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Aims and Scope

The Medical Bulletin of Haseki is the official scientific journal of the University of Health Sciences Haseki Training and Research Hospital. It covers subjects on general medicine, published both in Turkish and English, and is independent, peer-reviewed, international periodical and is published quarterly (January, March, June, September and November).

The aim of The Medical Bulletin of Haseki is to publish original research papers of highest scientific and clinic value on general medicine. Additionally, educational material reviews on basic developments, editorial short notes and case reports are published.

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The manuscript should not exceed 5000 words in total. All pages of the manuscript should be numbered at the top right-hand corner, except for the title page. Papers should include the necessary number of tables and figures in order to provide better understanding.

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Patients have a right to privacy. When not essential, identifying information, patient names and photographs should not be published, unless the written informed consent of the patient (parent or guardian) has been given.

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e) Journal on the Internet: Morse SS. Factors in the emergence of infectious disease. Emerg Infect Dis [serial online] 1995 1(1):[24 screens]. Available from:s URL:http://www/cdc/gov/ncidoc/EID/eid.htm. Accessed December 25, 1999.

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Original Article

Original Article

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Antibody False Positivity Among COVID-19 Convalescent Plasma Donors: A Comparative Study from the Turkish Red Crescent Blood Center

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Abstract

Aim: During our routine work at the Turkish Red Crescent (TRC) laboratories, human immunodeficiency virus (HIV) 1/2 antibody false-positive results were observed among Coronavirus disease-2019 convalescent plasma (CP) donors more frequently than healthy donors. We aimed to determine anti-HIV 1/2 antibody false-positivity rates among the CP donors and healthy blood donors.

Methods: The present study was designed as a cross-sectional study which was a type of observational study. Total 3689 donations from 2593 donors donated CP to the TRC between 11 April-06 July 2020, were screened by electrochemiluminescence immunoassay for the presence of antibody against HIV ½. The confirmation tests were performed with line immunoassay. All of the donors were non-remunerated CP donors between the ages of 18-60. For the control group, 411078 donations from 407363 healthy blood donors were received on the same days.

Results: Repeated reactivity rates (1.87%) were significantly higher than the control group (0.13%, $p<0.05$). However, there was not a statistically significant difference between the confirmed reactivity rates of the study group (0.03%) and the control group (0.01%, $p=0.217$).

Conclusion: In our study, it was determined that the false-positive results obtained from serologic HIV screening tests of CP donors were significantly higher when compared to the healthy blood donors.

Keywords: False HIV, convalescent plasma, serologic tests, immunoassay

Introduction

As a virus from the coronavirus family, the Severe Acute Respiratory syndrome-Coronavirus-2 (SARS-CoV-2), which was firstly defined in Wuhan -a sub-provincial city in China- towards the end of 2019 and assumed to be transmitted to humans from bats, has spread very fast and taken effect on global health, economy and social behavior around the world at short notice. During the writing of this paper, it was denoted that millions of people were infected with the virus and it led to the death of almost four and half million people in 235 countries/regions around the world (1). Naming the disease caused by this virus as Coronavirus disease-2019 (COVID-19) on 11 February 2020, World Health Organization (WHO) declared the outbreak as pandemic on 11 March 2020 (2).

There is no definite cure for the disease yet. The practice of CP, which comes up as a treatment choice and is received from the recovered patients, is an acquired passive immunity treatment. CP was used as post-exposure prophylactic for diseases such as viral hepatitis, measles, epidemic parotitis, and polio while it was used as the medical purpose for diseases such as influenza, bird influenza, SARS-CoV, Middle East Respiratory syndrome

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and Ebola (3-9). First practices related to the CP use in COVID-19 have come from the people's republic of China, the center of the disease. It was reported that the first CP was obtained in Wuhan on February 1st, 2020 and was given to a patient on February 9th, 2020 in this country (10). U.S. Food and Drug Administration (FDA) approved the use of the plasma received from people who were infected with and recovered from COVID-19 in the treatment of existing patients with a declaration of "COVID-19 Convalescence Research-Emergency" on March 24th, 2020 (11). Following all these news, it was decided by the Republic of Turkey Ministry of Health that the CP can be used in the treatment of COVID-19 patients. As part of this, in order to start receiving CP donations, a call was made by the TRC to those, who recovered from the disease and met the requirements of being a donor.

In the serologic tests conducted on the CP donors at the TRC laboratories, it was observed that anti-human immunodeficiency virus (HIV) ½ antibody was found to be false positive more often than other blood donors. In our study, it was aimed to show if there is any significant difference between the CP donors and healthy blood donors regarding the false positive HIV 1/2 test positivity rates.

Methods

Study design

The present study was designed as a cross-sectional study which was a type of observational study. This study was approved by the Turkish Red Crescent Ethical Committee (09.11.2020/2020-01). Total 3689 donations from 2593 donors, who donated CP to the TRC between 11 April-06 July 2020, were screened for the presence of anti-HIV ½ antibody. All of the donors in the study group were between the ages of 18-60 (median age 21.5) and were the voluntary and non-remunerated CP donors. The study group consisted of 2361 males (91.1%) and 232 females (8.9%). The clinical symptoms of CP donors in study group resolved at least 14 days before donation and in 48 hours before they had negative SARS-CoV-2 polymerase chain reaction test results for last consecutive two tests. For the control group, the test results of 411078 donations from 407363 healthy blood donors who donated within the same period were used. The blood donors in control group were between the ages of 18-60 (median age 27). The control group consisted of 350724 male (86.1%) and 56639 female (13.9%). The high male to female ratio in both groups was seen because the TRC does not accept plasma donations of any kind from women with a pregnancy history, including miscarriages or D/C, due to the risk of transfusion-related acute lung injury in the recipient. All the donors in the study and control groups gave the written consent before donation. These demographic data are summarized in Table 1.

Table 1. Demographic data of study and control groups

	Study group (n=2593)		Control group (n=407363)	
	Male	Female	Male	Female
Number (%)	2361 (91.1)	232 (8.9)	350724 (86.1)	56639 (13.9)
Median age (min-max)	21.5 (18-60)		27.0 (18-60)	
Donation number	3689		411078	

As part of the infectious serologic screening tests of blood donors, the anti-HIV 1/2 + p24 antigen tests were conducted on the electrochemiluminescence immunoassay (eCLIA) method and via the Cobas 8000 e801 (Roche, Germany) device and Elecsys HIV Duo (Roche, Germany) kits. In accordance with our test algorithm, the samples determined to be reactive in the first test were studied twice more and the results found to be reactive in at least two of three studies, were considered as "repeatedly reactive".

The confirmation tests were studied with the line immunoassay (LIA) method and via Auto-LIA 48 (Fujirebio, Belgium) device and INNO LIA HIV I/II score kits for the samples which were found to have repeated reactivity

Statistical Analysis

The data that used in our study were received from the digital archives of the TRC. The universe of our study consists of voluntary COVID-19 CP and healthy blood donors. The power of our cross-sectional study was calculated as 100%. For the statistical comparison of the reactivity rates of these two groups, Mid-P Exact test was used through OpenEpi v3.01 program, because it was recommended by the software for the actual distribution of data. The flow chart of the study is demonstrated on Figure 1.

Results

Sixty-nine (1.87%) of 3689 CP donations in the study group were found to have repeatedly reactive for anti-HIV 1/2 in the serologic tests. The confirmation test was negative (false positivity) in 68 donations (1.84%) among CP donors and 9 of them were female (0.35%), 59 of them were male (2.28%). In the study group confirmation test was positive (true HIV infection) in one male donor (0.03%). In the control group, 520 (0.13%) of 411078 blood donations were found to have repeated reactivity for HIV 1/2 antibodies in the serologic tests. The confirmation test was negative in 461 (0.12%), positive in 49 (0.012%) and indeterminate in 10 (0.002%) of them. In the control group, 84 (0.021%) of unconfirmed donors were female

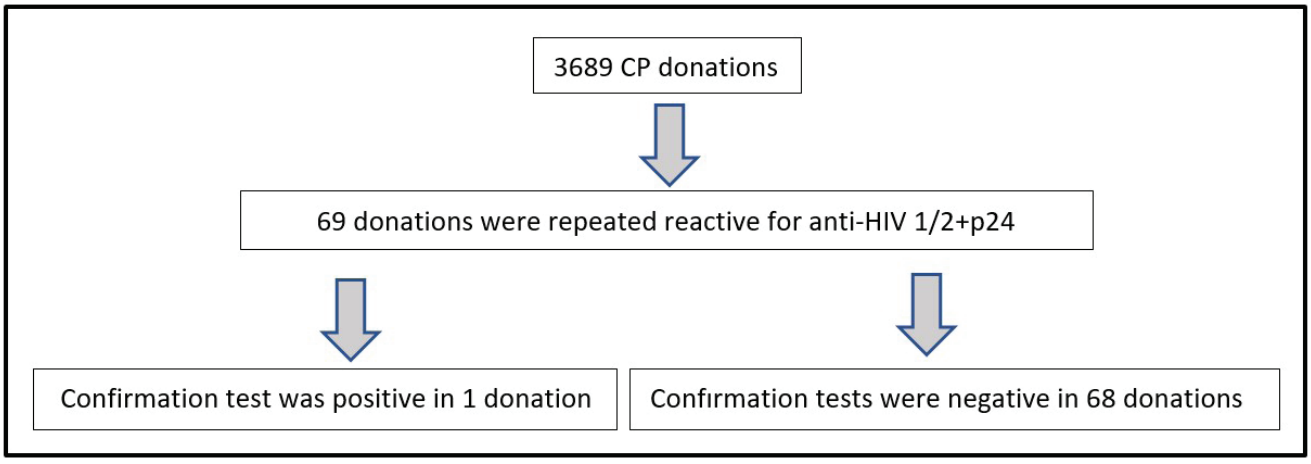

Figure 1. Flow chart of study

and 377 (0.093%) were male.

When the repeated reactivity rates of the study group (1.87%) and control group (0.13%) were compared, the difference was found to be statistically significant (p<0.05).

When the confirmed reactivity rates of the study group (0.039%) and the control group (0.012%) were compared, the difference was not statistically significant (p=0.217). In our study, any confirmed female donor was detected. Because of this reason, statistical comparison between genders was not calculated in the confirmed study group.

When the unconfirmed reactivities found in the study group (1.84%) and in the control group (0.12%) were compared, the difference was found to be statistically significant (p<0.05). In unconfirmed group, difference between female - male donors rates of the study group (0.35% and 2.28%, respectively) and control group (0.021% and 0.093%, respectively) were statistically significant (p<0.05). The findings are summarized in Table 2,3.

Table 2. Anti-HIV 1/2 reactivities of the CP and blood donations

	CP donations (n=3689)	Blood donations (n=411078)	p*
Repetitive reactivity % (n)	1.87 (69)	0.13 (520)	<0.05
Confirmed % (n)	0.03 (1)	0.012 (49)	0.217
Unconfirmed % (n)	1.84 (68)	0.12 (461)	<0.05
Indeterminate % (n)	0	0.002 (10)	-

*Mid-P exact test was used for comparison of two groups, HIV: Human immunodeficiency virus, CP: Convalescent plasma

Table 3. Unconfirmed anti-HIV 1/2 test results related to gender

	Male % (n)	Female % (n)	p*
CP donors (n=2593)	2.28 (59)	0.35 (9)	<0.05
Blood donors (n=407363)	0.093 (377)	0.021 (84)	<0.05

*Mid-P exact test was used for comparison of two groups, HIV: Human immunodeficiency virus, CP: Convalescent plasma

Discussion

The findings of our study support our hypothesis that COVID-19 patients might have a more false positive anti-HIV 1/2 test result than healthy blood donors in the serological methods. In our study, we found that false positivity rate in male donors was significantly higher than female donors. We think that this difference resulted from low number of female donors in study and control groups. Serologic tests for HIV 1/2, hepatitis B, C virus and syphilis are performed by the TRC to the plasma received from the CP donors due to biosafety reasons. As is known, in the tests based on the antigen-antibody interaction principle, cross-reactivity can be seen since the binding domain of each antibody or the molecular association may interact with more than one antigenic determinant or more than one antigen, respectively. In other words, the cross-reactivity can occur because of the antigen that shares single epitope or of the structural similarity of epitopes (12).

In the tests based on the SARS-CoV antigen-antibody interaction, cross-reactions similar to this can also be observed. For example, it was reported that dual antigenic cross-reactivity with N proteins was seen between SARS-CoV and swine group 1 CoVs [TGEVs (M6 and P115 and PRCV-ISU1] in a study conducted (13). Accordingly, there are studies showing that auto-antibodies in some autoimmune diseases can cross-react with the nucleocapsid protein of SARS-CoV and cause false positivity (14,15). Also, false positivities due to cross-reaction have been found between SARS-CoV and HCoV-229E & HCoV-OC43, which are among the other coronaviruses that cause common cold in humans (16). Similar cross-reactions have been observed for Human T-lymphotropic virus (HTLV) I

and II, which rank among the Retroviridae family just like HIV 1/2. It was suggested that these reactions can be associated with rgp46-1 and rgp46-2 antigens of HTLV-I and GD21, p19, p24, gp21 and gp46 antigens of HTLV-II (17). In a study conducted by Pradhan et al. (18), it was stated that the amino acid array of four domains located on the SARS-CoV-2 S glycoprotein shows similarity with HIV-1 gp 120 and gag glycoproteins. Finally Mannar et al. (19) reported that host-derived glycans on spike proteins displayed high levels of cross-reactivity with anti-HIV 1 gp120 antibodies. These findings support the idea that the significantly high false reactivity rate we encountered results from the similarity of antigenic epitopes. The false positive test results for anti-HIV 1/2 were reported with another device system that used eCLIA test method. Tan et al. (20) and Papamanoli and Prevdos (21) reported three acutely ill COVID-19 patients had false positive anti-HIV tests. In these patients negative test results were detected with repeated serologic tests with different devices and and with molecular techniques.

Study Limitations

The main limitation of our study was that only one device and kit system developed by one company was used in our study. The second limitation of our study was low percentage of female donors in study and control groups (8.9% and 13.9%, respectively), so our results are not generalizable to both genders. The third limitation of our study was indeterminate confirmation test results and difficulty of follow-up sample obtaining.

Conclusion

Despite these limitations our test systems are safe and accepted worldwide, due to the national blood-banking algorithm of our country. We think that the results of this study warn us to be careful about the serological HIV 1/2 tests for the COVID-19 patients. Because the number of patients who had experienced COVID-19 and recovered has been increasing day by day; false-positive anti-HIV 1/2 results might increase in hospital settings. HIV 1/2 serological tests are being ordered for many screening purposes so this cross-reactivity might be a real problem. It is needed to be investigated and reported for different device and kit systems. It seems that the manufacturers will need to study on and solve this cross-reactivity problem to avoid false positive results. We think that difference of false positivity rates between genders needs new studies including a higher female population than our study.

False-positive results in anti-HIV 1/2 tests might be observed in the patients recovered from COVID-19. Defined cross-reactivity should be taken into account both in blood banking, CP treatment process and routine clinical practice.

Authorship Contributions

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

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Original Article

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Diagnostic Value of Novel Presepsin and Inflammatory Biomarkers in Predicting the Clinical Course of COVID-19

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Abstract

Aim: The diagnostic value of inflammatory markers to determine the severity of Coronavirus disease-2019 (COVID-19) is postulated in some recent studies, but conclusions were inconsistent. Hence, we intend to examine the utility of presepsin, procalcitonin and C-reactive protein in predicting the severity of COVID-19 infection.

Methods: Single-center cross-sectional study was undertaken at the Intensive care unit of a university hospital. Eighty consecutive cases diagnosed with Severe Acute Respiratory syndrome-Coronavirus-2 RNA between October 2020 and July 2021 were classified according to the severity of the disease. Laboratory data related to Procalcitonin and C-reactive protein was retrieved from investigations coinciding with the day of admission. The stored plasma was subjected to an enzyme-linked immunosorbent assay to determine plasma presepsin levels. Statistical test for measures of screening was employed and a receiver operator curve was generated.

Results: We have determined that presepsin is the most sensitive prognostic indicator (93.3%) with a strong statistical association ($p<0.001$) for COVID-19. 15.99 ng/L could be used as a reference level to predict the progressive clinical course. Relatively lower sensitivity (88%) and positive statistical correlation ($p=0.049$) of C-reactive protein with high-risk infection were also observed. Procalcitonin showed limited diagnostic and prognostic value in our series.

Conclusion: Our findings seem to demonstrate the role of presepsin in providing prognostic information in COVID-19 patients. Therefore, we suggest that early monitoring of presepsin with routine marker profile might help in identifying patients suffering from a more severe disease.

Keywords: SARS-CoV-2 patients, biomarkers, presepsin, prognosis

Introduction

The Coronavirus disease-2019 (COVID-19) pandemic has become a major health concern across the globe resulting in at least 4.1 million deaths to date secondary to COVID-induced pneumonia and septicemia (1). According to CDC guidelines, a vast number of COVID-19 cases exhibit mild to moderate clinical course without the need for hospitalization. However, around 19% of cases suffer from severe disease reflected by dyspnea, hypoxia with up to 50% lung involvement. Unfortunately, 5% of these severe cases may progress to a critical stage, complicated by respiratory failure, shock, or multiorgan system dysfunction (2). Therefore, early diagnosis and predicting the severity of the disease is the key to implementing appropriate therapeutic interventions that would eventually improve outcomes in patient. In this regard, laboratory medicine lies at the core of diagnosing and monitoring the vast majority of human diseases including COVID-19 (3).

The tools presently available in laboratory medicine for diagnosing COVID-19 include molecular testing, serological testing and inflammatory biomarkers (4). Among these tools, molecular testing has certain limitations such as; anatomic variations in the patient, inadequate sample collection and storage, genetic changes in the virus and use of unsuitable reference range. Also, the biological source used to quantify the viral RNA, have a low detection rate and is not easy to obtain (5). Similarly, serological testing has the disadvantage of giving positive results after a month of the onset of symptoms (6). This leaves us with the choice of inflammatory biomarkers which are easy to

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quantify, cost-effective, have better accuracy, potential to predict disease severity and are measured in a biological material that is easy to procure such as blood (7).

In this regard, inflammatory biomarkers including procalcitonin (PCT), C-reactive protein (CRP) and recently discovered presepsin (P-SEP) has shown promising results to predict disease severity in recent studies (8). Hence, we have taken a novel initiative to conduct large-scale original research with the aim to determine the diagnostic value of P-SEP, PCT and CRP in predicting the clinical course of COVID-19 infection and also sought the association of these molecules with the progressive clinical course of infection. The outcome of this study may prove vital in identifying novel molecules that could serve as an indicator to predict rapidly progressive COVID-19 infection and also identify patients presented with advanced underlying disease.

Methods

Study Design and Ethical Considerations

A single-center, cross-sectional study on 80 clinically proven COVID-19 cases was undertaken at a tertiary care set-up in Karachi, Pakistan. Ethical approval was sought from Ethics Review Committee (1701219FHPAT) in February 2020 for the collection of the cases diagnosed with COVID-19 infection. Written informed consent was obtained from all participants.

Sampling

All patients were receiving in-patient care at ICU and isolation wards of various tertiary care hospitals from October 2020 to July 2021. The diagnosis of COVID-19 was done using sensitive PCR and the subjects were selected using a non-probability consecutive sampling model. The cases were characterized into 2 groups: Moderate, and severe to critical, based on the clinical course according to CDC guidelines. Patients with the outcome of death were excluded from this study.

Data Collection

The clinical data of lab investigation including blood culture, PCT and CRP at day 0 of admission and demographic data, age and gender were obtained from the hospital record, while the stored blood drawn at day 0 of admission for routine workup was also retrieved from specimen storage facility of the lab for P-SEP analysis.

Laboratory Assessments

The frozen plasma was allowed to melt and 3 cc was subjected to an enzyme-linked immunosorbent assay (ELISA) for P-SEP, using a commercially available kit (Bioassay technology; cat. no E3754Hu). The reaction was performed according to manufacturer protocol in a multidisciplinary BSL-2 category lab following WHO prescribed standard operating procedures (SOP) (9). Briefly, 3cc plasma was centrifugated at 3000 x g for 10 min. ELISA assay was done at room temperature by adding standard, streptavidin-HRP, sample, anti-IgA antibodies into the standard well. The well was then covered with sealer and the sample was incubated for 60 mins at 37 ℃. Then, the sealer was removed and the plate was washed with wash buffer. The plate was then blotted onto paper towels and substrate solutions A&B were then added to each well. Finally, for color change stop solution was added and the optical density at 450 nm was determined by a microplate reader.

Statistical Analysis

For statistical analysis, SPSS version 25.0 was used. The data were analyzed for normality by Kolmogorov-Smirnov test and the Shapiro-Wilk test and median was taken as the determinant of central tendency. Crosstabulation was performed for measures of screening i.e; sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Receiver operator curve (ROC) was generated and the area under the curve and cut-off levels which corresponds to severe infection were estimated. The association of biomarkers with COVID-19 was assessed and $p<0.05$ was considered statistically significant. 95% confidence interval (CI) was used for all statistical calculations.

Results

Overall, 60 participants were in a state of severe to a critical disease whereas the remaining 20 reflected a moderate pattern. Fever and dry cough were universally present in all subjects as the chief complaint. Severe to critically ill patients showed extensive lung damage on radiology and requires intubation or mechanical ventilation to deal with hypoxia while those suffering from moderate-intensity required minimal oxygen support to maintain saturation.

Table 1 presents statistical estimates of all participants included in this research. Among all patients, most subjects were men and aged above 60 years. The median age was recorded as 67.5 years. The median levels for P-SEP, PCT and CRP among patients within the severe disease group were observed as; 55.05 ng/L, 1.25 ng/mL and 136.95 mg/L respectively. On descriptive statistics, P-SEP proved to be the most sensitive marker (93.3%) for advanced COVID infection and showed the highest rate of identifying patients with underlying severe disease (76.7%), compared to CRP and PCT. The highest specificity rate was accounted for PCT (30%) in the current study.

On ROC analysis, a strong statistical association ($p<0.001$) of P-SEP with severe COVID infection was seen,

Table 1. Distribution of all participants with respect to clinicopathological characteristics and statistical estimates

Clinical characteristics	Distribution (n=80)							
Sex (male/female)	54/26							
Median age (years)	67.5 (33-90)							
Investigations (reference level) †	The severity of COVID-19		p (p=0.5)ǂ	Sensitivity (%)	Specificity (%)	PPV§ (%)	NPV‡ (%)	AUC ǂ
	Moderate ¶ (n=20)	Severe-to-critical ¶ (n=60)						
CRP (6 mg/L)	19	54	0.047 *	90	5	74	14.3	0.649
PCT (0.15 ng/mL)	14	47	0.549	78.3	30	77	31.6	0.455
P-SEP (5.5 ng/L)	17	56	0.001 *	93.3	15	76.7	42.9	0.778

†Prescribed laboratory reference levels for positivity ¶CDC Guideline for COVID-19 ǂ ROC indicator for null-hypothesis, *Statistically significant ROC values §Positive predictive value ‡Negative predictive value ǂArea under the curve, PCT: Procalcitonin, CRP: C-reactive protein, COVID-19: Coronavirus disease-2019

reflected by the area under the curve (AUC) of 0.778. Also, CRP levels were significantly higher ($p<0.05$) among critically ill patients accompanied by the ROC curve (AUC) of 0.649 (Figure 1). No significant difference was recorded for PCT. When 15.99 ng/L was used as a cut-off for P-SEP, we observed 93.3% sensitivity and 85% specificity for severe infection whereas when cut-off levels for PCT; 0.156 ng/mL and CRP; 7.95 mg/L was considered, 78% sensitivity and 70% specificity for PCT while 88% sensitivity and 95% specificity for CRP was recorded.

Discussion

Recent CDC guidelines describe that the core principle behind the management of Severe Acute Respiratory syndrome-Coronavirus-2 (SARS-CoV-2) infection (COVID-19) relies on monitoring respiratory rate, oxygen saturation & indicators of oxygenation such as; PaO_2/FiO_2. Unfortunately, the prescribed parameters are unreliable & subjective resulting in misdiagnosis, false interpretations & late diagnosis especially in cases with high-risk COVID-19 infection (10). So, there is an urgent need for novel bioassays that could identify the complex case or predict the clinical course of disease in order to improve outcomes among patients' by implementing appropriate treatment plans.

Measuring proinflammatory markers is considered as a cornerstone in risk assessment of patients with a wide variety of infectious diseases, due to their potential for predicting the clinical progression as well as guiding therapeutic decisions (8). Primarily, cytokine storm is the major factor behind the worst outcome among COVID-19 patients'. It is hypothesized that the interaction of macrophage and activated viral products triggers this release of cytokine and some immune mediators such as P-SEP (8). Therefore, measuring the concentration of P-SEP may provide valuable clinical information for

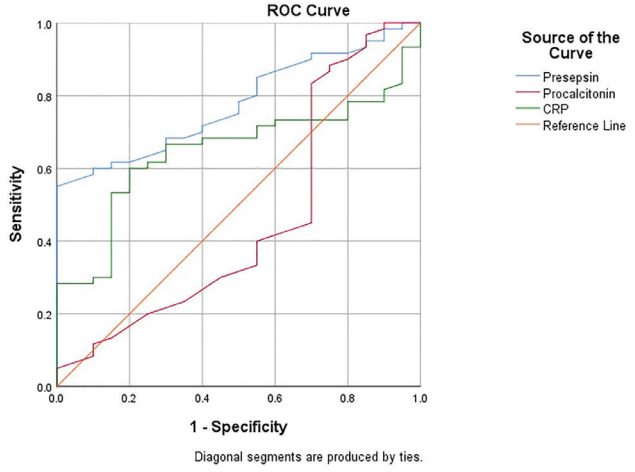

Figure 1. Receiver operating characteristic curve comparing levels of P-SEP (blue) and procalcitonin (red) and C-reactive protein (green) in severe to critical patients of COVID-19
COVID-19: Coronavirus disease-2019

risk stratification of COVID-19 patients'. In this context, recently, Domi et al. (11) and Ducastel et al. (12) showed the benefits of monitoring P-SEP in risk stratification of COVID-19 patients'. These findings are further strengthened by the results of a recent pooled analysis (8). Previously, Zaninotto et al. (3) and Fukada et al. (10) found a positive relationship of inflammatory biomarkers with COVID-19 and its clinical course in a limited set of cases and recommended further research. We, therefore, took a lead to investigate this association in a large group of patients with proven COVID-19 infection. To the best of our knowledge, this is the first original research conducted with the intent to determine the independent prognostic value of P-SEP, PCT and CRP for COVID-19 infection. Our findings may have long-lasting clinical implications. P-SEP is a recently identified biomarker which may facilitate in the

diagnosis of sepsis. Structurally, it is a truncated N-terminal soluble CD14 subtype (10). Whereas, PCT is a derivative of calcitonin, which is released primarily from C cells of the thyroid gland, monocytes as well as hepatocytes. PCT is a well-established molecule for identifying inflammatory response of infectious origin (13). Compared to P-SEP and PCT, CRP is produced by the liver in response to an ongoing inflammation anywhere in the body (14).

Numerous studies have reported the utility of P-SEP in predicting the severity and mortality in some inflammatory conditions related to infections (15). In the present research, we retrospectively compared the plasma levels of inflammatory markers including P-SEP between moderate and severe to critically ill COVID-19 patients'. The diagnosis of COVID-19 infection was performed by isolating SARS-CoV-2 RNA from nasopharyngeal swab specimens while patients' clinical status was determined in accordance with CDC guidelines (2). We have determined that P-SEP were significantly higher among severe to critically ill patients on admission than those who had a moderate disease. Furthermore, P-SEP has shown better sensitivity and specificity, which suggested that this molecule could serve as a useful prognostic indicator of COVID infection. Similar findings were reported in case series by Fukada et al. (10).

Despite no established mechanism of P-SEP elevation in COVID-induced pneumonia is documented to date, several studies have shown a strong relationship of P-SEP with short-term mortality in patients of acute respiratory distress syndrome (ARDS) (16). Because the potential of P-SEP to predict the progression of ARDS is well recognized, we believe this might assist clinicians to distinguish high-risk COVID-19 patients on admission and implement appropriate treatment strategies at an early stage.

In agreement with previous studies (17-19), we came across a weak statistical association of CRP accompanied by reasonably good sensitivity. We suggest that the observed relationship of CRP could be contributed by the inflammatory environment due to its natural proinflammatory characteristic, rather than the infection itself. However, this inference is subject to confirmation.

In a recent study by Tuncer et al. (20) PCT has shown the highest odds for predicting deterioration among COVID-19 patients', however, P-SEP status was not investigated either compared with other inflammatory markers. In contrast, some previous studies had shown limited or no role of PCT in the diagnosis and prognosis of COVID-19 (3,10). In the current research, although, PCT levels were elevated in the majority of cases, no difference between moderate and severe COVID-19 groups was seen. Moreover, the lowest sensitivity and specificity values were recorded for PCT in the present study. This observation is in agreement with Zaninotto et al. (3) and Fakuda et al. (10). We believe that steady and slow increase of PCT is the inherent property under infectious conditions and thus the values taken on day 0 of admission may have biased our findings. Therefore, further research is recommended in this regard.

Another important aspect of the present study is the proposed cut-off levels of inflammatory markers for risk stratification of COVID-19 infection. Previously, Zaninotto et al. (3) suggested a cut-off level of P-SEP for severe infection; however, the cut-off range of other inflammatory biomarkers was not evaluated and compared. Also, the study was unable to measure the sensitivity and specificity for the proposed P-SEP cut-off (3). Contrary to that, we suggest independent concentrations of P-SEP, PCT and CRP can be used to identify COVID cases with poor outcomes. Besides that, we also described sensitivity and specificity for corresponding cut-off concentration which gives further strength to our findings. However, we recommend more research using a larger sample size to establish precise cut-off limits for each of those molecules.

Study Limitations

Our study has some limitations. Firstly, due to consecutive sampling, the distribution of cases across two groups was not equal which might have influenced our findings. Secondly, we were not able to include cases with outcomes of death and hence were unable to assess the utility of these molecules to predict mortality. This limitation is contributed by the hospital policy of refraining the data of those who died from COVID-19. Thirdly, the study was conducted with the aim to determine the prognostic utility of inflammatory markers in COVID infection and hence only values corresponding to the day of admission were taken into account, thus preventing us to compare plasma levels with disease progression. Lastly, further studies are needed to better explain the mechanisms responsible for elevated levels of inflammatory markers especially; P-SEP in SARS-CoV-2 patients' and in particular its relation with multiorgan failure syndrome. Despite these limitations, there are certain strengths of the present research which are worth mentioning. This study is among a few studies to analyze the role of pro-inflammatory markers for the severity of COVID-19. We have conducted our research on a larger sample size compared to previous studies and performed a comprehensive comparative analysis of conventional markers and novel P-SEP. Overall, our findings may offer a useful strategy to stratify high-risk COVID-19 patients' in ICU admission who would benefit from intensive treatment.

Conclusion

The data obtained seems to demonstrate the role of the inflammatory biomarkers in providing prognostic

information in high-risk COVID-19 patients', as already described in several other diseases. Nevertheless, our findings showed that among all analyzed molecules; P-SEP proved to be the most useful tool in predicting the severity of COVID-19 infection. Further multicenter studies with a large number of subjects are warranted to confirm our findings.

Authorship Contributions

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

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Original Article

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Mental Health of the Refugee and Native Patients with End-Stage Renal Diseases Receiving Hemodialysis During COVID-19 in Istanbul: A Cross-Sectional Study from a Tertiary Center

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Abstract

Aim: Patients receiving hemodialysis (HD) are at high risk for developing psychiatric symptoms. The aim of this study is to investigate the levels and correlates of depression, anxiety, somatization and post-traumatic stress disorder (PTSD) symptoms of refugee HD patients and compare to native HD patients.

Methods: This is a descriptive study with a cross-sectional design. A total of 58 refugee (n=27) and native (n=31) end-stage renal disease patients receiving HD were included. The data were collected between 14th and 16th of July 2021. Patient health questionnaire-somatic, anxiety, and depressive symptoms (PHQ-SADS) scales and posttraumatic-stress disorder checklist for DSM-5 (PCL-5) were used for the clinical assessment.

Results: Refugee patients had similar sociodemographic, clinical and HD characteristics with native patients. Anxiety subscale and PCL-5 scores were significantly higher in refugee patients (p=0.04 and p=0.03, respectively), while depression and somatization subscales levels did not differ among groups. The age was negatively correlated with depressive symptom levels in refugee patients while somatic symptom levels were positively correlated with depression, anxiety and PTSD symptom levels in both groups.

Conclusion: The staff of HD centers should be trained in order to recognize psychiatric disorders and symptoms, and routine psychiatric assessment may contribute to improving the mental health and preventing adverse health outcomes in refugee HD patients.

Keywords: Renal dialysis, refugees, depression, mental health, COVID-19

Introduction

In consequence of the compelling treatment schedule, dietary restrictions, and impairment in physical and sexual functioning, living on hemodialysis (HD) is a perpetually challenging condition for patients with end-stage renal disease (ESRD) (1,2). Accordingly, depressive and anxiety disorders are the two most frequent psychiatric disorders in HD patients, the prevalence of depression and anxiety symptoms in HD patients are reported to be higher than the general population and range from 37% to 42% and from 38% to 53%, respectively (3). These two psychiatric conditions commonly co-occur but are frequently underdiagnosed and untreated, whereas both disorders were reported to be closely associated with poor treatment compliance and outcome, hospitalization, impaired quality of life and mortality in this population (3-6). Considering the Coronavirus disease-2019 (COVID-19) pandemic, the uncertainty and health-related anxiety could also increase

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the severity of mental disorders or may cause new-onset mental disorders in ESRD patients as individuals with severe chronic illnesses have higher rates of morbidity and mortality rates due to COVID-19 (7,8).

In the last decade, a total of 13 million refugees have been displaced due to the Syrian civil war in the Middle East (9). As of 2021, Turkey is the country hosting the largest number of refugees under "temporary protection" with approximately 3,6 million refugees, and many of those had to experience difficulties such as accessing health and social services, uncertainty around their legal rights, lack of social support, unemployment, financial issues, social isolation and xenophobia after forced migration (10,11). Therefore, post-traumatic stress disorder (PTSD) and other psychiatric conditions including depression, anxiety and somatization were reported to be at higher rates among refugees and have been found to persist over many years (12-14). As one of the most disadvantageous groups in society, refugees with ESRD could be expected to be highly vulnerable to psychological distress during the COVID-19 pandemic, as this population could be more adversely affected by negative consequences of the pandemic such as social isolation, economic hardship and unemployment, as well as uncertainty and health related anxiety (15). However, to date, there are still scant data before and during the COVID-19 pandemic on whether refugee HD patients have a higher risk of developing psychiatric symptoms than native HD patients. To the best of our knowledge, there are only two studies focusing on the mental health of refugee ESRD patients receiving HD (16,17).

This study set out to determine the mental health status of refugee and native ESRD patients receiving HD during the COVID-19. Our aim is to investigate and compare the levels of depression, anxiety, somatic anxiety and PTSD symptoms of refugee and native HD patients and we hypothesized that HD dependent ESRD patients have high comobidity of comorbidity of psychiatric conditions and refugee HD patients have higher depression, anxiety, somatic anxiety and PTSD levels compared to the native HD patients during the COVID-19.

