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CONTENTS / İÇİNDEKİLER

Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1094422

Duzce Med J, 2022;24(1):1-6
Düzce Tıp Fak Derg, 2022;24(1):1-6

Conscious and Correct Use of Biostatistical Methods in Medical Researches: From Planning to Reporting the Results - Part I

Tıbbi Araştırmalarda Biyoistatistiksel Yöntemlerin Bilinçli ve Doğru Kullanımı: Planlamadan Sonuçların Raporlanmasına - Bölüm I

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ABSTRACT

The principles and methods of biostatistics serve as a guide for healthcare practitioners in both their daily and scientific work. Biostatistics principles and methods should be considered at every stage. A scientific research requires a multidisciplinary teamwork and each team must include a biostatistics expert at the planning stage of the study. During the planning phase, a biostatistics expert can answer all questions about the design, conduct, data processing, data analysis, presentation of the results, and publication of the results. Reporting guidelines for almost all research designs were developed to improve the quality and transparency of research reports. When the research results are presented in a structured and standardized way using these guidelines, it will allow the readers to interpret the findings more easily and accurately. It is now easier for researchers to gain access to a variety of software or web applications that allow them to perform basic and advanced statistical analyses. However, erroneous/misleading results are produced when these software are used unconsciously or incorrectly by those with insufficient statistical knowledge. Health professionals should also have sufficient statistical knowledge to identify errors in articles related to their field. Therefore, they are expected to have knowledge of research designs and basic statistical concepts. Knowing the basic concepts such as population, sample, sampling methods, random assignment of volunteers to groups, parameters, statistics, data types, etc. will help the readers while evaluating the material and method sections of the articles.
Keywords: Biostatistics; medicine; research; planning; reporting guidelines.

ÖZ

Biyoistatistik ilke ve yöntemleri, sağlık çalışanlarına hem günlük hem de bilimsel çalışmalarında rehberlik eder. Biyoistatistik ilke ve yöntemleri araştırmaların her aşamasında dikkate alınmalıdır. Bilimsel bir araştırma, multidisipliner bir ekip çalışmasını gerektirir. Her ekipte mutlaka bir biyoistatistik uzmanı bulunmalıdır ve çalışmanın planlama aşamasından itibaren ekibe dahil edilmelidir. Planlama aşamasında; araştırmanın tasarımı, yürütülmesi, verilerin işlenmesi, verilerin analizi, sonuçların sunumu ve yorumlanarak yayın haline getirilmesi aşamalarıyla ilgili tüm sorular biyoistatistik uzmanın yardımı ile cevaplanabilir. Araştırma raporlarının kalitesini ve şeffaflığını artırmak için hemen hemen tüm araştırma tasarımları için raporlama kılavuzları geliştirilmiştir. Bu kılavuzlar kullanılarak araştırma sonuçları yapılandırılmış ve standart bir şekilde sunulduğunda, bulguların okuyucular tarafından daha kolay ve doğru yorumlayabilmelerine olanak tanıyacaktır. Günümüzde araştırmacıların temel ve ileri düzey istatistiksel analizleri yapabilecekleri birçok yazılıma ya da web uygulamalarına ulaşımları kolaylaşmıştır. Ancak, istatistik bilgi düzeyi yetersiz olanlar tarafından bu yazılımların bilinçsiz ve yanlış kullanılmaları hatalı/yanıltıcı sonuçların elde edilmesine neden olmaktadır. Sağlık çalışanlarının kendi alanları ile ilgili makaleleri okurken yapılmış belirgin hataları belirleyebilecek düzeyde istatistik bilgisine sahip olmaları da gerekir. Bu nedenle, araştırma tasarımları ve temel istatistiksel kavramlar hakkında bilgi sahibi olmaları beklenir. Popülasyon, örneklem, örnekleme yöntemleri, gönüllüleri gruplara rastgele atama yöntemleri, parametre, istatistik, veri tipleri vb. temel kavramları bilmeleri, makalelerin materyal ve metot bölümlerini değerlendirirken okuyuculara kolaylık sağlayacaktır.
Anahtar kelimeler: Biyoistatistik; tıp; araştırma; planlama; raporlama kılavuzları.

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INTRODUCTION

The principles and methods of biostatistics serve as a guide for healthcare professionals in both their daily and scientific work. Conscious use of this guide will take health professionals in the right way, and unconscious use will lead to the wrong way.

There is a misconception among researchers that the biostatisticians were solely responsible for analyzing the data from the completed study. However, the task of biostatistics in research is to ensure that every study is organized, conducted, and completed according to scientific methods. Therefore, biostatistics principles should be considered at every stage from the planning of the study to the report writing.

It is against the nature of science for a person to be an expert in every subject, no matter how broad his knowledge and experience. Therefore, a scientific research cannot be a one-man operation. It requires a multidisciplinary teamwork. Each research should be planned, conducted, analyzed, and finalized by writing a report by a team of experts from different branches related to the study. Every research team must have a biostatistician and he/she will be included in the team at the beginning of the study. If this is not possible, biostatistics consultancy should be obtained from the beginning of the study.

Researchers perceive research and statistics as separate sciences and consider themselves as a researcher and a biostatistician as a person who will apply appropriate methods to the data they have collected. For this reason, he organizes the stages of the research (planning and data collection) that he considers to be of interest to him and compiles his data. Since he thinks that biostatistics methods will be used in the future, he applies to a biostatistics expert to evaluate the data he has collected or to apply some significance tests (1).

If the research is not well organized, in other words, it has no scientific value, applying even the most advanced biostatistics methods to this research will not bring a solution. Andrew Lang's (1905) quote explains this very well: “He uses statistics as a drunken man uses lamp-posts... for support rather than illumination.” (2). The contribution of a biostatistician to a research at the planning stage is more important than what he/she will do at the evaluation stage.

The unconscious and wrong use of statistics in published articles is a problem that we constantly encounter. Statistical errors can be made at any, some, or all stages of the planning of the research, designing, data collection, analysis of the collected data, the presentation of the obtained results with appropriate tables and graphics, and the interpretation of the results based on the research findings.

