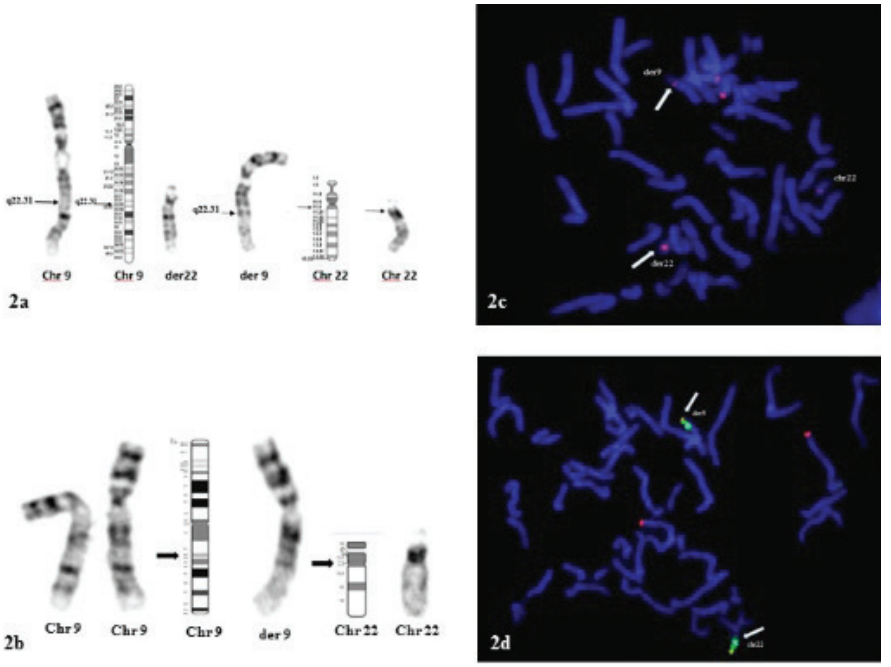

Figure 2: Partial karyotypes and FISH images of mother and proband. Partial karyotype of the mother showing the breakpoints of normal and derivative (der) chromosome 9s and 22s **(a)**, partial karyotype of the proband showing two normal and one derivative chromosome 9 and one normal chromosome 22 **(b)**. Arrows indicate the breakpoint regions. In **figure 2c**, the cep14/22 (red) painting of mother's metaphase is demonstrated. The occurrence of centromere region on der 9 (up) is confirmed via cep14/22 probe. Arrows indicate der9 (up), normal 22 (down) and der22 (right middle). In **figure 2d**, proband's chr 22q11 (LSI bcr: blue), qter regions of ch22 (yellow) and whole chromosome paintings of ch22 (green) are demonstrated on der9 (up) and normal chr22 (down) (the red signals show the 3qter regions and green signals show the 3pter regions as control probes). Arrows indicate der 9 (up) and normal 22 (down).

external hydrocephalus. An echocardiogram at ten months revealed a mitral valve prolapse and secundum atrial septal defect (Table 1).

Cytogenetics and molecular cytogenetics

Cytogenetic analysis was performed in the proband and later in the parent with their consent (4). Proband karyotype was 46,XX,+der(9)(9pter->9q22.31::22q11.1->22qter),-22. Paternal cytogenetic analysis revealed a normal result (46,XY), while the mother had a reciprocal translocation between Chr.9 and Chr.22 [46,XX,t(9;22)(q22.31;q11.1)] (Figure 2a, 2b) (Table 1).

FISH analyses were carried out for both the proband and the mother using the centromere, telomere (9p: pVYS234B, 9q: pVYS235B) and whole chromosome painting probes of Chr.9 and Chr.22 (Cytocell, Cambridge UK), and ToTelVysion™ Multi-colour DNA probe mixture 3 [(22q11 (LSI bcr), 22qter (pVYS207M)] with chromosome 3pter and 3qter as controls)] (Vysis, Downer's Grove, IL, USA) (Figure 2c, d). The existence of centromere region of Chr.22 (p11.1q11.1) on derivative Chr.9 was detected via FISH analysis (Figure 2c, d), and the final karyotype of the proband with dicentric chromosome was 46,XX,+9,dic(9;22)(9pter→9q22.31::22p11.1→22qter) mat,.ish dic(9;22)(9pter+,9qter-, D14Z1/D22Z1+,LSI BCR+,22qter+,wcp22+) (Figure 2b, d). Array-CGH (Human Genome G3 SurePrint 8x60K ISCA Array; Agilent Technologies, Santa Clara, California) confirmed the breakpoints of the alteration and revealed a duplication in size of 61.75 megabases of Chr.9, consisting of the *BICD2, NTRK2, HNRNPK* and *SMARCA2* genes with no gain or a loss of chromosome 22 [(arr[hg19] 9p24.3p13.1(211,086-38,741,437)x3,9q21.11q22.31(71,069,763-94,291,138)x3] (Figure 3).