Methods

Study Design and Sample

The study was conducted in accordance with the Declaration of Helsinki and its later amendments. Ethics committee approval was obtained from University of Health Sciences Turkey, Haseki Training and Research Hospital Ethics Committee (approval number: 2021-59) and COVID-19 Scientific Review Board of Ministry of Health of the Republic of Turkey. This study is a cross-sectional study conducted in a tertiary center, which enrolled 69 refugee and native patients who were receiving HD in the HD Center of University of Health Sciences Turkey, Haseki Training and Research Hospital, which which a have a high percentage of refugee dialysis patients. The term "native" was used to describe native Turkish patients. The data of the study were collected between 14 and 16 July 2021. Patients younger than 18 and older than 75 years old, those with history of HD less than three months, those undergoing home HD, those who were not able to fill out questionnaires in Turkish and Arabic language, those with intellectual disability that would limit the ability to answer the questionnaires and those who refused to give informed consent were excluded from the study. Finally, a total of 58 adult patients, consisting of 27 Syrian refugees and 31 native patients receiving HD, who met the study criteria and gave informed consent, were included in the study.

Sociodemographic and Clinical Questionnaire

This questionnaire was prepared by the researchers for this study. The following demographic and clinical data were extracted from from the electronic records: Age, gender, education level, comorbidity, primary cause of kidney disease, duration of HD, residual renal functions, urea reduction rate, vascular access route, Kt/V, and also hemoglobin, albumin, urea and creatinine levels obtained at the beginning of July 2021. The data including monthly household income per capita, the number of people living together and duration of refugee status were obtained from the participants.

Patient Health Questionnaire-Somatic, Anxiety, and Depressive Symptoms (PHQ-SADS)

Patient health questionnaire-somatic, anxiety, and depressive symptoms (PHQ-SADS) is a self-administrated tool designed to measure the rates of psychiatric morbidity (18). The scales can be applied together or separately as PHQ-15 (somatization), GAD-7 (anxiety), PHQ-9 (depression), and 5-item panic modules. Cut-off scores of 5, 10 and 15 represent mild, moderate and severe symptom levels on all three scales. Validated forms in Turkish and Arabic language were used for the refugee and native patients (19,20).

Posttraumatic Stress Disorder Checklist for DSM-5 (PCL-5)

The PCL-5 is a 20-item self-report scale developed to assess the severity of PTSD symptoms of DSM-5 (21). The rating scale ranges between 0 (not at all) and 4 (extremely) for each symptom. A cut-off value of ≥31 was suggested by the authors as the optimal PCL-5 score for PTSD diagnosis. Validated forms in Turkish and Arabic languages were used for the refugee and native patients (22,23).

Statistical Analysis

The analyses were performed using the IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as numbers

and percentages for categorical variables and as mean, standard deviation for numerical variables. The conformity of variables to normal distribution was assessed using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). The chi-square test was used for two-group comparisons of categorical variables. For the comparison of two and multiple groups the Mann-Whitney U and Kruskal-Wallis tests were used for the comparison of quantitative data, respectively. Spearman's rho correlation coefficients were used for correlation analysis. A p-value of less than 0.05 was considered statistically significant.

Results

There was no significant difference between refugee and native patients regarding; age, gender, education level, primary cause of kidney disease, monthly household income per capita, the number of people living together, duration of HD, residual renal functions, vascular access route, hemoglobin, albumin, Kt/V, urea and creatinine levels (p>0.05). The data regarding sociodemographic, clinical and HD characteristics are provided in Table 1.

The comparison of PHQ-9, GAD-7, PHQ-15 and PCL-5 between refugee and native HD patients indicated

Table 1. Characteristics of the refugee and native patients receiving HD

Characteristics		Refugee patients (N=27)	Native patients (N=31)	p
Demographic and clinical characteristics				
Age, mean ± SD		48.0±15.2	49.5±15.8	0.62*
Gender, n (%)	Male Female	12% (44.4) 15% (55.6)	17% (54.8) 14% (45.2)	0.43**
Education status, n (%)	Illiterate	4% (14.9)	2% (6.5)	0.12***
	Primary school	12% (44.4)	13% (41.9)	
	High school	6% (22.2)	9% (29)	
	Graduate school	5% (18.5)	7% (22.6	
Primary kidney disease, n (%)	Diabetic kidney disease	8% (29.6)	10% (32.3)	0.78***
	Hypertensive nephrosclerosis	7% (25.9)	9% (29)	
	Glomerulonephritis	1% (3.7)	1% (3.2)	
	Autosomal dominant polycystic kidney disease	0% (0)	1% (3.2)	
	Others	2% (7.4)	4% (12.9)	
	Unknown	9% (33.3)	6% (19.4)	
Presence of residual renal function, n (%)	-	11% (40.7)	13% (41.9)	0.96**
The number of people living together, mean ± SD	-	6.1±1.8	4.1±1.8	**<0.001***
Monthly household income per capita (Turkish Liras), mean ± SD	-	603±337	568±307	0.89*
Duration of refugee status (year)	-	7.3±1.2	-	-
Hemodialysis characteristics				
Hemodialysis frequency, n (%)	Once weekly	1% (3.7)	1% (3.2)	0.97***
	Twice weekly	3% (11.1)	4% (12.9)	
	Thrice weekly	23% (85.2)	26% (83.9)	
Duration of hemodialysis (year), mean ± SD	-	4.4±4.3	3.7±3.6	0.44*
Vascular access, n (%)	Arteriovenous fistula	24% (88.9)	24% (77.4)	0.24**
	Tunneled catheter	3% (11.1)	7% (22.6)	
Kt/V, mean ± SD	-	1.82±0.34	1.77±0.54	0.38*
Urea reduction rate (%),mean ± SD	-	76±6	76±7	0.43*
Initial serum creatinine (mg/dL), mean ± SD	-	8.2±2.3	7.1±2.3	0.09*
Initial serum urea (mg/dL), mean ± SD	-	123±28	132±36	0.29*
Albumin (g/dL), mean ± SD	-	3.6±0.6	3.9±0.6	0.24*
Hemoglobin(g/dL), mean ± SD	-	9.8±1.8	9.9±1.4	0.91*

*Mann-Whitney U test, **chi-square test, ***Kruskal-Wallis test, bold print indicates statistical significance at 0.05 level

that GAD-7 and PCL-5 scores were significantly higher in refugee patients (p<0.05 for both) while PHQ-9 and PHQ-15 scores did not differ among groups. The data regarding the comparison of the scales between refugee and native patients are provided in Table 2.

When the participants were investigated regarding the symptom severity of psychiatric disorders using suggested cut-off values of the scales; depression and somatic anxiety were found to be the most frequent psychiatric conditions (29.3% for each). Among refugees, somatic anxiety and PTSD were the most frequent psychiatric conditions (33.3% for each), while depression was more frequent among the native patients. The data regarding the symptom severity of depression, anxiety, somatic anxiety and PTSD are provided in Table 3.

Regarding the correlates of PHQ-9, GAD-7 and PCL-5 in refugee and native HD patients; there was a strong negative correlation between the age and PHQ-9 scores in refugee patients (r=-0.611; p<0.01). Duration of refugee status had a moderate and positive correlation with PHQ-9 scores in the refugee group (r=0.475, p<0.05). Somatic anxiety had a strong positive correlation with PHQ-9 scores in both refugee and native patients (r=0.737; p<0.001; r=0.650, p<0.001, respectively); strong positive correlation with GAD-7 scores in refugees (r=600, p<0.01) and weak positive correlation with GAD-7 (r=0.381, p<0.05) in native patients; and moderate positive correlation with PCL-5 scores in refugee patients (r=0.419, p<0.05) and weak positive correlation in native patients (r=0.384, p<0.01). The data on the correlates of PHQ-9, GAD-7 and PCL-5 are provided Table 4.

Table 2. Comparison of depression, anxiety, somatic anxiety and PTSD levels between refugee and native patients receiving HD

Scales	Refugee patients (n=27)	Native patients (n=31)	p
PHQ-9	5.96±5.11	7.29±5.19	0.32*
GAD-7	7.20±3.89	4.83±4.54	**0.04***
PHQ-15	7.71±4.43	7.32±4.53	0.75*
PCL-5	26.65±15.84	16.52±13.09	**0.03***

*Mann-Whitney U test, PHQ-9: Patient health questionnaire-9, GAD-7: Generalized anxiety disorder-7, PHQ-15: Patient health questionnaire-15, PCL-5: Posttraumatic stress disorder checklist for DSM-5, Bold print indicates statistical significance at 0.05 level

Table 3. Depression, anxiety and PTSD symptoms in refugee and native patients

Symptom severity	Refugee patients (n=27)	Native patients (n=31)
PHQ-9≥10	6 (22.2%)	11 (35.4%)
GAD-7≥10	8 (29.6%)	4 (12.9%)
PHQ-15≥10	9 (33.3%)	8 (25.8%)
PCL-5≥31	9 (33.3%)	5 (16.1%)

PHQ-9: Patient health questionnaire-9, GAD-7: Generalized anxiety disorder-7, PHQ-15: Patient health questionnaire-15, PCL-5: Posttraumatic stress disorder checklist for DSM-5

Discussion

This study aims to investigate and compare the levels of depression, anxiety and PTSD symptoms of refugee and native HD patients during COVID-19. The main findings of the study are as follows: (i) refugee HD patients had similar sociodemographic, clinical and HD characteristics to native patients native patients; (ii) regarding the comparison of the PHQ-SADS subscales scores; anxiety and traumatic stress levels were significantly higher in refugee patients while depression and somatic anxiety levels did not differ among groups; (iii) regarding psychiatric comorbidity; anxiety and PTSD were more frequent among refugee patients and depression was more frequent among native patients; (iv) the age was negatively correlated with depressive symptom levels in the refugee group, while somatic symptom levels were positively correlated with depression, anxiety and PTSD symptom levels among both refugee and native patients.

In a multicenter study conducted in Europe, refugees were found to represent 1.5% of the dialysis population in several European and Middle Eastern countries with a very skewed geographic distribution, and only a limited number of centers treat >20 refugees due to non-reimbursement of the treatment costs (24). In Turkey, all health expenditures of refugees who are under legal "temporary protection" were covered by the Government of The Republic of Turkey. This was probably the main reason of the relatively higher rate of refugee HD patients in our sample. Language and social work assistance for refugee patients in University of Health Sciences Turkey, Haseki Training and Research Hospital could also have contributed to the increased admission rates of the refugees to the HD center of the hospital.

When the rate of participants by gender was examined, the rate of women and men was found to be equal. The majority of the HD patients were middle-aged and with low household income per capita according to the "Income and Living Conditions Survey" of the Turkish Statistical Institute (25). The most common causes of ESRD were diabetic kidney diseases and hypertensive nephrosclerosis as previously reported in other countries and Turkey (16,26). Our results indicated that there was no significant difference regarding sociodemographic, clinical and HD characteristics regarding age, gender, education level, among refugee and native patients.

The presence of comorbid psychiatric disorders in HD patients is a challenging condition for nephrologists as it negatively influences treatment compliance and is

Table 4. Correlates of PHQ-9, GAD-7 and PCL-5 in refugee and native patients receiving HD

	PHQ-9				GAD-7				PCL-5			
	Refugee patients (n=27)		Native patients (n=31)		Refugee patients (n=27)		Native patients (n=31)		Refugee patients (n=27)		Native patients (n=31)	
	r	p	r	p	r	p	r	p	R	p	r	p
Age	-0.611	**<0.01**	-0.175	0.37	-0.316	0.16	-0.192	0.31	-0.169	0.44	-0.347	0.08
Monthly household income per capita	-0.019	0.93	-0.316	0.25	0.007	0.98	-0.083	0.75	-0.205	0.38	-0.299	0.30
Duration of HD	0.171	0.46	0.080	0.68	-0.030	0.89	0.054	0.78	-0.037	0.87	-0.059	0.77
Duration of refugee status	0.475	**0.03**	-	-	0.264	0.25	-	-	0.197	0.40	-	-
Kt/V	0.173	0.45	0.057	0.77	-0.030	0.89	-0.005	0.98	-0.212	0.33	-0.126	0.53
Albumin	0.366	0.10	-0.002	0.99	0.079	0.73	0.145	0.45	-0.063	0.78	-0.022	0.92
Hemoglobin	0.011	0.96	-0.320	0.09	0.017	0.94	-0.060	0.75	-0.266	0.21	-0.226	0.26
Urea	0.222	0.33	0.076	0.69	-0.107	0.64	0.276	0.14	-0.179	0.415	0.005	0.97
Creatinine	0.119	0.607	0.076	0.70	0.049	0.83	0.276	0.14	-0.034	0.88	0.007	0.97
Somatic symptoms (PHQ-15)	**0.737**	**<0.001**	**0.650**	**<0.001**	**0.600**	**<0.01**	**0.381**	**0.04**	**0.419**	**0.04**	**0.513**	**<0.01**

r: Spearman's rho correlation coefficient, HD: Hemodialysis, PHQ-9: Patient health questionnaire-9, GAD-7: Generalized anxiety disorder-7, PHQ-15: Patient health questionnaire-15, PCL-5: Posttraumatic stress disorder checklist for DSM-5, Bold print indicates statistical significance at 0.05 level

associated with adverse outcomes such as hospitalization and mortality (27). HD requires regular attendance for several hours per week and dietary restrictions, restricts the patient's autonomy and may lead to a feeling of being machine-dependent. These conditions commonly result in exacerbation of the comorbid psychiatric disorders such as depression and anxiety as well as the emergence of new-onset mental conditions (28). Furthermore, patients with ESRD were found to have higher prevalence rates of depression than those with other chronic diseases (29). When it comes to the nationality and refugee status; in a recent study, the authors have reported that adverse clinical outcomes associated with depressive symptoms differ among ethnic groups, and ethnicity is suggested to be an important factor that could influence the adverse clinical outcomes and depression in HD patients (30). Immigrant HD patients were reported to be more prone to develop depressive and anxiety symptoms than the native patients (31). Accordingly, authors from Jordan have reported that among Syrian refugees with HD dependent ESRD; 36% had severe depression, 74% were anxious about their illness and 43% had feelings of being a burden to their families (17). In another very recent study conducted in Turkey, it was reported that Syrian refugee HD patients had higher depressive symptom levels compared to native HD patients (16). Our results indicated that our sample had higher rates of anxiety, depression and somatic anxiety. The most frequent psychiatric conditions were somatic anxiety and PTSD in refugee patients, while one-third of native patients reported moderate or higher depressive symptoms. Our results indicated similar depression and anxiety levels in HD patients compared to the literature (2,28,32). However, on the contrary to the results of previous studies conducted in Turkey and other countries reporting higher depression levels in refugee populations (16,31,33), depression rates were lower in refugee patients than in native patients. This discrepancy probably resulted from the different methodology of these studies, and it could be explained as follows: (i) different self-report scales with different sensitivity and specificity levels such as Beck depression inventory and hospital anxiety and depression scale were used by the researchers in some of these studies and this could be associated with the discrepancy of the rates of depression; (ii) in our study, we used the cut-off values ≥10 for PHQ-9 indicating moderate or higher depression severity and did not take into account those with mild symptoms in order to obtain more accurate rates of depression, (iii) these studies were conducted in different countries and psychiatric symptom levels of refugees could vary according to the host country (12).

The somatic symptoms of depressive disorders have similar characteristics with the symptoms of uremia such as sleep disturbances, fatigue, anorexia, gastrointestinal symptoms, aspects of volume overload and pain, which may complicate complicate recognizing the underlying psychiatric illness (34). Somatic symptoms and distress are also associated with mental disorders, particularly in the refugee population facing traumatic experiences and economic difficulties, which was also the case in our sample (13,35). Hence, investigating somatic distress in both native and refugee HD patients appears to appers to provide substantial benefit for the detection and management of the mental disorders, and also psychological trauma following humanitarian crises. Besides, patients receiving HD were also found to have higher somatic complaints

than the general population (36). In our study, one-third of the participants reported moderate or higher somatization and refugee patients had significantly higher levels of somatic symptoms than native patients and this was consistent with studies conducted in Turkey and other countries (14,37,38).

Somatic complaints and distress are also closely associated with psychological trauma and PTSD was previously reported to be a frequent mental health issue among Syrian refugees in Turkey (13,39). Accordingly, in our study, the comparison of PTSD symptom levels revealed that refugee HD patients were having significantly higher PTSD levels than native patients, as expected. As this study is conducted during COVID-19, the pandemic could be another factor which has contributed to the exacerbation of PTSD and somatic distress as previously reported (40,41). These results suggest that HD dependent refugee ESRD patients may be at high risk of somatic anxiety which is associated with the disease itself and psychological trauma.

The age was found to be negatively associated with levels of depression in refugee patients with ESRD in our study. Younger age is reported to be associated with higher depression levels in patients receiving HD (42). However, there are contradictory findings in the literature on the association of the age with psychiatric symptoms among refugee and native HD populations (13,43,44). This discrepancy was probably associated with the different life conditions of refugees in host countries where these studies were conducted. Another explanation is that, the high risk of depression for younger populations populations could be resulted from the negative impact of COVID-19 and related preventive measures on social life, and this probably is also the case for the younger patients with ESRD (40,45,46). The duration of refugee status was positively correlated with levels of depression, and this result suggests that being under refugee status for a longer duration with a chronic illness such as ESRD could be associated with higher depression levels. Somatic symptoms correlated with depression, anxiety and PTSD levels in both refugee and native patient groups. Individuals with comorbid depression and anxiety are at high risk for somatic symptoms (47). As a considerable amount of the refugees have experienced traumatic events, traumatization may also be an important etiologic factor for somatization in the refugee population. Regarding HD characteristics, laboratory levels of the parameters such as Kt/V, hemoglobin, albumin, urea and creatinine did not correlate with PHQ-9, GAD-7 and PCL-5 scores.

Study Limitations

Our study is conducted in a single center and has a relatively small sample size. Thus, the findings of this study cannot be generalized. Due to the cross-sectional design, the effect of the psychiatric conditions on the outcomes of HD could not be investigated. The psychological assessment was conducted via self-rating scales instead of clinical interviews and this may have resulted in increased levels of psychiatric symptoms. Finally, refugee patients in our sample were only registered refugees, and our results does not represent the unregistered Syrian refugees with ESRD which may have higher psychiatric symptom levels and probably could not reach the HD treatment. However, given the growing concerns about the mental health of refugees around the world, our study may contribute to the literature as it is one of the first studies focusing on the mental health of refugee patients with ESRD which may cause a significant psychological burden.

Conclusion

Our results indicated a high rate of comorbidity of psychiatric conditions in ESRD patients receiving HD, refugee patients appear to have higher comorbidity of anxiety, somatization and PTSD than native HD patients. Hence, refugee HD patients should be closely monitored in terms of psychiatric symptoms. Therefore, routine psychiatric assessment of these patients in order to achieve early diagnosis and more effective management, may contribute to improve the mental health and prevent adverse health outcomes in HD patients. Policy-makers should promote the implementation of formal screening programs for psychiatric disorders among ESRD patients receiving HD. Physicians, nurses and social workers of HD centers should be trained and supported in order to recognize psychiatric symptoms and disorders and to provide psychoeducation programs for the patients and their relatives. HD centers should establish a solid collaboration with consultation-liaison psychiatrists.

Authorship Contributions

Concept: M.Y., Design: M.Y., E.C., Data Collection or Processing: Y.B., Analysis or Interpretation: M.Y., E.C., S.A., H.K., Literature Search: M.Y., S.A., H.K., Writing: M.Y. Y.B.

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The Relationship Between Intrahospital Mortality and P-Wave Dispersion in COVID-19 Patients

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Abstract

Aim: P-wave dispersion (PWD) is a noninvasive electrocardiographic (ECG) marker of atrial remodeling. Inflammations are likely to cause atrial remodeling. This study aims to determine the relationship between PWD and intrahospital mortality in Coronavirus disease-2019 (COVID-19) patients.

Methods: One hundred eighty-nine patients who tested positive for polymerase chain reaction for a diagnosis of COVID-19 between March 2020 and January 2021 were included in the cross-sectional study. PWD was calculated from the pre-treatment ECG of all patients at the time of hospitalization.

Results: Mean PWD values were numerically and statistically significant in the group who died in hospital compared to the group discharged after recovery (46.37±19.00 ms vs. 31.86±11.08 ms, p<0.001). Regardless of gender, regression analysis found an independent relationship between PWD values and mortality in COVID-19 patients. In the receiver operating characteristic curve analysis, PWD values above 34 ms had 73% sensitivity and 69% specificity in predicting all-cause mortality in COVID-19 patients (area under the curve: 0.737, confidence interval: 0.666-0.808, p<0.001).

Conclusions: PWD was associated with in-hospital mortality in COVID-19 patients. The findings of this study demonstrate that the PWD values calculated at the time of hospitalization can be helpful in determining the prognosis of COVID-19 patients.

Keywords: P-wave dispersion, SARS-CoV-2, COVID-19, electrocardiography, mortality

Introduction

Despite our increasing experience with Coronavirus disease-2019 (COVID-19) and the diversity of medical treatment options, its mortality remains high (1). As the number of cases increases, cardiovascular diseases are increasingly reported (2). Multiorgan involvement occurs as a result of both the direct effect of the virus and the cytokine storm. Decreased cell perfusion, hypoxia, and increased inflammatory responses during the course of the disease are the main mechanism of many clinical conditions, and as the clinical condition worsens, there are serious difficulties with the accompanying differential diagnoses (3).

Electrocardiography (ECG) has been confirmed to be useful in many clinical situations outside cardiology practice. Each wave seen on the ECG strip (such as P, QRS, and T-wave) gives clues to the clinician about the patient's clinical situation (4).

The P-wave is an indicator of atrial transmission and reflects the subclinical conditions (e.g. fibrosis and atrial inflammation) of the atria (5). In the Framingham Heart Study, in which P-wave duration, amplitude, and length were examined, prolongation of the PR interval was shown to be associated with increased mortality and atrial fibrillation (6). According to what we know from previous studies, myocardial damage can occur as a result of the direct effect of the viral pathogen causing the infection or systemic inflammation (7). To the best of our knowledge, although PWD has been revealed to be associated with atrial fibrillation in COVID-19 patients, its relationship with mortality has not been examined.

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The aim of this study was to analyze the usability of P-wave dispersion in predicting in-hospital mortality in patients diagnosed with COVID-19.

Methods

Patients and Study Design

Before the study, approval was obtained from the COVID-19 Science Committee of the Ministry of Health (reference number: 2021-03-11T13_34_23) and our Canakkale Onsekiz Mart University's Ethics Committee (date: 31.03.2021 decision no: 2011-KAEK-27/2021-E.2100040583). Due to the design of the study, informed consent was not received from the participants. This study was carried out in accordance with the Declaration of Helsinki.

This study is a single-center cross-sectional study and was conducted in a tertiary health center between 15 March 2020 and 1 January 2021. Patients newly diagnosed with a positive polymerase chain reaction test were included in the study for the diagnosis of COVID-19. The PWD of the patients was calculated from the ECG taken before starting treatment. Patients were divided into two groups: Group 1, those who lost their lives in the hospital, and group 2, those who were discharged after recovery.

Demographic data, laboratory results and cardiac risk factors were obtained from electronic medical records and recorded for both groups.

This study did not include those with active infections other than COVID-19, those with acute cerebrovascular disease, severe renal and liver failure, those with a permanent pacemaker and pace rhythm, those with any kind of branch blocks, those with malignant disease, those who took antiarrhythmic drugs that can cause PR prolongation, those with application rhythm atrial fibrillation and patients under 18 years of age.

Definitions

Hypertension was defined as systolic blood pressure above 140 mmHg and diastolic blood pressure above 90 mmHg or the use of antihypertensive drugs. Diabetes mellitus was defined as fasting blood glucose above 126 mg/dL or the use of antidiabetic medication. Smoking was identified as the use of cigarettes for more than the previous six months.

The ECGs (Fukuda Denshi, Tokyo, Japan) of all the patients included in the study, taken in the supine position with 12 leads, 25 mm/s paper speed and 1 mV/cm width, were taken during hospitalization and recorded in the hospital's electronic database. The ECGs were evaluated in a computer environment and enlarged when necessary during the measurement.

In evaluating ECGs in the sinus rhythm, an isoelectric line was determined in which all patients initially accepted the start and end of the P-wave. Two R-R distances were used to calculate heart rate. Then the P wave with the longest duration in 12 derivations was considered the P-maximum (Pmax) and the P-wave with the shortest duration was considered the P-minimum (Pmin). The difference between Pmax and Pmin was considered to be the PWD (8).

The PR interval was defined as the length between the beginning of the P wave and the QRS complex; the P-wave duration (PWD) was defined as the length between the start and end of the P-wave. The QRS complex, on the other hand, was defined as the end point of the S wave from the end point of the PR. All ECGs were evaluated twice on two different days by two different specialists who did not know the clinic of the independent patients. Interclass and intraclass correlations achieved reliabilities of 96% and 94% respectively.

Statistical Analysis

The Kolmogorov-Smirnov test was used to evaluate the distribution of continuous variables. Continuous variables obtained as a result of the analysis were expressed as mean ± standard deviation, whereas the data that did not conform to normal distribution are expressed as median and interquartile range. Categorical variables were expressed as percentages and numbers. The t-test and Mann-Whitney U test were used to compare parameters that were compatible with normal distribution and those that were not. The chi-square test was used when comparing the odds ratios of categorical variables. The relationship between PWD and all-cause mortality was shown in separate box-plot graphs. Pearson correlation analysis was used for correlation between PWD values and demographic and laboratory variables. The effects of variables such as age, DM, HT, Platelet, Max P and Min P wave duration, PR interval and PWD on predicting intrahospital mortality in patients with COVID-19 were evaluated separately for males and females with backward stepwise regression. ROC analysis was performed for the usability of PWD to predict mortality and the results are shown on an ROC curve. P-values below 0.05 were considered statistically significant. Statistical data were obtained using the SPSS 19.0 (SPSS Inc, Chicago, IL, USA) application.

G* power analysis (effect size 0.50, alpha error: 0.05 and to have 80% power) was used to calculate the results were calculated for 128 patients (64 patients in group 1 and 64 patients in group 2), and a total of 189 patients (94 in group 1 and 95 in group 2) was included in the study.

Results

One hundred-eighty-nine people, comprising 80 female and 109 male patients, were included in the study. The patients were divided into two groups. Those who lost their lives in the hospital formed group 1, while those who were discharged after recovery made up group 2. The mean age of the patients in group 1 was 73.5±12.4 years and it consisted of 51 male and 43 female patients. The mean age of the patients in group 2 was 73.9±13.5 and it consisted of 58 male and 37 female patients. C-reaktif protein (CRP), CRP/albumin ratio, serum ferritin, D-dimer, and Hs-TnT values were higher in the group 1 patients, i.e. those who died in hospital. There was no statistically significant difference between the groups in terms of fever and basal oxygen saturation at the first admission (p=0.329 and p=0.111, respectively) (Table 1).

Table 1. Demographic and laboratory data of the COVID-19 patients

	Patients		
Clinical characteristics	**Non-survivors (n=94)**	**Survivors (n=95)**	**p**
Age, years Mean ± SD	73.5 ±12.4	73.7 ±13.5	0.901
Gender, n (%)			
Males	51 (54)	58 (61)	0.503
Females	43 (45.7)	37 (39)	0.502
Smoking, n (%)	22 (23)	14 (15)	0.129
Co-morbidities, n (%)			
DM	26 (28)	21 (22)	0.377
HTN	37 (39)	30 (32)	0.263
Fever, n (%)	35 (37)	42 (44)	0.329
Basal oxygen saturation, %	91 (89-93)	92 (90-93)	0.111
Laboratory data, mean ± SD		149.40±56.19	0.085
Glucose, mg/dL	165.61±71.44	0.96±0.28	0.290
Creatinine, mg/dL	1.01±0.29	12.63±4.40	0.886
White blood cell, (x10^3 µL)	12.53±4.48	12.26±2.16	0.242
Hemoglobin, g/dL	11.89±2.16	244.58±77.58	**0.028**$^{\alpha}$
Platelet, (x10^3 µL)	218.26±85.19	6.98±5.43	0.265
Neutrophil, (×10^3/L)	7.84±5.14	0.67±0.78	0.089
Lymphocyte (×10^3/L)	0.89±0.99	6.59±10.92	**<0.001**$^{\alpha}$
CRP, mg/dL	12.78±10.92	6.21±0.91	0.252
Total protein, (g/dL)	6.06±0.94	3.10±0.77	0.398
Albumin, (g/dL)	3.01±0.73	8.26±12.66	0.393
Procalsitonin	6.38±17.08	2.33±4.10	**<0.001**$^{\alpha}$
CRP/albumin raito	4.58±4.10		
Laboratory data, median (per 25-75)			
Fibrinogen	646 (505-860)	526 (434-667)	0.007$^{\beta}$
Serum ferritin	520 (38-232)	115 (38-232)	<0.001$^{\beta}$
D-dimer, ng/Ml	1512 (652-2592)	179 (67-179)	<0.001$^{\beta}$
Hs-TnT, ng/L	103 (27-211)	66 (35-169)	0.038$^{\beta}$
Medications, n (%)			
Hydroxychloroquine	51 (54)	44 (46)	0.275
Oseltamivir	32 (34)	28 (30)	0.534
Azithromycin	35 (37)	32 (34)	0.650
Favipiravir	28 (30)	20 (21)	0.184

SD: Standard deviations, DM: Diabetes mellitus, HTN: Hypertension, CRP: C-reactive protein, Hs-TnT: high-sensitivity troponin T, $^{\alpha}$: T-test, $^{\beta}$: Mann-Whitney U-test. Blood parameters may differ between groups in COVID-19 patients

While a positive correlation was observed with PWD, CRP (r=0.145, P=0.047) and CRP/albumin ratio (r=0.162, P=0.026) in the Pearson correlation analysis, a negative correlation was observed between platelets (r=-0.147, P=0.044) and PWD was observed (Table 2).

In electrocardiographic examinations, the PR interval (p=0.025), QRS width (p<0.001), PWD viewed from D2 derivation (p=0.015), max P-wave duration (p<0.001) and PWD (p<0.001) variables were numerically and statistically significant in the group that died in the hospital compared to the group that was discharged after full recovery (Table 3).

As a result of the backward stepwise regression analysis, while the Pmax wave duration (p=0.021), PR interval (p=0.048), PWD (p=0.024) and PWD (p=0.004) in male patients with COVID-19 diagnosis, platelet in female patients (p=0.012) max PWD (p=0.003) and PWD (p<0.001) were independent predictors of mortality (Table 4).

Table 2. Correlation analysis between PWD and different clinical variables

Variables	r	p
Heart rate	0.011	0.881
Age	0.006	0.938
Female	0.013	0.854
DM	-0.070	0.338
HTN	-0.081	0.266
Total protein	-0.111	0.129
Albumin	-0.081	0.268
Procalcitonin	-0.083	0.255
CRP	0.145	0.047$^{\alpha}$
CRP/albumin ratio	0.162	0.026$^{\alpha}$
Platelet	-0.147	0.044$^{\alpha}$
Fibrinogen	0.043	0.557
Serum Ferritin	0.112	0.123
D-dimer	0.091	0.215
Hs-TnT	-0.029	0.691

PWD: P-wave dispersion, DM: Diabetes mellitus, HTN: Hypertension, CRP: C-reactive protein, Hs-TnT: high-sensitivity troponin T, $^{\alpha}$: Pearson correlation analysis. PWD is correlated with CRP/albumin ratio which is one of the infection parameters

Table 3. Electrocardiographic characteristics of COVID-19 patients

	Patients		
Parameters	**Non-survivors**	**Survivors**	**p**
Heart rate (bpm)	88.77±15.85	92.65±18.68	0.125
PR interval (ms)	137.27±37.44	122.63±50.53	0.025$^{\alpha}$
QRS width (ms)	107.15±21.38	93.05±22.22	<0.001$^{\alpha}$
P wave duration, lead II, ms	127.98 ±40.60	111.63 ±50.53	0.015$^{\alpha}$
Max P-wave duration (ms)	98.63±24.01	82.06±13.32	<0.001$^{\alpha}$
Min P-wave duration (ms)	52.26±15.18	50.31±11.23	0.316
PWD (ms)	46.37±19.00	31.86±11.08	<0.001$^{\alpha}$
PWD ≥34 ms, n (%)	69 (73.4)	30 (32)	<0.001$^{\beta}$

PWD: P-wave dispersion
$^{\alpha}$: T-test, $^{\beta}$: Chi-square test, PWD is significantly higher in COVID-19 patients who died in hospital

As shown in Figure 1, the mean PWD values were numerically and statistically significant in non-survivors compared to survivors (46.37±19.00 ms v31.86±11.08 ms, p<0.001).

PWD values over 34 ms in the ROC curve analysis had 73% sensitivity in estimating all-cause mortality in the COVID-19 patients, and a 69% specificity (area under curve: 0.737, confidence interval: 0.666-0.808, p<0.001) (Figure 2). The distribution of patients with PWD ≥34 ms is shown in Figure 3.

Discussion

To our knowledge, our study is the first to show that PWD has been found to be a predictor of intrahospital mortality in COVID-19 patients. The main results of the study were that PWD values were higher on surface ECG in those COVID-19 patients who lost their lives. Another important result was that PWD was associated with all-cause deaths in both females and male patients diagnosed with COVID-19.

Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) causes serious mortality and morbidity with its multisystemic organ involvement. COVID-19 has a poor prognosis despite new treatments and vaccines (9). Although laboratory parameters such as troponin, serum ferritin and D-dimer are predictors of a poor prognosis, the criteria for giving a poor prognosis are still not sufficient (10). Underlying diseases such as HT, DM, chronic kidney failure, and ischemic heart disease are common in those who die due to COVID-19 (11). Also, CRP elevation, hypoalbuminemia, and thrombopenia are markers of poor prognosis in COVID-19 patients (12). Although laboratory findings and additional underlying diseases indicate a poor prognosis, these are common results seen in many infectious cases and are not specific for a particular disease.