The most important stage of the research is the planning stage. At this stage, very important mistakes are encountered. The main reason for these mistakes is that a biostatistician was not included in the team at the beginning of the study or professional statistical consultancy was not received. The best time a statistician is needed is during the planning phase of the study. At this stage, it is much easier to identify and correct any deficiencies.

At the planning stage of the study;

- Forming a research question
- Creating research hypotheses in accordance with the objectives of the study
- Determining the research design (observational, experimental, meta-analysis, etc.)
- Determining what measure is most appropriate for measuring the phenomenon of interest, when and with what measuring instrument, who will measure it, etc.
- Defining the population and sample of the study
- Evaluation of the availability of a source from which data can be obtained
- Which statistical methods will be used to test the research hypotheses
- Calculation of the minimum number of subjects required for the study
- Whether the control group will be used in the study
- Which sampling method will be used in the study
- How to assign participants to groups
- Whether blinding will be used in the study, and if so, who will be blinded
- How to handle missing data problem
- How to handle outliers/extreme values
- Are there any ethical issues related to conducting the study
- What kind of tables and graphics will be utilized to summarize the findings (draft tables and graphs can be prepared)
- What interpretations and inferences will be made
- Who will be the author in the study, their responsibilities, and the order of the authors

etc. many more questions need to be addressed. All of these questions can only be answered with the help of a biostatistician. Statistics can be considered as the common language of all fields of science. It is also a fact that the results obtained from research processes in which this common language is not used appropriately are not accepted in the scientific community (3-5).

The following statement, cited by Altman (6) from Mainland (1950), highlights the importance of statistical principles in scientific research: “Finally, it must be stressed again that, whatever sources of help are found and whatever techniques are employed, the investigator himself has to grasp the principles of statistical reasoning... modern statistical principles are not something that we can take or leave as we wish, for they comprise the logic of the investigation in all fields, including the field of clinical research”.

Biostatistics education for health professionals at the undergraduate and graduate levels is critical. Because, the perspective of a health professional who understands these principles and methods adequately and can use them with skill expands on the individual and community events. As a result, health professionals develop the ability and skill of perceiving and thinking in multidimensional, rather than one-dimensional, ways. Health professionals are aware that the only way for the findings to be valid and reliable is the scientific approach, and the only factor that will ensure this is the appropriate and correct use of biostatistics principles and methods. This awareness gives health professional the ability and skill to always approach events with a scientific view, and to solve problems.

Today, the increase in the number of statistical package programs and the easy accessibility of these softwares offer researchers some advantages, but on the other hand, it has become an important problem. Researchers can utilize both univariate and multivariate statistical methods with applications available as free softwares or web pages. Statistical package programs have been developed to make mathematical calculations easier. The unconscious use of these packages by people with insufficient biostatistical knowledge leads to erroneous results. Hofacker (7) expressed this situation in his article as follows: "The good news is that statistical analysis is becoming easier and cheaper. The bad news is that statistical analysis is becoming easier and cheaper".

Various measures have been taken to reduce the sources of bias and statistical error in research as much as possible and to standardize the processes. In particular, Bradford Hill`s study titled "The time to allow for statistical factors is when an inquiry is planned, not when it is completed" published in The Lancet since the 1930s drew attention to this issue and statistical errors in studies published in scientific journals began to be examined. Although significant progress has been made over the years, it is observed that serious statistical errors continue to be made in scientific studies in recent years. In fact, the use of complex statistical methods is increasing, and this causes readers to be more disadvantaged (6,8).

REPORTING GUIDELINES

Editors/referees would benefit from a list of features to consider when evaluating a publication. However, depending on the type of research design, there are many different points to consider, and these can be overlooked. Furthermore, people's perspectives on various issues may differ depending on their areas of expertise. As a result, working groups comprised of journal editors, reviewers, authors, and other stakeholders developed reporting guidelines for all research designs in order to improve the quality and transparency of research reports (9).

These guidelines state point by point what to consider in the study title, abstract, introduction, material-method, results, and discussion sections. However, there are also problems with the use of these guides. Caulley et al. (10) examined 200 articles that reported using the guidelines for four different study designs and found that only 39% of those articles used the guidelines appropriately.

Almost 500 improved guidelines and their extensions can be accessed on the Enhancing the QUAlity and Transparency Of health Research (EQUATOR)-network (https://www.equator-network.org/) web page. A brief list of available guidelines for the main study designs is given in Table 1 (11).

It would be helpful to list the features to be considered while reading or reviewing a scientific publication. Ideally, an article should be expected to answer "yes" to all the questions on the list. But very few articles can fulfill this expectation. Naturally, not all questions on the list are of equal weight. The first consideration should be given to the design of the study and the risk of bias. Because, if the design of the study is unacceptably wrong, it doesn't matter which statistical method is applied in what way and whether the results are interpreted correctly. Secondly, the appropriateness of the statistical analysis and the correct presentation and interpretation of the results come. The presentation of the findings is also important, but statistical analysis and correct interpretation is not as important as the methodology of the study.