DISCUSSION

After the first example of trisomy 9 shown via quinacrine mustard fluorescence and trypsin banding techniques by Feingold and Atkins in 1973, numerous cases of partial trisomy 9 with a concomitant partial monosomy/trisomy

were reported (Table 1) (3, 5-9). In those cases, the phenotypic effects of the concomitant partial monosomy/trisomy could not be excluded. However, the present case was derived from the adjacent-2 of 2:2 segregation in meiotic disjunction of maternal translocation and her clinical findings were demonstrative for pure duplication of 9pter to 9q22.31 without any other concurrent imbalance. Contrary to what is known about the dicentric chromosomes, which are almost always unstable in the cell division with the centromeres pulled to the opposite poles of the cell at anaphase, dicentric chromosome [dic(9;22)] of our case was stable in the cell cycles. The steadiness of this chromosome might be because of the formation of a pseudo dicentric chromosome in which only one centromere is active, and presumptively the active centromere belongs to Chr.9 (10).

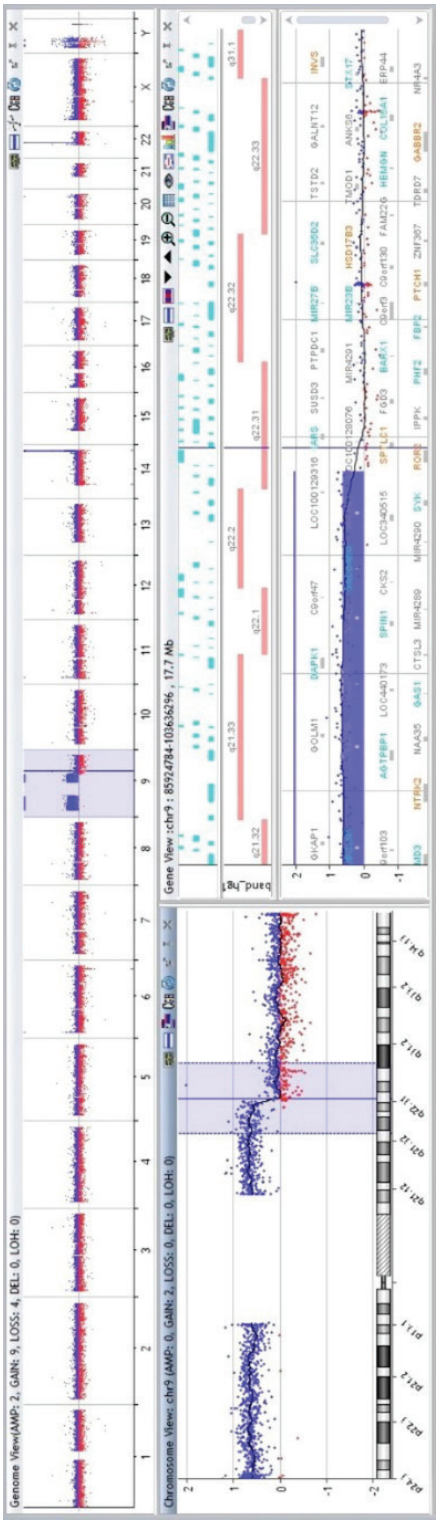

Figure 3: Array-CGH image of the proband. The breakpoint region is 9q22.31. There is a duplication of chromosome 9 (61,751 kilobases) but no gain or loss of chromosome 22 (Human Genome G3 SurePrint 8x60K ISCA Array)

The present case (adjacent-2 segregation product) is one of the few options for the infant to be born alive with dysmorphic signs as adjacent-1 segregation will be ended with the trisomy/monosomy of Chr.22 with a partial deletion/duplication of chromosome 9. In 3:1 segregation, there are two options of disomy 22 with duplication and deletion of Chr.9 derived from tertiary monosomy/trisomy segregation pattern, while the vast majority of the gestational products will be monosomic or double trisomic for the chromosome 9 and 22 (interchange monosomy/trisomy products). The family was informed about all the options of the segregation via videotaping consultation, and notified about the preimplantation and prenatal diagnosis in future pregnancies (11).

Low-set/malformed ear is a common facial abnormality seen in almost all patients with the regular or partial trisomy 9 cases, concordant with ours (Figure 1a) (2, 7, 12-14). While our case and the Sutherland GR *et al*. case were presented with microcephaly, the Dhangar S *et al*. case had macrocephaly (Table 1) (3, 6). Different extremity deformities, most frequently talipes or rocker bottom feet, were also reported in cases with complete or partial trisomy 9 (2, 3, 6, 15). The present case had laterally deviated feet, joint laxity, clinodactyly of fifth finger, and relatively shorter second toes (2, 3). Hearth defects (valve defects and atrial septal defects) were presented in those cases and a mitral valve prolapse and a secundum atrial septal defect were detected in our infant's echocardiogram at 10 months of age (2, 13).

The genes located on the long arm of chromosome 9, like *BICD2, NTRK2, HNRNPK,* and *SMARCA2,* which are mostly associated with autosomal dominant developmental delay and growth retardation, were found to be duplicated in our case. The *BICD2* gene is associated with childhood-onset muscle weakness and atrophy, while the *HNRNPK* gene is associated with a complex syndromic neurodevelopmental disorder, which explains hypotonia, delayed psychomotor development, and the sacral dimple in our patient. *SMARCA2* and *NTRK2* genes are related to microcephaly, and our case also has this finding (Table 1).

In conclusion, this case with pure partial trisomy of chromosome 9 has allowed us to describe the accurate phenotype-genotype correlation of this duplication and the proper counselling. It must be kept in mind that the identification of the breakpoints, the size of the duplication or the deletion and the identification of the genes encompassed in the imbalances are important, and all laboratory techniques, such as cytogenetic analysis, FISH and array-CGH studies, should be applied to get a reliable result.

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

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