It is known that the cardiovascular system is retained either directly or indirectly due to SARS-CoV-2 (13). An ECG analysis by McCullough and colleagues showed that T-wave inversion and nonspecific repolarization

Table 4. Regression analysis for PWD and clinical variables of males and females

	Males			Females		
Parameters	**Exp**	**95%**	**p**	**Exp**	**95%**	**p**
Age	0.983	0.953-1.013	0.256	1.033	0.944-1.074	0.098
DM	0.939	0.384-2.296	0.890	0.571	0.208-1.568	0.277
HTN	0.455	0.200-1.032	0.060	1.286	0.524-3.154	0.583
Platelet	1.002	0.997-1.006	0.452	1.008	1.002-1.014	0.012[α]
Max P-wave duration	0.964	0.941-0.988	0.003[α]	0.911	0.869-0.956	<0.001[α]
Min P-wave duration	0.985	0.959-1.012	0.283	0.994	0.958-1.031	0.741
PR interval	0.992	0.984-1.000	0.048[α]	0.993	0.982-1.005	0.272
P-wave duration	0.991	0.983-0.999	0.024[α]	0.994	0.982-1.006	0.319
PWD	0.960	0.933-0.987	0.004[α]	0.877	0.825-0.932	<0.001[α]

DM: Diabetes mellitus, HTN: Hypertension, PWD: P-wave dispersion, [α]: Backward stepwise regression analysis, regardless of gender, increased PWD values are predictors of mortality in COVID-19 patients who died in the hospital

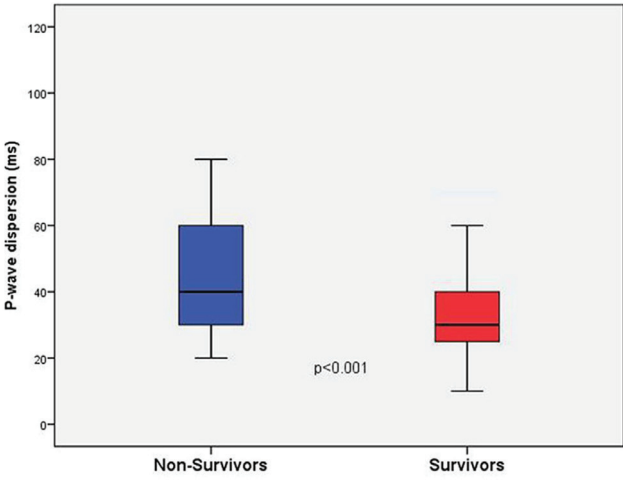

Figure 1. Comparison of PWD for COVID-19 non-survivors and survivors
PWD: P-wave dispersion, COVID-19: Coronavirus disaese-2019

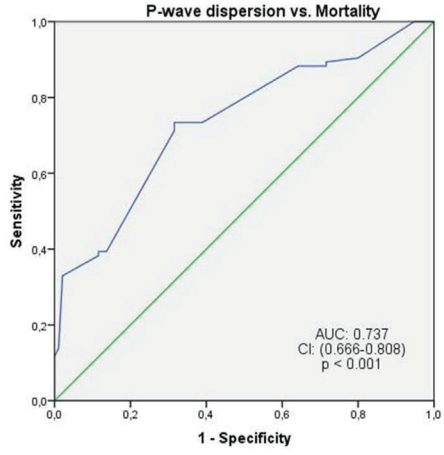

Figure 2. Receiver operating characteristic curve of PWD for predict in-hospital mortality in patients with COVID-19 disease
PWD: P-wave dispersion, COVID-19: Coronavirus disaese-2019, CI: Confidence interval, AUC: Area under the curve

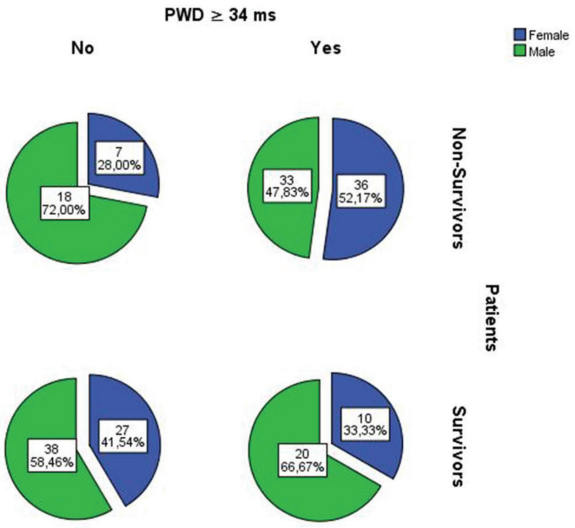

Figure 3. Distribution of patients with PWD ≥34 ms between groups
PWD: P-wave dispersion

abnormalities were associated with increased mortality rates in COVID-19 patients (14). Despite this, the relationship of PWD to intrahospital mortality in COVID-19 patients has not been investigated.

PWD is an important indicator for atrial transmission and can be easily calculated from ECG samples. Prolonged periods are called interatrial blocks and have not been adequately investigated in clinical practice (15). Although the underlying cause is not certain, intercellular fibrotic changes have been pointed to as the reason. Although fibrotic changes were monitored as secondary to an increase in the left atrium diameter, in animal models prolonged PWD values have also been shown, regardless of left atrium diameters (16). In the current study of patients hospitalized with a diagnosis of COVID-19, PWD values were found to be both prolonged and associated with increased mortality in the group who died.

In cases of systemic inflammation, atrial remodeling causes delays in depolarization along with changes to membrane potential. These result in changes in the duration of the P wave in the clinic (17). As seen in various infection tables for COVID-19, data such as increased CRP levels, neutrophilia and lymphopenia have been identified as markers indicating the severity of inflammation. It is known from previous studies that inflammation can have effects on ECG (18). In the current study, a positive correlation was observed between CRP values and PWD. Autopsy studies conducted in COVID-19 patients found that heart muscle cells were infiltrated and there was cardiomyocyte damage (19). Although atrial fibrosis can be demonstrated by biopsy and radiological methods, these methods are expensive and can be difficult to access. In the current research, as a result of the ROC curve analysis, PWD values of 34 and above had predictive values for mortality in COVID-19 patients, and it is possible to demonstrate this with a noninvasive method such as ECG.

Study Limitations

Although the current study obtained significant results, it has some limitations. First, this was a single-center study with a small number of patients. Second, since only the ECGs of the patients at the time of hospitalization were evaluated, we did not have information about the change in PWD during follow-up. Third, we did not have access to information about the left atrium diameter, since echocardiography could not be performed on most patients due to the risk of exposure to the infection. Despite these limitations, the study had strengths. Although our study was a single center and the number of patients was small, it had 80% power as a result of G* power analysis. Additionally, our inter-observer and inter-observer measures showed minimal variation. PWD was still significantly higher in those who died in-hospital compared to those who were discharged after recovery. Therefore, the increase in PWD assessment will give the clinician an idea about the prognosis of patients with COVID-19 patients.

Conclusions

PWD can be calculated by easy and noninvasive methods. Increased PWD values correlate with inflammation indicators, suggesting that transmission in atrial tissue is affected secondary to inflammation. In addition, the current study shows that PWD can help in determining the prognosis of COVID-19 patients.

Authorship Contributions

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

Conflict of Interest: No conflict of interest was declared by the authors.

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Treatment Outcome and Mortality Among Geriatric Patients Diagnosed with Multiple-Drug Resistant Tuberculosis: A Comparative Analysis from a Tertiary Referral Center

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Abstract

Aim: Multidrug-resistant (MDR) tuberculosis (TB) and drug-sensitive-TB have long treatment periods which affect patient compliance and treatment outcomes. We planned to determine the factors that will affect the management of this disease in the elderly population.

Methods: In a period of two years, a total of 82 elderly patients with bacteriologically proven TB enrolled into our retrospective study between 2011-2018. The patients' demographic features, laboratory findings, and hospital records were analyzed.

Results: We enrolled 67 (87.7%) patients with drug-sensitive TB and 15 (18.2%) MDR-TB in our study. In the study population, 73.2% (n=60) were male and the mean age was 75±9 years. When we compare treatment complications of MDR-TB and drug-sensitive-TB, we found out the rate of electrolyte imbalance, nephrotoxicity, and ocular toxicity more common among the MDR-TB group (p=0.008, p=0.008, and p=0.032, respectively). When we compare the mortality rate, cure, and treatment success between MDR-TB and drug-sensitive TB, there were no statistically significant results (p=0.898, p=0.549, p=0.488; respectively).

Conclusion: However, we think that this was due to the low sample size. Nevertheless, we should be careful in terms of complications management of the geriatric population.

Keywords: Geriatric, mortality, pulmonary, tuberculosis, multidrug-resistant

Introduction

Tuberculosis (TB) continues to be a serious health problem with increasing human immunodeficiency virus infection, substance abuse, poverty, and immigration in our globalizing world. While there is a decrease in TB cases, mortality rates are still high. The average life expectancy is getting longer as the health care facilities increase in the world and as a result elderly population is increasing. TB is 30% more frequent in elderly people in the United States (1). In spite of all difficulties, the world has done a great job in its struggle with TB. In addition to that, a new question arisen in the literature whether the geriatric population is a new sensitive group for TB treatment or not. The variability in clinical presentation and mortality rates in the elderly population differ from the young population. So these should be handled differently (2-4).

Both isoniazid and rifampicin resistance and rifampicin monoresistance (RR) are defined as multidrug resistance (MDR) according to the World Health Organization (5). MDR-TB patients' compliance and treatment outcomes are affected by potentially toxic drugs and longer treatment period compared to drug- sensitive TB (6-9). In elderly patients, polypharmacy due to comorbidities and physiological changes due to aging may make treatment management more difficult (10).

Besides the studies searching geographical and sociocultural differences in TB, there are small-scale studies on the elderly patients with TB in the literature.

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In addition to the difficulties in the management of the patients with TB in itself, we evaluated the treatment results of the elderly patients in our TB clinic in order to determine the factors that would affect the management of the disease and to determine the measures to be taken in this direction.

Methods

Study Design

The study was undertaken in accordance with the principles of the Helsinki Declaration and had the approval of the institutional commitee. It was designed as a cross-sectional study. The retrospective design study hasn't got local ethical approval however it has got hospital scientific data usage approval (no: E-66628377-929). A retrospective patient informed consent form is available.

Patient Evaluation

In our study, we enrolled elderly patients who were ≥65 years old and hospitalized in drug-sensitive TB and MDR-TB clinics in two years of periods (24 months). Patients with at least two sputum smear positivity or any culture positivity for TB were diagnosed microbiologically as TB and they were classified according to the results of the drug susceptibility test (DST). We retrospectively recorded our patients' demographic data, clinical features, comorbidities, and laboratory results. The treatment complications of our patients who were diagnosed and treated by pulmonologists specialized on TB were recorded. Treatment and follow-up data were obtained from tuberculosis dispensary registries. Mortality data were obtained from the death notification system of the government database. Patients who were lost to follow-up were recorded as missing.

Hepatic toxicity was considered as an increase in transaminase value to 1.5 times than normal or basal value and/or the increase in bilirubin value above normal of the laboratory reference level. Nephrotoxicity was defined as serum creatinine value that was 1.5 times higher than the normal or basal value. According to the patient's baseline values, the electrolyte change that reached the lower and upper limits of the laboratory reference during the treatment was defined as the electrolyte imbalance. Endocrine dysfunction is defined as hypothyroidism not detected after TB drug use and evaluated by internal medicine specialist as not related to any other organic pathology. Gastrointestinal intolerance was recorded as stomach and bowel disorders not associated with hepatic dysfunction. Arthralgia was recorded as pain in at least one joint during the use of medical therapy. Skin rash was recorded as various skin reactions associated with drug use or drug allergy. Anemia, leukopenia, and thrombocytopenia associated with drug use were recorded as hematological disorders. Ototoxicity was recorded as a permanent auditory or vestibular abnormality associated with the use of aminoglycoside. Ocular toxicity was defined as impaired visual acuity or drug-related ocular pathology recognized by ophthalmologist. Complications of the central nervous system were defined as signs of systemic dysfunction such as dizziness, vertigo, insomnia, convulsion, and paralysis related to drug use.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 24.0 for Windows (IBM SPSS Statistics Data Editor) was used for statistical analysis of the data. Descriptive data were given as number of participants and frequency. Categorical variables were expressed as the number of patients and the percentage value. The comparison of categorical variables was performed using the chi-square and Fisher's Exact tests. Continuous variables were given as mean and standard deviation. The Shapiro-Wilk test was used to determine whether the continuous variables were normally distributed. For continuous variables, Student's t-test and Mann-Whitney U test were used relative to the normality of distribution of the variables. A p-value of <0.05 was considered statistically significant.

Results

For two years follow-up periods, 82 patients with ≥65 years of age who were hospitalized in the drug-sensitive TB and MDR-TB clinics were included in our study. The mean age of our patients was 75±9 years (range 65-94 years). According to the DST results, 18.2% (n=13) of our cases were MDR-TB and 81.7% (n=64) were drug-sensitive TB (Table 1). Among the study population, 26% (n=20) of our cases had a prior history of- TB. Of the cases, 89.6% (n=69) were smear-positive pulmonary TB (For the distribution of TB involvement site go to Figure 1). The mean follow-up period was 810±525 days and 8 patients (10.4%) were loss to follow-up. The rate of comorbid diseases in our study population was 68.8%. When we compared the patients with comorbidities, there were no significant differences between the patients with the MDR and sensitive TB in terms of treatment success, rate of mortality, and treatment adverse effects ($p>0.05$ for all parameters).

According to DST results, our patients had 19.5% isoniazid, 16.9% rifampicin, 11.7% ethambutol, 11.7% streptomycin, and 1.3% quinolone resistance. In the comparison of MDR and drug-sensitive TB patients, the MDR-TB group had higher electrolyte disturbance, nephrotoxicity, and visual impairment during the treatment period ($p=0.02$, $p=0.02$, and $p=0.03$, respectively). Besides, hypothyroidism, psychosis, ototoxicity, and neuropathy

were more common in the MDR-TB group, the difference was not statistically significant (p>0.05) (Table 1).

When treatment success was evaluated, it resulted in 53.6% cure. Treatment failure was present in 1 case (1.3%) and treatment abandonment was 6 (7.8%). During follow-up, 9 (11.7%) cases were exitus during hospitalization and 13 (16.9%) patients were exitus during follow-up as outpatient. In the comparison of the TB groups, no statistically significant difference was found between mortality, cure, treatment success, and abandonment of treatment (p=0.89, p=0.55, p=0.49, p=0.27, respectively).

Table 1. Details of treatment adverse effects related to TB† groups

	Drug sensitive TB, n (%)	Multidrug resistant TB, n (%)	p*
Electrolyte imbalance	9 (14.1%)	6 (46.2%)	0.02*
Nephrotoxicity	9 (14.1%)	6 (46.2%)	0.02*
Ototoxicity	1 (1.6%)	2 (15.4%)	0.07
Ocular toxicity	2 (3.1%)	3 (23.1%)	0.03*
Gastric intolerance	25 (39.1%)	6 (46.2%)	0.64
Psychosis	3 (4.7%)	2 (15.4%)	0.20
Depression	2 (3.1%)	0 (0%)	>0.99
Artralgia	1 (1.6%)	1 (7.7%)	0.31
Hepatotoxicity	38 (59.4%)	4 (30.8%)	0.06
Skin rash	10 (15.6%)	0 (0%)	0.20
Convulsion	1 (1.6%)	1 (7.7%)	0.31
Vertigo	4 (6.3%)	1 (7.7%)	>0.99
Neuropathy	4 (6.3%)	3 (23.1%)	0.09
Hypothyroidism	1 (1.6%)	2 (15.4%)	0.07

*Significant p-value is set as p<0.05 and significant p-value are given as bold
†TB: Tuberculosis

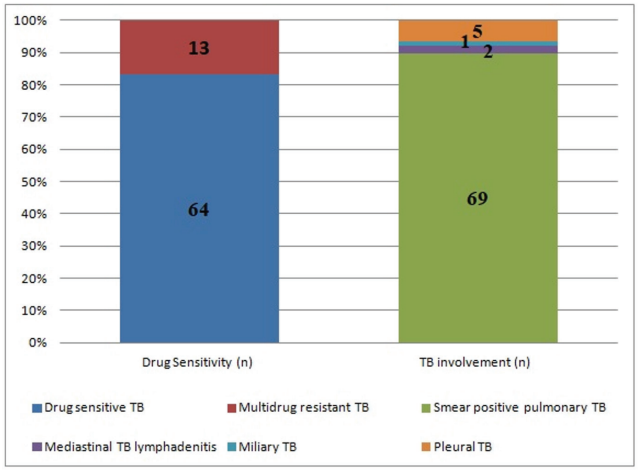

Figure 1. Distribution of the patients according to TB† involvement
TB: Tuberculosis

Discussion

When the results of TB treatment were evaluated in elderly patients, there were limited number of studies in the literature. According to these data, the mortality rate of TB in elderly is increased compared to the younger population (11). While treatment results about drug-sensitive TB and MDR-TB were available in younger population, we wondered about the treatment results and the factors affecting that in the elderly. There were no differences in treatment success and cure rates between drug-sensitive TB and MDR-TB in our study. However, we found out that the treatment complications rates are increased in MDR-TB in the elderly.

In studies from Far Eastern countries, the mortality rate in the presence of TB in the elderly was reported 3.9% higher than the general population (12). Increased mortality in the elderly is associated with reduced immunity and increased comorbidities. Although TB susceptibility of this population increases, there are also delays in diagnosis due to different clinical presentations in this group of patients (5,13). Moreover, low body mass index, smoking history, and poorly controlled diabetes increase TB susceptibility in elderly (14-17). TB frequency is increased in impaired nutritional status (18). Because of all these, we aimed to increase the awareness of clinicians on this issue by our study.

It is mentioned that the incidence of drug side effects in the treatment process of elderly TB patients with comorbidities is high in the literature (19). The most serious side effect is reported as hepatotoxicity (20). Hepatotoxicity is frequently associated with isoniazid, rifampin, and pyrazinamide, while follow-up of medical therapy requires precision. We reported that patients who were on MDR-TB treatment were found to be more sensitive to drug side effects compared to the patients with drug-sensitive TB. We found out that the most common adverse effects of anti-TB treatment were hepatotoxicity and gastric intolerance. In addition to that, clinicians should be more careful to detect and manage nephrotoxicity and electrolyte imbalance, because these are common and may affect the vital functions of the patients.

In the literature, it was found that radiological patterns could be a sign of mortality in TB patients, but we could not find any relation in the elderly in our study. We think that it might be due to our small sample size (11). Another possible reason is that, besides the non-specific clinical presentations in elderly patients, also radiological patterns may not be guiding for the prognosis. For instance, decreased appetite decreased daily functional activity, and subfebrile fever may occur frequently in elderly patients (21).

Study Limitations

Because our study was designed to be retrospective, our sample size was not so large. The drug history of patients after discharge could not be questioned and this information could not be accessed from the system. This was missing data in our study. Another limitation is the scarce diversity of ICD codes related to mortality which was accessed from the electronic mortality system. Despite the small number of geriatric MDR-TBC patients in the community, our study is a valuable study because it has a sufficient number of cases, is one of the few studies conducted in the geriatric group, and contributes to the literature.

Conclusion

The results of treatment success, cure, and mortality rates of drug-sensitive TB and MDR-TB in the geriatric population were not different. However, it is recommended to be more careful in terms of drug side effect management and treatment interventions in MDR-TB patients with increased fragility. Especially, close monitoring of electrolyte and liver function tests and periodic eye examination may facilitate the management of complications by clinicians. Further studies are needed to generalize our results.

Authorship Contributions

Concept: M.G.O., S.T.O., Design: M.G.O., S.T.O., Data Collection or Processing: F.A., M.G.O., S.T.O., Analysis or Interpretation: S.T.O., S.I., Literature Search: S.T.O., F.A., S.I., Writing: S.T.O., S.I.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

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Diagnostic Value of Diffusion Magnetic Resonance Imaging in Detecting Malignant Axillary Lymph Nodes in Breast Cancer Patients

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Abstract

Aim: The prognostic value of apparent diffusion coefficient (ADC) measurement to detect axillary malignant lymph nodes in breast cancer is not clear. We aimed to investigate the prognostic importance of diffusion weighted imaging (DWI) in the differentiation of metastatic and non-metastatic lymph nodes.

Methods: Magnetic resonance imagings (MRIs) of 102 breast cancer patients were reviewed from the hospital PACS and automation systems between July 2018 and March 2021. Cortical thickness, short, long-axis diameters, long/short axis ratios and ADC measurements were done for ipsilateral and contralateral axillary lymph nodes. Univariate and multivariate regression analyses were performed.

Results: Pathology revealed that 64 patients had ipsilateral metastatic lymph nodes while 38 patients did not. When grouped into metastatic and non-metastatic groups, breast tumor size, mean diameter of the short axis, long axis and cortical thickness were significantly higher in the metastatic group. Ipsilateral metastatic lymph nodes' mean ADC values were significantly lower than non-metastatic ones with a calculated value of $0.972x10^{-3}$. The ipsilateral ADC value of the lymph node and loss of fatty hilum were the two significant factors to predict metastatic lymph nodes in multivariate analysis.

Conclusion: DWI is a valuable noninvasive tool in the diagnosis of metastatic axillary lymph nodes.

Keywords: Breast neoplasm, axilla, lymph nodes

Introduction

Breast cancer is the most common type of malignancy and the second most common cause of death among women in Turkey as well as in the world (1,2). The presence of lymph node metastasis is the most important prognostic factor for long-term and disease-free survival in patients (3,4). Axillary imaging of newly diagnosed breast cancer patients is currently done by either ultrasonography (US) or magnetic resonance imaging (MRI). MRI has advantages over US as being non-operator dependent, enabling it to evaluate even the deeply located lymph nodes and compare with contralateral axilla simultaneously. Conventional MRI features suspicious for metastatic axillary nodes are reported as cortical thickening, loss of fatty hilum, round shape and heterogeneous contrast enhancement after Gadolinium injection (5). Diffusion-weighted imaging (DWI) is an advanced functional MRI method that apparents the free motion of the water molecules in tissues (6). Apparent diffusion coefficient (ADC) can quantify the aforementioned motion of the water molecules and provide information about the microscopic cellular changes like cellular membrane integrity or cell proliferation (7,8).

So far, the prognostic value of ADC measurement of the lymph nodes in various malignities of head and neck, uterus and cervix have been shown (9). However, it is not clear whether it contributes to detecting axillary malignant lymph nodes in breast cancer. In the review of De Cataldo et al. (10) they concluded that DWI could be used in patients with low-intermediate risk of lymph node involvement. In another study of Liu et al. (11) they didn't find significant differences regarding ADC values of metastatic and nonmetastatic axillary lymph nodes in T1 and T2 stage breast cancer patients. In this study,

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we aimed to investigate the prognostic importance of DWI in the differentiation of ipsilateral metastatic and non-metastatic lymph nodes and determine the optimal combination of the MRI features to detect nodal status in breast cancer patients.

Methods

Study Design

This study was conducted in accordance with the 1964 Helsinki declaration and approval of the Ethics Committee of the hospital was obtained. Informed consent was waived. The cohort of the study was 114 newly diagnosed breast cancer patients at our radiology clinic who underwent preoperative breast MRI and US-guided ipsilateral axillary lymph node biopsy between July 2018 and March 2021. Patients who underwent axillary lymph node biopsy before breast MRI (n=4), patients whose DWI had artefacts which impede appropriate interpretation (n=2) and patients whose histopathological data couldn't be recollected (n=6) were excluded from this study. After inclusion and exclusion criteria were applied, 102 patients with 102 ipsilateral lymph nodes were enrolled in this study. All patients underwent tru-cut biopsy from breast masses and sentinel lymph node biopsy (SLNB) or axillary dissection from the ipsilateral axilla.

Breast MRI Protocol

All breast MRIs were acquired using 1.5 Tesla Siemens scanner (Avanto, Erlengen Germany) with patients positioned prone in an 8-channel breast array coil. The conventional MRI protocol was applied as T1 weighted fast spin echo axial sequence (TR = 650, TE = 112, Matrix 448 × 224, FOV = 320 x 320 mm, NEX = 1, Thickness = 3.0 mm) and pre- and post-contrast T1 weighted three-dimensional fat-suppressed axial sequence (TR = 485, TE = 10, Matrix 350 × 350, FOV = 320 x 320 mm, NEX = 1, FA = 10.0, Thickness 3.00). Images were taken before contrast administration and five times after contrast injection with 80s intervals. Gadopentetate dimeglumine contrast medium was injected with a dose of 0.1 mmol kg-1. Diffusion-weighted images [TR/TE = 1000/83, NEX = 2 and Thickness = 2 mm, FOV = 320 mm, Matrix 180x238] were obtained before contrast and ADC maps were attained. To interpret DWI, b0 and b1000 sn/mm^2 were used.

Evaluation of Conventional and Diffusion Weighted MRIs

All of the MRI images were analyzed and reported by consensus readings of two radiologists with 8 years and 4 years of experience in breast radiology. The interpreters were blinded to histopathological results. In conventional MRI sequences, presence or loss of fatty hilum, cortical thickness, long-axis diameter, short-axis diameter, long-axis to short-axis ratios were evaluated. While loss of fatty hilum was evaluated in precontrast T1 and T2 weighted images, other features were evaluated in precontrast T2 weighted images. The index lymph node was selected in consensus if there was no suspicious lymph node in axilla. While calculating ADC values, freely selected minimum size regions of interests (ROIs) were used and located to the cortex of each lymph node. Three measurements from the same and most suspicious lymph nodes were done and the minimum values were noted. Attention was given while placing ROIs not to include fatty hilar or adjacent soft tissues (Figure 1,2). The same calculation method was used to compare the contralateral axillary lymph nodes in normal morphology.

Histopathologic Evaluation

Histopathology results were obtained by fine needle aspiration biopsy of the most suspicious lymph nodes, SLNB or axillary curettage. In patients with suspicious metastatic lymph nodes in MRI images, care was taken to biopsy this lymph node. In patients with normal morphological lymph nodes, preoperative biopsy was not planned and SLNB was used as the histopathologic indicator.

Statistical Analysis

For statistical analysis, SPSS 15.0 for Windows program was used. Continuous data (short axis length, long axis length, long axis-short axis ratio, maximal cortical thickness, and ADC value) are given as mean, standard deviation, minimum, maximum, median, interquartile range. Categorical variables (sex, loss of fatty hilum) are

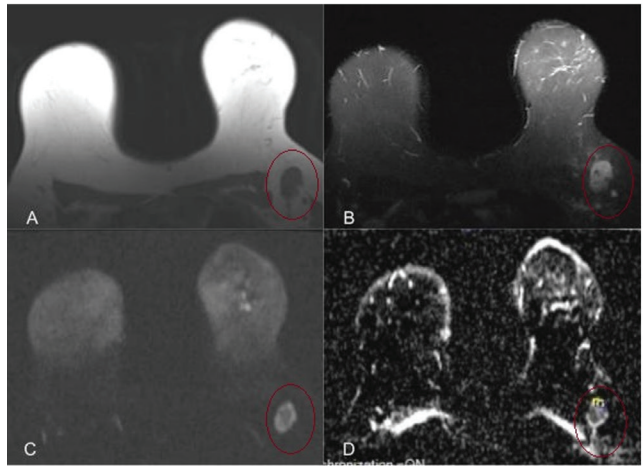

Figure 1. Sixty-eight year-old female patient diagnosed with invasive ductal carcinoma of the left breast with axillary metastasis. A) T1- weighted, B) T2- weighted, C) Diffusion -weighted imagings and D) ADC map. An enlarged metastatic lymph node is seen in the left axilla which shows prominent diffusion restriction
ADC: Apparent diffusion coefficient

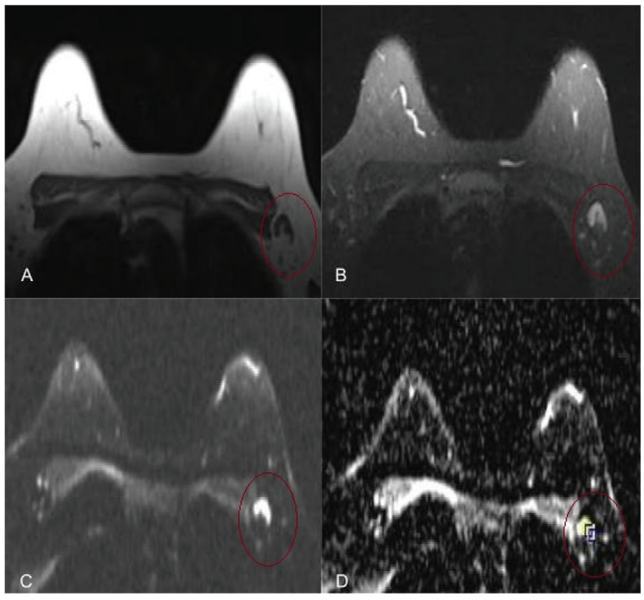

Figure 2. Forty-three year-old female patient diagnosed with tubular carcinoma of the left breast. There is a lymph node in the left axilla. A) T1-weighted and B) T2 weighted imaging) which is proven as reactive pathologically. No diffusion restriction is seen in C)DWI and D) ADC map

ADC: Apparent diffusion coefficient, DWI: Diffusion weighted imaging

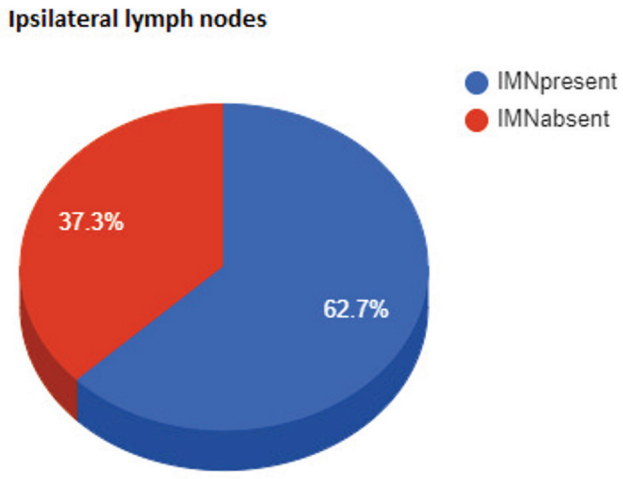

Figure 3. Schematic representation of ipsilateral metastatic nodes present (IMNpresent) and absent (IMNabsent) patients

given as numbers and percentage. Independent two-group comparisons were achieved with the Student's t-test when the numerical variables met the normal distribution condition, and the Mann-Whitney U-test was used when the condition was not met. Relationships between numerical variables were performed using Spearman correlation analysis since parametric test conditions were not met. To compare independent samples, the Wilcoxon test was used. To obtain cut-off values and compare the diagnostic performance of variables, receiver operating characteristics (ROC) was used. The optimal cut-off value was determined according to the highest Youden index (J), and the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for each variable. Determinative factors were examined by logistic regression analysis. Statistical significance level of alpha was accepted as $p<0.05$.

Results

The mean patient age was 52.0±11.9 (range between 32 and 85). The most common pathological subtype was invasive ductal carcinoma (n=73, 71.57%) followed by invasive lobular carcinoma (n=9, 8.82%). FNAB or surgical final pathology revealed that 64 patients had ipsilateral the metastatic lymph nodes while 38 patients did not (Figure 1). Loss of fatty hilum was present in 38 ipsilateral lymph nodes (37.3%), whereas 64 (62.7%) of them had normal hilar structure.

When grouped into metastatic and non-metastatic ipsilateral lymph nodes, breast tumor size (p=0.004), mean diameter of the short axis (p=0.001), long axis (p=0.003) and cortical thickness (p<0.001) were significantly higher in metastatic group. There were no statistical differences between the age of the patients and long/short axis ratios between the two groups (Table 1)

Ipsilateral metastatic lymph nodes' mean ADC values were significantly lower than ipsilateral non-metastatic lymph nodes (p<0,001), as expected. The mean ADC value of contralateral lymph nodes was significantly higher in the ipsilateral metastatic lymph node group (p<0,009). There was a statistically significant difference in the metastatic lymph node group between ipsilateral and contralateral ADC values (p<0.001) whereas not in the non-metastatic group (p<0.08) (Table 2).

ROC curve analysis was performed and area under curves (AUC) were calculated for ADC values and each conventional MRI feature to predict metastatic ipsilateral lymph nodes. The highest Jouden indices and AUC values were calculated in ipsilateral ADC value and cortical thickness parameters (Figure 3). The cut of the value of ADC was found 0.972 x 10 -3 with 84.4% sensitivity and 86.8% specificity, 91.5% PPV, 76.7% NPV (J: 71.22, AUC: 0.929). The cut off value of cortical thickness was found 5.5 millimeters with 76.56% sensitivity and 81.58% specificity, 87,5% PPV, 67.39% NPV (J: 57.77 AUC: 0.828). The lowest AUC and sensitivity, specificity, PPV and NPV were observed in the long axis-short axis ratio (Table 3).

Fatty hilum loss was the only descriptive parameter in our study. The number of fatty hilum loss in lymph metastatic group lymph nodes was significantly higher

than ones in the non-metastatic group. Even the sensitivity was relatively low (54.7%), the specificity (92.1%) and PPV (92.1%) were significantly high.

Multiple regression analysis was performed to investigate the effect of calculated cut-off value on other conventional MRI features. The ipsilateral ADC value of the lymph node and loss of fatty hilum were the two significant factors to predict metastatic lymph nodes (Table 4).

Discussion

Axillary lymph node status is the most important prognostic factor in newly diagnosed breast cancer patients (9). Detecting axillary metastasis in breast cancer patients is crucial as it has a primary role in staging and optimal treatment decision. The diagnostic values of physical examination, ultrasound-guided biopsy, mammography and ultrasonography were evaluated in the prediction of axillary involvement in breast cancer patients and the results were not satisfactory (12). Nowadays; SLNB is the gold standard technique with high sensitivity of 91.2% and high specificity of almost 100% for the detection of metastatic lymph nodes (13). However; it is an invasive procedure with possible complications.

At this point, as being a noninvasive alternative, conventional MRI with dynamic contrast enhancement

Table 1. Age, tumor size of the patients and short diameter, long diameter, long to short diameter ratio and cortical thickness parameters in ipsilateral metastatic and non-metastatic lymph nodes

	Lymph node status				
	Metastatic		Non-metastatic		
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	p*
Age	51.6±11.3	50 (43.25-59)	52.6±13.1	50 (42.75-61)	0.928
Tumor size	34.0±19.5	31 (20-40)	24.8±13.9	23.5 (15-29.5)	0.004
Short axis diameter	12.86±5.64	11 (9-17)	9.29±4.37	8.5 (6-11)	0.001
Long axis diameter	21.39±9.12	19 (14.25-27.5)	16.16±6.42	16 (12-18)	0.003
Long/short axis diameter	1.73±0.49	1.6 (1.43-1.87)	1.79±0.37	1.75 (1.55-2.02)	0.156
Cortical thickness	10.59±6.06	9 (6-14)	4.97±2.54	5 (4-5)	<0.001

*by Mann-Whitney U test, SD: Standard deviation, IQR: Interquartile range

Table 2. Mean and Median ADC values of ipsilateral metastatic and non-metastatic lymph nodes and the contralateral lymph nodes of all patients

	Lymph node status				
	Metastatic		Non-metastatic		
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	p*
Ipsilateral ADC ($x10^{-3}$)	0.77±0.19	0.76 (0.62-0.90)	1.17±0.20	1.15 (1.07-1.31)	<0.001
Contralateral ADC ($x10^{-3}$)	1.27±0.21	1.27 (1.09-1.40)	1.15±0.18	1.12 (1.04-1.27)	0.009
p**	<0.001		0.080[a]		

*Ipsilateral ADC p<0.001 by 2-sample t-test, Contralateral ADC p=0.009 by Mann-Whitney U test, **by Wilcoxon test, [a] There was no significant difference only when ipsilateral and contralateral ADC values were compared in non-metastatic group, SD: Standard deviation, IQR: Interquartile range, ADC: Apparent diffusion coefficient

Table 3. Calculated Area under curve, confidence interval, p-value, cut-off value. Youden index, sensitivity, specificity, positive predictive value and negative predictive value of each variable

Test result variable (s)	AUC	95% CI		p	Cut-off value	Youden index	Se (%)	Sp (%)	PPV (%)	NPV (%)
Primary tumor size	0.673	0.567	0.779	0.004	24.5	16.12	63.49	52.63	68.97	46.51
Cortical thickness	0.828	0.746	0.910	<0.001	5.5	57.77	76.56	81.58	87.5	67.39
Short axis diameter	0.701	0.599	0.804	0.001	9.5	30.37	70.31	60.53	75.00	54.76
Long axis diameter	0.670	0.565	0.775	0.004	16.5	25.61	65.63	60.53	73.68	51.11
Long axis/short axis diameter	0.421	0.306	0.535	0.183	1.696	11.02	53.13	57.89	68.00	42.31
Ipsilateral ADC ($x10^{-3}$)	0.929	0.018	0.124	<0.001	0.972	71.22	84.38	86.84	91.53	76.74

ADC: Apparent diffusion coefficient, AUC: Area under curve, CI: Confidence interval, Se: Sensitivity, Sp: Specificity, PPV: Positive predictive value, NPV: Negative predictive value

stands out to evaluate the nodal status. Preoperative conventional MRI gives the opportunity to staging,

Table 4. Multiple regression analysis of the features to predict metastatic lymph nodes

	p	OR	%95 CI (min-max)	
Ipsilateral ADC ($x10^{-3}$) <0.972	<0.001	77.896	11.838	512.588
Loss of fatty hilum	0.006	65.541	3.260	1317.507
Cortical thickness	0.736	0.920	0.568	1.492
Short axis diameter	0.542	0.736	0.275	1.973
Long axis diameter	0.289	1.409	0.748	2.653
Long/short axis diameter	0.304	0.053	0.000	14.281

CI: Confidence interval, OR: Odds ratio, Min-max: Minimum-Maximum

detecting and measurement of axillary lymph nodes and evaluation of morphological features like cortical thickness, shape, fatty hilum obliteration, and contrast enhancement (14,15). In recent years; DWI has been added to routine breast MRI protocols to increase diagnostic performance. DWI is widely used in diagnosis and treatment response in breast cancer (16-18). The value of DWI in axillary lymph node evaluation is still controversial.