Table 1. Reporting guidelines for main study types (11)

Study Design	Guideline
Randomized trials	CONSORT
Observational studies	STROBE
Systematic reviews	PRISMA
Study protocols	SPIRIT
	PRISMA-P
Diagnostic/prognostic studies	STARD
	TRIPOD
Case reports	CARE
Clinical practice guidelines	AGREE
	RIGHT
Qualitative research	SRQR
	COREQ
Animal pre-clinical studies	ARRIVE
Quality improvement studies	SQUIRE
Economic evaluations	CHEERS

CONSORT: CONsolidated Standards Of Reporting Trials
STROBE: STrengthening the Reporting of OBservational studies in Epidemiology
PRISMA: Preferred Reporting Items for Systematic reviews and Meta-Analyses
SPIRIT: Standard Protocol Items: Recommendations for Interventional Trials
PRISMA-P: Preferred Reporting Items for Systematic reviews and Meta-Analyses Protocols
STARD: STAndards for Reporting of Diagnostic Accuracy
TRIPOD: Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis
CARE: CAse REports
AGREE: Appraisal of Guidelines for Research and Evaluation
RIGHT: Reporting Items for practice Guidelines in HealThcare
SRQR: Standards for Reporting Qualitative Research
COREQ: COnsolidated criteria for REporting Qualitative research
ARRIVE: Animal Research: Reporting of In Vivo Experiments
SQUIRE: Standards for QUality Improvement Reporting Excellence
CHEERS: Consolidated Health Economic Evaluation Reporting Standards

RESEARCH REGISTRY SYSTEMS

In order to prevent researchers from causing bias in the study at any stage of the research, clinical research registries, in which protocols can be uploaded, have been started to be established since 2000. It is recommended that the protocol of the study be uploaded to these registration systems before the first patient/subject is included in the study. Some journals require that the protocols of the articles sent to them be uploaded to one of the public registration systems. Journal editors and referees can evaluate whether there is deviation from the protocol at any stage of the study, which would cause bias. Protocols are commonly recorded in registries such as World Health Organization's International Clinical Trials Registry Platform (https://www.who.int/clinical-trials-registry-platform/the-ictrp-search-portal), EU Clinical Trials Register (https://www.clinicaltrialsregister.eu/), and The United States (https://clinicaltrials.gov/) to provide transparency in studies. Clinical studies conducted in our country are required to be registered on the Turkish Medicines and Medical Devices Agency's clinical research portal (https://kap.titck.gov.tr). PROSPERO (https://www.crd.york.ac.uk/prospero/) and Cochrane Collaboration (https://www.cochrane.org/) are the most

widely used registry systems for systematic review and meta-analysis studies. On the other hand, the protocols accessible from these registry systems should not be considered 100% correct.

In fact, within the scope of the concept of reproducible research, which has become popular approach in recent years, some journals have begun to request the data of the studies and the commands of the statistical methods used in the analysis as supplementary materials.

When health professionals read the articles published in literature, they should have sufficient biostatistical knowledge to understand whether the research is carried out in accordance with biostatistics principles and scientific research methods. In the following part, some basic statistical principles will be presented for this purpose.

BASIC STATISTICAL CONCEPTS

Statistics covers the methods used in planning, conducting, obtaining data, organizing, summarizing, analyzing, interpreting and reaching a decision for scientific purposes.

Population - Sample

A population is a collection of all observations/individuals/objects that have common characteristics related to the subject under study. Scientific research is not conducted on a population, but on a small subset, which is representative of that population and contains fewer observations, and is called a sample. For the sample to be representative of the population, the sample size must be sufficient and the sample should be drawn using one of the appropriate probability sampling methods. If the sampling is biased, there is no statistical method to correct this, and it is inevitable that the results will be misleading.

Parameter - Statistic

The value obtained from the sample is called statistic, and the value obtained from the population is called parameter. The main purpose of statistical methods is to estimate the unknown population parameter with the statistic calculated from the sample. Since it is studied on samples, there is always the possibility of making mistakes depending on chance, no matter what decision is made. The main purpose of complying with the principles of biostatistics in all steps of the research is to minimize this error as much as possible and to ensure that it is at the desired level.

Variable

A variable is defined as any characteristic that can take different values, such as anthropometric measures, cholesterol, hemoglobin level, and treatment duration. It's called a variable because it has values that vary from person to person or from situation to situation within the same person. Variables can be classified as dependent variables (output, explained, predicted) and independent (explanatory, predictor, exposure, covariate) variables. The dependent variable is the variable that can occur with the effect of one or more independent variables and whose relationship with the independent variables is examined. The independent variables are the variables that are thought to affect the dependent variable. The primary variable(s) of the studies should be clearly stated in the material-method section. The research design and the minimum number of samples required in the study are determined according to the primary variable(s).

Data

Data are materials collected in order to explain the study questions or to solve a problem. Data are raw materials obtained by measurements, surveys, observations, biochemical tests, biomedical imaging, etc. Data is transformed into information by processing with statistical methods. In order to achieve the targeted goals in research, it should be decided from at the beginning of the study which data will be collected, how and in which format it will be collected. It is a wrong approach to collect data that is not directly related to the study purpose in case it will be useful to us in the future. For example, adding extra questions that are not necessary to the questionnaire will reduce the reliability of the answers to other questions. If scale forms are to be used to measure the behavior/attitudes of individuals, validity and reliability studies of these scales must be carried out in our country.

Data Type

The type of data determines which descriptive statistics and statistical methods are used in the study. Data are basically divided into two groups as quantitative and qualitative (or numerical and categorical). Quantitative data can be categorized and converted into qualitative data, but it should be taken into account that there will be a loss of information in this case. While classifying the data, cut-off points should not be determined arbitrarily (to obtain statistically significant results), cut-off values should be chosen according to criteria that can be commonly accepted by everyone objectively. Arbitrarily setting cut-off points or creating new variables from variables in a dataset to obtain statistically significant results is an unethical use of statistics.

Qualitative Data

Data describing the characteristics of the individual, which do not require measurement or counting, such as gender (male-female), blood type (AB-AB-O), geographical regions, response to treatment (complete response, partial response, no response), educational status (low, medium, high), disease stages (stage I, stage II, stage III, stage IV) are called qualitative data.

Qualitative data is divided into two groups: nominal (unordered) and ordinal. Blood type, marital status, geographical area, etc. variables are nominal data, and educational level, disease stages, staging, etc. variables are ordinal data. Qualitative data can be entered into statistical software by giving numerical codes. Since the given numeric codes are not used to indicate a measured quantity, but to describe the characteristics of the subjects, arithmetic operations should not be performed on these variables. In addition, since there is no proportionality between categories in ordinal qualitative data (for example, the difference between stage I and stage II and the difference between stage III and stage IV are not equal), arithmetic operations are not performed in such data. Frequencies and percentages are used to summarize qualitative data. If only percentage values are to be given in the tables, the row or column totals should be given to know how many subjects were used in the calculation.