This study showed the diagnostic performance of DWI and conventional MRI characteristics of ipsilateral lymph nodes to predict malignancy in breast cancer patients. Regarding conventional MRI characteristics; long axis, short axis and cortical thickness of the malignant lymph nodes were significantly higher in the metastatic group. The ratio of long axis to short axis sizes did not differ between groups. In previous studies, no significant correlation was found between lymph node diameter and the presence of metastasis (19-22). However, Atallah et al. (23) and Yoshimura et al. (24) found that metastatic lymph nodes had larger long-axis size with a threshold of 12 and 10, respectively. In our study, we calculated the short and long-axis diameters as 9.5 mm and 11.5 mm. In another study, these values were found 9.3 and 11.3 mm which are very similar to our results (9). We found that the cortical thickness was the best diagnostic parameter among conventional MRI features. Kim et al. (9) and Scaranelo et al. (25) also found the maximal cortical thickness as the most discriminative parameter among conventional MRI features.

As being the only descriptive parameter; fatty hilum loss was observed in 3 (7.89%) cases among the non-metastatic group and 35 (54.69%) cases among the metastatic group. Fatty hilum loss showed the highest specificity (92.1%) that loss of fatty hilum is the best parameter to exclude the metastatic lymph nodes. This result was concordant with the literature (9,24,26).

In this study, the cut-off ADC value of the ipsilateral lymph node <0,972 $X10^{-3}$ showed highest sensitivity (84.3%), specificity (86.4%), PPV (91.53%) and NPV (76.74%) to predict the nodal status. In terms of ADC value measurement, our results were concordant with the literature (27-29). Guvenc et al. (27) found the cut-off value of ADC value as 0.985 $X10^{-3}$ mm^2/sec with a sensitivity of 83%, specificity of 98%, PPV of 95%, and NPV of 93%. They calculated the AUC for ipsilateral lymph nodes as 0.96. Kim et al. (9) founded the threshold ADC value of 253 lymph nodes in 252 breast cancer patients as 0.986 × 10^{-3} mm^2/sec with sensitivity, specificity, PPV, and NPV values as 75.8%, 83.9%, 72.6% and 86.0%, respectively. Fornasa et al. (29) reported the sensitivity and specificity to be 82.22% and 82.35%, respectively. These results are similar to our percentages that show the reproducibility of ADC measurement in axillary lymph nodes.

We found a significant difference between ipsilateral metastatic lymph nodes and contralateral non-metastatic ones that; ipsilateral metastatic lymph nodes had lower ADC value (mean ± SD: 0.77±0.19 × 10^{-3}) than contralateral non-metastatic group (mean ± SD: 1.27±0.21 × 10^{-3}). Another study conducted by Ramirez et al. (30) found a similar result. The multivariate logistic regression analysis showed that ADC value lower than 0.972 ×10^{-3} and loss of fatty hilum had the strongest associations with axillary metastasis (Table 4). In the study conducted by Guvenc et al. (27) they also found the strongest associations with lower ADC value (<0.985×10^{-3}) and axillary metastasis.

Study Limitation

Our study has some limitations. First of all; due to its retrospective design, the study results couldn't be utilized in clinical diagnosis. A prospective study with a larger number of patients would reveal more trustworthy results. Secondly; all the ADC calculations were obtained by two radiologists by consensus MRI readings which resulted in lack of information about interobserver variability. Lastly; we performed institutional standard protocol to obtain MRIs. As a known dilemma, the calculated ADC values are affected by the scanning parameters (TR and TE) and b value used for DWI. Even so, we believe that this study will contribute to literature regarding the high diagnostic value of diffusion-weighted imaging of axilla in breast cancer patients.

Conclusion

Diffusion-weighted imaging is significantly valuable in the noninvasive diagnosis of metastatic axillary lymph nodes in breast cancer. In terms of conventional MRI parameters, higher values of the short axis, long axis diameters and loss of fatty hilum are significantly correlated with axillary metastasis. Lower ADC value and loss of fatty hilum had

strongest associations with axillary metastasis. Therefore; DWI should be included in routine MRI protocols in breast cancer staging, since ADC values of the ipsilateral lymph nodes could help to differentiate the metastatic ones in order to prevent unnecessary biopsies.

Authorship Contributions

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

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Original Article

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Evaluation of the Obstetrical Brachial Plexus Injuries with Forensic Perspective

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Abstract

Aim: Obstetrical brachial plexopathy (OBP) is the loss of function of the brachial plexus due to a traumatic, idiopathic, or iatrogenic reason since birth. OBP mostly occurs with a traumatic background that is secondary to a difficult birth. In this study, we aimed to investigate the medical characteristics of the OBP cases, which were evaluated for malpractice reasons by the Forensic Medicine and define the features that should be considered in the forensic evaluation of OBP.

Methods: The medical files of all cases with OBP evaluated in the 2nd Specialization Board of the Council of Forensic Medicine between 2013 and 2017 due to malpractice were included in the study. The dates of birth, maternal ages, comorbid diseases of the mother, the number of pregnancies, birthplaces and birth weeks, birth weights, and additional comorbid pathologies were investigated retrospectively.

Results: A total of 287 cases were evaluated in this study. The mean age of forensic evaluation was found to be 2.9±2.3 years. All patients were born with vaginal delivery except one caesarean case. In 55% of cases had shoulder dystocia, 16% clavicle fracture, 4% humerus fracture and humeral head dislocation were detected. 23.3% of the cases had a history of a difficult birth and 11.5 % had assisted delivery. In 45% of cases had developmental pathologies accompanied by hypotonia. None of the cases were evaluated as medical malpractice.

Conclusion: Standard medical records of cases with OBPs contained sufficient information for neurological and forensic evaluation. Our study shows the OBP's does not only originate from the traumatic etiology; about half of the cases have different comorbid pathologies, especially neurodevelopment problems. In order to achieve standardization and understand the comorbidities of plexopathy better, it would be appropriate to expand the medical data in detail.

Keywords: Brachial plexus neuropathies, brachial plexus, parturition, obstetric, malpractice

Introduction

The brachial plexus is a complex peripheral neural network that provides innervation to the upper limb. The brachial plexopathies that occur with damage of this network lead to loss of muscle strength in the flask type of upper extremity. Pathologies seen in this neural network in newborns are called obstetrical brachial plexopathies (OBP). OBPs are rarely seen about 1-2 per 1,000 live births (1,2). OBP can develop in the prenatal or intra-natal processes. The majority of OBPs are obstetric complications due to traumatic peri or intra-natal causes. Shoulder dystocia is the leading known risk factor (2). Other risk factors are high birth weight (macrosomia) and other comorbidities seen with birth trauma, such as assisted birth and breech presentation (3,4).

Although OBP occurs due to the stress in the brachial plexus during childbirth, it can also be seen in cases that do not have any risk in the obstetric process and without any problems during the delivery (3). For instance, uncomplicated caesarean delivery is expected to decrease

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the risk of OBP. However, OBP can also be seen in infants born with caesarean delivery. In these cases, maternal uterine malformation and familial obstetrical brachial plexus palsy may be considered among the prenatal processes. Besides, comorbid conditions such as hypotonic neonate or transient hypotonia of the newborn may be created by caesarean delivery, and perinatal asphyxia is considered another factor for OBP (3). It is interpreted that as a risk factor, hypotonia makes both the baby and plexus structures susceptible to stretching and creates a predisposition to brachial plexopathy (3,5).

Brachial plexopathies are an important cause of disability in the young population, and improvement with treatment is very limited in this pathology (2,6). Neurological evaluation and electrophysiological examination help understand the time of occurrence of the lesion and the type of involvement at the brachial plexus. Determining the kind of involvement is vital for planning the treatment (5,7,8). In evaluating a newborn with OBP, accompanying conditions such as humerus or clavicle fracture, diaphragm paralysis should be considered. This approach should be carried out by a multidisciplinary team of pediatricians, neurologists, orthopedists, neurosurgeons, and physiotherapists. In this respect, OBP creates a financial and moral burden on families.

A large number of lawsuits are filed against OBP cases with malpractice allegations. When there is a medical malpractice claim to physicians or assisted health professionals, especially the course of pregnancy, the birth processes and the features that may predispose to plexopathy, such as macrosomia are investigated in detail with medical documents. In this study, we aimed to investigate the medical characteristics of the OBP cases evaluated for malpractice by the Council of Forensic Medicine, 2nd Specialization Board and define the features that should be considered in the forensic evaluation of OBP.

Methods

In this study, all OBP cases with claims of malpractice brought to the 2nd Specialization Board of the Council of Forensic Medicine between 2013 and 2017 were scanned from the medical files retrospectively with personal data protection. The Scientific Committee of the Forensic Medicine ethically approved this study (23.06.2017 with the number 21589509/256). Only the cases with signs of brachial plexopathy since birth were included in this study. All features of the detected cases were systematically noted and documented; i.e.; their birth dates, maternal ages, and diseases of the mother if any, how many pregnancies the mother had, who assisted the mother during the labor, birthplaces of the cases, birth weeks and weights, Electromyography (EMG) findings and applied treatments. In this retrospective study, descriptive statistical method was used as a statistical method. Basic statistical methods; mean value, percentage calculation and standard deviation were used to demonstrate the all the features of the data.

Results

A total of 287 OBP files were evaluated between 2013 and 2017, with an average of 57 per year (Figure 1). One hundred-fifty-six (54.3%) of the cases were male and 131 (45.6%) were female. According to file dates of the cases, the average age of forensic evaluation was determined as 2.9±2.3 years (minimum: 0 and maximum: 17). The detailed evaluation of the files showing OBP was evaluated as a complication in all cases. Apart from one patient with caesarean birth, all of the other cases were born with normal delivery. Two hundred eighty-five babies were born in the hospital, and there was only one birth at home (Figure 2). In twenty-three cases out of 264, we were able to determine the health professional who assisted the delivery. In 77.3% (204) of the 264 cases, a physician-assisted the labor (Figure 3).

Among the 251 cases of which the birth weeks were detected; 89.6% of them were born in term (225), 5.6% were preterm (19), and 2.4% were post-term (6). The

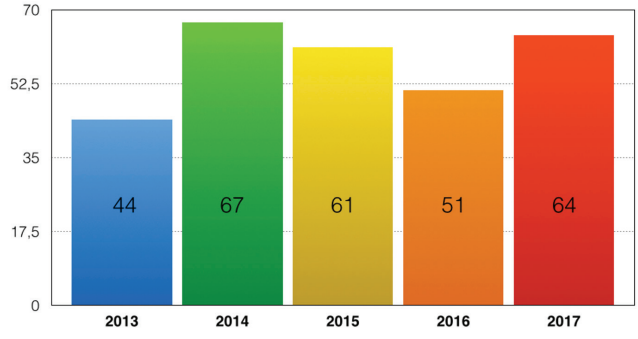

Figure 1. Distribution of the cases by years between 2013 and 2017

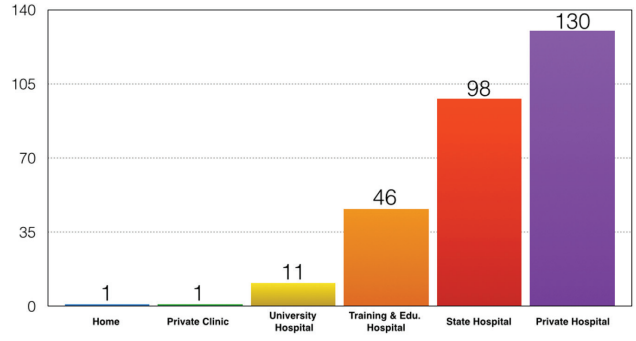

Figure 2. Distribution of birth places of the cases

mean birth weight of the cases was 4.03±508.6 kg (Figure 4). There were 4 cases known to be born in the term but whose birth weight could not be determined from the file information. The frequency of the delivery presentation of the cases was cephalic (255), breech (2), and footling breech presentations (2). The delivery presentation could not be detected in 27 cases. Among the 175 mothers whose given birth age was seen, the average age was calculated as 29.7±5.6 years. From the medical records, the medical history of 61 mothers were available. It was learned that 18 of the 61 mothers were healthy, 24 had diabetes, 8 had hypertension, 7 had obesity, and 3 had thyroid disease. Previous pregnancy information can be reached in 75 mothers, and 47 of them gave their first birth. Only four mothers had a history of a baby with plexopathy in her previous births. The EMG evaluation data and treatment of the cases were summarized in Figure 5.

Despite 25 cases that no information could be found about comorbid pathology in the files, the accompanying pathologies observed in OBP cases were very diverse. It was noted that there was more than one accompanying pathology in 138 of 262 cases. The most common accompanying pathology was found to be shoulder dystocia in 158 patients. The most frequently observed comorbid pathologies detected in the rest of 130 cases were patent ductus arteriosus, pulmonary hypertension, microcephaly, mental and motor developmental delay,

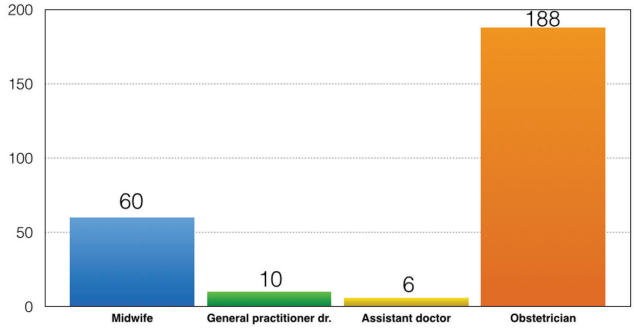

Figure 3. Distribution of health professionals for assisted delivery

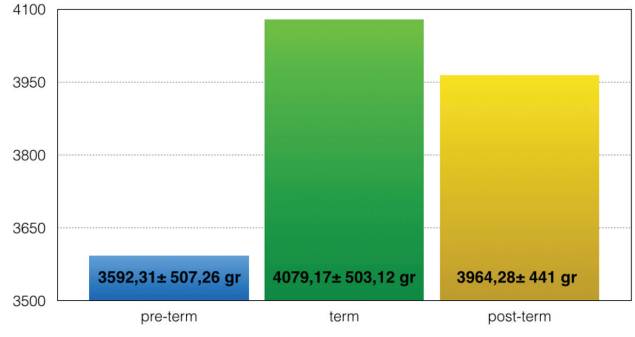

Figure 4. Average birth weight distribution by birth weeks

	first EMG (141 cases)	second EMG (38 cases)
The average age (month)	8.6 ± 14.5	13.6 ± 15.7

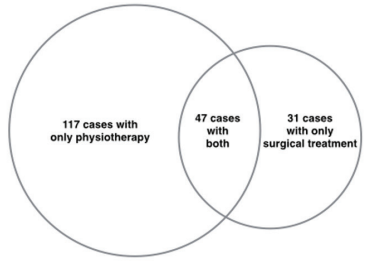

Figure 5. Age of EMG evaluation and treatment distribution of the cases. The average age of the first EMG study was calculated as 8.6±14.5 months (min: 0 to max:108 months). Thirty-six patients underwent EMG study for the second time. The second EMG study's average age was 13.6±15.7 months (min: 1 to max: 70 months). The 117 of 195 cases were treated with physical therapy only, 31 cases surgical only, and 47 cases both with physical therapy and surgically

hypotonia, oligo and polyhydramnios, neonatal convulsion, fetal bradycardia, hyperbilirubinemia, asphyxia, cyanosis, intracranial bleeding, anemia, infection, and caput succedaneum. There was clavicle fracture in 47 cases, humerus fracture or humeral head dislocation in 9 cases, and diaphragmatic paralysis in 3 cases. History of a difficult delivery, use of assisted delivery methods, prolonged labor, maternal insufficiency were noted in 67, 33, 9, and 2 cases, respectively.

Discussion

In this study, OBP cases on forensic evaluation within five years were systematically documented. The information about medical factors such as a history of difficult delivery, complications of delivery, shoulder dystocia, fetal macrosomia, gestational diabetes, material obesity, advanced maternal age, male fetus or post-term delivery, which are known to be associated with OBP in the literature, were investigated through the information from the medical documents. Traumatic damage of the brachial plexus during delivery may cause OBP, or it may also be seen for an undetectable reason (1,3,4,9-11). Therefore the damage at the plexus should be distinguished whether it is a result of traumatic obstetric complications or it is developed for a different reason. Complication management of the case is important where there are clues that OBP develops on a traumatic background,

especially in complicated birth history, clavicle fracture. Examination notes, consultations, tests, and treatment of the case are essential for understanding complication management.

The most critical risk factor for OBP is stretching plexus structures during delivery (3,4). Fetal macrosomia, abnormal birth presentation, difficult and prolonged delivery are important predisposing factors in OBP (9,10). Except for one case in our series, all of them were born in the hospital. Also, in all of them, childbirth was directed by trained healthcare professionals. In our series, there was no case under 2,500 g and 53 cases are macrosomic. It is noteworthy that the distribution of the average birth weight of preterm cases is 3592.3±507.2 gr. These findings once again point out the association of macrosomia with OBP (Figure 4).

In our series, the clues that OBP might derive from traumatic reasons and frequencies are shoulder dystocia in 55%, clavicle fracture in 16.4%, humerus fracture and humeral head dislocation in 4.2%, history of difficult delivery in 23.3%, assisted delivery methods in 11.5% of the cases. However, in 130 patients, who constituted 45% of the cases, there were different comorbid pathologies. These pathologies were patent ductus arteriosus, pulmonary hypertension, microcephaly, mental and motor developmental problems, hypotonia, oligo and polyhydramnios, and neonatal convulsion, which may indicate many comorbid heterogeneous different diseases. Our study showed that OBP might occur in healthy developing infants with traumatic background and as well as in cases with developmental problems. Although maternal and natal factors are discussed in the foreground in previous studies, it is predicted that OBP cases may be of genetic and intrauterine origin (9). In our study about half of the cases have neurodevelopmental problems supports literature. In about half of the cases, brachial plexopathy with developmental problems requires detailed further documentation and investigation.

When our series is examined in terms of documentation of the main predisposing factors that may cause OBP, it is seen that basic information such as birth weight, birth week, place of birth, and the assisted health professional of the delivery are well documented in almost all of the files. There are a few cases whose birth weight (1) assisted labor (23) and birth presentation (27) were not available. The cases are well documented in terms of basic predisposing factors. On the other hand, some basic information, such as the maternal factors known to pose a risk for OBP, is not well documented. In most of the files, mothers' comorbid diseases, how many births the mother had, or whether the mother has another child with plexopathy are not included. This situation is an obstacle in the standard evaluation of these cases.

In this study, in addition to the basic medical information of cases with OBP, EMG study and treatments were also documented in terms of complication management. In this respect, EMG information is not available in approximately half of the cases (50.8%). In the remaining cases, there is great heterogeneity in terms of EMG study practice. Especially timing of EMG studies showed a wide range. This situation can be interpreted in terms of neurologists' having no standardization in the electrophysiological approach to OBPs (8). EMG should be considered as an extension of the neurological examination. The EMG study provides important information about the duration, spread, and degree of pathology. Therefore, EMG is important in terms of proper management of the cases and complication management. In our study, 110 of 141 cases who underwent EMG study were treated. It was also determined that 110 (57.6%) of 191 patients referred to physiotherapy with or without plexus surgery had EMG. It is understood that further investigation with EMG study is associated with a high rate (78%) of treatment.

Obstetrics and gynecology is the most common branch of all malpractice lawsuits in Turkey (12). It has been reported that psychosocial factors and lack of communication between the doctor and the family have an effect on malpractice lawsuits in which newborns are affected, rather than the severity of the involvement in the brachial plexus (13-15). For this reason, it is recommended that the obstetrician inform the family first-hand, that the involvement of the babies should be examined in detail with tests (especially EMG, standardized quality of life scales and functional upper limb scales) and detailed documentation of the comorbid factors including rare diseases of the infant (14,16,17).

Study Limitations

The retrospective method is the major limitation of the study. Depending on the retrospective evaluation, it was not possible to reach the result of every parameter determined in every file. For instance, maternal medical history detected in %21 (61/287) of the cases and this is the least informed parameter of the study. Despite the limitations in maternal medical information, the characteristics of the cases were well documented and our study revealed important results about characteristics of the OBP cases. The OBP cases with malpractice claim have been discussed for the first time in the literature. Our study will contribute to the literature with these features.

Conclusion

Etiopathology is a critical issue in cases that are evaluated for malpractice claims. The OBP has a

multifactorial etiopathology. The OBP can occur as a comorbid to a developmental pathology as well as the well-known predisposing factors. This study also shows the OBP's does not only originate from the traumatic etiology; about half of the cases have different comorbid pathologies, especially neurodevelopment problems. Our study showed that the medical records of patients with OBPs contained sufficient information for neurological and forensic evaluation. However, to achieve standardization and to understand the comorbidities of plexopathy better, it would be appropriate to expand the medical data in detail.

Authorship Contributions

Concept: E.K.O., H.A., Design: E.K.O., N.H.A., Data Collection or Processing: M.S.C., A.S., Analysis or Interpretation: N.H.A., H.A., K.G., E.K.O., Literature Search: E.K.O., M.S.C., Writing: N.H.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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Cognitive Emotion Regulation Strategies and Cognitive Flexibility Levels in High School Students Subjected to Peer Bullying

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Abstract

Aim: Peer bullying is thought to be negatively affected by high school students in many respects such as cognitive, emotional, psychological and physical. It is aimed to examine the relationship between cognitive flexibility levels and cognitive emotion regulation strategies of high school students who were exposed to peer bullying. Furthermore, it is examined whether these variables differ according to sociodemographic differences.

Methods: The sample of the study consists of 400 high school students reached via the internet from different cities of Turkey in 2020. The data in the study were obtained using "the peer bullying scale", "cognitive flexibility scale", "cognitive emotion regulation scale" and "sociodemographic information form" prepared by the researcher.

Results: It was found statistically significant that boys were subjected to more bullying on the peer bullying scale in the subscales of terror, teasing, and open attack. The rates of bullying students in vocational and technical high schools, who had poor school success and friendship relationships, were also found to be statistically significant ($p<0.005$). Students with good school achievement and friendship relationships had higher levels of cognitive flexibility, while boy students and those with very good friendship relationships scored highly on the "refocusing on planning" subscale of the cognitive emotion regulation scale ($p<0.05$).

Conclusion: According to our research, cognitive flexibility decreases as peer bullying levels increase. The use of maladaptive cognitive emotion regulation strategies also appear to increase.

Keywords: Bullying, cognition, flexibility, emotion regulation

Introduction

Peer bullying negatively affects children's cognitive, emotional, psychological, and physical development in many factors. Children's bullying behavior affects not only their developmental processes but also their school and daily life, emotional and behavioral processes. In recent years, it is seen that research on peer bullying, which is common both in our country and in other countries, has increased.

Exposure to peer bullying has an impact on cognitive coping skills and emotional arrangements. Cognitive flexibility is defined as the awareness of alternative ways and options to adapt to a situation (1). Problem-solving involves the process of finding solutions to problems encountered when heading towards a goal (2). Accordingly, problem-solving skills and cognitive flexibility are parallel in looking for alternative solutions to problems (2-4).

Emotion regulation consists of internal and external processes used to monitor, evaluate and alter a person's emotional responses, which are particularly intense and transient for the person in achieving their goals (4-6). Cognitive emotion regulation is the cognitive way to express the cognitive part of coping skills and manage

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emotionally stimulating information intake. Emotional and behavioral responses caused by peer bullying may be associated with children's cognitive flexibility levels and emotion editing methods, thus adversely affecting their cognitive flexibility and cognitive emotion regulation strategies (7).

In this study, we aimed to the level of peer bullying in high school students, the cognitive flexibility levels, and cognitive emotion regulation strategies of peer bullying, based on the assumption that high school freshmen and sophomore students who are trying to adapt to a new environment and school environment may be more affected.

Methods

Study Design

The present study was approved by Istanbul Gelisim University Ethics Committee (date: 12.03.2020, number: 2020-07). In this study, 400 students between the ages of 14 and 16 who attended high school one and high school sophomore education in different cities of Turkey were included. The cases consist of high school students reached via the internet from different cities of Turkey in 2020. "peer bullying scale", "cognitive flexibility scale", "cognitive emotion regulation scale" and "sociodemographic information form" were used. Exclusion criteria were psychiatric diseases and neurological diseases and cases with poor course success. The scales were filled by mutual interviews by the psychologist via the Internet. Informed consent forms of children and families were obtained via e-mail.

Sociodemographic Information Form

It consists of questions prepared by the researcher to obtain the demographic information of the students. At the beginning of the form, there is informed consent that introduces the research and includes approval for voluntary participation.

Peer Bullying Scale

The peer bullying scale (PBS) used in the study was adapted to Turkish by Gültekin and Sayil (8) based on the scale developed by Mynard and Joseph (9) to determine the exposure of school children to peer bullying and was developed as a suitable scale for Turkish children and adolescents (10).

The original scale is of the type of self-notification and can be applied individually or in groups. Students who participate are asked to choose "never", "once" and "more than one" for each item on the scale. The selected options are scored by giving 2 for "more than", 1 for "once" and 0 for "never". There are 16 items on the original scale, and the highest score from the scale in total is 32 and the lowest score is 0. As scores from the scale rise, the frequency of peer bullying increases, and as scores drop, it is rarely thought that they are bullied or not exposed at all. As a result of the analysis of the basic components applied to the data on the original scale, the substances were collected in 4 factors specified as physical, verbal, social, and psychological bullying. The scale of Gultekin and Sayil (8) consists of 27 items as a result of the development work and analysis for Turkish children and adolescents. As a result of the factor analysis carried out on the scale, 5 factors defined as error, overt victimization, teasing, relational victimization, and attacks on property were obtained.

Cognitive Flexibility Scale

The cognitive flexibility scale (CFS) was developed by the Scholar to measure adolescents' cognitive flexibility (11). This scale allows us to understand how flexible individuals are to themselves, others, and their environment. The scale is a scale of semantic differences and consists of 19 items. Scaled scoring is calculated by giving a score of "5-4-3-2-1" from positive to negative (e.g. I Can't Succeed). As the scores are increasing, the level of cognitive flexibility increases.

Cognitive Emotion Regulation Scale

The cognitive emotion regulation scale (CER) was developed by Garnefski et al. (12) to measure the cognitive emotion regulation strategies people use against stressful and threatening life events and was adapted to Turkish by Ataman (13) and validity and reliability studies were carried out. It consists of 36 items and nine subscales on a scale. Each of the subscales has 4 items. CER is a scale self-declaration type that can be applied to people 12 years and older. The substances are of type 5 lichen and are evaluated between 1 (none) to 5 (always). The score of each of the subscales ranges from 4 to 20 and is evaluated with ratings from the subscales. It is understood that the higher the score from the subscale, the more the strategy indicated by that subscale is used (12,14).

Statistical Analysis

The data obtained were entered into the computer environment as a numerical expression and statistical analysis was carried out using the statistical package program (SPSS 25.0) for social sciences. Before starting the analysis, the data were examined in terms of normal distribution. In small samples ($n<30$), the Kolmogorov-Smirnov test was used because the Shapiro-Wilk test produced stronger results in detecting normal non-dispersing conditions, and in large samples ($n>30$) it produced the best results for deciding by avoiding type I errors. A p-value of 0.05 or less was considered statistically significant.

Results

The study consisted of 114 (28.5%) boys and 286 (71.5%) girls attending the first year of high school one and sophomore year (Table 1).

Nine percent of the sample was found to be subjected to peer bullying, 7.5% to terror, 15.3% to tease, 13.3% to overt victimization,14.5% to relational victimization, and 8.3% to attacks on property (Table 2).

The PBS and relational victimization, attack on property scores on the item do not differ significantly by gender variable ($p>0.05$). There was a statistically significant difference in the subscale of terror, teasing, open attack compared to girls ($p<0.05$) (Table 3).

When differences in CFS and subscales were examined by school type, students in Vocational and Technical High Schools scored higher than students in other high schools ($p<0.005$) (Table 4).

There is a moderate negative correlation between the PBS and CFS score ($r=-0.315$ $p<0.01$). Terror ($r=-0.122$ $p<0.01$), tease ($r=-0.098$ $p<0.01$), open victimization ($r=-0.176$ $p<0.01$), relational attack ($r=-0.264$ $p<0.01$), attack on property ($r=-0.122p<0.01$) and CFS score found to be negatively weak. Refocusing on plan subscale scores differ significantly by gender variable ($p<0.05$). There was a positively weak relationship between self-blame ($r=0.208$, $p<0.01$), rumination ($r=0.249$, $p<0.01$), putting into perspective ($r=0.114$, $p<0.01$), catastrophing ($r=0.164$ $p<0.01$), and other-blame ($r=0.215$, $p<0.01$) and the PBS score (Table 5).

Table 1. Distribution by sociodemographic variables

		n (%)
Sex	Boy	114 (28.5%)
	Girl	286 (71.5%)
	Total	400 (100%)
Class	9th grade	223 (55.8%)
	10th grade	177 (44.2%)
School type	Anatolian	180 (45%)
		101 (25.3%)
	Vocational and Technical	91 (22.8%)
	Science	28 (7%)
Success at school	Poor	27 (6.8%)
	Middle	188 (47%)
	Good	157 (39.3%)
	Very good	28 (7%)
Friendship relations	Poor	15 (3.8%)
	Middle	66 (16.5%)
	Good	164 (41%)
	Very good	155 (38.8%)
Parental marital status	Married	367 (91.8%)
	Divorced	33 (8.3%)
Education status-mother	Elementary school graduate	189 (47.3%)
	Middle school graduate	84 (21%)

Discussion

In this study, high school students' exposure to peer bullying and their cognitive flexibility levels, cognitive emotion regulation strategies and their relationship were investigated. Olweus also conducted a large sample in Norway, which found that 15% of students (aged 7 to 16) experienced bullying and victimization, 9% were victims of bullying and 7% were those who were bullying others (15). In this study, peer bullying was found to be 9%. Another study in the United States found that the prevalence of being a victim of peer bullying or bullying at school at least once in the last two months was 20.8% physically, 53.6% verbally, 51.4% relationally, and cyberbullying 13.6% (16). A study of high school students in Turkey found that all students were bullied at least once during their student life, of which 33.5% were verbal, 35.5% physical, 28.3% psychological, and 15.6% sexual assault (17).

In our study, it was determined that the most common exposure in terms of bullying was teasing, relational

Table 2. Distribution of sample group by peer bullying cutting score

	n (%)
Group not subjected to peer bullying	364% (91)
Peer bullying group	36% (9)
Group not subjected to terror	370% (92.5)
Group subjected to terror	30% (7.5)
Group not subjected to teasing	339% (84.8)
Group subjected to teasing	61% (15.3)
Group not exposed to overt victimization	347% (86.8)
Overt victimization group	53% (13.3)
Group not subjected to relational victimization	342% (85.5)
Group subjected to relational victimization	58% (14.5)
Group not attacks on property	367% (91.8)
Group attacks on property	33% (8.3)

9% of the sample was found to be subjected to peer bullying, 7.5% to terror, 15.3% to tease, 13.3% to overt victimization,14.5% to relational victimization, and 8.3% to attacks on property

Table 3. Comparison of peer bullying scale subscale scores by gender variable of the sample

		N	s.o	K.T	U	Z	p
Peer Bullying	Boy	114	212.35	24207.50	14951.50	-1.311	0.190
Scale	Girl	286	195.78	55992.50	-	-	-
	Total	400	-	-	-	-	-
Terror	Boy	114	221.47	25247.50	13911.50	-3.715	0.000*
	Girl	286	192.14	54952.50	-	-	-
Tease	Boy	114	217.78	24826.50	14332.50	-2.026	0.043*
	Girl	286	193.61	55373.50	-	-	-
Overt Vict.	Boy	114	230.04	26224.00	12935.00	-3.741	0.000*
	Girl	286	188.73	53976.00	-	-	-
Relational Vict.	Boy	114	195.06	22237.00	15682.00	-0.650	0.516
	Girl	286	202.67	57963.00	-	-	-
Attac. on prop	Boy	114	211.09	24064.00	15095.00	-1.390	0.165
	Girl	286	196.28	56136.00	-	-	-

*p<0.05, Mann Whitney-U test
There was a statistically significant difference in the subscale of terror, teasing, open attack compared to girls

Table 4. Comparison of cognitive flexibility scale by school type variable of the sample group

	N	S.O	X^2	SD	p
CFS Anatol. HS	180	216.19	13.608	3	0.226*
Anatol.Imam Hatip HS	101	191.00	-	-	-
Vocational and Technical HS	91	168.82	-	-	-
Science HS	28	236.86	-	-	-

*p<0.05. Kruskal-Wallis H-test. Student's t-test in Vocational and Technical High Schools scored higher than students in other high schools, CFS: Cognitive flexibility scale, Anatol: Anatolian, HS: High school

victimization, open victimization, attacks on property, and terror respectively. A study (18) with high school students also found that the most common form of bullying was "teasing" followed by terror, relational victimization, open victimization, and attacks on property, respectively. In our study boys are bullied more in the subscales of terror, teasing, and overt victimization. Consistently with our findings (19) boys were more likely to be bullied by peers than girls. Consistent with these findings, studies in the literature show that boys are more involved in physical and verbal bullying and girls are more involved in relational bullying and girls exhibit bullying behaviors such as teasing and relational victimization more often than boys rather than physical bullying (16,20).

Students with poor school achievement were found to be more bullied than those with good school achievement (21). It is thought that poor school achievement of students may be a factor in being subjected to peer bullying.

In terms of friendship relations, we found that students with good friendships are less bullied. Poor friendship relations increase the rate of peer bullying, and good friendship relation is a protective factor in terms of bullying. Consistent with the findings of this research in the literature, victims report that they have failed to relate to their peers in particular and have established fewer social relationships. As a result of these studies, those who are bullied are socially isolated and have poorer social skills. Social isolation of those who are bullied is often both the result and the cause of victimization (20). Bullying has recently been conceptualized as a relationship problem, suggesting that this aggressive behavior occurs in the context of a relationship between peers (22).

There are significant differences in cognitive flexibility according to the type of high school, which may be explained by factors such as the socioeconomic level of students, their academic achievements, the physical and social conditions of schools, and the provision of adequate support in and out of school-related to compliance (23). As school success increases, so does the level of cognitive flexibility (24).

In the subscale of "refocusing on the plan", it was found that boys used the strategy of regulating this feeling against stress and negative situations more often than girls (25). In the literature, boys are more used to the "other-blame" strategy of cognitive emotion regulation than girls (26). Other studies concluded that (27) the biggest differences between boys and girls were in using "putting into perspective", "rumination" and "other-blame" strategies. Girls used the "rumination" and " putting into perspective" strategies more often when faced with a stressful event, while boy concluded that they used the "other-blame" strategy more often.