Advanced statistical analysis performed with qualitative data requires the use of different regression models, depending on the type of the dependent variable

(binary/dichotomous, nominal, or ordinal). While qualitative independent variables with more than two categories were included in the model; dummy variable coding is required for nominal variables. For ordinal independent variables, either a dummy variable can be coded or it can be included in the model as a numerical variable (if it is thought to be a proportional increase or decrease).

Quantitative (Numerical) Data

Data such as age, hemoglobin, leukocyte count, number of children, number of patients, and body mass index expressed as numerical values obtained as a result of measurement or counting are called quantitative data. Quantitative data is divided into discrete numerical data and continuous numerical data. Discrete numerical data are values obtained by counting and expressed as an integer number. Examples of discrete numerical data include the number of patients, the number of lymph nodes, and the number of embryos transplanted. Continuous numerical data is data that changes within a specific range and can take any value. Age, FSH, LH, hemoglobin, B12 level, etc. are examples of continuous numerical data.

When summarizing the numerical data, it should be decided which central tendency (mean, median, geometric mean, trimmed mean) and dispersion measures (standard deviation, range, interquartile range, etc.) to use according to the distribution of the data. For example, it is not appropriate to use the arithmetic mean and standard deviation in a skewed data set or in the presence of outlier/extreme values. It is also not appropriate to give the standard deviation as a measure of dispersion together with the median. Different advanced analysis methods/models are used depending on whether the quantitative dependent variables are continuous or count data, whether the relationship between the variables is linear or not, and whether parametric assumptions are satisfied or not.

All statistical methods are applied under the assumption that data are measured without error. Therefore, researchers should be sure that the data they obtained are correct, complete, valid, and reliable (1,3).

Presenting the Results with Tables and Graphs

The tables are useful tools to present the results in a compact and readily comprehensive form. When the results are given in a table, readers can easily understand the message and get an idea about the variable under study without reading the text. Constructing an appropriate table is not an easy task. It requires knowledge, skills, and experience. How the results are displayed in the table depends on what should be shown and how it should be shown to the readers. The best way to create a good table is to create several tables and choose the one that looks the best and is easy to understand. A simple table is better than a complex one. Complex tables can make it difficult to inspect and understand the information given in a table. Tables should be self-explanatory. Instead of preparing a complex table, it may be more appropriate to prepare a few simple tables.

Each table should have a title that clearly defines the contents of the table. Row and column labels must be clearly indicated. The units of measurement for the variables that appear in the rows and columns should be written as well.

The information given in the table can also be displayed graphically if desired. The main purpose is to make the information in the table clearer and easier for the reader to understand. The exact values of the results can be read from the tables, but this is not possible in the graphics. For this reason, in scientific research, it is not recommended to display the results in a graph without displaying them in a table.

The graph requires a title, a horizontal axis label, and a vertical axis label. Titles are usually written above the table and below the graph. Different graphs are used depending on the data type. Qualitative data uses bar and pie charts, while quantitative data typically uses histograms, scatter polygons, box line charts, line charts, and error bar charts.

Researchers must be careful when drawing or interpreting graphs. Changing the scale of the graphs drawn with the same data may cause different interpretations of the results. Researchers sometimes deliberately change the scale of the graphs in order to display the results as they wish. Sometimes, because their statistical knowledge level is not sufficient, they may draw the scales incorrectly (software can automatically adjust the scales) without realizing it.

The graphics' colors and resolutions should be adjusted according to their intended use. If a book or journal is to be printed in black and white, for example, shades of gray can be used instead of color graphics. To make the graphics look better in the articles, they would be drawn at 300 dpi resolution.

CONCLUSION

Researchers should be aware that research is a too difficult process to be carried out by a single person and should be conducted by a multidisciplinary team comprised of all experts who may be required. Since there is a possibility of making mistakes and biases at every stage of the research, they can only anticipate these issues and take the necessary precautions only in this manner. A biostatistics expert should join this multidisciplinary team at the beginning of the study. His knowledge will be required during the planning and executing studies, analyzing data, and presenting and reporting results. Researchers have easy access to software that can perform various statistical analyses. They should be aware, however, that if they use these software correctly and consciously, they can achieve accurate results.

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The Role of the Urine Dipstick Test in the Detection of Abnormal Proteinuria Using Different Cut-off Levels in Hypertensive Pregnancies

Hipertansif Gebeliklerde Farklı Kesim Değerleri Kullanılarak Anormal Proteinüri Saptanmasında Spot İdrar Protein Ölçümünün Rolü

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ABSTRACT

Aim: The aim of this study was to determine the diagnostic accuracy of different urine dipstick protein threshold levels in predicting the presence of abnormal proteinuria in pregnant women with hypertension.
Material and Methods: A total of 326 singleton pregnant women who underwent 501 urine protein tests and who had suspected preeclampsia were included in this retrospective study. Patient data was taken including medical and obstetric history. The results of dipstick urinalysis and concurrent 24-hour urine protein excretion measurements were compared to determine the accuracy of urinalysis.
Results: A dipstick result of 1+ was found to be the best cut-off to predict 500 mg of protein excretion per day, with sensitivity and specificity of 62.09% and 88.97%, respectively. A 2+ proteinuria dipstick cut-off had high specificity and positive predictive value (PPV) (99.05% and 98.84%, respectively) for the prediction of 300 mg of protein excretion per day; this cut-off had low sensitivity (21.46%). A cut-off of 1+ also provided satisfactory specificity and PPV (91.43% and 94.48%, respectively) for the detection of 300 mg of protein excretion per day, but sensitivity was compromised (38.89%). Among 301 patients with negative dipstick results, 212 had a 24-hour urine protein extraction greater than 300 mg, with a false negative rate of 70.43%.
Conclusion: The results suggest that the urine protein dipstick measurement has limited quantitative ability for the prediction of abnormal proteinuria. Additionally, the use of 500 mg 24-hours protein excretion as a cut-off value for abnormal proteinuria may provide useful data.
Keywords: 24-hour urine protein; dipstick urinalysis; preeclampsia; pregnancy; proteinuria.