Table 5. Examining the relationship between peer bullying scale and subscale scores and cognitive emotion regulation scale and subscale scores

	PBS	Terror	Tease	OV	RV	AOP
Self-blame	0.208	0.112*	0.209**	0.167	0.160	0.141
Acceptance	-0.216		-0.195**	0-.192	-0.161	-0.121*
Rumination	0.249	0.072	0.229**	0.169"	0.232**	0.125*
Planning	0.033	0.005	0.029	0.078	0.012	0.045
Positive refocus. Positive refocusing	0.056	0.029	0.057	0.078	0.055	0.069
Putting into pers.	0.114*	0.011	0.099*	0.115*	0.105*	0.116*
Catastrophe	0.164	0.085	0.153	0.137	0.140	0.149
Other-blame	0.215	0.065	0.162	0.146	0.232**	0.090

**p<0.01, *p<0.05. There was a positively weak relationship between self-blame, rumination, putting into perspective, catastrophing, and other-blame and the PBS score.
PBS: Peer bullying scale, OV: Overt victimization, RV: Relational victimization, AOP: Attacks on property

Students in Vocational and Technical High Schools use the subscales of "self-blame" and "Catastrophe" more than students in other high schools. It turns out that students in Vocational and Technical High Schools are more inclined to use "Self-blame" and "Catastrophe" strategies from cognitive emotion regulation strategies when faced with negative, threatening events. A study of 9-11-year-olds in the literature (28) found significant differences in the subscales of self-blame, positive reappraisal, rumination, positive refocusing, and planning from cognitive emotion regulation strategies in favor of private school students.

When the relationship between PBS and CFS is examined, it shows that the level of cognitive flexibility decreases as peer bullying exposure increases. As PBS subscales such as "Intimidation/Intimidation", "tease", "overt victimization", "relational victimization" and "attacks on property" increase, exposure to peer bullying appears to reduce cognitive flexibility (29). From this point of view, as the level of cognitive flexibility increases, social abilities develop, and happiness level increases (30).

When the subscales of the CER scale with PBS are examined; As cognitive emotion-regulation strategies such as "self-blame", "putting into perspective", "rumination", "other-blame" and "Catastrophe" increase, so do the types of bullying such as "terror", "tease", "overt victimization", "relational victimization" and "attacks on property". Cognitive maladaptive coping strategies increase as bullying increases. In studies with adolescents found that the choice of cognitive emotion-regulation strategies played a partial agent variable role on cognitive flexibility and emotional autonomy (31,32). It was also observed that the level of use of the "acceptance" cognitive emotion regulation strategy decreased as the exposure to bullying increased. Acceptance and positive refocusing from cognitive emotion-regulating strategies reduce depression (33); terror and rumination strategies have been shown to explain the increase in anxiety and the acceptance strategy explains the decrease in the level of anxiety (34).

Cognitive flexibility decreases as "self-blame", "rumination", "catastrophe" and "other-blame" strategies are used (35). It can also be interpreted that the more cognitive flexibility increases, the more likely it will be to use the "acceptance" strategy. In a study that investigated the effect of cognitive flexibility on cognitive emotion regulation; As the level of "alternative" subscale, which is expressed on the CFS as "measuring the ability to perceive that there are alternatives to situations encountered in life and one's behavior, and the ability to produce solutions to difficult situations", increases, so does the level of using "other-blame", "catastrophic", "positive refocusing", "refocusing on planning" and "positive reappraisal" strategies from cognitive emotion regulation (36-38).

Study Limitations

One of the limitations of our study is that our research is carried out with scales filled over the internet. Taking the sample from across the country increases diversity but impairs homogeneity. The high number of cases and the filling of scales with the psychologist are the strengths of our study.

Conclusion

It was observed that students' cognitive flexibility levels decreased as peer bullying exposure increased and they preferred to use maladaptive cognitive emotion regulation strategies. With the prevention of peer bullying, it is thought that students can use harmonious cognitive emotion regulation strategies by developing alternative

methods to be more flexible and adaptable in the face of the situations they encounter.

*This study was produced from the first author's master's thesis.

Authorship Contributions

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

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Original Article

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Evaluation of Internal Nasal Valve Using Computed Tomography After Le Fort I Osteotomy: A Cross-Sectional Study from a Tertiary Center

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Abstract

Aim: Internal nasal valve, which has been the most resistant region of the nasal airway, is affected by Le Fort I osteotomy. This study aimed to investigate the changes in the internal nasal valve (INV) after Le Fort I osteotomy.

Methods: A retrospective computed tomography (CT) data of thirty-two patients who underwent Le Fort I surgery alone or combined with mandibular surgery from 2018 to 2020 were evaluated. INV area, INV angle, external nasal valve area, and interalar width were measured at preoperative (T1) and postoperative period (T2) on CT images.

Results: CT assessment showed that the INV area was increased for both the right and left side (p1=0.005, p2=0.007). Right and left INV angle was increased from 16.15±3.24^0 to 19.63±5.21^0 and from 15.93±3.26^0 to 19.17±4.43^0 respectively (p1=0.000, p2=0.007). Interalar width was increased at the postoperative period (p=0.000). Also, the correlation between interalar width and INV area was found borderline significant (p=0.051, r=0.814). Right and left external nasal valve areas were increased after surgery (p1=0.000, p2=0.003).

Conclusion: Maxillary surgery and surgical procedures affecting interalar width have an impact on the internal nasal valve.

Keywords: Internal nasal valve, airway resistance, dentofacial deformities, tomography, orthognathic surgery

Introduction

Airflow resistance during breathing is essential for good pulmonary function (1). The nasal airway resistance constitutes about 50% of total airway resistance, and the most resistant region of the nasal airway is the internal nasal valve (INV) (2). The narrowest part of the nasal passage, INV, where the turbulence of inspiratory and expiratory flow occurs, is the most critical valve that adjusts the airflow (3). The INV is located at the inferior aspect of the upper lateral cartilage and is surrounded by inferior nasal concha and nasal septum (4). Small changes in the INV can significantly impact airflow resistance, affecting nasal function (5). A previous study stated that the average INV area was less than 0.38 cm^2 in obstructed airway patients, while the normal INV was measured as 0.51cm^2 (4). Theodore (6) stated it should be between approximately 40 to 60 mm^2. Many studies reported an increase in the INV area correlated with a higher patient satisfaction level for breathing (7,8). The factors affecting the nasal valve area are septal deviation, turbinate hypertrophy, skeletal irregularities, iatrogenic collapse, and mucosal hypertrophy (4). One possible factor affecting the INV is Le Fort I osteotomy, a commonly used method for treating dentofacial deformities in adult patients. As a result of maxillary impaction or advancement, nasal airway resistance can be decreased because enlargement of interalar width affects an increase in nasal valve angle (9).

Most studies assessing the nasal airway resistance following maxillary surgery used anterior rhinomanometry (AR) and acoustic rhinometry. However, the efficiency of these devices in the determination of nasal airway resistance and clinical applicability is limited (8,10,11). Computed tomography (CT) is an objective imaging

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technique in evaluating the INV anatomy. In previous studies, CT findings were highly correlated with patients' complaints and clinical examination findings (11,12). To the best of our knowledge, there has been no study examining changes in internal nasal valves using CT following Le Fort I surgery.

The purpose of this study was to explore changes in the INV area, INV angle and investigate associated parameters with the internal nasal valve, such as interalar width and external nasal valves.

Methods

Study Design

Ethics committee approval of this retrospective study was obtained from Istanbul Medipol University Ethics Committee (date: 07/01/2021, no: E-10840098-772.02-947). A written consent form was collected from each patient. The sample consisted of thirty-two patients who underwent Le Fort I surgery between 2018 and 2020 in the Oral and Maxillofacial Department of Istanbul Medipol University Dentistry faculty. Patients who had pre and postoperative CT (at least six months) were included in this study. The exclusion criteria were as follows: 1- Patients who underwent multi-piece Le Fort I osteotomy, 2- Patients with congenital deformities such as cleft lip and palate, 3- Patients with a previous history of surgery in internal and external nose 4- Patients with incomplete pre and postoperative CT records.

Measurement Methodology

On the cephalometric tracing module of NemoFab software (Nemotec S.L., Madrid, Spain), the horizontal distance between A point to Nasion Perpendicular was defined as maxillary advancement amount, the vertical distance between A point to Nasion point defined as anterior impaction amount. The rotation amount of maxilla was recorded by 3D surgical simulations on preoperative CT using NemoFab software. Positive values were given for maxillary advancement, superior movement of the maxilla, and left maxillary rotation. Negative values were given for maxillary setback, inferior movement of the maxilla, and right maxillary rotation.

A multi-slice CT device (MSCT, Philips Brilliance ICT 256; Philips Medical Systems, Eindhoven, The Netherlands) (scan setting 120 kV, 150 mAs) was used slice thickness 0.75 to 1.25 mm. For measurements on internal nasal valves, pre and postoperative coronal CT scans were reformatted perpendicular to the anterior aspect of the estimated acoustic axis described by the previous study (Figure 1a) (13). They showed a correlation between acoustic rhinometry (AS) findings and coronal CT slice obtained at the acoustic axis. Slice of CT was obtained by RadiAnt DICOM 2.2.9 Viewer (Medixant, Poznan-Poland). Interalar width and external nasal valve area were measured on a surface model obtained from CT.

Following measurements were taken:

Right and left INV angle (r-INV angle-l-INV angle): Defined as the angle between two lines passing from medial and lateral nasal airway lumen margins (Figure 1b).

Right and left INV area (r-INV area-l-INV area): Defined as the inner surface area of soft tissues of the nasal airway (Figure 1c).

Right and left external nasal valve area (r-ENV area-l-ENV area): Inner surface area of right and left nostrils (Figure 2).

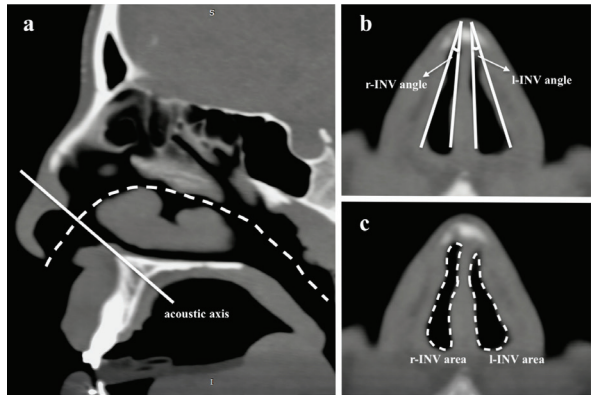

Figure 1. Measurements of internal nasal valve area at the acoustic axis. a: acoustic axis; b: right and left internal nasal valve angle (r-INV and l-INV angle); c: right and left internal nasal valve area (r-INV and l-INV area)
INV: Internal nasal valve

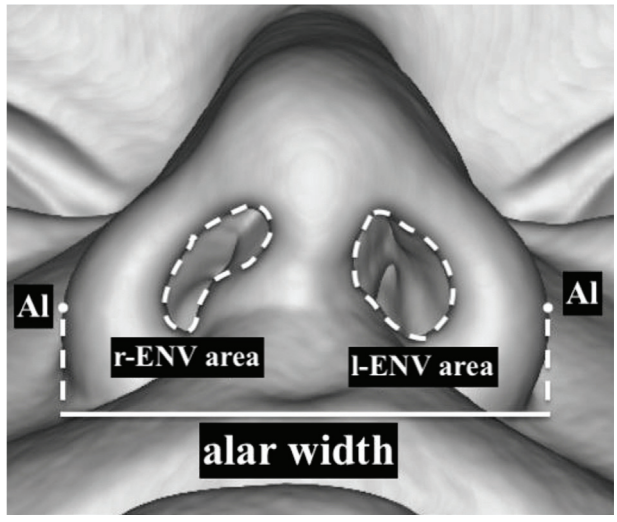

Figure 2. Measurements of the external nasal valve and interalar width.
r-ENV area: right external nasal valve area, l-ENV area: left external nasal valve area, Al: most lateral point of alar curvature
r-ENV: R-external nasal valve

Interalar width: Distance between the right and left alar wing's farthest points (Figure 2).

Surgical Technique

Under general anesthesia, local anesthetic solution (2% lidocaine and epinephrine 1:1000,000 IU) was applied to the maxillary vestibular sulcus. Horizontal incision at vestibular sulcus was performed at the level of the mucogingival junction from the first premolar to the contralateral first molar. After incision, the anterior nasal spine, pyriform aperture, and lateral surface of the maxillary sinus were exposed. Le Fort I osteotomy was performed. Maxilla was fixed in the new position by four 2.0 mm mini plates (KLS Martin, Tuttlingen, Germany) and screws. Alar base cinch suture passing from the right and left alar base to a drilled hole on the anterior nasal spine was performed with 2.0 Nylon. This suture was tightened up to pre-measured alar base width. The wound edges were sutured with a 4.0 vicryl suture (Ethicon; Johnson and Johnson Medical, Norderstedt, Germany).

Statistical Analysis

The paired samples t-test was performed to compare pre and postoperative measurements using Statistical Package for the Social Sciences (SPSS for Windows, version 18.0, SPSS Inc., Chicago, USA). Pearson's correlation test was used for correlations. P<0.05 was considered significant.

Results

This retrospective study included 32 patients (21 females, 11 males) with an average age of 24.6±6.71 years. The mean anterior movement, maxillary superior-inferior movement and rotation amount were 3.08±2.47 mm (r=1 to 8 mm), 0.45±2.92 mm (r=-4.85 to 5.90 mm) and -0.32±1.38 mm (r=-3.65 to 2.2 mm) respectively (Table 1). Table 2 shows descriptive data for INV area, INV angle, ENV area, and interalar width. The mean INV area was 90.34±22.26 mm^2 for the right and 89.32±25.24 mm^2 for the left before surgery. This area was increased to 104.12±31.75 mm^2 for the right, and 101.18±31.31 mm^2 for the left following the surgery. The difference between pre and postoperative values was significant for both the right and left INV area (p1=0.005, p2=0.007). Right INV angle was increased in twenty-five patients (r=0.710 to 11.20), whereas it was decreased in seven patients (r=-6.940 to -0.190 to) (Figure 3). Left INV angle was increased in twenty-six patients (r=0.210 to 13.100), and it was decreased in five patients (r=-7.030 to -1.100) (Figure 3). The mean right and left INV angle was increased from 16.15±3.240 to 19.63±5.210 and from 15.93±3.260 to 19.17±4.430 respectively. These changes were also found significant for the right and left sides (p1=0.000, p2=0.007). The correlation between the left INV area and the left internal nasal angle was significant (p=0.000, r=0.583). Correlation between the right INV area and the right internal nasal angle was also significant

Table 1. Demographic variables of the study

Variable	
Subjects	32
Sex	
Female	21
Male	11
Mean age (year)	24.6±6.71
Maxillary movement amount (mm)	
Advancement	3.08±2.47 (r=1 to 8)
Superior-inferior direction	0.45±2.92 (r=-4.85 to 5.90)
Rotation	-0.32±1.38 (r=-3.65 to 2.2)

Table 2. Comparison of investigated values at two timepoints (values shown as mean ± SD)

Variable	T1	T2	p*
Right internal nasal valve angle (0)	16.15±3.240	19.63±5.210	0.000
Left internal nasal valve angle (0)	15.93±3.260	19.17±4.430	0.007
Right internal nasal valve area (mm^2)	90.34±22.26	104.12±31.75	0.005
Left internal nasal valve area (mm^2)	89.32±25.24	101.18±31.31	0.007
Total internal nasal valve area (mm^2)	179.66±34.81	205.30±45.18	0.000
Right external nasal valve area (mm^2)	114.97±36.11	123.52±34.04	0.000
Left external nasal valve area (mm^2)	107.82±29.76	115.20±30.24	0.003
Interalar width (mm)	35.87±4.15	39.15±3.59	0.000

*paired samples t-test: It was used to determine whether the changes in the pre and postoperative periods were significant. T1: Preoperative, T2: Postoperative, SD: Standard deviation

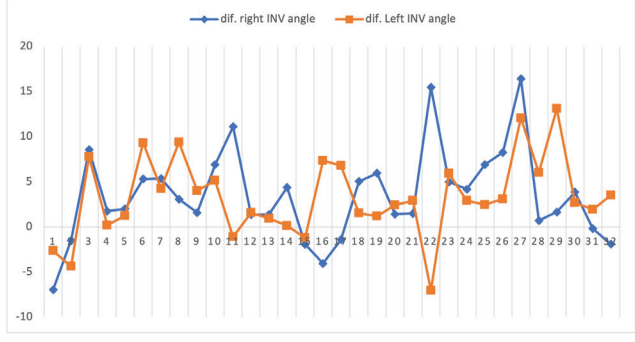

Figure 3. Difference of pre and postoperative INV angle for the right and left side. Dif. Right INV angle: right internal nasal valve angle difference; dif. left INV angle: left internal nasal valve angle difference
INV: Internal nasal valve

(p=0.011, r=0.444). The total INV area was increased from 179.66±34.81 mm^2 at the preoperative period to 205.30±45.18 mm^2 at the postoperative period. These changes were found significant (p=000).

For the external nasal valve area, the preoperative area was 114.97±36.11 for the right side and 107.82±29.76 for the left side. Postoperative values were 123.52±34.04 for the right side and 115.20±30.24 for the left side. There was a significant difference between pre and postoperative values for the right and left sides (p1=0.000, p2=0.003).

Inter-alar width was increased from 35.87±4.15 mm preoperatively to 39.15±3.59 mm postoperatively, which was statistically significant (p=0.00). Correlation between inter-alar width and changes in total INV area was found borderline significant (p=0.051, r=0.814). Correlation between inter-alar width and maxillary impaction was found borderline significant (p=0.058, r=0.401).

Discussion

Le Fort I osteotomy has been routinely performed in patients with dentofacial deformity to correct malocclusion, and it has varying degrees of effect on nasal airway form and function (14). Due to that, the maxilla can be moved in any direction by Le Fort 1 osteotomy; total maxillary impaction can increase nasal airway resistance and interalar width in certain cases, which may cause a decrease in nasal airflow (15,16). A previous study has shown a higher tendency to nasal airway resistance and nasal obstruction seen in patients with maxillary constriction (17,18). The results presented here prove an increase in the nasal valve area, ENV, INV angle, and interalar width following Le Fort I surgery. This study's most significant finding was a positive correlation between total INV area and interalar width. From this positive correlation, it can be commented that maxillary movements and supplementary surgical procedures such as alar cinch suture may affect INV area and angle by changing interalar width.

Many studies assessed nasal valves following Le Fort I surgery, and these studies used other methods such as AS and AR. Guenthner et al. (9) stated that nasal airway resistance following maxillary repositioning was assessed by rhinomanometry, and they found a negative correlation between impaction and airflow resistance. Erbe et al. (19) evaluated changes in nasal airway using rhinomanometry and found that the cross-sectional area at isthmus nasi increased significantly and correlated with interalar width. Haarmann et al. (20) evaluated nasal airway resistance in cases performed both rhinoplasty and orthognathic surgery and found that nasal airway resistance was decreased after combined surgery. Pourdanesh et al. (21) analyzed the effect of three different maxillary surgical movements on nasal airflow, volume, and resistance after Le Fort I osteotomy. They stated that maxillary advancement and impaction associated with nasal resistance reduction, leading to improved nasal airflow. Oliveira et al. (22) found that nasal resistance was decreased, and the minimum nasal cross-sectional area was increased significantly after maxillary expansion. Yoon et al. (23) reported that INV angle and area were increased following rapid palatal expansion in pediatric patients. They also showed that the expansion amount of the maxilla had been positively correlated with nasal obstruction scores and INV angle. Similarly, another study stated a positive correlation between maxillary expansion and INV angle in adults (24). Doruk et al. (25) also found that nasal resistance decreased significantly after maxillary expansion. However, Baraldi et al. (26) assessed thirteen patients with maxillary transversal deficiency after surgically assisted maxillary expansion, and they found that the anterior minor cross-sectional area of nasal passage was not statistically different after the operation. Data from CT findings in the present study showed that widening of interalar distance increased INV area by opening nasal valves so nasal airway resistance could be reduced.

Measurements were made on a coronal slice of CT, which was perpendicular to the estimated acoustic axis on CT. Because it was stated in a previous study that there were strong correlations between CT slice obtained perpendicular to the acoustic axis and AS findings, while there was a weak correlation between AS findings and CT image that is perpendicular to the nasal floor (13).

Study Limitations

Due to the retrospective design of the present study, it has not been investigated the relationship between physical examination of nasal airway function and INV changes. The study's strength was to examine the effect of Le Fort I osteotomy on the INV. Further prospective studies assessing the relation of physical examination of nasal airway function with INV changes following Le Fort I osteotomy could be carried out.

Conclusion

According to our findings, INV area and angle were affected to some degree by changes in interalar width, so movement direction of the maxilla or supplementary surgical procedures such as alar cinch suture, which are affecting interalar width should be kept in mind that it may change nasal airway resistance.

Authorship Contributions

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

Conflict of Interest: No conflict of interest was declared by the authors.

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

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Original Article

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Long-Term Relapse Outcomes of Smoking Cessation in Older Smokers

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Abstract

Aim: Limited data are available in the literature about the long-term results of smoking cessation and the relapse rate in older smokers. We aimed to demonstrate the efficacy of short- and long-term smoking cessation behaviors in individuals over 65 years of age.

Methods: Patients' data were analyzed retrospectively from hospital records between 2014 and 2018. All patients received individual behavioral therapy and were prescribed the drugs that were best suited for their individual circumstances, such as nicotine replacement therapy, bupropion, and varenicline. Patients who quit smoking during the three-month period of outpatient follow-up were contacted by phone at least two years later and asked whether they still smoked or not.

Results: In total, 68 older participants (13 female and 56 male) were included. In the initial three-month follow-up period, 29 of the participants (42.6%) dropped out of the smoking cessation program after the first interview. Eleven of the smokers (16.2%) did not quit smoking, and 28 (41.1%) did quit smoking. Fourteen participants (20.6%) achieved long-term abstinence (i.e., were not smoking at the two-year follow-up), while the remaining 10 participants (14.7%) had started to smoke again. No statistically significant difference was found between quitters and non-quitters in terms of age, gender, duration of the smoking habit, the number of cigarettes smoked daily, the reason for starting smoking, the reason for the desire to quit smoking, and the treatment methods used.

Conclusion: Smoking cessation strategies should be tailored and constantly re-evaluated in elderly people for safe management, and they should be followed up closely to avoid relapse risk.

Keywords: Aged, smoking cessation, recurrence

Introduction

The rate of using tobacco and tobacco products among people aged 65 and older in Turkey is 17.9% for men and 2.6% for women (1). It is known that smoking is associated with an increased risk of chronic heart and lung diseases, cerebrovascular diseases, and cancer, and these diseases are most prominent in the aged population. Thus, increased risk of morbidity and mortality attributed to tobacco exposure can be prevented through smoking cessation. In the elderly population, mortality is approximately three-fold greater among smokers, while a decreased mortality risk with smoking cessation has been found, despite the high mortality rates in this age group (2). Mortality rates are lower in former smokers who have abstained longer, suggesting that the best results are achieved when people stop smoking early. It was observed that male smokers who quit at age 65 gained 1.4 to 2.0 years of life expectancy, and women gained 2.7 to 3.7 years of life expectancy. Additionally, the health benefits of smoking cessation that have been demonstrated for older adult smokers include a lower risk of stroke, respiratory tract disease, and cancer. Thus, quitting smoking even at an older age can yield substantial benefits (3).

It has been determined that the results of smoking cessation behavioral therapy and pharmacotherapy are as effective in the elderly as in the young (4,5). However, limited data are available in the literature about the long-term results of smoking cessation and the relapse rate in older smokers. In this study, we aimed to demonstrate the short- and long-term efficacy of smoking cessation behaviors in individuals over 65 years of age.

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Methods

Data Collection

Our study was approved by the local ethics committee University of Health Sciences Turkey, Istanbul Training and Research Hospital Ethics Committee (date: 08.05.2020, no: 2264). Owing to the retrospective nature of the study with no available informed consent.

In total, 68 older participants (13 female and 56 male) were included in our study from among 550 patients who applied voluntarily to the smoking cessation clinic at Yedikule Chest Diseases and Chest Surgery Training and Research Hospital, Istanbul, Turkey between January 2014 and September 2018. Patients' data were analyzed retrospectively and they were contacted by phone and asked whether they were smoking or not in January 2020.

Participants' demographic information was recorded, and they completed the six-item Fagerström test for nicotine dependence test at the first visit to the smoking cessation program. A score of 5 or more indicates significant dependence, while a score of 4 or less shows a low to moderate dependence (6). All patients received individual behavioral therapy and were prescribed the drugs that were best suited for their individual circumstances, such as nicotine replacement therapy (NRT), bupropion, and varenicline.

Participants who smoked ≥20 cigarettes daily received 21 mg nicotine/24 h nicotine patches for four weeks, followed by patches with a reduced dose (14 mg nicotine/24 h) for two weeks, and then by patches with a further reduced dose (7 mg nicotine/24 h) for two more weeks. For individuals who smoked <20 cigarettes daily, we offered 14 mg nicotine/24 h patches for six weeks followed by 7 mg nicotine/24 h patches for two weeks.

Participants were given either bupropion 150 mg/day for the first three days and 300 mg/day for 12 weeks from the fourth day or varenicline 0.5 mg/day from the first to the third day, 1 mg/day on the fourth to the seventh day, and 2 mg/day from the second to the 12th week. All participants were encouraged to revisit the clinic every 1-2 weeks after the first session. Combined therapies were provided to patients who were not successful with their first treatment or their medications were changed at follow-up visits.

Besides the pharmacological treatment, all patients received individual behavioral therapy from a physician and a smoking cessation nurse. Non-quitters included participants who did not provide follow-up data or were lost to follow-up and those who failed to quit smoking. Patients who quit smoking during the three-month period of outpatient follow-up were contacted by phone at least two years later and asked whether they still smoked or not.

Statistical Analysis

The data were analyzed using IBM SPSS Statistics 18© SPSS Inc. The compliance of continuous variables with normal distribution was examined with the Kolmogorov-Smirnov test. Categorical variables are presented as frequency and percentage, and continuous variables as mean, standard deviation, median, and the smallest and largest values. Associations between categorical values were calculated by chi-square tests and Fisher's Exact tests. Significant differences between groups for parametric continuous values were determined with the Student's t-test, while the Mann-Whitney U test was used for non-parametric continuous variables. A p-value of 0.05 was considered significant.

Results

A total of 68 participants (13 female and 55 male; mean age 67.35±3.04 years) were included in the study. The demographic variables are shown in Table 1.

Although 28 (41.1%) of the smokers were able to quit smoking in the first three months following the smoking

Table 1. Smoking cessation status by sociodemographic characteristics

	Quitter (n=14)	Non-quitter (n=54)	p
Age (mean ± SD)	68.07±3.56	67.16±2.95	0.41¶
Weight (kg)	68.21±11.71	67.16±2.95	0.15¶
Gender n (%)			
Female	1 (7.7%)	12 (92.3%)	0.20* ¥
Male	13 (23.6%)	42 (76.4%)	
Marital status n (%)			
Married	12 (22.2%)	42 (77.8%)	0.75* ¥
Single	0 (0.0%)	1 (100.0%)	
Widow	2 (15.4%)	11 (84.6%)	
Children n (%)			
Yes	13 (21.0%)	49 (79.0%)	0.91* ¥
No	1 (50.0%)	1 (50.0%)	
Educational level n (%)			
Primary school	9 (18.8%)	39 (81.2%)	0.20* ¥
Juniour high school	4 (36.4%)	7 (63.6%)	
High school	0 (0.0%)	7 (100.0%)	
University	1 (50.0%)	1 (50.0%)	
Job n (%)			
Non-working	1 (9.1%)	10 (90.9%)	0.35* ¥
Active working	0 (0.0%)	3 (100.0%)	
Retired	13 (24.1%)	41 (75.9%)	
Comorbidities n (%)			
Cardiovascular diseases	5 (35.7%)	14 (25.9%)	0.69** ¥
Cerebrovascular diseases	0 (0.0%)	3 (5.6%)	0.86
COPD	5 (35.7%)	9 (16.7%)	0.23
Malignancy	2 (14.3%)	4 (7.4%)	0.78
Liver diseases	0 (0.0%)	1 (1.9%)	0.60
Depresion	0 (0.0%)	3 (4.4%)	0.86
Alcohol consumption	0 (0.0%)	2 (3.7%)	0.46

*Row percentage, **Column percentage, ¶ t-test, ᵘ Mann-Whitney U test, ¥ Chi-square test, COPD: Chronic obstructive pulmonary disease, SD: Standard deviation

cessation intervention, only 14 achieved long-term abstinence (20.6%) after two years, while the remaining 10 (14.7%) had started to smoke again (Figure 1). The smoking cessation rate was 7.7% for women and 23.6% for men.

Participants' smoking history and cessation behaviors are shown in Table 2.

Participants' reasons for their intention to quit smoking included current illness (55.8%), doctor's advice (47%), fear of illness (45.5%), social pressure (30.8%), harm to the environment (29.4%), desire to be a good example for their family (29.4%), disturbance of smell (25%), shame (13.2%), economic concerns (10.2%), religious beliefs (7.3%), and workplace pressure (1.47%). No significant difference was observed in terms of their reasons for starting smoking or for their desire to quit smoking and their smoking cessation behavior (p>0.05).

Eight (18.1%) of the 44 participants who applied to the smoking cessation program voluntarily quit smoking, while 26.1% of the 23 participants who applied to the program on the advice of a doctor quit smoking.

Treatment methods and relationships showed Graphic 1 for quitters and non-quitters. No significant relationship was observed between the treatment methods used and smoking cessation results (p>0.05) (Graphic 1).

The most common side effects of NRT were dry mouth, attention deficit, dyspepsia, palpitations, and anorexia. Of the patients who used bupropion, 15 (46.6%) had dry mouth, four (26.6%) experienced itching, and four (26.6%) had insomnia. Among the patients who used varenicline, nine (26.4%) experienced dry mouth, eight (23.5%) reported colorful dreams, eight had (23.5%) insomnia, five (14.7%) experienced nausea, and five (14.7%) experienced personality change.

Seven of these participants had started smoking again in the first three to six months following the smoking cessation program, one of them relapsed 18 months after the program, and one of them started smoking in the third year after the program. No statistically significant difference was found between quitters and those who restarted smoking in terms of age, gender, duration of smoking, the number of cigarettes smoked daily, the reason for starting smoking, the reason for the desire to quit smoking, and the treatment methods used.

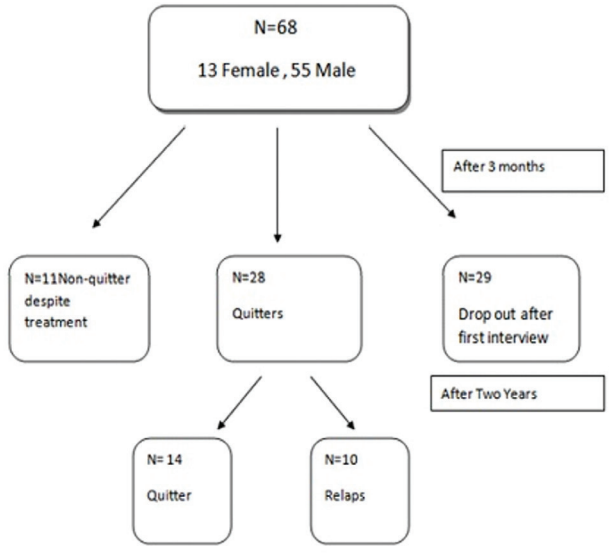
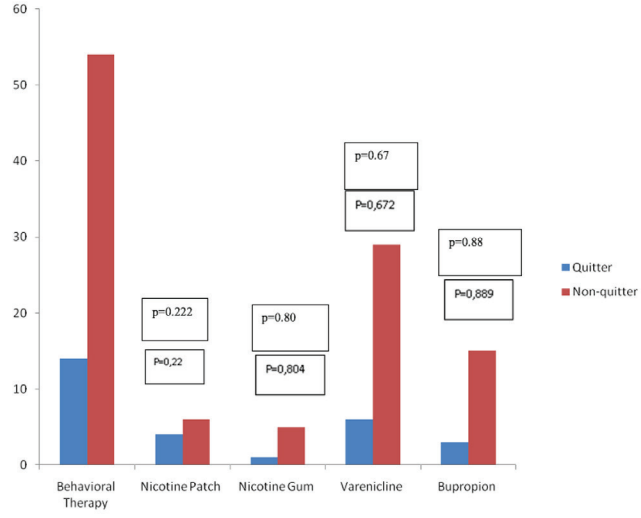


Figure 1. Scatter diagram of participants



Graphic 1. The relationship between treatment methods used and smoking cessation behavior

Table 2. Smoking history and cessation behavior of participants

	Quitter (n=14)	Non-quitter (n=54)	p
Smoking duration	46.93±8.59	44.02±8.74	0.27
Daily amount of cigarette	21.36±10.94	27.80±19.06	0.15
Number of attemps to quit	3.07±2.64	2.20±4.20	0.06
Fagerstrom nicotine dependence	5.36±2.27	5.24±2.67	0.77
t-test			

Discussion

In our study, the quit rate at three-month following a smoking cessation program for individuals over 65 years of age was 41.1%, but at the two-year long-term follow-up, 10 patients had started smoking again, and thus, the long-term quit rate was 20%.

Both short- and long-term smoking cessation rates in the elderly are at least as high as in younger people (7). In a study conducted in Brazil, the cessation rate was

reported as 41.1% in elderly participants, while 35.4% of participants younger than 60 years old were smoke-free at a one-year long-term follow-up. In a Taiwan cohort, the rate of short-term smoking cessation at a three-month follow-up was 48.1%; the long-term results of this study have not been reported (8,9). Moreover, the smoking cessation rate of older smokers was found to be 37.3% at a three-year follow-up in another study (10). In our study, the drop-out rate after the first interview conducted as part of the smoking cessation program was 42%; this is compatible with the results of previous studies that included participants of all ages. Previous results indicated that older age smokers had lower dropout rates compared with younger smokers (11). In a previous study conducted in our clinic, the quit rate after six months was found to be 24% for all ages, but long-term data was not available (12). Our present data showed that older smokers can quit smoking at almost the same rate as the general population as indicated by our historic controls. Considering the long-term results (i.e., two-year follow-up), older smokers seem to be less successful according to our results, but this needs more clarification due to the limited data in the literature. Some studies have demonstrated that older people quit smoking at a higher rate than younger adults (13,14). Kim et al. (4) showed that the rate of quitting smoking in the elderly was two-fold higher than in their younger counterparts. This may be related to the absence of comorbid diseases associated with smoking in younger age groups. However, although elderly smokers are more likely to succeed in quitting than their younger counterparts, attempting a smoking cessation program could be more challenging for elderly people (13). The motivation to quit in the elderly might be low due to several reasons, but success rates are higher once they are admitted to a program. Our cohort of smokers aged >65 years demonstrated an acceptable quit rate both in the short and long term.

Studies have indicated that more chronic illness, being a nondrinker, being married to a non-smoking spouse, living in a household with no other smokers, having smoked for a shorter duration, consuming more cigarettes daily, experiencing depression, being more educated could be predictors of smoking cessation among the elderly (4,15). However, our study, similar to previous studies, showed that all the socio-demographic variables studied as well as smoking duration, the number of pack-years, nicotine dependence and comorbidities were not significantly correlated with quitting smoking (16). Several studies have suggested that compared to men, women may be at greater risk for smoking-related diseases and may also have greater difficulty quitting smoking, which may result from a combination of biological, psychological, and social factors along with reduced access to smoking cessation treatment (17,18). Whitson et al. (16) reported higher rates of quitting in females, while other studies have demonstrated that males were more successful (17). In our study, no statistically significant difference was found between women and men.

Previous studies have found that elderly patients most frequently want to quit smoking due to existing illness or fear of illness (10,19). In our study, the factors that most often motivated attempts to quit smoking were similar to previous data. However, it is noteworthy that sensitivity to environmental harm and a desire to be a good example for their family seems to be prominent motivators in Turkish society, and this could be associated with the cultural and social characteristics of the population. In fact, Gunay et al. (14) found the thought of harming the environment was the most common reason for quitting smoking. Older Turkish smokers were also motivated to succeed in smoking cessation to provide a better role model for their children and grandchildren (14). It is also known that advice and encouragement from doctors and other healthcare professionals increase the motivation to quit smoking, and that older smokers are more sensitive to doctors' advice (20,21). In our study, it was seen that those who applied to the smoking cessation program under the advice of a doctor had a higher rate of quitting in both the short and the long term. Therefore, doctors should ask their elderly patients whether they smoke or not, regardless of their complaint, and elderly patients should be advised by their doctor to quit smoking.

In our study, no difference was observed between the treatment methods in terms of smoking cessation success either at the three-month (short-term) or two-year (long-term) follow-up. In the literature, some studies have found that pharmacotherapy increases smoking cessation success up to four-fold. Behavioral therapy might play an additive role in increasing quit rates (22). In some studies, NRT alone was significantly more effective or equally effective as varenicline and bupropion and would be safe for older smokers (9,23). However, the effectiveness of treatment strategies for smoking cessation in the elderly is controversial. Recently published a real world cohort study, effectiviness of cessation strategies were not superior than unassisted quitting (7). We obtained reasonable results with a 20% long-term quit rate at the two-year follow-up in older smokers; the choice of treatment had no impact on the quit rate. During our follow-up, no side effects that could cause serious mortality or morbidity were observed. While the most common side effects were dry mouth in all groups, colorful dreams and insomnia were observed frequently in varenicline users without any need for drug cessation. Generally, pharmacotherapy for smoking cessation can be used safely in the elderly; however,

specific metabolic characteristics, comorbidities, and multiple medicines use should be taken into consideration (24). Therefore smoking cessation strategies should be tailored and constantly re-evaluated in elderly patients for safe management.

Few studies have explored the limits of recidivism in older smokers by providing long-term follow-up data. A detailed analysis of factors that contribute to recidivism and the development of protocols associated with reducing the relapse rate in elderly people would enhance the success of smoking cessation programs. It is known that the rate of resumption of smoking is 35-40% for all ages in one year, but few studies have been conducted specifically on the elderly (10,25,26). In two cohort studies, the resumption of smoking rate was reported as 1% and 16%, respectively. Both studies provide longitudinal follow-up results without any data on treatment effect and the results are given cross-sectionally (16). Pekel et al. (26) found a relapse rate of 50.1% for all ages without any specific analysis of the elderly population and nicotine dependency was shown to be associated with higher rates of relapse; this is similar to the results of previous studies (27). Esen et al. (28) found relapse in males (12.9%) was significantly lower than in females (20.9%) and no significant difference in relapsing between the mean age groups. In our study, the recidivism rate was 35.7%; no association was found between recidivism and age, gender, duration of smoking, the number of cigarettes smoked per day, the reason for starting smoking, the reason for quitting, and the treatment methods. In previous studies, neither behavioral treatments nor bupropion nor NRT prevented relapses. However, varenicline decreased the rate of relapse (29). In our study, those who resumed smoking most often started again within six months. Therefore, it can be concluded that older patients should be followed periodically for at least six months or a year to avoid relapse risk.

Study Limitations

Our study has some limitations. First, we intentionally prescribed the drugs that were best suited for each participants' individual circumstances. This would create bias in comparing the effect of different medications on smoking cessation success. Second, data on our long-term abstinence rate was collected by phone and based on patient self-reports, which were not confirmed biochemically. Therefore, participants may have misreported whether they quit smoking or not. Finally, our sample size was relatively small, and further large studies are needed to confirm our results. Although this cohort has a limited number of participants, it may contribute to the literature due to the limited data evaluating long-term relapse outcomes in the elderly.

Conclusion

Smoking cessation increases the quality of life by reducing mortality and morbidity in the elderly as well as in all ages. Older smokers are willing to quit smoking as much as young adults and they are successful at least young counterparts. All elderly patients should be questioned about their smoking status, regardless of their complaints, and directed to a smoking cessation programme for appropriate treatment by physicians. Tailored cessation programs and drugs should be program according to their metabolic characteristics, comorbidities, and medication use. It is also extremely important to follow up, question whether they smoke or not and motivate successful quitters regularly to avoid recidivism.

Authorship Contributions

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

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

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The Association Between Erectile Dysfunction and Subclinical Hypothyroidism in Males with Type 2 Diabetes Mellitus

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Abstract

Aim: Overt hypothyroidism is known to affect sexual functions, but data on subclinical hypothyroidism (SCH) are insufficient. We aimed to investigate the relationship between erectile dysfunction (ED) and SCH in men with type 2 Diabetes Mellitus.

Methods: This cross-sectional study included 117 diabetic patients aged between 45-70 years who applied to our outpatient clinic between March and June 2018. Biochemical blood tests and levels of hormones were analyzed. International erectile function index-5 (IIEF-5) questionnaire was applied for the assessment of ED. According to the IIEF-5 questionnaire scores, patients were grouped as severe ED (n=47), moderate ED (n=46), and no ED (n=24). Patients were also grouped according to the level of thyroid-stimulating hormone (TSH) into 3 groups; 0.27-2.49 mU/l (n=58), 2.5-4.2 mU/l (n=33), and >4.2 mU/l (n=26). Statistically significance level was set at 0.05.

Results: 40% of the patients had severe ED and 39% moderate ED, while 21% had no ED. The TSH levels were significantly different between the ED groups ($p<0.001$). A significant negative correlation was found between the IIEF-5 score and the TSH levels ($p<0.001$, $r=-0.453$). The IIEF-5 score, and duration of ED were significantly different between the TSH groups (both; $p<0.001$).

Conclusions: SCH is closely associated with ED in diabetic men. So, we recommend conducting thyroid function tests in diabetic men with ED and screening for ED in men with SCH.

Keywords: Male, thyrotropin, diabetes mellitus, type 2, erectile dysfunction, hypothyroidism

Introduction

Type 2 Diabetes Mellitus (T2DM), characterized by hyperglycemia, is a metabolic disorder resulting from insulin resistance, insufficient insulin secretion, or excessive glucagon secretion (1). There are two main polygenetically formatted basic defects in T2D, which is a progressive disease. These are insulin resistance and insulin secretion defect in beta cells (2).

Erectile dysfunction (ED), which is defined as the persistence of insufficient erection and/or failure to maintain an adequate erection for successful sexual intercourse is more common in men with T2DM (3). Diabetic ED resulting from endothelial dysfunction is known to be associated with cardiovascular diseases, obesity, hypertension, metabolic syndrome, and aging (4-6).

Hyper and hypothyroidism are the main thyroid diseases that have negative effects on the male reproductive system (7). Short-term hypothyroidism has no significant effect in men, but long-term hypothyroidism has been shown to impair male reproductive functions (8). Subclinical hypothyroidism (SCH) is a biochemical definition in which high levels of thyroid stimulating hormone (TSH) are detected when serum-free thyroid hormone levels are normal (9). It has been shown that SCH, which affects many metabolic systems, is also associated with insulin

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resistance (10). The pathophysiology of SCH and ED in these patients is multifactorial (3,9). Both SCH and ED are conditions that are increasingly prevalent, especially in the middle-aged and elderly diabetic male population, and cause metabolic and sexual dysfunction and reduce the quality of life.

In our study, we aimed to evaluate the possible relationship, which has not been evaluated before, between ED that occurs because of endothelial dysfunction and SCH that causes microscopic endothelial oedema and atherosclerosis in patients with T2DM.

Methods

Study Design

This study was designed as a cross-sectional study and was approved by the Local Ethics Committee (University of Health Sciences Turkey, Umraniye Traning and Research University Hospital Ethics Committee date: 23.02.2018; number: B, 10,1, TKH.4.34.H.GP.0.01/20). It was carried out in accordance with the Helsinki Declaration. Written informed consent was obtained from all participants. One hundred twenty-two male patients who applied to the diabetes outpatient clinic of our hospital were included in the study in order of admission according to the power analysis results. The study was conducted with 117 patients, as five patients left voluntarily after being included in the study. Patients between the ages of 45 and 70 years with a diagnosis of T2D, who have not received thyroid replacement therapy, have normal kidney and liver functions, and have a normal lifestyle with regular physical activity were included. T2DM was diagnosed according to the criteria of the American Diabetes Association (11). The exclusion criteria of the study include; type 1 diabetes mellitus, history of treated or untreated thyroid disease, taking medications that may affect thyroid functions (amiodarone, furosemide, glucocorticoids, iodine etc.), malignancy, hyperprolactinemia, major pelvic surgery, prostate cancer, abnormal rectal examination findings, prostatectomy, severe cardiovascular and neurovascular disease, acute cerebrovascular accident, acute or chronic infection, uncontrolled diabetes, major psychiatric diseases, and diabetic neuropathy.

A detailed anamnesis was taken from all patients and physical examinations were performed [weight, height, body mass index (BMI), waist circumference and blood pressure]. BMI was calculated by dividing weight (kg) by height in meters squared (m^2). Duration of diabetes and erectile dysfunction, daily number of cigarettes smoked, and use of antihypertensive drugs and anti-hyperlipidemic drugs of all participants were recorded. The patients were divided into 3 groups according to their TSH levels as 0.27-2.49 mU/l (n=58), 2.5-4.2 mU/l (n=33), and>4.2 mU/l (n=26) (Figure 1).

Evaluation of Erectile Dysfunction

The international index of erectile function (IIEF-5) questionnaire was applied to all patients to evaluate ED (12). IIEF-5 questionnaire scores range between 5 and 25. This questionnaire has been translated into Turkish and its validity and reliability have been previously tested in Turkey (13). Patients were divided into 3 groups according to their IIEF-5 questionnaire scores. The groups were defined as the following: no ED between 21 and 25 (n=24), moderate ED 11 and 20 (n=46) and severe ED between 5 and 10 (n=47) (Figure 1).

Evaluation of Subclinical Hypothyroidism

SCH is defined as the detection of high TSH levels while free thyroid hormone levels in the serum are normal (14). The normal range was defined for serum-free thyroxine (FT4) as 7-18 pg/mL, for serum-free triiodothyronine (FT3) as 2.60-4.80 pg/mL, and for TSH as 0.3-4.0 mU/l. The inter-test variation for FT4, FT3, and TSH was 3.6%, 4.1%, and 4.3%, respectively.

Metabolic Parameters

Several types of tests were used for different parameters: Plasma glucose was measured by enzymatic test; glycosylated hemoglobin A1c (HbA1c) by HPLC method; calcium, phosphate, alanine transaminase, aspartate transaminase, gamma glutamyl transferase alkaline phosphatase, amylase, albumin, total cholesterol, high density lipoprotein (HDL) and triglyceride concentrations by enzymatic colorimetric test; creatinine by Jaffe` method; c-reactive protein by an immunoassay; blood urea nitrogen by spectrophotometer; potassium, sodium and chlorine levels by ion-selective electrode analysis with an Architect plus (Abbott Park, Illinois, USA) instrument. Serum luteinizing hormone (LH), free testosterone, FT4, TSH and prolactin (PRL) levels were measured by using the radioimmunoassay method (Brahms, Hennigsdorf, Germany). The normal range for LH was 1-9 IU/L, for free

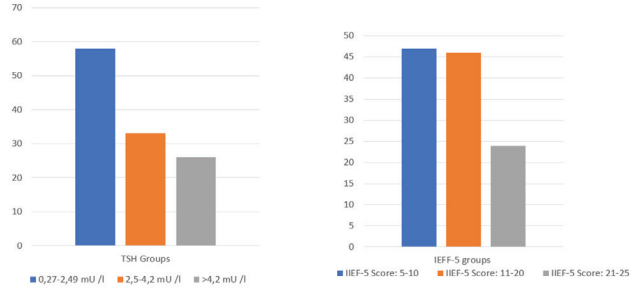

Figure 1. IEFF-5 and TSH groups
IIEF-5: International index of erectile function, TSH: Thyroid-stimulating hormone

testosterone was 8.7-54.7 pg/mL, and for PRL was 60-400 µIU/mL. The inter-test variation for LH, free testosterone, and PRL was 3.9%, 4.7% and 5.1%, respectively. Patients or controls with abnormal levels of these parameters were excluded from the study. Biochemical blood tests were carried out and levels of hormones were measured. Blood samples were taken from the patients on an empty stomach between 08:00 and 10:00 am. Samples were analyzed simultaneously and in the same laboratory.

Statistical Analyses

Descriptive statistics (mean, standard deviation, minimum, median, maximum) were used to define continuous variables. The comparison of two independent and normally distributed continuous variables was made with the student's t-test, and the comparison of two independent variables that are not normally distributed was performed with the Mann-Whitney U test. The Pearson correlation coefficient was calculated to determine the relationship between two normally distributed continuous variables, and the Spearman's rho correlation coefficient to determine the relationship between two non-normally distributed continuous variables. Chi-square (or Fisher's Exact test where appropriate) was used to examine the relationship between categorical variables. The statistical significance level was set at 0.05. The analyses were performed using the MedCalc Statistical Software version 12.7.7 (MedCalc Software Bvba, Ostend, Belgium; http://www.medcalc.org; 2013) Program.

Results

While 40% of the patients participating in the study met the criteria for severe ED and 39% moderate ED, ED was not present in 21% of the patients. SCH was detected in 26 of 117 patients included in the study. The demographic data, anthropometric measurements, clinical and biochemical parameters of the patients are summarized in Table 1. The mean age of the patients was 56±8 years, the IIEF-5 score was 13±6, the TSH level was 2.70±1.76 mU/l, HbA1c level was 8.95%±2.30, and the duration of diabetes was 8±7 years. 49.2% of the patients participating in the study were smokers. In terms of using antidiabetics, while 32.4% of the patients were using only oral antidiabetic, 18.7% were using only insulin. The percentage of patients using both insulin and oral antidiabetic was 48.9%. The patients were first compared according to their IIEF-5 questionnaire scores: 21-25 points, 11-20 points, and 5-10 points (Table 2). SCH was detected in 17 of 47 patients with severe ED. There was a statistically significant difference between the 3 groups in terms of age, TSH, and duration of ED ($p<0.01$ for all). There was no difference between the IIEF-5 score and parameters such as PRL, testosterone, FSH, HbA1c, glucose, diabetes duration, waist circumference and BMI.

In the correlation analysis between the IIEF-5 score and other parameters; there was a weak negative correlation between the IIEF-5 score and hemoglobin, and a modest negative correlation between IIEF-5 age, TSH, and duration of ED (Table 3). In the regression analysis, a 1-unit change in age decreased the IIEF-5 score 0.141-fold, and a 1-unit change in TSH decreased the IIEF-5 score 0.814-fold.

When the patients were divided into 3 groups according to their TSH levels as 0.27-2.49 mU/l, 2.5-4.2 mU/l and >4.2 mU/l, a significant difference was found between

Table 1. Demographic data, anthropometric measurements, clinical and biochemical parameters of the patients

n=117	Mean ± standard deviation
Age (years)	56±8
Duration of diabetes (years)	8±7
DED (years)	5±4
IIEF-5 Score	13±6
TSH (0.3-4.0 mU/l)	2.7±1.76
FT3 (2.60-4.80 pg/mL)	2.68±0.43
FT4 (7-18 pg/mL)	1.07±0.22
Hba1c (%4.7-%5.6)	8.95±2.3
Glucose (70-100 mg/dL)	196±95
Prolactin (60-400 µIU/mL)	8.86±5.21
Free testosterone (3.7-54.7 pg/mL)	4.6±1.68
FSH (1.3-19.3 mIU/m)	5.94±4.27
Luteinizing hormone (1-9 IU/L)	4.63±2.52
Sodium (135-145 mEq/L)	139±3
Potassium (3.5-5.5 mmol/L)	4.55±0.42
Creatinine (<1 mg/dL)	0.96±0.29
Blood urea nitrogen (10-20 mg/dL)	35.7±10.86
AST (15-50 IU/L)	21±9
ALT (10-40 U/L)	30±29
C-reactive protein (<3 mg/L)	1.41±3.22
Hemoglobin (12.4-14.8 g/L)	14.01±1.76
Neutrophil (1.5-8.0 10^3/uL)	4.638±1.506
Triglyceride (<150 mg/dL)	184±137
HDL (40-60 mg/dL)	38.3±9.7
LDL (<130 mg/dL)	147±115
Total cholesterol (<200 mg/dL)	207±49

DED: Duration of erectile dysfunction, IIEF-5: international index of erectile function, TSH: Thyroid-stimulating hormone, FT3: serum-free triiodothyronine FT4: Serum-free thyroxine, HbA1c: Glycolyzed hemoglobin A1c, AST: Aspartate aminotransferase, FSH: Follicular stimulant hormone, ALT: Alanine aminotransferase, HDL: High-density lipoprotein, LDL: Low-density lipoprotein

the groups in terms of age, the IIEF-5 score, duration of ED and hemoglobin levels (Table 4). The IIEF-5 score was statistically lower in the SCH group.

In the correlation analysis between the TSH level and other parameters (Table 3); a weak statistically significant positive correlation was found between the TSH level and age, hemoglobin, and alanine aminotransferase (r= 0.210, p=0.023; r=0.217, p=0.019, r=0.202, p=0.029, respectively).

Discussion

In this study, we investigated a possible relationship between ED and SCH in men with T2DM. We found a clear relationship between ED and SCH in men with the diagnosis of T2DM. In this patient group, we found that while the TSH level increased, the IIEF-5 score decreased and as the IIEF-5 score decreased the TSH level increased.

ED, which is defined as the persistence and/or failure of sufficient penile erection for successful sexual intercourse, is more common in men with a diagnosis of T2DM (15,16). In epidemiological studies, it has been shown that approximately 30 to 90% of men with T2DM have ED (17,18). In the Massachusetts Male Aging Study, ED was shown to be 3 times higher in diabetic men compared to non-diabetics (19). The prevalence of severe ED in diabetic patients included in our study was 40% and moderate ED was 39%. Our study had a higher prevalence of diabetic ED than previously reported studies. This may be due to the differences in demographic and clinical characteristics of the patients in this study from patients in previous studies. The pathophysiology of ED is multifactorial. Mainly, hypogonadism, diabetic neuropathy and endothelial dysfunction are blamed (20). However, endocrine disorders can also cause ED. Low serum testosterone,

Table 2. Comparison of parameters according to IIEF-5 score

	IIEF-5 Score: 5-10 (n=47) Avr ± SD	IIEF-5 Score: 11-20 (n=46) Avr ± SD	IIEF-5 Score: 21-25 (n=24) Avr ± SD	p
Age (years)	59.1±7.6	56.1±7.2	51.5±5.9	<0.001*
Duration of diabetes (years)	9.2±8.4	8.4±7.1	6.1±4.7	0.852
DED (years)	6.9±4.1	4.9±3.6	0	<0.001*
TSH (0.3-4.0 mU/L)	3.3±1.4	2.5±1.9	1.7±1.4	<0.001*
FT3 (2.60-4.80 pg/mL)	2.7±0.4	2.7±0.4	2.7±0.4	0.435
FT4 (7-18 pg/mL)	1.1±0.1	1.1±0.3	1.02±0.2	0.406
Hba1c (%4.7-%5.6)	8.9±2.1	8.6±2.3	9.4±2.7	0.424
Glucose (70-100 mg/dL)	198.7±101.1	192.3±84.7	197.6±109	0.915
Prolactin (60-400 µIU/mL)	9.4±6.1	8.4±4.7	8.7±4.4	0.693
Free testosterone (3.7-54.7 pg/mL)	4.8±1.9	4.7±1.5	4.01±1.2	0.241
FSH (1.3-19.3 mlU/mL)	6.5±4.06	5.4±4.8	5.9±3.4	0.706
Luteinizing hormone (1-9 IU/L)	4.7±2.2	4.6±2.9	4.7±2.2	0.84
Sodium (135-145 mEq/L)	138.9±2.6	139.8±2.9	138.4±4.2	0.091
Potassium (3.5-5.5 mmol/L)	4.5±0.4	4.6±0.3	4.6±0.5	0.829
Creatinine (<1 mg/dL)	1.007±0.4	0.9±0.2	0.9±0.2	0.366
Blood urea nitrogen (10-20 mg/dL)	35.8±9.7	36.05±10.6	35.1±13.7	0.974
AST (15-50 IU/L)	21.04±6.5	20.3±8.7	22.8±7.6	0.159
ALT (10-40 U/L)	27.6+16.5	28.8+22.4	35.9+51.5	0.168
C-reactive protein (<3mg/L)	1.36±1.7	1.1±1.1	0.8±0.8	0.222
Hemoglobin (12.4-14.8 g/l)	14.4±1.2	14.2±1.6	13.3±1.7	0.117
Neutrophil (1.5-8.0 10^3/uL)	4.8±1.7	4.5±1.2	4.7±1.6	0.897
Triglyceride (<150 mg/dL)	188.4±178.3	163.3±92.1	206.9±113.1	0.404
HDL cholesterol (40-60 mg/dL)	40.1±10.2	37.9±8.5	36.4±9.6	0.53
LDL cholesterol (<130 mg/dL)	156.3±166.3	129.1±37.6	166.2±93.2	0.22
Total cholesterol (<200 mg/dL)	208.9±59.9	200.8±44.8	215.2±35.03	0.613

Avr: Average, SD: Standard deviation, DED: Duration of erectile dysfunction, IIEF-5: International index of erectile function, TSH: Thyroid-stimulating hormone, FT3: Serum-free triiodothyronine FT4: Serum-free thyroxine, HbA1c: Glycolyzed hemoglobin A1c, AST: Aspartate Aminotransferase, FSH: Follicular stimulant hormone, ALT: Alanine aminotransferase, HDL: High density lipoprotein, LDL: Low density lipoprotein

*Kruskal-Wallis test was used. There was a statistically significant difference between the IEFF-5 groups in terms of age, TSH, and duration of ED

Table 3. Correlation of IIEF-5 score and the TSH level with all parameters

		IIEF-5 score	TSH level
Age (years)	r	-0.431*	0.21*
	p	<0.001	0.023
Duration of Diabetes (years)	r	-0.079	0.111
	p	0.398	0.232
DED (years)	r	-0.642*	0.404*
	p	<0.001	<0.001
IIEF-5 Score	r	1	-0.453*
	p		<0.001
TSH (0.3-4.0 mU/l)	r	-0.453*	1
	p	<0.001	
FT3 (2.60-4.80 pg/mL)	r	-0.028	-0.002
	p	0.768	0.986
FT4 (7-18 pg/mL)	R	-0.067	-0.014
	p	0.476	0.88
Hba1c (%4.7-%5.6)	r	-0.027	0.017
	p	0.775	0.856
Glucose (70-100 mg/dL)	r	-0.064	0.139
	p	0.493	0.136
Prolactin (60-400 µIU/mL)	r	-0.054	0.019
	p	0.564	0.839
Free testosterone (3.7-54.7 pg/mL)	r	-0.164	0.092
	p	0.077	0.324
FSH (1.3-19.3 mlU/mL)	r	-0.015	-0.05
	p	0.874	0.595
Luteinizing Hormone (1-9 IU/L)	r	0.033	-0.146
	p	0.721	0.117
Sodium (135-145 mEq/L)	r	0.022	-0.088
	p	0.811	0.347
Potassium (3.5-5.5 mmol/L)	r	0.039	-0.119
	p	0.678	0.202
Creatinin (<1 mg/dL)	r	-0.144	0.056
	p	0.121	0.545
Blood Urea Nitrogen (10-20 mg/dL)	r	-0.035	-0.092
	p	0.708	0.326
AST (15-50 IU/L)	r	0.019	-0.017
	p	0.836	0.853
ALT (10-40 U/L)	r	-0.061	0.202
	p	0.516	0.029
C-reactive protein (<3 mg/L)	r	-0.11	0.148
	p	0.237	0.11
Hemoglobin (12.4-14.8 g/l)	r	-0.231*	0.217*
	p	0.012	0.019
Neutrophil (1.5-8.0 103/uL)	r	0.052	-0.088
	p	0.576	0.348
Triglyceride (<150 mg/dL)	r	0.078	0.115
	p	0.405	0.216
HDL cholesterol (40-60 mg/dL)	r	-0.102	-0.121
	p	0.274	0.193
LDL Cholesterol (<130 mg/dL)	r	0.085	-0.055
	p	0.363	0.555
Total Cholesterol (<200 mg/dL)	r	0.095	0.019
	p	0.306	0.841

DED: Duration of erectile dysfunction, IIEF-5: International index of erectile function, TSH: Thyroid-stimulating hormone, FT3: Serum-free triiodothyronine FT4: Serum-free thyroxine, HbA1c: Glycolyzed hemoglobin A1c, AST: Aspartate aminotransferase, FSH: Follicular stimulant hormone, ALT: Alanine aminotransferase, HDL: High-density lipoprotein, LDL: Low-density lipoprotein
*Spearman's rho p<0.05 (For the correlation between two continuous variables that are not normally distributed)

hyperprolactinemia, and hypothyroidism were the most common endocrine abnormalities in patients with sexual dysfunction (21).

In general population screenings, it has been shown that the prevalence of SCH varies between 4% and 10% (9,10,22). The prevalence of SCH in the patients included in our study was 22.2%, and it was slightly higher than the general population. We think that this is since the patients included in our study were diabetic and SCH was a common endocrine disorder in diabetic patients. Although SCH is generally an asymptomatic condition, it is known to affect many organs in the body (23). SCH has been shown to be involved in the pathophysiology of atherosclerosis and cardiovascular diseases by causing endothelial dysfunction (23).

SCH and ED, both of which have multiple mechanisms in their pathogenesis, are increasingly prevalent especially in middle-aged and elderly diabetic male populations and are conditions that decrease physical function, sexual life quality, and quality of life. SCH and ED constitute an important social problem both in our country and in the world, due to their treatment process, costs, complications they cause, and their increasing prevalence in recent years. The association between SCH and T2DM is well known (24). However, the relationship of SCH to the microvascular complications of diabetes is not clear. Various mechanisms may play a role in the relationship between thyroid dysfunction and microvascular complications in diabetes. First, it has been shown that insulin resistance is associated with clinical and SCH (24). One possible mechanism may be defective fibrinolysis or impaired vasodilation associated with insulin resistance (24). There are several studies evaluating the relationship between thyroid function and microvascular complications such as diabetic retinopathy, neuropathy, and nephropathy in patients with T2DMM, but the results are controversial (25-27). To the best of our knowledge, there are no studies

investigating the relationship between SCH and diabetic ED to date. Our results showed that SCH was associated with diabetic ED in 117 type 2 diabetic patients.

Free testosterone levels drop by approximately 40% in men between the ages of 25 and 75 (3). This condition can cause ED in this age group. However, in our study, we could not find a relationship between SCH and androgenic hormones such as testosterone. This situation makes us think that SCH causes hormone-independent ED in this patient group. At this point, as shown in our study, the IIEF-5 score in the SCH group was lower than the normal group. Therefore, we believe that there are different common pathways in the pathophysiology of SCH and ED in diabetic men.

Thyroid dysfunction can affect body growth metabolism and the synthesis and secretion of sex steroids (28). Numerous clinical studies have shown that both hyperthyroidism and hypothyroidism cause sexual and reproductive problems (28,29). Additionally, it is known that thyroid hormone level affects Leydig cells, Sertoli cells and spermatogenesis. It has been shown that the risk of developing ED is higher in men with hypothyroidism (29). Our study revealed that this condition occurs when SCH is present.

It has been shown that SCH suppresses antioxidative capacity by reducing paraoxonase and arylesterase activities (30). Increased oxidative stress may play an important role in the pathogenesis of diabetes-related

Table 4. Comparison of parameters according to the TSH levels

	TSH level: 0.27-2.49 mU/l (n=58)	TSH level: 2.5-4.2 mU/L (n=33)	TSH level >4.2 mU/L (n=26)	p
	Avr ± SD	Avr ± SD	Avr ± SD	
Age (years)	54.4±6.9	59.4±8.1	57.3±7.7	0.015*
Duration of diabetes (years)	7.2±5.8	9.8±8.4	8.6±8.6	0.384
DED (years)	2.8±3.5	7.7±4.2	5.2±3.8	<0.001*
IIEF5 score	16.2±5.7	10.1±5.8	9.6±5.8	<0.001*
FT3 (2.60-4.80 pg/mL)	2.7±0.4	2.7±0.4	2.6±0.5	0.932
FT4 (7-18 pg mL)	1.1±0.2	1.02±0.1	1.1±0.2	0.222
Hba1c (%4.7-%5.6)	8.9±2.4	9±2.2	9.1±2.3	0.821
Glucose (70-100 mg/dL)	186.3±92.7	212.1±112.1	198.6±81.1	0.422
Prolactin (60-400 µIU/mL)	8.7±4.8	9.1±3.8	8.9±7.5	0.301
Free testosterone (3.7-54.7 pg/mL)	4.4±1.7	5.1±1.9	4.5±1.2	0.311
FSH (1.3-19.3 mIU/mL)	5.3±2.8	5.9±2.8	6.3±5.1	0.434
Luteinizing hormone (1-9 IU/L)	4.6±1.9	4.7±2.5	3.9±1.9	0.305
Sodium (135-145 mEq/L)	139.2±3.5	139.1±2.8	139.04±2.9	0.544
Potassium (3.5-5.5 mmol/L)	4.6±0.4	4.6±0.4	4.4±0.3	0.254
Creatinin (<1 mg/dL)	0.9±0.2	1.08±0.4	0.9±0.1	0.461
Blood urea nitrogen (10-20 mg/dL)	35.8±11.2	36.4±12.1	34±8.7	0.769
AST (15-50 IU/L)	21.2±8.7	21.4±6.9	22.1±10.8	0.924
ALT (10-40 U/L)	29.3±37.6	31.1±17.9	31.4±20.5	0.212
C-reactive protein (<3 mg/L)	1.1±1.2	1.01±1.4	2.6±6.3	0.299
Hemoglobin (12.4-14.8 g/L)	13.7±1.7	14.4±1.4	14.2±2.2	0.036*
Neutrophil (1.5-8.0 103/ul)	4.6±1.3	5.1±1.9	4.1±1.05	0.078
Triglyceride (<150 mg/dL)	170.3±97.1	210.5±214.6	177.3±75.9	0.522
HDL cholesterol (40-60 mg/dL)	39±9	39.7±11.3	35.1±8.6	0.171
LDL cholesterol (<130 mg/dL)	141.9±58.6	177.8±198.5	122.04±37.8	0.113
Total cholesterol (<200 mg/dL)	205.9±39.3	219.2±66.8	196.4±41.5	0.23

Avr: Average, SD: Standard deviation, DED: Duration of erectile dysfunction, IIEF-5: International index of erectile function, TSH: Thyroid-stimulating hormone, FT3: Serum-free triiodothyronine FT4: Serum-free thyroxine, HbA1c: Glycolyzed hemoglobin A1c, AST: Aspartate aminotransferase, FSH: Follicular stimulant hormone, ALT: Alanine aminotransferase, HDL: High density lipoprotein, LDL: Low density lipoprotein

*Kruskal-Wallis test was used. There was a significant difference between the TSH groups in terms of age, the IIEF-5 score, duration of erectile dysfunction and haemoglobin levels

complications. SCH can also cause endothelial dysfunction by causing thickening of the basement membrane (30). In our study, we believe that SCH causes ED by affecting cardiac function, peripheral vascular resistance, endothelial function, and renal hemodynamics.

In addition, it has been proven that high TSH concentration causes endothelial dysfunction by reducing the formation and availability of nitric oxide (NO) (16). It is well known that NO plays an important role in the relaxation of the corporal smooth muscle and vascular system to initiate and maintain erection (3). This pathway may be one of the possible mechanisms between SCH and ED in our study.

Study Limitations

Our study had some limitations. First, our study was single center cross-sectional analysis. We could not establish a causal relationship between SCH and diabetic ED. Second, thyroid function was evaluated at a single time point. Third, the definition of ED was based on a one-time measurement. Despite all these limitations, to the best of our knowledge, there is no study in the literature evaluating the relationship between ED and SCH in men with T2DM so that the present study is valuable for being the first study in the literature on this subject.

Conclusion

This study revealed that SCH is closely associated to ED in diabetic men. We recommend conducting thyroid function tests in diabetic men with ED and screening for ED in men with SCH. However, large randomized controlled clinical studies are needed to determine whether there is a true relationship between SCH and ED in diabetic patients.

Authorship Contributions

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

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Original Article

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The Efficacy of Combination Regime in *Helicobacter Pylori* Eradication: A Cross-Sectional Study from an Experienced Endoscopy Center

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Abstract

Aim: The success of antibiotics used for eradication in the treatment of *Helicobacter pylori* (Hp)-positive gastritis is controversial. This study aims to evaluate the post-treatment eradication success/failure of Hp-positive gastritis cases, discuss antibiotic resistance, and what we can do additionally.

Methods: The data of 1,471 patients who underwent upper gastrointestinal endoscopy between January 1 and December 31, 2019, were retrospectively evaluated through the hospital digital recording system. Of these, data of 126 patients who have been diagnosed with HP-positive gastritis and treated with a trio of clarithromycin, amoxicillin, and omeprazole were analyzed. Initial and control endoscopic pathologies and Hp-positivity were compared.

Results: In the control of 126 patients, 87 (69%) patients had a normal endoscopic appearance, but only 46 (36.5%) patients were normal in the histopathological examinations of biopsies. Regardless of the severity, complete eradication of Hp was possible in 31 (24.6%) patients. There was no statistically significant difference in Hp detection rates after treatment (p=0.719 for 1+, p=0.583 for 2+, p for 3+)=0.980).

Conclusion: In gastritis cases, Hp positivity continues despite the treatment, although the pathological severity changes. It is appropriate to discuss the effectiveness of routine treatments and to take culture/antibiogram, even bismuth including preparations to the forefront.

Keywords: *Helicobacter pylori*, gastritis, antibiogram

Introduction

Helicobacter pylori (Hp) is a gram-negative, spiral-shaped flagellated bacterium that causes gastroduodenal inflammation. Carriers are observed in half of the world population and 80% in developing countries (1,2). In Turkey, in 2013, the national prevalence study with the urea breath test, the ratio was determined as 81.6% (3). It is most commonly transmitted by oral-oral or fecal-oral route; In societies with low socioeconomic status and crowded families, it has been reported that it can be transmitted by Hp contaminated water and foods, even kissing and droplet infection (4).

The stomach was considered sterile due to its acidic environment. However, the detection of Campylobacter-like microorganisms in the stomach by Mashall et al. (5) in 1983, and then proving that this bacterium causes gastritis when taken orally by Marshall et al.(5) in 1985, the use of antibiotics in treatments came to the fore.

Inflammation and superficial epithelial damage are the main pathological mechanisms in gastritis; depending on the degree and duration, edema, hyperemia, erosion, regeneration, ulcer, cicatrix, and atrophy can be seen-acute or chronic gastritis patients present with epigastric pain, burning, dyspepsia, nausea, and vomiting. The definitive diagnosis can be made by showing the bacteria by endoscopy and biopsy and by breath urea test, antibody tests, and stool antigen tests (6).