ÖZ

Amaç: Bu çalışmanın amacı, hipertansif gebelerde anormal proteinüri varlığını öngörmede farklı idrar ölçüm çubuğu protein eşik seviyelerinin tanısal doğruluğunu belirlemektir.
Gereç ve Yöntemler: Bu geriye dönük çalışmaya, preeklampsiden şüphelenilen toplam 326 tekiz gebe kadından elde edilen 501 idrar protein testi sonucu dahil edilmiştir. Hastaların tıbbi ve obstetrik geçmiş verileri kaydedildi. İdrar tahlilinin doğruluğunu belirlemek için idrar ölçüm çubuğu protein tahlili ve eşzamanlı 24 saatlik idrar protein atılım ölçümlerinin sonuçları karşılaştırıldı.
Bulgular: 1+ spot idrar protein ölçüm sonucu, 500 mg günlük protein atılımını sırasıyla %62,09 duyarlılık ve %88,97 özgüllük ile öngörmede en iyi kesim değeri olarak bulundu. Spot idrarda 2+ proteinüri değeri; 300 mg günlük protein atılımını öngörmede yüksek özgüllük ve pozitif tahmin değerine (PTD) (sırasıyla %99,05 ve %98,84) sahipken, bu kesim değerinin duyarlılığı düşüktür (%21,46). 1+ kesim değeri; 300 mg günlük protein atılımının saptanması için tatmin edici özgüllük ve pozitif tahmin değerine sahipti (sırasıyla %91,43 ve%94,48), ancak duyarlılığı düşüktü (%38,89). Spot idrar protein ölçümü negatif olan 301 hastadan 212'sinde günlük 300 mg üzeri 24 saatlik idrar protein atılımı tespit edildi (%70,43 yanlış negatiflik).
Sonuç: Sonuçlar, spot idrar protein ölçümünün anormal proteinüriyi tahmin etmek için sınırlı niceliksel kabiliyete sahip olduğunu göstermektedir. Ek olarak, anormal proteinüri için kesme değeri olarak 500 mg 24 saatlik protein atılımının kullanılması yararlı veriler sağlayabilir.
Anahtar kelimeler: 24 saatlik idrar proteini; daldırma çubuğu idrar tahlili; preeklampsi; gebelik; proteinüri.

INTRODUCTION

Pregnancy-related hypertensive disorders are one of the leading causes of maternal and perinatal mortality globally. Preeclampsia is a pregnancy-specific hypertensive disease with multi-system involvement. It affects 3-8% of all pregnancies worldwide and is also a financial burden on healthcare systems and society (1,2). Formerly, preeclampsia was recognized as hypertension with proteinuria. Although an abnormal level of protein excretion was known to be a hallmark of preeclampsia, the Task Force on Hypertension eliminated the dependence of the diagnosis on proteinuria in 2013 (3). Recent guidelines noted that, in the absence of proteinuria, acute onset hypertension with evidence of end-organ dysfunction is adequate for the diagnosis of preeclampsia (4,5). Although proteinuria is no longer required for diagnosis, it still occurs in approximately 75% of cases (6) and is associated with more severe neonatal outcomes (7-9). The accurate detection of proteinuria in pregnant women with suspected preeclampsia is therefore still valuable in daily practice.

Three methods are available for the assessment of urinary protein excretion: 1) dipstick urinalysis, 2) urine protein-to-creatinine ratio (UPCR), and 3) 24-hour urine protein testing. During gestation, physiological limits of urinary protein excretion may increase up to 150-250 mg per day and the recommended threshold value to define clinically significant proteinuria is 300 mg per day (10). 24-hour urine collection is the gold standard method for evaluation of the level of proteinuria (11). However, this test has some limitations: firstly, the technique is time-consuming, which hampers rapid diagnosis, and secondly, it can be cumbersome for ambulatory patients and it presents practical difficulties associated with urine collection. In current practice, many clinicians opt for dipstick urinalysis for the evaluation of abnormal proteinuria. This method is fast, easy, and cheap, but provides limited quantitative information. Urinary protein excretion is variable throughout the day and hydration or diuresis may influence the accuracy of the test. The American College of Obstetricians and Gynecologists (ACOG) recommends urine dipstick testing using ≥2+ (100 mg/dL) as the discriminant value only if other quantitative methods are not available (4). On the other hand, the International Society for the Study of Hypertension in Pregnancy (ISSHP) suggests initial assessment using dipstick urinalysis. If the protein level is found to be ≥1+ (30 mg/dL), then other quantitative methods are applied (5).

The current study was undertaken to determine the diagnostic accuracy of different urine dipstick protein threshold levels in predicting the presence of abnormal proteinuria in pregnant women with hypertension.

MATERIAL AND METHODS

This single-center, retrospective cohort study was conducted in a tertiary referral university hospital between January 2010 and January 2018 to assess the validity of dipstick urinalysis. The study protocol was approved by the local institutional ethics committee (Bezmialem Vakıf University Faculty of Medicine, 22.12.2020, 21/400) and was carried out in accordance with the principles set out in the Helsinki Declaration 2008.

A total of 326 singleton pregnant women who underwent 501 urine protein tests and had suspected preeclampsia were recruited. All patients had new-onset hypertension with greater than 140/90 mmHg blood pressure after 20 weeks of gestation. Patients younger than 18 years old, patients with bacteriuria, and patients with both a urine volume under 400 mL/day and a duration of over four days between dipstick urinalysis and 24-hour urine collection were excluded from the study. A detailed medical and obstetric history was taken, including age, gravida, parity, previous history of preeclampsia, associated pathologies, the results of dipstick urinalysis and 24-hour urine protein test, gestational age at the time of dipstick screening, and the time interval between dipstick urinalysis and 24-hour urine protein test.