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Standardized treatment in gastritis is antibiotics against Hp and acid-reducing proton pump inhibitors. Hp eradication is the basis of the treatment. Many people are asymptomatic carriers; changes in Hp intensity in lesions, antibiotic resistance, and frequent recurrence of the disease point to the difficulty in eradication and studies carried out on these handicaps and how to treat which patients.

This study aims to evaluate the post-treatment eradication success/failure of Hp positive gastritis cases, discuss antibiotic resistance, and what we can do additionally.

Methods

This study was conducted with approval from the Ethics Committee of the University of Health Sciences, Haseki Training and Research Hospital (ref no: 2020/294 date: 29.07.2020). Informed consent forms were obtained from all patients.

The study was conducted at the Surgical Endoscopy unit of Haseki Training and Research Hospital with patients diagnosed with antral gastritis, pangastritis, erosive gastritis, gastric ulcers between January 1 and December 31, 2019. One thousand four hundred seventy-one patients were retrospectively evaluated through the hospital digital recording system. Those diagnosed with bulbitis, duodenitis, alkaline reflux, gastric polyp, and gastric cancer were excluded from the study. Patients with a diagnosis of bulbitis, duodenitis, and alkaline reflux were not included in the study, as it would impair the study group's homogenization due to the bile effect. One hundred twenty-six patients were Hp positive and were treated with combined drugs containing clarithromycin, amoxicillin, and omeprazole for 14 days and then used omeprazole for 28 days, and whose pathological samples were collected with control endoscopies within the first three months after the end of treatment, were studied. The patients received triple clarithromycin therapy, which was recommended as the first-line treatment of Hp infection by the American College of Gastroenterology (7). Hp severity was evaluated with the updated Sydney classification in endoscopic biopsies, and it was expressed as "+" mild, "++" moderate, and "+++" severe Hp infection (8).

Statistical Analysis

Data analysis was performed by the SPSS 15.0 for Windows program. Categorical variables were presented as numbers and percentages, while numerical variables were presented as mean, standard deviation, minimum and maximum values. The chi-square test and McNemar test were used in dependent groups and independent groups, respectively. Analysis results with a $p<0.05$ were accepted as significant.

Results

In 2019, 1,471 patients with gastritis and related diagnoses were scanned, and 126 (8.6%) patients were included in the study. Seventy-seven (61.1%) were female, and 49 (38.9%) were male. The mean age was 45.2 years, ranging from 21 to 80 years.

Hp positivity change in endoscopic and pathological diagnoses before and after the treatment is in Table 1. In 87 (69%) of 126 patients diagnosed with antral gastritis, erosive gastritis, pangastritis, and ulcer, endoscopic appearance was normal after treatment. However, pathologically, only 46 (36.5%) patients were regular; the other patients had acute gastritis, chronic gastritis, erosion, and ulceration. Complete eradication of Hp regardless of the severity was possible in 31 (24.6%) patients.

Although 87% of the patients had a regular appearance in the endoscopic evaluation after the treatment, only 46% of the patients were evaluated as regular in the pathological examination. On the other hand, success in Hp eradication remained at 24.6%.

Endoscopic diagnoses, pathological diagnoses, and the severity of Hp are in Table 2-4, compared with post-treatment in the context of the same variables. The statistical relationship of Hp detection before and after treatment according to the diagnosis groups is shown in Table 5.

Although Hp eradication was achieved in 31 patients after treatment and was statistically significant, Hp positivity continues in 95 patients.

There was no statistically significant difference in Hp eradication rates according to pre-treatment diagnosis groups (Table 6) (0.719 p=0.583 p=0.980).

Table 1. Pre and post-treatment diagnoses results

		Before treatment	After treatment
		n (%)	n (%)
Endoscopic diagnosis	Normal view	0 (0.0)	87 (69.0)
	Antral gastritis	84 (66.7)	34 (27.0)
	Erosive gastritis	14 (11.1)	1 (0.8)
	Pangastritis	17 (13.5)	4 (3.2)
	Ulceration	11 (8.7)	0 (0.0)
Pathological diagnosis	Normal mucosa	0 (0.0)	46 (36.5)
	Acute gastritis	42 (33.3)	34 (27.0)
	Chronic gastritis	59 (46.8)	45 (35.7)
	Chronic gastritis with erosion and ulceration	25 (19.8)	1 (0.8)
Pathological *Helicobacter pylori* intensity	Hp negative	-	31 (24.6)
	1 +	37 (29.4)	55 (43.7)
	2 +	51 (40.5)	31 (24.6)
	3 +	38 (30.2)	9 (7.1)

Table 2. Comparative evaluation of endoscopic diagnoses before and after treatment

		Before treatment			
		Antral gastritis	Erosive gastritis	Pangastritis	Ulceration
		n (%)	n (%)	n (%)	n (%)
After treatment	Normal view	73 (86.9)	6 (42.9)	4 (23.5)	4 (36.4)
	Antral gastritis	11 (13.1)	7 (50.0)	11 (64.7)	5 (45.5)
	Erosive gastritis	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)
	Pangastritis	0 (0.0)	1 (7.1)	2 (11.8)	1 (9.1)

Table 3. Comparative evaluation of pathological diagnoses before and after treatment

		Before treatment		
		Acute gastritis	Chronic gastritis	Chronic gastritis with erosion and ulceration
		n (%)	n (%)	n (%)
After treatment	Normal mucosa	26 (61.9)	20 (33.9)	0 (0.0)
	Acute gastritis	14 (33.3)	20 (33.9)	0 (0.0)
	Chronic gastritis	2 (4.8)	19 (32.2)	24 (96.0)
	Chronic gastritis with erosion and ulceration	0 (0.0)	0 (0.0)	1 (4.0)

Table 4. Comparative evaluation of *Helicobacter pylori* intensity before and after treatment

		Before treatment		
		+	++	+++
		n (%)	n (%)	n (%)
After treatment	No colonization	9 (24.3)	13 (25.5)	9 (23.7)
	+	24 (64.9)	25 (49.0)	6 (15.8)
	+ +	4 (10.8)	11 (21.6)	16 (42.1)
	+++	0 (0.0)	2 (3.9)	7 (18.4)

When we evaluated which group was more successful in Hp eradication, it was seen that there was no significant difference between the groups.

Discussion

Even if Hp is asymptomatic, it can cause gastritis, gastric ulcer, gastric cancer, mucosa-related lymphoid tissue lymphomas (9,10). It is also associated with extra-gastric neurological, hematological, dermatological, cardiovascular, ocular, hepatobiliary, metabolic, and allergic diseases and is studied in the literature (11). Treatment is important. There are problems in its eradication due to antibiotic resistance globally and in our country (6,12-14).

Table 5. Post-treatment Hp positivity rates

		Before treatment Hp positivity	After treatment Hp		
			Negative	Positive	
		n=126	n (%)	n (%)	p
Total		n=126	31 (24.6)	95 (75.4)	<0.001*
Endoscopic diagnoses before treatment	Antral gastritis	n=84	22 (26.2)	62 (73.8)	<0.001*
	Erosive gastritis	n=14	4 (28.6)	10 (71.4)	0.125
	Pangastrit	n=17	4 (23.5)	13 (76.5)	0.125
	Ulceration	n=11	1 (9.1)	10 (90.9)	1.000
Pathological diagnoses before treatment	Acute gastritis	n=42	9 (21.4)	33 (78.6)	0.002*
	Chronic gastritis	n=59	17 (28.8)	42 (71.2)	<0.001*
	Chronic gastritis with erosion and ulceration	n=25	5 (20.0)	20 (80.0)	0.063
Hp intensity Before treatment	+	n=37	9 (24.3)	28 (75.7)	0.004*
	+ +	n=51	13 (25.5)	38 (74.5)	<0.001*
	+ + +	n=38	9 (23.7)	29 (76.3)	0.004*

*McNemar test, Hp: *Helicobacter pylori*

Table 6. Hp eradication rates after treatment in pre-treatment diagnosis groups

		Post-treatment Hp eradication	
		n (%)	p*
Endoscopic diagnoses before treatment	Antral gastritis Erosive gastritis Pangastrit	22 (26.2) 4 (28.6) 4 (23.5)	0.719
	Ulceration	1 (9.1)	
Pathological diagnoses before treatment	Acute gastritis Chronic gastritis	9 (21.4) 17 (28.8)	0.583
	Chronic gastritis with erosion and ulceration	5 (20.0)	
Hp intensity before treatment	+ + +	9 (24.3) 13 (25.5)	0.980
	+ + +	9 (23.7)	

*chi-square test, Hp: *Helicobacter pylori*

In our study, Hp was detected in 95 (75.4%) patients in control endoscopy and biopsies performed within the first three months after treatment of 126 patients treated for Hp positivity. Although the normal endoscopic appearance was obtained in 87 (69%) patients, the presence of Hp continued with a decrease in its severity. Our results were

found to be + in 55 (43.7%) patients, + + in 31 (24.6%) patients, and in 9 (7.1%) + + + is reflected in the form of detection. In all patients before treatment (p<0.001), patients with antral gastritis before treatment (p<0.001), patients with a pathological diagnosis of acute (p=0.001) and chronic (p<0.001) gastritis, in all groups regarding Hp severity (1+ for p=0.004, for 2+ p<0.001, for 3+ p=0.004) Hp eradication was found to be statistically significant in terms of its increase and decrease (McNemar test). There was no statistically significant difference in Hp eradication rates in the endoscopic diagnosis, pathological diagnosis and pathological Hp intensity groups before treatment (Table 6) (p=0.719 p=0.583 p=0.980).

Our results show the failure of the trio of clarithromycin, amoxicillin, and omeprazole which are widely used in Turkey. Most patients stop treatment when they become asymptomatic and do not come for control. Asymptomatic, untreated Hp infection continues. The success of eradication with these handicaps is controversial.

Although antibiotic resistance varies from country to country, in Hp eradication, treatment regimens are changing considering resistance, and new protocols are recommended. Culture and antibiogram stand out as the gold standard. To give an example of regimen changes suggested in the literature; the addition of bismuth to triple therapy was included in the Maastricht consensus in 1997, in regions where clarithromycin resistance is above 15-20%, and quinolone resistance is below 10%, levofloxacin instead of clarithromycin in triple therapy, adding metronidazole to triple therapy or hybrid therapies are recommended (7,15,16).

In a study conducted in Turkey, 18.2% clarithromycin, 45.5% metronidazole, 18.2% levofloxacin resistance were detected, while amoxicillin resistance was not observed (14).

Methods such as the detection of Hp in endoscopic sampling, rapid urease test, polymerase chain reaction, urea breath test, specific antigen detection in stool samples are used to diagnose Hp. However, none of these methods provide information about the antibiotic resistance that determines the treatment result. Since the culture antibiogram is now considered the gold standard, it is appropriate to shift the investigations in this direction (17). In the literature, culture positivity is around 30%, sensitivity is 45-89%, and specificity is 97-100%. Utku et al. (18) in a study conducted in Turkey they reported the culture positivity was 38.3%, sensitivity 60.3%, and specificity 100%. The aim of this article, which we wrote as a surgical team, is not to discuss the sensitivity and specificity of various tests or the effective methods of culture/antibiogram but to raise awareness. The difficulty of eradication of Hp, which can be isolated even from 6000-year-old mummies since ancient times should also be understood (19). The addition of bismuth salts to classical treatment is on the agenda again with successful results in resistant cases (20).

Study Limitations

The study was conducted by surgeons based on the data of the surgical endoscopy unit, and it does not include detailed gastroenterological evaluations. One of the limitations of the study is that it is retrospective. Our study once again reveals the failure of the triple regimen, which is widely used in our country in primary care. The data of our study can contribute to the literature in terms of reviewing traditional treatment regimens and using antibiotic susceptibility tests more widely.

Conclusion

In cases of gastritis, Hp positivity continues despite treatment, although the pathological severity changes. It would be appropriate to discuss the effectiveness of routine treatments and to emphasize the culture/antibiogram. In fact, it would be appropriate to use preparations containing bismuth more prominently into account.

Authorship Contributions

Concept: M.A., A.K.K., M.C., Design: A.H., A.K., Data Collection or Processing: M.S.D., O.M.A., Analysis or Interpretation: D.Y., M.S.D., O.M.A., Literature Search: M.C., D.Y., Writing: M.C., M.A.

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Original Article

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Detailed Analysis of Rare Causes of Secondary Duodenitis in Patients Diagnosed with Endoscopic Duodenitis: A Cross-Sectional Study from a Tertiary Referral Center

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Abstract

Aim: Findings compatible with duodenitis are not rare in patients who have undergone endoscopy. Although the final histopathological diagnoses in most of these cases are chronic primary non-specific duodenitis, less commonly, there are also diseases that cause secondary duodenitis, too. We aimed to examine the causes of secondary duodenitis in our patients in detail.

Methods: Upper gastrointestinal endoscopic procedures of 3.776 adult patients performed for various reasons in our endoscopy unit (Istanbul Yeni Yuzyıl University, Medical Faculty, Gaziosmanpasa Hospital, Department of Gastroenterology) between 2017 and 2021 were reviewed retrospectively by scanning the hospital automation system. The demographic, clinical, endoscopic, and histopathological features of the patients who underwent endoscopic biopsies with a prediagnosis of duodenitis were examined.

Results: Biopsies with a pre-diagnosis of duodenitis were performed on 231 (6.12%) of 3776 adult patients during the endoscopic procedures. The mean age of the patients was 42.03 years (18-89 years); 42.4% were males and 57.6% were female. The most common symptoms/signs of the patients were dyspepsia (45%), chronic diarrhea (38%), and iron deficiency anemia (34%), respectively. The two main histological diagnoses were primary non-specific duodenitis (58.44%) and celiac disease (29.87%). In the remaining cases, various causes of secondary duodenitis such as graft versus host disease, eosinophilic duodenitis, Brunner's gland adenoma, polyps, giardiasis, Crohn's disease, cytomegalovirus duodenitis, and amyloidosis were detected.

Conclusion: Although the most common etiology of duodenitis we encountered in our study was primary non-specific duodenitis, a considerable number, and variety of other etiologies of secondary duodenitis were also detected. Among many causes of secondary duodenitis, celiac disease should be kept in mind especially in female patients with younger age, presenting with dyspepsia.

Keywords: Duodenitis, endoscopy, histopathology

Introduction

Duodenitis occurs as a result of epithelial damage and inflammation of the duodenal mucosa. The pathognomonic diagnosis of duodenitis is achieved through histopathological examination of biopsies obtained from abnormal appearing duodenal mucosa samples composed of uniform, multifocal, superficial duodenal erosions, mucosal edema, and hyperemia, during endoscopic examination. Duodenitis is divided into acute and chronic in terms of clinical course. Chronic duodenitis is divided into primary (non-specific duodenitis: NSD) and secondary (specific) duodenitis. Histological findings with duodenal mucosal damage as a result of prolonged exposure to increased gastric secretion are also called peptic duodenitis or chronic NSD. Duodenitis may also occur secondary to increased acid secretion, increased bile reflux, viral, bacterial, fungal, and parasitic infections, chemical agents, radiation, drugs i.e. non-steroidal anti-inflammatory drugs, alcohol, allergens, and autoimmune diseases (1-3).

In some studies, it was stated that gastric pathologies caused by *H. pylori* may induce NSD. In another study, it

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was reported that intestinal metaplasia may contribute to NSD. In another study, the authors stated that duodenal ulcer disease may be one of the clinical stages of NSD (4-7). Non-peptic secondary duodenitis occurs in the younger age group with distal duodenal involvement as panduodenitis or postbulbitis (8,9). There are many different local or systemic causes of secondary duodenitis such as inflammatory bowel diseases (IBD), celiac disease (CD; gluten enteropathy), eosinophilic gastroenteritis, acute and chronic infections [tuberculosis, giardiasis, cytomegalovirus (CMV), HSV, HIV, candidiasis etc], vasculitis (Behcet's disease, Henoch-Schönlein purpura etc) and autoimmune diseases. Among these, CD is the most common cause of secondary duodenitis.

We investigated the histopathological diagnoses of the biopsies taken from abnormal mucosal areas in the second part of the duodenum, which were endoscopically diagnosed as duodenitis, with giving more emphasis on secondary nonspecific causes of duodenitis.

In clinical practice, NSD and CD come to mind when endoscopic duodenitis is mentioned. However, there are many secondary causes of duodenitis other than CD. We found that few studies have been conducted on secondary causes of duodenitis in the literature. Therefore, in the present study, we aimed to investigate the causes of secondary duodenitis in our clinic.

Methods

Study Design

The present study was approved by the Istanbul Yeni Yuzyil University of Medicinal Ethics Committee (number; 13.08.2020/031) and conducted between from September 2017 to February 2021 in accordance with the Declaration of Helsinki. A consent form was filled out by all participants.

We investigated upper gastrointestinal endoscopic (UGE) procedures of 3.776 adult patients performed for various reasons in the endoscopy unit of a tertiary referral center (Istanbul Yeni Yuzyil University, Medical Faculty, Gaziosmanpasa Hospital, Department of Gastroenterology) between 2017 and 2021 were retrospectively screened.

The demographic data, main complaints, UGE findings, and histopathological diagnoses were obtained from the hospital automation system.

The patients that had biopsies taken from the second part of the duodenum with a pre-diagnosis of duodenitis were included. Lesions in the duodenal bulbus and stomach such as ulcer, erosion, gastritis, bulbitis were excluded and only the lesions localized in the second part of the duodenum were examined.

Endoscopic and Histopathologic Procedure

UGE examinations were performed with a Fujinon EG 590 WR video gastroscopy device (Japan) and forceps-guided biopsy. For histological examination, biopsy specimens fixed with 10% formalin were obtained after routine tissue processing. Serial sections of 4 µm were prepared from the specimens embedded in paraffin blocks. Hematoxylin & Eosin stain was used for histological examination of the deparaffinized tissue sections. Anti-CD3 immunohistochemical antibody was applied to biopsy materials to detect intraepithelial lymphocytosis, (DAKO, Clone F.7.38, USA). In histopathological evaluation, biopsies were evaluated mainly in terms of intraepithelial lymphocytosis (>40 lymphocytes per 100 enterocytes), crypt hyperplasia, and villous atrophy and divided into 5 different categories according to the classification defined by Marsh and modified by Oberhuber (Modified Marsh Classification) (10).

Statistical Analysis

The research data were worked up into Excel sheets. Statistical Package for the Social Sciences Version 23.0 (SPSS 23.0 Chicago, USA) for Windows was used for statistical analysis. The defining statistics are presented as numbers and percentages for categorical variables, and a mean, standard deviation (SD), and median are included for numeric variables. The categorized groups were compared using the chi-square test. A statistical significance level of alpha was accepted as $p<0.05$.

Results

UGE procedures performed on 3.776 adult patients in the gastroenterology endoscopy unit of our hospital between 2017 and 2021 were retrospectively screened. In 231 of these procedures (6.12%), biopsies were taken from the duodenum via forceps with a preliminary diagnosis of duodenitis.

The mean age of the patients was 42.03 years (18-89 years). Ninety-eight (42.4%) patients were male, one hundred thirty-three (57.6%) patients were female (Table 1).

The most common symptoms/findings in the pre-endoscopic evaluation of patients were dyspepsia (45.02%), chronic diarrhea (38.10%), and iron deficiency anemia (34.20%) respectively (Table 1).

According to the histopathological results of biopsies (primary and secondary) with duodenitis in our study, the two most common diagnoses were NSD and CD. NSD was detected in 135 (58.44%) patients. The second most common cause of duodenitis was CD, which was seen in 69 (29.87%) patients and was the most common cause of secondary duodenitis. All histopathological diagnoses are summarized in Figure 1.

Table 1. Demographic characteristics of patients and endoscopic procedure indications		
Age (years)	All patients (mean ± SD)	42.03±13.9 (18-89)
	Male (mean ± SD)	41.14±11.8 (19-82)
	Female (mean ± SD)	42.89±15.7 (18-89)
Gender (n, %)	Male	98 (42.4%)
	Female	133 (57.6%)
Endoscopic procedure indications (complaints and findings) (n, %)	Dyspepsia	104 (45.02%)
	Chronic diarrhea	88 (38.10%)
	Iron deficiency anemia	79 (34.20%)
	Vitamin B12 deficiency	44 (19.05%)
	Abnormal weight loss	42 (18.18%)
	Gastrointestinal bleeding	24 (10.39%)
SD: Standard deviation		

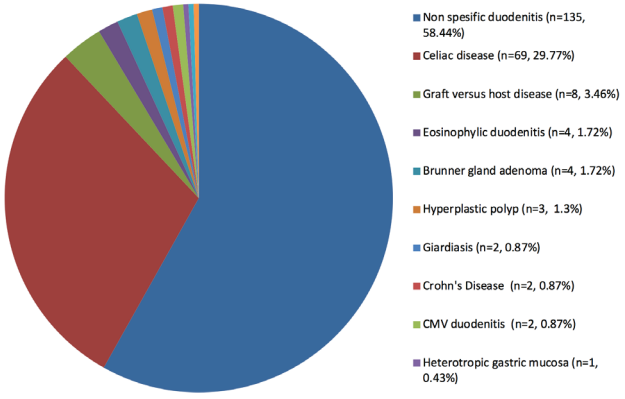

Figure 1. Histopathological results of endoscopic biopsies with a preliminary diagnosis of duodenitis (primary: Non-specific duodenitis, secondary: Other diseases) (n, %)

The mean age of 69 patients whose histopathological results were compatible with CD was 29.66 years (18-56). The female/male ratio of those patients was 2.83. According to the modified MARSH classification, 8 (11.6%) patients were Marsh I, 19 (27.5%) patients Marsh IIIa, 32 (46.4%) patients Marsh IIIb, 10 (14.5%) patients Marsh IIIc.

Discussion

Chronic duodenitis occurs with long-term epithelial damage and inflammation of the duodenal mucosa and is diagnosed endoscopically and histopathologically. In duodenal biopsies taken with UGE examinations, many different diagnoses can be revealed under the title of chronic secondary duodenitis (1,2,11). Various systemic and gastrointestinal system (GIS) diseases are localized in the small intestine and these diagnoses are reached by endoscopic biopsies. However, although taking biopsies from normal mucosal areas is not recommended according to some study results, there are studies showing that especially in the diagnosis of iron deficiency anemia, duodenal biopsies taken from non-lesional normal mucosa can be useful in reaching a diagnosis, even to a small extent (12-15). We examined the results of biopsies taken from mucosal areas that had abnormal endoscopic findings and reported as duodenitis. Most of the patients who had the endoscopical pre-diagnosis of duodenitis and underwent duodenal biopsy had a history of malabsorption, diarrhea, and anemia (16). The most common symptoms/findings in our patients who underwent biopsy with the pre-diagnosis of duodenitis were dyspepsia, anemia, and chronic diarrhea (Table 1). It was confirmed that 58.44% of the duodenal mucosal anomalies that were reported as duodenitis were histopathologically NSD (Figure 1).

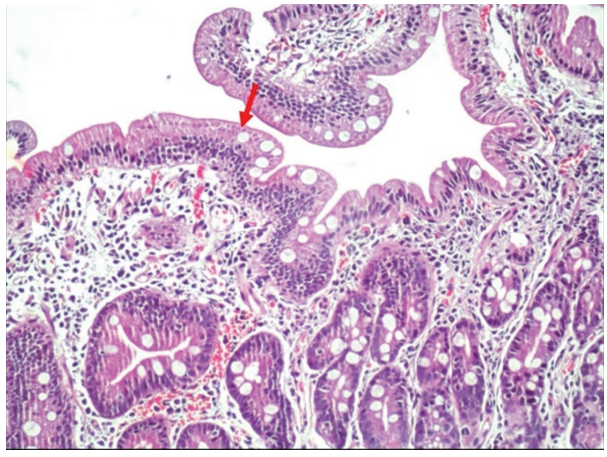

Figure 2. Non-specific duodenitis: Moderate villous blunting and mildly increased lymphocytic inflammatory cell invasion and congestion in lamina propria (H&E, 200X)
H&E: Hematoxylin and Eosin

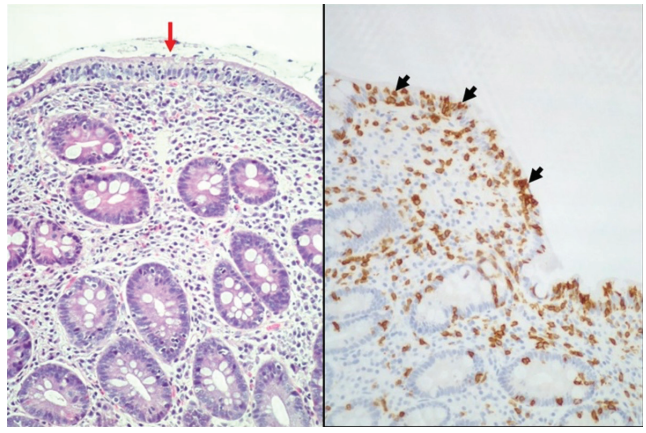

Figure 3. Celiac Disease: a-Complete villous atrophy in a duodenal biopsy of a patient with Celiac Disease (red arrows), (H&E, 200 X). b- Intraepithelial lymphocytosis highlighted with anti-CD3 immunohistochemistry (black arrowheads), (anti-CD3 immunohistochemical antibody, 200X)
H&E: Hematoxylin and Eosin

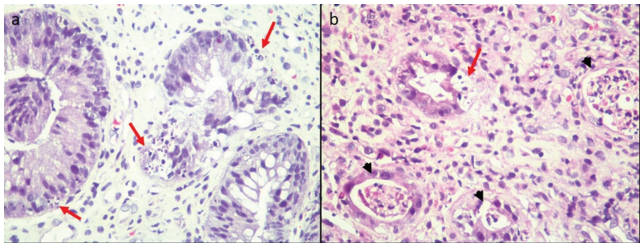

Figure 4. Graft versus host disease (GvHD): a- Apoptotic changes in colonic crypt epithelium, (red arrows, H&E, 400X). b-Prominent crypt denudation and apoptotic crypt abscess, (black arrowheads), (H&E, 200X)
H&E: Hematoxylin and Eosin

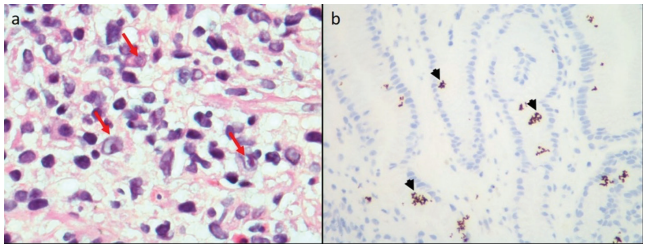

Figure 5. Cytomegalovirus (CMV): a-Nucleomegaly and basophilic intranuclear inclusions in vascular endothelial cells, (red arrows), (H&E, 1000X). b- Positive reaction with anti-CMV immunohistochemical antibody, (black arrowheads), (anti-CMV antibody, 400X)
H&E: Hematoxylin and Eosin

Several microscopic images of duodenitis cases belonging to our patients are shown in Figures 1-5.

The most common secondary histopathological duodenitis etiology was CD, which is a global disease. The prevalence of CD in the general population is about 0.5 % to 1%. According to the histopathological results of our patients who underwent biopsies with a preliminary diagnosis of duodenitis, CD was the most common secondary cause of duodenitis with 29.87% and was the second most common cause in all cases with duodenitis (primary and secondary) (Figure 1). The incidence of CD may be partially related to better recognition of the disease, more testing opportunities, and an increase in immune-mediated diseases (17,18). It has been reported that the prevalence of CD has increased approximately 4 times in the last 3 decades. When the literature is examined, it is stated that CD can be diagnosed at a younger age thanks to the advanced endoscopic and clinical facilities but many cases have been reported in advanced ages and most of the patients were female. A striking result from our study was that for CD, younger mean age and female gender were predominant (19). In CD, in many patients, the first complaint in presentation is dyspepsia. It is an immune-mediated enteropathy that develops in genetically susceptible individuals. In CD, the antibodies are formed mainly against gluten in wheat and other gluten-like cereal proteins in cereals such as barley, rye, and oats (20-26). Endoscopic findings suggestive of CD include disappearance or decrease in the number of duodenal plicae, combing findings, nodular mucosal appearance with the mosaic pattern but the sensitivity and specificity of these endoscopic findings vary between 59-94% and 92-100%, respectively. Similar endoscopic findings can also be seen in giardiasis, autoimmune enteropathy, and HIV infection, so histopathology remains the gold standard in diagnosing CD. In order to make an accurate histopathological diagnosis of chronic duodenitis, the pathologists should evaluate certain histopathological criteria such as villus to crypt ratio, crypt hyperplasia, damaged/flattened enterocytes, the number of intraepithelial lymphocytes, and ruling out the presence of microorganisms such as Giardia and CMV (27,28). When the endoscopic reports were examined, the number of cases in which the gastroenterologist specified CD as the second preliminary diagnosis besides duodenitis was 63, and in this context, the rate of predicting CD by the endoscopist was 91.3%. This rate is somewhat higher than that of in similar studies in the literature (16). Modified MARSH classification is used in histopathological diagnosis. If there is a clinical suspicion, even if the endoscopist sees a normal-appearing mucosa in the duodenum, taking multiple biopsies from many areas is recommended (14,29-32).

Aside from these two predominant etiologies (NSD and CD), many secondary chronic duodenitis causes such as graft versus host disease (GvHD), Crohn's disease, giardiasis, eosinophilic duodenitis, and CMV duodenitis were also detected (Figure 1). In a study conducted by Han et al. (8) in which 6334 patients' UGE were examined; 475 cases of duodenitis were detected. Twenty-one (4.4%) patients were diagnosed with secondary duodenitis, histopathologically. The most common diagnoses of secondary duodenitis were IBD in 7 patients, CMV duodenitis in 3 patients. These two major etiologies were followed by Behcet's disease in 2 patients, radiation-induced duodenitis, Henoch-Schönlein purpura, candidiasis, tuberculous enteritis, eosinophilic enteritis, and parasitic infestation respectively (8).

GvHD is a disease that often involves the skin, liver, and GIS, presenting with organ dysfunction due to a severe immunological reaction caused by T-lymphocytes passing from the donor to the recipient patient (33,34). The risk of developing chronic GvHD within two years after allogeneic bone marrow transplantation is 50-60%. The diagnosis of GvHD is made by endoscopic examination and the histopathological interpretation of the endoscopic mucosal biopsies (35,36). In the hematology clinic of our hospital, bone marrow transplantations are frequently

performed and 3.46% of our biopsies with a pre-diagnosis of duodenitis were diagnosed as GvHD (Figure 4). The average age of our patients was 32 years, and the female/male ratio was 1.66.

Eosinophilic gastroenteritis (EG) is a disease whose pathogenesis is not fully understood, involving genetic and environmental factors, and is associated with allergy and food hypersensitivity. Eosinophilic duodenitis and gastritis are characterized by persistent symptoms and elevated eosinophil leucocytes in the GIS (37). In a study conducted with 220 cases diagnosed with EG, gastric involvement was the most common (43%). Duodenal involvement was reported at a rate of 31%. In the present study, EG was determined to be the fourth most common secondary duodenitis etiology with a ratio of 1.72% (38-40).

Brunner's gland adenoma is a rare benign duodenal neoplasia that occurs as a result of hyperplasia of exocrine glands in the proximal duodenal mucosa. It constitutes 10.6% of benign tumors of the duodenum and is usually asymptomatic (41,42). In the present study, it was detected at a rate of 1.72%.

In the present study, 2 female patients with 22 and 35 years of age were diagnosed with Crohn's disease by duodenal biopsy. Although duodenal involvement is common in IBD compared to the general population, the low rate of H. pylori infection in these patients is remarkable. Granulomatous inflammation is rarely seen in Crohn's patients with duodenal involvement, and the patients are generally asymptomatic. Therefore, the diagnosis might be challenging. Crohn's disease should be kept in mind in patients with H. pylori negative duodenitis (8,43,44).

CMV causes reinfection by reactivation, especially in immunocompromised hosts, which becomes latent after the primary infection. CMV often involves the GIS. Colonic involvement is the most common. Duodenal involvement is rare. Demonstrating inclusions specific to CMV histopathologically is the gold standard in diagnosis. Performing immunohistochemical staining on tissue samples aids in diagnosis. In our case, we both demonstrated the inclusion bodies and immunohistochemical reaction in detecting CMV duodenitis (Figure 5) (45,46).

Polypoid lesions in the duodenum are rare. Hyperplastic polyps are more common in the duodenum, with a prevalence of ≤0.5%. Tubular adenomas are also extremely rare in the duodenum. In a study investigating 25.000 EGD procedures, the incidence of duodenal adenomas was found to be 0.4% (47). In another study, the incidence of duodenal adenomas was demonstrated to be 0.03% (48). Sometimes endoscopic polypoid appearing lesions do not represent actual polyps. In a study investigating 19560 endoscopic procedures, 5 duodenal polypoid lesions were detected, all located in the second part of the duodenum, and histopathologically reported as NSD (49,50). In our study, 3 hyperplastic polyps and 1 tubular adenoma without high-grade dysplasia were detected.

Giardia duodenalis (lamblia, intestinalis) is an acute and chronic protozoal infection. It can cause sporadic or epidemic diarrhea, usually in children and young adults who travel internationally and who are immunocompromised. In the chronic form, vitamin, protein, and energy malnutrition can be seen due to malabsorption. The diagnosis of the disease can easily be made by the detection of cysts or trophozoites in the stool. The presence of protozoa in the duodenal aspirate or biopsy also leads to a definitive diagnosis (51). In the present study, we detected Giardia infection in two patients.

Congenital remnants of the gastric mucosa can be seen in any part of the gastrointestinal tract (esophagus, duodenum, ileum, such as Meckel's diverticulum). This condition is called heterotopic gastric mucosa (HGM). It is usually asymptomatic and diagnosed incidentally. In one study, duodenal HGM was reported in 8.9% of 28.210 patients who underwent endoscopic procedure. In another study, the incidence of HGM was found to be 0.43%. HGM should be histologically differentiated from gastric metaplasias since the latter is an acquired disease that presents following duodenal ulcer and duodenitis. In gastric metaplasia, an incomplete form of gastric tissue is observed in the duodenum which is composed of either foveolar epithelium or pyloric glands only (52,53).

GIS involvement in systemic amyloidosis is frequent. It can involve all parts of the GIS. In autopsy studies, the amyloid accumulation rate in the GIS is 70-100%. In a study conducted in our country, amyloid deposits were also found in the duodenum in all 14 patients diagnosed with amyloidosis in the kidney (54,55). In the present study, amyloid accumulation was demonstrated by Congo red stain in the duodenal biopsy of one patient with the Familial Mediterranean Fever that presented with signs of chronic diarrhea and malabsorption.

Study Limitations

The number of patients who underwent duodenal biopsy in our study was relatively small and our study was retrospective. We think that these situations create limitations for our study. In the future, multicenter and prospective studies conducted with a larger number of patients will yield more valuable results.

However, in a substantial number of our patients, we identified rare causes of secondary duodenitis. The rare causes of secondary duodenitis are not well known to clinicians, so our study may contribute in this aspect. Similarly, we have seen that similar studies have not been

conducted in the literature recently. For this reason, we think that the results of our study will contribute to the literature.

Conclusion

We examined the secondary causes of the lesions endoscopically defined as duodenitis. A sparse number of studies about this topic are present in the literature. In the present study we determined considerable number and variety of causes of secondary duodenitis. In the presence of malabsorption symptoms such as anemia and diarrhea in patients with younger age and female gender, causes of secondary duodenitis such as CD and IBD may be considered. GVHD should be included in the differential diagnosis in the presence of sudden-onset diarrhea in patients with allogeneic bone marrow transplantation.