Dipstick urinalysis was performed by an H-800 automatic urine analyzer (Dirui Industrial, Co. Ltd. China) on freshly evacuated midstream urine samples at any time during the day except the first voided morning specimen or before bedtime. The grades of proteinuria as provided by the manufacturers were presented as 0 (negative), trace (0-30 mg/dL), 1+ (30-100 mg/dL), 2+ (100-300 mg/dL), 3+ (>300 mg/dL). 24-hour urine samples were collected from outpatients or inpatients, in accordance with written instructions which were given to patients for proper collection. Urine collection started at 8.00 am in the morning after discarding the first urine sample of the day. 24-hour quantitative proteinuria was carried out according to the colorimetric method using an Architect C16000 clinical chemistry analyzer (Abbott Laboratories, Abbott Park, IL, USA).

Statistical Analysis

The descriptive statistics are given as mean±standard deviation or median (min-max) for numerical variables and frequency, percentage were given for categorical variables. The chi-squared test was used to compare distribution of categories to give sensitivity, specificity and predictive values (PPV, NPV, +LR, -LR) of the dipstick urinalysis. The statistical analysis were performed using SPSS, version 22 (IBM SPSS Statistics for Windows, Armonk, NY; IBM Corp., Released 2013) and Medcalc (MedCalc Software, Ostend, Belgium).

RESULTS

In this study, 501 urine samples were collected from 326 pregnant women who had suspected preeclampsia. The characteristics of the population are presented in Table 1. The mean age of the study group was 33.3±5.8 years. The median gravida was 2 (range, 1-9) and the median parity was 1 (range, 0-6). Almost half of the cases were nulliparous (149 out of 326, 45.7%) and 3.6% (n=12) of patients had previous history of preeclampsia. Furthermore, type 1 diabetes (1.8%, 6 cases); type 2 diabetes (6.4%, 21 cases) and gestational diabetes (14.7%, 48 cases) were observed as associated pathologies in the study group. The mean gestational age at the time of dipstick screening was 224.9±32.1 days. The median time interval between dipstick urinalysis and 24-hour urine protein testing was 2 (range, 0-4) days.

Table 2 indicates the diagnostic accuracy of dipstick urine analysis in predicting the presence of significant proteinuria levels in 24-hour urine collection. 1+ was found to be the best cut-off to predict 500 mg of protein excretion per day, with sensitivity and specificity of 62.09%

Table 1. The baseline demographic and clinical parameters

Characteristics	**(n=326)**
Maternal age (years)	33.3±5.8
Gravida	2 (1-9)
Parity	1 (0-6)
Nulliparous	149 (45.7%)
Previous history of preeclampsia	12 (3.6%)
Gestational age at the time of dipstick screening (days)[a]	224.9±32.1
Time interval between dipstick urinalysis and 24-h urine protein test (days)[a]	2 (0-4)
Associated pathologies	
Type 1 diabetes	6 (1.8%)
Type 2 diabetes	21 (6.4%)
Gestational diabetes	48 (14.7%)
24-h protein excretion range[a]	
Less than 300 mg/day	105 (20.9%)
300-500 mg/day	185 (36.9%)
500-5000 mg/day	197 (39.3%)
More than 5000 mg/day	14 (2.7%)
Dipstick proteinuria[a]	
Negative	301 (60%)
Trace	37 (7.3%)
1+	77 (15.3%)
2+	58 (11.5%)
3+	28 (5.5%)

[a]: n=501, values are expressed as n (%), mean±standard deviation or median (min-max)

Table 2. Diagnostic ability of dipstick urine analysis in predicting the presence of significant proteinuria levels in 24-hour urine collection

	24-h urine protein (mg/day)			
	≥300 (n=396)	**<300 (n=105)**	**≥500 (n=211)**	**<500 (n=290)**
Dipstick Proteinuria				
≥1+	154	9	131	32
<1+	242	96	80	258
		95% CI		**95% CI**
Sensitivity	38.89	34.06-43.89	62.09	55.17-68.66
Specificity	91.43	84.35-96.01	88.97	84.78-92.33
PPV	94.48	89.78-97.44	80.37	73.43-86.17
NPV	28.40	23.65-33.53	76.33	71.43-80.76
LR (+)	4.54	2.40-8.58	5.63	3.99-7.93
LR (-)	0.67	0.61-0.74	0.43	0.36-0.51

	24-h urine protein (mg/day)			
	≥300 (n=396)	**<300 (n=105)**	**≥500 (n=211)**	**<500 (n=290)**
Dipstick Proteinuria				
≥2+	85	1	76	10
<2+	311	104	135	280
		95% CI		**95% CI**
Sensitivity	21.46	17.52-25.84	36.02	29.54-42.89
Specificity	99.05	94.81-99.98	96.55	93.75-98.33
PPV	98.84	93.69-99.97	88.37	79.65-94.28
NPV	25.06	20.96-29.52	67.47	62.73-71.96
LR (+)	22.54	3.18-159.96	10.45	5.54-19.71
LR (-)	0.79	0.75-0.84	0.66	0.60-0.73

CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value, LR(+): positive likelihood ratio, LR(-): negative likelihood ratio

and 88.97%, respectively. A 2+ proteinuria dipstick cut-off had high specificity and positive predictive value (PPV, 99.05% and 98.84%, respectively) for the prediction of 300 mg of protein excretion per day; this cut-off had low sensitivity (21.46%). A 1+ cut-off also provided satisfactory specificity and PPV (91.43% and 94.48%, respectively) for the detection of 300 mg of protein excretion per day, but sensitivity was compromised (38.89%). Among patients with negative dipstick results (n=301), 212 had a 24-hour urine protein extraction greater than 300 mg, with a false negative rate of 70.43%.

DISCUSSION

The results of the present study verify the limited quantitative ability of dipstick urine analysis for the prediction of proteinuria in pregnant women with hypertension. For all comparisons, the specificities were high (>85%), but sensitivities differed. The best correlation was observed between the 1+ dipstick threshold and 500 mg of protein excretion per day, with an overall accuracy of 77.6%. In addition, both 1+ and 2+ dipstick thresholds showed a better correlation with proteinuria of 500 mg/day compared with proteinuria of 300 mg/day.

The classical threshold established for the diagnosis of significant urine protein excretion in pregnancy is 300 mg per day. Although this dividing line is commonly accepted, this is solely based on expert opinion and the findings of previous studies with small sample sizes (10,12). Current studies indicate proteinuria of 500 mg/day as an appropriate cut-off for abnormal proteinuria, especially in healthy primiparous women during late pregnancy when carrying twins (13,14). Also, with a diagnostic cut-off value of 300 mg/day, the incidence of isolated proteinuria may reach 8% during pregnancy, whereas preeclampsia affects 3-8% of pregnancies (15). Given this uncertainty surrounding the cut-off value for the 24-hour urine protein test, in the present study, we investigated the association between dipstick proteinuria results and daily proteinuria levels at various grades. According to our results, both 1+ and 2+ proteinuria measured using the dipstick test are more highly correlated with proteinuria of 500 mg/day than with proteinuria of 300 mg/day. The overall accuracy of 1+ proteinuria measured using the dipstick test increased from 49.9% to 77.6% and that of 2+ proteinuria increased from 37.7% to 70.8%. At this threshold, the urine dipstick test provides better overall accuracy and may replace UPCR analysis. Given the wide availability, ease of use, and low cost, the urine dipstick test may still play an important role in clinical practice.

A brief review by ISSHP advised initial use of dipstick urine protein analysis in cases of suspected preeclampsia, and they recommend no further evaluation if the dipstick test is negative (5). However, our results do not entirely support that a negative dipstick reading can rule out abnormal proteinuria. In 212 out of 301 patients with negative dipstick urine protein analysis, the 24-hour urine protein test results were found to be positive, with a false negative rate of 70%. Previous studies have also shown widely varying sensitivity and specificity with this cut-off level for predicting abnormal proteinuria (16-22). In parallel with our results, Meyer et al. (16) also reported a false negative rate of 66% in 123 patients. A review from

Waugh et al. (23) showed sensitivity and specificity for this test range of 59% and 28%, respectively. They also indicated the usefulness of this threshold in informing clinical decision-making. In only one study, the dipstick test provided both sensitivity and specificity above 80% for reference standard testing (19).

Based on guidelines from the ACOG, the reference standards for screening urinary protein excretion are the 24-hour urine protein test and UPCR. They also recommend, in the absence of these, that a dipstick reading of 2+ alone is sufficient for diagnosis (4). Previous studies have displayed suitable sensitivity, but some lack specificity for defining abnormal protein excretion, or vice versa (16-18,20). In the present study, a dipstick reading of 2+ accurately predicted 85 of the 86 cases, with a PPV of 98.84%, with only one false positive case detected. Therefore, the threshold of 2+ for the dipstick test could be beneficial and practical for women requiring a rapid diagnosis, especially in patients with high-risk pregnancies.

The most important limitation of this study is its retrospective design and the disadvantages inherent to it. In addition, our study does not provide insight into the relationship between dipstick test results and perinatal outcomes, which would be the ideal outcome measure. However, the study also has important strengths. First, it includes one of the largest cohorts on this subject in the literature, with the trial conducted in a single center. Second, the short time interval between dipstick urinalysis and 24-hour urine protein test provided a reliable analysis and enabled us to assess the correlation between these two tests.

CONCLUSION

Our findings indicate that urine protein dipstick measurement has limited quantitative ability for the prediction of abnormal proteinuria. Given the growing body of evidence against the use of 300 mg 24-hours protein excretion as a cut-off value for abnormal proteinuria, our results may provide useful data for those selecting 500 mg 24-hours as the cut-off, as we do in our clinical practice. Additionally, given the uncertainty of the laboratory criteria for establishing proteinuria during pregnancy, it is important to encourage the accumulation and presentation of local data.

Ethics Committee Approval: The study was approved by the Clinical Researches Ethics Committee of Bezmialem Vakıf University (22.12.2020, 21/400).

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Measurement of Tracheobronchial Angles of COVID-19 Patients on Computed Tomography and Correlation with Pneumonia Severity in Turkish Population

Türk Popülasyonunda Bilgisayarlı Tomografide COVID-19 Hastalarının Trakeobronşiyal Açılarının Ölçümü ve Pnömoni Şiddeti ile Korelasyonu

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ABSTRACT

Aim: This study aimed to evaluate the values of tracheobronchial angles on computed tomography (CT) and to investigate the relationship between angles and CT severity scores (CT-SS) of coronavirus disease 2019 (COVID-19) patients. There is no available literature measuring tracheobronchial angles of adult COVID-19 patients and investigating the relationship of angles with pneumonia severity.

Material and Methods: This study was a single-center retrospective analysis of 92 RT-PCR positive patients, aged between 18-40 years, who underwent CT between May and October 2020. The right bronchial angles (RBA), left bronchial angles (LBA), subcarinal angles (SCA), and interbronchial angles (IBA) were measured by a radiologist on coronal CT images with the measurement model used in past. CT-SS was calculated by using a visual scoring system with a global score of 0-25.

Results: Thirty-seven (40.2%) patients had normal CT imaging and 55 (59.8%) patients had pulmonary involvement. The CT-SS were ranged from 0 to 24, with a median value of 2.5. The mean IBA was calculated as 81.67±15.20°, mean SCA 77.65±15.78°, mean RBA 39.26±7.51°, and mean LBA 43.35±8.43°. No statistically significant difference was found in SCA, IBA, RBA, and LBA between the groups with and without COVID-19 pneumonia (p=0.277, p=0.389, p=0.218, and p=0.227, respectively). Also, no significant correlation was found between tracheobronchial angles and pneumonia CT-SS of the patients.

Conclusion: We calculated the distribution range of tracheobronchial angle values in the adult Turkish COVID-19 patients. According to our study, tracheobronchial angles don't affect the disease severity and clinical outcome of COVID-19 patients.