Authorship Contributions

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

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

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Evaluation of Pupil Diameter and Midline Shift in Patients Undergoing Decompressive Craniectomy

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Abstract

Aim: Intracranial pressure may increase due to reasons such as head trauma, intracranial hematoma, cerebral infarction, and cerebral edema. Decompressive craniectomy (DC) may be preferred in the treatment. Our study aims to examine the time of DC and the effect of pupil diameter and midline shift on mortality.

Methods: Data from the hospital automation system and patient file records were retrospectively reviewed between 2017 and 2019, and 57 patients who underwent DC for raised intracranial pressure. Fifty-seven patients who underwent DC, whose neurological examination and pupil diameter were recorded after increased intracranial pressure, were evaluated.

Results: The mean age of 57 patients was 50.38±19.91 years. The patients' mean preoperative Glasgow coma score (GCS) was 8.85±3.6 and the mean GCS at discharge was 8.96±6.0. The mean cranial midline shifts of the patients were 7.88±5.35. 49.1% of the patients who underwent DC survived.

Conclusion: Mortality rate is lower in patients who underwent DC compared to patients with isochoric pupil, anisocoric, and mid dilated patients. The timing of DC is important in terms of its effect on mortality.

Keywords: Decompressive craniectomy, intracerebral hemorrhage, intracranial hypertension, trauma

Introduction

Intracerebral hemorrhage can cause an increase in intracranial pressure (ICP) as a result of traumatic brain injury, cerebral infarction, and cerebral edema. Early intubation, hyperventilation, hyperosmolar therapy, barbiturate coma, and external ventricular drainage system are recommended for reducing intracranial pressure. Decompressive craniotomy (DC) can be planned in cases that do not respond to treatment (1,2). DC is a surgical procedure performed by removing a large portion of the skull and opening the dura mater. With DC, a sufficiently large bone flap should be removed to reduce intracranial pressure and increase compliance. It has been shown that functional outcomes are better with adequately large DC sizes (3). Except for three; subtemporal, bifrontal, frontotemporoparietal types, unilateral and bilateral DC has been defined. While it is frequently performed in the frontotemporoparietal area in cerebral infarction cases, bifrontal decompressive surgery is frequently performed in cases of frontal contusion cerebri and edema (4,5).

Decompressive hemicraniectomy and bifrontal craniectomy are the most commonly used surgical procedures. DC can be applied in early and late periods. However, the application time of DC is still controversial (6-8). In addition, there are few articles in the literature reporting the effect of pupil diameter, midline shift status on DC application time, and mortality.

Our study aimed to evaluate the effect of DC on the time and mortality of pathology differences that increase intracranial pressure by examining GCS, midline shift, pupil diameter.

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Methods

Study Design

Ethical approval was obtained from the Ethics committee of University of Health Sciences Turkey, Haseki Training and Research Hospital (protocol number: 149, date: 17.6.2019). Data from the hospital automation system and patient file records were retrospectively reviewed between 2017-2019 and 57 patients who underwent DC for raised intracranial pressure (cerebrovascular infarction, post-traumatic subarachnoid hemorrhage, epidural hemorrhage, subdural hemorrhage, intracranial hemorrhage), abscess, postoperative tumor and aneurysm) were included in the study. Patients who did not undergo DC and whose file information and examinations were missing were excluded from the study. Age, gender, hypertension, diabetes mellitus, anticoagulant usage status, neurological examination and pupil diameters, Glasgow coma scale (GCS), pupillary light reflex, cranial midline shift, and survival were recorded.

DC was performed unilaterally in all patients. Frontotemporoparietal DC was performed in 48 patients, subtemporal in 3 patients and bifrontal DC in 6 patients.

Surgical Technique

Surgery was performed under general anesthesia. The surgical field was closed sterile. The periosteum was resected with a skin flap from the incision line. The burr hole was opened with a hand motor. Bone tissue was removed by joining around the burr holes with the hand motor cutting edge and craniectomy was performed. The dura was opened with a scalpel. The dura was suspended from the sides to the bone tissue. The hematoma was evacuated in patients with hematoma. Bleeding control was performed in a controlled manner at these stages. A drain was placed in the subdural space. The layers were closed in accordance with the anatomical structure.

Statistical Analysis

SPSS® for Windows v20.0 (SPSS Inc., Chicago, IL, USA was used for statistical analysis. The frequency, arithmetic mean and the standard deviation of the variables were shown in descriptive data. The distributions were calculated using the Kolmogorov-Smirnov normality test and the differences among the independent groups were calculated by independent samples (Student's t-test) for normal distribution.

Results

The mean age was 50.38±19.91 years old. Twenty-three (40.4%) patients had hypertension, 7 (12.3%) patients had diabetes mellitus. The anticoagulant use rate was 28.1%. The mean GCS before decompressive surgery was 8.85±3.6 and 8.96±6.00. Twenty-nine (50.9%) after decompressive surgery (Table 1). Twenty-eight (49.1%) of the patients were survived.

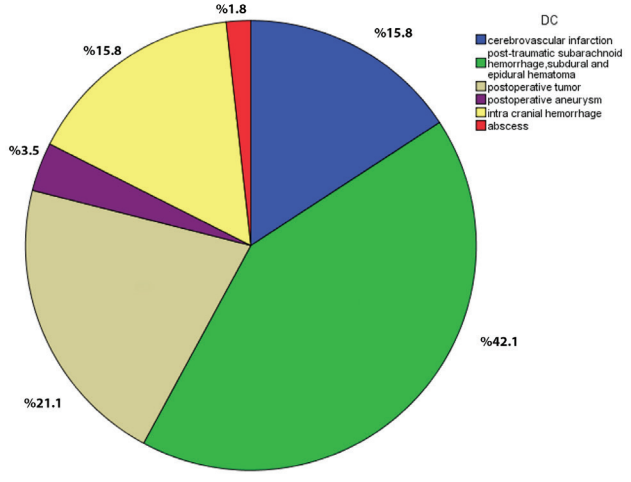
Nine (15.8%) of the operated patients had cerebral infarction. Twenty-four patients (42.1%) had a subarachnoid hemorrhage, epidural hemorrhage, and subdural hemorrhage due to trauma. There were 12 (21.1%) bleeding complications after tumor surgery, 2 (3.5%) bleeding after aneurysm surgery, 9 (15.8%) primary intracranial hemorrhage, and 1 (1.8%) cranial abscess (Graphic 1).

Decompressive surgery direction of the patients; right side 40.4%, left side 50.8%, frontal or occipital 7%, and bilateral 1.8%. In 70% of the patients, galeal duraplasty was performed after durotomy during surgery. In the pupil examination of the patients; 12.3% (n=7) pupils were fix or mid-dilated, 38.6% (n=22) had anisocoria, 49.1% (n= 28) were isochoric. The distribution of ages was 33.3% (n=19) under 40, 29.8% (n=17) between 40 and 60, 36.8% (n=21) over 60 years old respectively. When the

Table 1. Distribution of demographic (n=57)

		Mean ± SD	Minimum	Maximum
Age (years)	Male	48.17±20.13	32	70
	Female	55.58±18.95	36	75
Vital sign	Saturation (%)	95.98	85	100
	Systolic blood pressure (mmHg)	151.58	85	220
	Diastolic blood pressure (mmHg)	86.98	45	140
	Heart rate *(/dk)	93.36	50	135

Independent samples t-test was used for normal distributions, SD: Standard deviation



Graphic 1. Etiological diagnosis of patients who underwent decompressive craniectomy

patient age grouping was made, when the mortality rates above and below the cut-off value of 40 years and 60 years were compared, it was statistically significant (p<0.05). The death rate was found to be statistically significant due to the comparison of patients under the age of 40 with the patient group over 60 years old (p<0.05).

The patients are classified as GCS. Patients with GCS between 3 and 7 were 35.1% (n=20), 36.8% (n=21) for GCS between 8 and 11, 28.1% (n=16) for GCS between 12 and 15. Compared with the GCS group between 3 and 7, the mortality is statistically significantly lower in GCS 12-15 (p<0.05) (Table 2).

The mean of midline shift in cranial CTs of all patients was 7.88±5.35 mm. The average midline shift amount is 8.94±5.21 mm in the mortality group (n=29) and was 6.79±5.23 mm in the survivor group (n=28). Patients who did not develop shift were statistically significantly lower mortality (p<0.05) than others who developed shifts, however, there was no statistically significant difference between groups that developed shifts in terms of mortality (p>0.05).

Table 2. Glaskow coma scale classification and shift status; midline shift was found to be higher in patients with GCS <8 compared to other groups

	GCS	n	Mean	SD	95% Confidence interval	
					Lower bound	Upper bound
Midline shift	GCS: 3-7	20	8.67	4.77	37.62	108.87
	GCS: 8-11	21	7.87	5.19	78.26	143.54
	GCS: 12-15	16	6.92	6.34	97.69	168.05
Overall					82.93	124.78

Independent t-test was used, GCS: Glaskow coma scale, SD: Standard deviation

Table 3. Pupil, midline shift, exitus cross table; patients with isochoric pupils have less midline deviation and lower mortality rates compared to other groups

Exitus			Midline shift					
			No shift	Less than 5 mm	From 5 to 10	From 10 to 15	More than 15 mm	Total
Yes	Pupil	Anisocoric	0	2	3	2	3	10
		Fixdilate or middilate	0	0	4	1	2	7
		Isochoric	0	4	4	4	0	12
	Total		0	6	11	7	5	29
No	Pupil	Anisocoric	1	1	5	4	1	12
		Fixdilate or middilate	0	0	0	0	0	0
		Isochoric	5	4	3	2	2	16
	Total		6	5	8	6	3	28
Total (n)			**6**	**11**	**19**	**13**	**8**	**57**

Independent t-test was used

The amount of shift was higher in elderly patients and it was statistically significant (p<0.05). Under 40 years old (5.55±4.40 mm) midline shifts were found less and statistically significant compared to 40-60 years old (9.40±5.44 mm) (p<0.05). When the shift rates under 40 years old (5.55±4.40 mm) and over 60 years old (8.76±5.57 mm) were compared, p=0.05 was found. Under 40 years of age (5.55±4.40 mm) midline shifts were less and statistically significant (p<0.05) than over 40 years old (9.05±5.45 mm). Shift rates above 60 years old (8.76±5.57) and below (7.32±5.2) were not statistically significant (p>0.05).

Statistical correlations were made between age, gender, preop and postop GCS of patients undergoing decompression surgery, surgical side, and presence of hypertension, development of midline shift, diabetes, anticoagulant use, duraplasty, and exitus. There was a statistical significance between anticoagulant use and GCS status, hypertension, and exitus of the group who underwent decompressive surgery (p<0.05). In the case of anisocoric and fixed dilated pupils, the GCS of the patients were found to be low and statistically significant (p<0.05).

As the patients age groups increase, the rate of using antihypertensive and anticoagulant use increases with a statistical correlation (p<0.05). It was found that the patients with shift were statistically significantly higher between pupil status, all groups that went to decompressive surgery regarding mortality.

A statistical significance was found between fixed and mid-dilated pupils and exitus (p<0.05). All patients with fixed or mid-dilated patients (n=7), twelve patients (42.85%) with pupillary isochoric and light reflexes, ten (45.45%) of the patients with anisocoria have died.

The midline shifts of the patients with anisocoria were 8.64±5.00 mm, and in the patients with fix dilated or mid-dilated were 10.27±5.98 mm. Patients with isochoric pupils have a midline shift of 6.70±5.34 mm. A statistical significance was found between fixed or mid-dilated in terms of mortality (p<0.05). No statistical significance was found when the patients with and without anisocoria in terms of mortality (p>0.05). Pupil states of the patients were compared with each shift group. There was no statistical significance (p>0.05) (Table 3).

Discussion

DC has been increasingly applied recently (9). DC is frequently applied to MCA infarcts. The rate of infarction cases is 10-20 per hundred thousand per year. Mortality rates range from 41% to 79%, according to literature. While the mortality rate is 16% in patients with early

surgery, it reaches 34.4% in the late period and 78% in the non-surgical group (10,11). In addition, it has been stated that right hemisphere involvement, advanced age, and presence of pupil dilation will increase the mortality rate in MCA infarct patients who have been applied DC (12). The average age of our patient group with cerebral infarction was 64.55. DC was applied after the patients had neurological and clinical deterioration who did not improve despite medical treatments. The mortality rate was 55.5%.

DC can be applied early or late. Early DC is usually performed in the acute phase, and hemorrhage is intervened to reduce intracranial pressure. Late DC is applied to patients who do not benefit from medical treatment, who have increased intracranial pressure or do not decrease pressure levels (13). Studies show that DC has a better prognosis than medical treatment and is more effective in controlling ICP levels (1). It has been shown that late DC has a lower prognosis effect than early DC (14). Early DC and hemorrhage evacuation were performed in patients with intracranial hemorrhage after head trauma. The mortality rate of trauma patients was 58.3%. In recent studies, it has been stated that despite the decrease in acute DC applications to trauma patients, there may be a decrease in mortality rates with medical treatment and good care (15). Compared to infarct patients, the mean age of trauma patients was low, had a lower midline shift, and the mortality rate was higher despite early DC application. This result may be due to brain damage, cerebral edema, and the extent of brain ischemia due to decreased perfusion pressure. Pupils of six of the trauma patient group were fixed and mid-dilated. The pupils of six of the trauma patient group were fixed and moderately dilated. The amount of midline deviation was significantly higher in these patients compared to the other patient groups. Early DC was applied, and all six patients died. Pupillary was fix-dilated, and the exitus rate was high due to the mass and edema effect and the excess amount of shift. In the literature, it has been reported that patients with intracerebral hemorrhage have a poor prognosis if their mean GCS is <8 and the average age is >58.18±14.22, and the amount of shift is >4 mm (16). However, it was stated that the mortality rate of patients with GCS<5 and bilaterally fixed dilated pupils would be extremely high (17). In our patient group with intracerebral hemorrhage, the mean GCS is 8.2, and the average age is 49.77±17.59. The shift was 9.08±6.27. Five patients died (55.5%). Despite the early application of DC to these patients, GCS showed a poor prognosis due to low and high midline shifts. Bleeding occurred in 12 patients after intracranial tumor surgery; early DC applied. The mortality rate was 33.3%. After aneurysm surgery, bleeding developed in two patients and one patient died. The mortality rate was 50%. The mortality rate was found to be lower than bleeding after intracranial tumor surgery and bleeding after aneurysm surgery. Due to the low number of patients with hematoma after aneurysm surgery, the mortality rate may have been high. One patient was operated on for intracranial abscess, and DC was applied due to hemorrhage development, and the patient recovered completely. Mortality rates of secondary intracranial hemorrhages were found to be lower than primary intracranial hemorrhages.

In the patient groups, the amount of shift was lower in those under 40 years of age compared to other age groups (5.55±4.40 mm versus 9.05±5.45 mm). Because young patients have good brain compliance and elastance, they are resistant to lesions, and their success rate increases with faster diagnosis and early application of DC because they show clinical signs in fewer shift conditions. In elderly patients, the brain tissue's compliance and elastance are low and show less resistance to lesions. When the amount of shift reaches advanced levels, clinical findings emerge. Therefore, it causes late diagnosis and initiation of late treatment. In our study, exitus did not develop in patients who did not develop midline shift. Edema in the brain tissue causes an increase in intracranial pressure, decreased perfusion pressure, and ischemia. When DC is performed, intracranial pressure decreases with brain tissue expansion, and cerebral perfusion improves. Although DC effectively controls intracranial pressure, the administration time to patients is controversial (18,19). In our study, we evaluated pupil diameters and DC application times and results. The mean midline level of the group with pupil status, fix dilated or mid-dilated, is 10.27±5.98 mm. Early-term DC was applied to the patients in this group. All patients with fixed or mid dilated pupils have died.

The absence of light reflex indicates a poor prognosis. Twelve (42.85%) of the patients with pupillary isochoric and bilateral light reflexes and 10 (45.45%) of the patients with anisocoria died. Midline shifts are higher in anisocoric patients. It will be insufficient to decide on DC time by evaluating pupil diameters alone. Rapid and early application of DC in anisocoric pupils may affect decreasing mortality. Besides, low GCS, high age, and comorbid diseases were factors affecting mortality. In one study, patients with brain damage causing increased intracranial pressure were evaluated by comparing those who received DC and medical treatment. Patients' mortality after decompression surgery was found to be lower than those who received conservative treatment. However, he had high vegetative survival and bedriddenness (20,21). In our study, mortality rates of patients with isochoric pupils

treated with DC were found to be lower than those with middilate pupils.

Study Limitations

The low number of patients caused us to make limited evaluations. A larger number of patients are needed for a more comprehensive assessment. There is heterogeneity between intracranial pathologies. Therefore, the timing of the operations may have caused differences in the preoperative and postoperative neurological examination results. Despite these limitations, our study will contribute to the literature in terms of providing an idea about mortality and decompressive surgery time by evaluating pupil diameter and midline shift conditions.

Conclusion

The mortality of patients after decompression surgery is lower than those who received conservative treatment. However, it has high vegetative survival and bed dependency. As the time of DC, it will be beneficial to decrease mortality if it is preferred in the early period without waiting for asymmetry in pupil diameters or loss of light reflex.

Authorship Contributions

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

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Original Article

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Can Hematological Parameters be Used for Differential Diagnosis in Rotavirus Infection at any Age of Children? A 6-year Outcomes from a Tertiary Referral Center

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Abstract

Aim: Routine blood parameters are widely used to detect of infectious diseases. Our study aimed to investigate the hematological parameters changes in rotavirus (RV) acute gastroenteritis and to evaluate the value of these parameters in children.

Methods: In our study, the hematological data of patients diagnosed with RV-positive acute gastroenteritis (RPAGE) and RV-negative acute gastroenteritis (RNAGE) were analyzed between 2015-2020. In addition to the data automatically measured by complete blood count, lymphocyte/monocyte ratio (LMR), neutrophil/lymphocyte ratio (NLR), mean platelet volume/platelet ratio (MPVPR) were calculated. All cases were evaluated in three different age groups (<1-year-old, 1-to 5-year-old, and >5-year-old).

Results: The present study included 2,144 patients (340 children with RPAGE, 1,804 children with RNAGE). Red blood cell count, hemoglobin, and hematocrit were higher in cases over 1 year of age with RPAGE. The MPVPR was lower in all age groups with RPAGE. The cut-off values of MPVPR for predicting Rotavirus infection (RI) were 0.023 (<1-year-old group), 0.026 (1-to 5-year-old group), and 0.032 (>5-year-old group). The LMR was lower and NLR was higher in cases over 1 year of age with RPAGE. The cut-off value of LMR for predicting RI were 1.99 (1-to 5-year-old group) and 0.96 (>5-year-old group). The cut-off value of NLR for predicting RI were 1.41 (1-to 5-year-old group), and 3.79 (>5-year-old group).

Conclusion: The low MPVPR can be used as a hematological biomarker for the identification of RPAGE cases in all age groups. Low LMR and high NLR can indicate RPAGE cases in over 1 year of age.

Keywords: Gastroenteritis, rotavirus, neutrophils, lymphocytes, monocytes, mean platelet volume, platelets

Introduction

Diarrhea is the major leading cause of death among children younger than 5-year old worldwide (1). Rotavirus (RV) is one of the most common causes of acute gastroenteritis (AGE) in childhood and the leading worldwide cause of acute diarrhea-related death in children under the age of five (2,3). RV-associated annual morbidity rates among children under five years of age ranged from 0 to 112/100,000 with an average mortality rate of 39/10,000 per year in The Eastern Mediterranean region, as defined by the World Health Organization (WHO-EMRO region) (4). Deaths from rotavirus infection (RI) still continue despite efforts to make better hygiene conditions, widespread vaccination, and improvements in treatments (5,6).

Rotaviruses belong to the Reovirus family, usually, type A RV is responsible for infections in children. Apart from RV, the other most common causes of AGE in childhood are Noroviruses, enteric types of the adenovirus, and astroviruses (7). The diagnosis of RI depends on the detection of RV antigen in stool by serological methods.

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However, these tests can often be time-consuming, sometimes expensive and inaccessible. Routine complete blood count (CBC) test is performed in pediatric patients, especially in many febrile diseases, because of its easy availability and cheaper.

In recent years, in addition to routine CBC parameters, new hematological markers such as lymphocyte/monocyte ratio (LMR), neutrophil/lymphocyte ratio (NLR), mean platelet volume/platelet ratio (MPVPR) have been used in inflammation (8,9).

This study aimed to evaluate the routine blood parameters in children with AGE and investigate the easily accessible new hematological markers to use in the differential diagnosis of RI.

Methods

Study Design

The present study was approved by the Local Ethics Committee of Medipol University Hospital (approval no: 957/2020) and designed as a cross-sectional study which is one of the types of observational studies. Children with AGE aged 0 to 16 years who presented at the department of pediatrics from January 2015 to December 2020 were included in this study. Medical information for demographic data and laboratory parameters were extracted from patient files.

During the study period, RV antigen test was performed in 5,134 children with gastrointestinal complaints and in 831 of these patients (16.2%) RV antigen was detected in the stool. In the present study, 340 children with RV-positive acute gastroenteritis (RPAGE), 1,804 children with RV-negative acute gastroenteritis (RNAGE) were included. The patients without hematological data were excluded from the study (n=2,990) (Figure 1). All children were evaluated in three different age groups: the <1-year old group, the 1-to 5-year-old group and the >5-year-old group. Informed consent has been received.

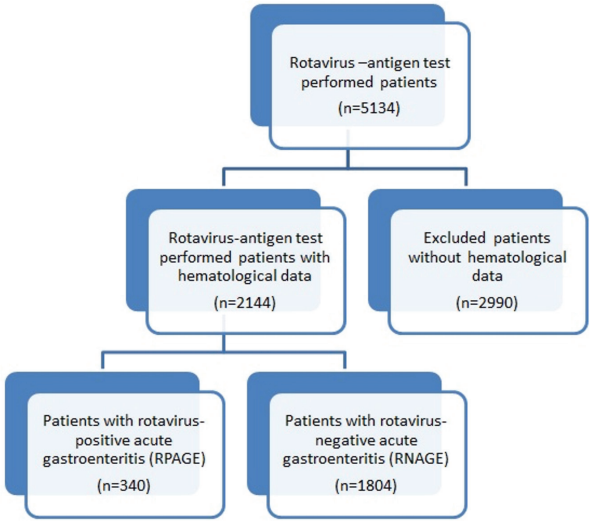

Figure 1. Enrollment algorithm of patients

Laboratory Assessments

Routine CBC analysis was performed from venous whole blood samples with EDTA taken from patients (Sysmex XN-1000, Sysmex R&D Center Europe GmbH, Germany). In addition to the routine hematological parameters measured with the device, LMR, NLR and MPVPR were calculated. The diagnosis of RI was made by examining RV antigen in fresh stool samples (NADAL RV test, Germany).

Statistical Analysis

SPSS 16.0 software was used for statistical analysis. Among the continuous variables, data conforming to the normal distribution were expressed as mean ± standard deviation. Categorical variables were specified as frequency. Comparisons of groups of continuous variables were made by t-test. ANOVA test was used to analyze the differences between groups. Pearson test was used for the analysis of categorical variables. Receiver operating characteristic analysis was performed to evaluate the diagnostic value of LMR, NLR and MPVPR in RI. $p<0.05$ was considered statistically significant.

Results

There was no statistically significant difference in terms of demographical data between RPAGE and RNAGE cases ($p>0.05$) (Table 1).

In the <1-year-old group, except MPVPR, the red blood cell count (RBC), hemoglobin level, hematocrit level, mean corpuscular volume (MCV), white blood cell count (WBC), neutrophil count, lymphocyte count, monocyte count,

Table 1. Demographical characteristics of study groups

		RPAGE (n=340)	RNAGE (n=1804)	p
<1 year old	Male	47	295	0.46
	Female	32	213	
	Mean age (month)	0.6±0.3	0.6±0.4	0.81
1-5 years old	Male	123	563	0.09
	Female	89	386	
	Mean age (year)	2.3±1.5	2.5±1.6	0.75
>5 years old	Male	21	201	0.52
	Female	28	146	
	Mean age (year)	9.2±2.7	10.6±3.0	0.08

RPAGE: Rotavirus-positive acute gastroenteritis, RNAGE: Rotavirus-negative acute gastroenteritis

platelet count, MPV, LMR, and NLR were well matched between study groups (Table 2). In the <1-year-old group RPAGE cases; the cut-off value of MPVPR was 0.023, the area under the curve (AUC) was 0.44 (0.37-0.51), sensitivity and specificity were 60% and 32%, respectively. There was no difference between LMR and NLR rates in this age group (Figure 2 A).

In the 1-to 5-year-old group, while the RBC, hemoglobin level and hematocrit level were statistically significantly higher in RPAGE cases, MCV was low. The WBC, monocyte count and platelet count were similar between RPAGE and RNAGE cases. While the neutrophil count and NLR were statistically significantly higher in RPAGE cases, MPV, LMR, and MPVPR were low (Table 3). In the 1-to 5-year-old group RPAGE cases; the cut-off value of MPVPR was 0.026, AUC was 0.42 (0.38-0.46), sensitivity and specificity were 62% and 32%, respectively. The cut-off value of LMR was 1.99, AUC was 0.37 (0.32-0.41), sensitivity and specificity were 55% and 26%, respectively, and the cut-off value of NLR was 1.41, AUC was 0.61 (0.56-0.65), sensitivity and specificity were 70% and 40%, respectively, in this age group with RPAGE (Figure 2 B).

In the >5-year-old group, the RBC, hemoglobin level, and hematocrit level were statistically significantly higher in RPAGE cases. There was no statistically significant difference among the WBC, monocyte count, platelet count, MPV, and MCV between RPAGE and RNAGE cases. While the neutrophil count and NLR were statistically significantly higher in RPAGE cases, LMR and MPVPR were low (Table 4). In the >5-year-old group RPAGE cases; the cut-off value of MPVPR was 0.032, AUC was 0.41 (0.33-0.49), sensitivity and specificity were 64% and 30%, respectively. The cut-off value of LMR was 0.96, AUC was 0.29 (0.22-0.36), sensitivity and specificity were 69% and 26%, respectively, and the cut-off value of NLR was 3.79, AUC was 0.69 (0.61-0.76), sensitivity and specificity were 81% and 53%, respectively, in this age group with RPAGE (Figure 2 C).

Table 2. Hematological parameters of the <1-year-old group

	RPAGE (n=79)	RNAGE (n=508)	p
RBC (10^{12}/L)	4.39±0.73	4.25±0.71	0.13
Hemoglobin (g/dL)	11.48±2.21	11.39±2.27	0.73
Hematocrit (%)	33.92±6.01	33.44±6.32	0.50
MCV (fL)	78.22±11.65	79.13±10.16	0.91
WBC (109/L)	11.51±5.89	12.20±6.27	0.34
Neutrophil (109/L)	4.80±3.62	5.08±4.26	0.54
Lymphocyte (109/L)	4.70±2.82	5.27±2.95	0.10
Monocyte (109/L)	1.35±0.79	1.44±0.94	0.37
Platelet (109/L)	391.25±151.42	368.76±145.06	0.22
MPV (fL)	9.60±0.67	9.74±0.92	0.12
Lymphocyte/ monocyte ratio	4.22±3.06	4.66±3.68	0.25
Neutrophil/ lymphocyte ratio	1.70±1.90	1.52±3.12	0.50
MPV/platelet ratio	0.028±0.014	0.032±0.026	0.04#

#The RPAGE group was compared with the RNAGE group (t-test)
RPAGE: Rotavirus-positive acute gastroenteritis, RNAGE: Rotavirus-negative acute gastroenteritis, MPV: Mean platelet volume, MCV: Mean corpuscular volume, WBC: White blood cell, RBC: Red blood cell)

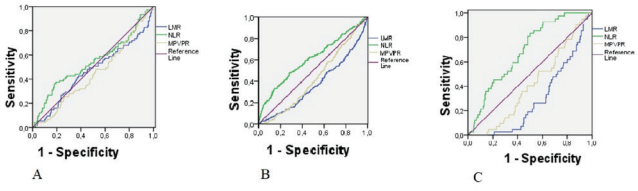

Figure 2. ROC curves of LMR, NLR, and MPVPR values in patients with RPAGE A. <1-year-old group, B. 1-to 5- year-old group, C. >5-year-old group
ROC: Receiver operating characteristic, LMR: Lymphocyte/ monocyte ratio, NLR: Neutrophil/lymphocyte ratio, MPVPR: Mean platelet volume/platelet ratio, RPAGE: Rotavirus positive acute gastroenteritis

Table 3. Hematological parameters of the 1-to 5-year-old group

	RPAGE (n=212)	RNAGE (n=949)	p
RBC (10^{12}/L)	4.72±0.42	4.50±0.67	0.001#
Hemoglobin (g/dL)	11.82±1.01	11.56±1.56	0.02#
Hematocrit (%)	34.64±2.69	33.84±4.28	0.01#
MCV (fL)	73.58±4.77	75.68±6.47	0.001#
WBC (10^9/L)	10.67±4.52	10.76±8.61	0.83
Neutrophil (10^9/L)	6.96±4.34	6.07±4.39	0.007#
Lymphocyte (10^9/L)	2.61±1.97	3.18±2.06	0.001#
Monocyte (10^9/L)	1.05±0.44	1.06±1.54	0.80
Platelet (10^9/L)	331.57±101.28	300.49±125.83	0.22
MPV (fL)	9.21±0.75	9.35±0.95	0.01#
Lymphocyte/ monocyte ratio	2.75±2.23	3.96±5.02	0.001#
Neutrophil/ lymphocyte ratio	5.03±5.93	2.89±3.64	0.002#
MPV/platelet ratio	0.031±0.016	0.042±0.052	0.001#

#The RPAGE group was compared with the RNAGE group (t-test)
RPAGE: Rotavirus-positive acute gastroenteritis, RNAGE: Rotavirus-negative acute gastroenteritis, MPV: Mean platelet volume, MCV: Mean corpuscular volume, WBC: White blood cell, RBC: Red blood cell

Discussion

It may not always be possible to reach specific serological detection tests used in the diagnosis of RI. The use of more accessible and cheaper hematological parameters may provide advantages in the identification

Table 4. Hematological parameters of the >5-year-old group			
	RPAGE (n=49)	RNAGE (n=347)	p
RBC (10^{12}/L)	4.86±0.65	4.28±0.97	0.005#
Hemoglobin (g/dL)	13.17±1.46	11.70±2.28	0.001#
Hematocrit (%)	38.06±3.75	34.23±6.38	0.001#
MCV (fL)	78.94±7.43	81.18±8.66	0.07
WBC (10^9/L)	10.17±4.72	9.69±12.39	0.63
Neutrophil (10^9/L)	8.33±4.36	6.18±4.86	0.004#
Lymphocyte (10^9/L)	1.14±0.54	1.78±1.86	0.02#
Monocyte (10^9/L)	0.87±0.32	0.80±0.61	0.22
Platelet (10^9/L)	256.44±95.63	230.92±177.80	0.15
MPV (fL)	9.68±0.66	9.85±1.09	0.15
Lymphocyte/monocyte ratio	1.40±0.70#	3.37±5.17	0.001#
Neutrophil/lymphocyte ratio	9.40±6.93#	6.07±8.01	0.005#
MPV/platelet ratio	0.041±0.019#	0.079±0.102	0.001#

#The RPAGE group was compared with the RNAGE group (t-test)
RPAGE: Rotavirus-positive acute gastroenteritis, RNAGE: Rotavirus-negative acute gastroenteritis, MPV: Mean platelet volume, MCV: Mean corpuscular volume, WBC: White blood cell, RBC: Red blood cell

of RI. Many studies show that NLR, LMR, and platelet/lymphocyte ratios are used in the evaluation of morbidity and mortality in critical diseases due to bacterial, viral infections, and sepsis were evaluated in a meta-analysis and the importance of these new markers was emphasized (10). There are also studies supporting the use of these markers in cancer patients and coronary diseases (11,12).

It is known that lymphopenia can be a good predictor of bacterial infections, and both lymphocyte and neutrophil count should be considered rather than WBC in adult patients with suspected bacteremia (13). Monocytes, an essential component of peripheral blood, are considered an indicator of systemic inflammation (14). The lymphocyte/monocyte ratio, a new inflammatory biomarker, is more sensitive in showing the balance between lymphocytes and monocytes (15). While the ratio of lymphocytes, which play a critical role in destroying viruses, is expected to increase in traditional viral infections, the opposite may occur in some viral diseases (16,17). Also, leukocyte subgroups in peripheral blood in infections may show age-related variability (10).

Proinflammatory cytokines cause an increase in cytoplasm volume of platelet cells in patients with the inflammatory condition. But these large platelet cells rapidly migrate to the site of inflammation where they undergo activation, and MPV may drop in proceeding inflammation (18,19). There are data in the literature about changes of platelets and MPV in inflammatory processes and neoplastic diseases and its possible role as a biomarker (20,21).

In childhood, the platelet count and MPV have been recognized as a hematological marker, and their roles were demonstrated in various conditions such as severe sepsis in children (22), patent ductus arteriosus in preterm infants (23), and sepsis in very low birth weight neonates (24). Some studies evaluated the prognostic value of NLR (25) and LMR (26) in retinopathy of prematurity. Most of these studies conducted <1-year-old age belong to the neonatal period. The <1-year-old subgroup in our study has a large number of cases that examined the entire 0-12-month period.

Children less than 1-year-old are at high risk for both developing RI and complications (27). The use of easily accessible new hematological parameters in the differential diagnosis of RI becomes more valuable in this age group.

Fei et al. (16) reported that high MPVPR can be used as an indicator in patients <6 years of age with influenza A infection. In another study, it was found that the lymphocyte count, platelet count, and LMR were lower, the NLR and MPVPR were higher in children with influenza infection (28). In the study of Mete et al. (20) a constant decrease in MPV was found in RPAGE and RNAGE cases compared to the healthy control group, and it was stated that MPV acted as a negative acute phase reactant. In our study, MPV was low only in the 1-to 5-year-old group with RPAGE. Besides, MPVPR was statistically significantly lower in RPAG cases in all age groups.

A study in RPAGE patients less than five years of age found a decrease in the lymphocyte count and MPV, an increase in the neutrophil count and monocyte count. In the same study, it was stated that low LMR and high NLR could indicate RI in children with AGE (29). In another study conducted in a group of pediatric patients <36 months old, an increase in NLR was found in acute pyelonephritis cases together with other acute phase reactants (30). In our study, MPV was similarly low in the 1-to 5-year-old group RPAGE cases, but it was not observed any difference in the <1-year old group or the >5-year-old group RPAGE cases.

In the present study, the neutrophil count and NLR were higher, while the lymphocyte count and LMR were lower in patients with RPAGE over 1 year of age.

Study Limitations

Our present study has some limitations. It could not be reached enough data to determine the clinical severity of gastroenteritis in patients. RNAGE cases have not been differentially diagnosed with other infectious agents. It should be noted that these limitations arise due to the retrospective nature of our study. Despite all these limitations, it is thought that our study will make important contributions to the literature as it provides

the opportunity to evaluate a large number of patients in different age groups separately.

Conclusion

New hematological parameters may potentially useful for the identification of RI in childhood. The present study emphasized that low MPVPR can be used as a biomarker in the differential diagnosis of RI in all age groups. It was shown that low LMR and high NLR had a predictive value for RI in children older than one year of age, but further studies are needed for children the <1-year-old.

Authorship Contributions

Concept: M.A.O., H.T., M.O., Design: M.A.O., H.T., M.O., Data Collection or Processing: M.A.O., H.T., A.I.T., Analysis or Interpretation: M.A.O., H.T., M.O., A.I.T., Literature Search: M.A.O., H.T., Writing: M.A.O., H.T., M.O., A.I.T.

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