Keywords: COVID-19; computed tomography; chest CT severity score; bronchial angle; subcarinal angle; interbronchial angle.

ÖZ

Amaç: Bu çalışma, koronavirüs hastalığı 2019 (coronavirus disease 2019, COVID-19) hastalarının bilgisayarlı tomografide (BT) trakeobronşiyal açı değerlerini hesaplamayı ve bu açılar ile BT şiddet skorları (BT-ŞS) arasındaki ilişkiyi araştırmayı amaçlamaktadır. Yetişkin COVID-19 hastalarının trakeobronşiyal açılarını ölçen ve açıların pnömoni şiddeti ile ilişkisini araştıran mevcut bir literatür bulunmamaktadır.

Gereç ve Yöntemler: Bu çalışma, Mayıs ve Ekim 2020 arasında BT çekilen 18-40 yaş arasında 92 RT-PCR testi pozitif hastanın tek merkezli retrospektif analizidir. Sağ bronş açıları (sağ BA), sol bronş açıları (sol BA), subkarinal açılar (SKA) ve interbronşiyal açılar (İBA), geçmişte kullanılan ölçüm modeli ile koronal BT görüntülemelerinde bir radyolog tarafından ölçüldü. BT-ŞS, global skoru 0-25 olan bir görsel skorlama sistemi kullanılarak hesaplandı.

Bulgular: Otuz yedi (%40,2) hastanın BT görüntülemeleri normal iken, 55 (%59,8) hastada akciğer tutulumu vardı. BT-ŞS 0 ile 24 arasında olup ortanca değeri 2,5 idi. Ortalama İBA 81,67±15,20°, ortalama SKA 77,65±15,78°, ortalama sağ BA 39,26±7,51° ve ortalama sol BA 43,35±8,43° olarak hesaplandı. SKA, İBA, sağ BA, sol BA değerleri bakımından COVID-19 pnömonisi olan ve olmayan gruplar arasında istatiksel olarak anlamlı bir farklılık saptanmadı (sırsıyla, p=0,277; p=0,389; p=0,218 ve p=0,227). Ayrıca trakeobronşiyal açılar ile pnömoni BT-ŞS arasında istatiksel olarak anlamlı bir korelasyon bulunmadı.

Sonuç: Çalışmamızda, erişkin Türk COVID-19 hastalarında trakeobronşiyal açı değerlerinin dağılım aralığını hesapladık. Çalışmamıza göre trakeobronşiyal açılar, COVID-19 hastalarında, hastalığın şiddetini ve klinik sonucunu etkilememektedir.

Anahtar kelimeler: COVID-19; bilgisayarlı tomografi; toraks BT şiddet skoru; bronş açısı; subkarinal açı; interbronşiyal açı.

INTRODUCTION

The trachea extends from the inferior aspect of the cricoid cartilage to the carina and its craniocaudal length is measured usually 10-12 cm (1,2). Trachea branches to the left and right main bronchus (LMB and RMB) at the 4-5th thoracic vertebra level generally (3).

Conventional chest radiographs (posterior-anterior, PA) and lateral chest radiographs are the imaging modalities used for the initial evaluation of the trachea and central airways. Computed tomography (CT) scan shows the anatomy of the airways better and allows evaluation in three planes as axial, coronal, and sagittal. Coronal images of CT are very useful for imaging the trachea in a single plane (2).

Tracheal bifurcation angle means both subcarinal angles (SCA) and interbronchial angles (IBA). The angle measured between the central axes of the right and left main bronchi is called the IBA. The angle formed at the intersection of the lines drawn along the lowest surfaces of the right and left main bronchi is defined as SCA (4-7). Tracheobronchial angles may differ depending on race, age, and individual and angle measurement techniques (1,8-15). Therefore, it is difficult to decide the range of "normal" tracheobronchial angles.

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which was firstly reported in Wuhan, China (16). The patients with COVID-19 mostly have a good prognosis and mild symptoms. But, severe pulmonary involvement, pulmonary edema, acute respiratory distress syndrome (ARDS), multiple organ failure, and death were observed in some patients (17). While some patients with COVID-19 have no pulmonary involvement, some patients have extensive pulmonary involvement and a poor prognosis.

In our study, we investigated whether tracheobronchial anatomy features may be a risk factor for the occurrence of severe pneumonia in patients with COVID-19. To our knowledge, no study in the literature measured tracheobronchial angles on thorax CTs of adult COVID-19 patients and investigate the relationship of angles with pneumonia severity. The aim of this study was to evaluate the values of tracheobronchial angles on CT and to investigate the correlation between angles and CT severity score (CT-SS) of COVID-19 patients.

MATERIAL AND METHODS

This study was approved by the Ethics Committee of Kanuni Training and Research Hospital (dated 26.11.2020 and no: 2020/71), and was conducted according to the Declaration of Helsinki and Good Clinical Practice.

Study Group and Data Collection

Our study was a single-center retrospective analysis of 92 patients with a positive COVID-19 polymerase chain reaction (PCR) test. Chest CT examinations of all study patients were performed in Kanuni Training and Research Hospital at the time of admission. Patients who underwent chest CT scans between May and October 2020 were included in the current study. We included only laboratory confirmed patients with RT-PCR positivity in samples from the oropharyngeal and nasopharyngeal regions. The RT-PCR tests were performed in the Microbiology Laboratory at the same hospital. RT-PCR tests were repeated in patients with a high radiologic and clinic suspicion of COVID-19 when the initial PCR test was negative. We collected the data for retrospective analysis including demographic characteristics, PCR test results, and initial CT imaging. Exclusion criteria include being younger than 18 years or older than 40 years, having any comorbidities, having a history of heart disease, mediastinal abnormalities, musculoskeletal deformity, vascular anomalies, or any previous thoracic injury, or a history of tracheobronchial surgery that may affect the normal anatomy of trachea and bronchus.

Computed Tomography Protocol

All patients underwent chest CT examinations on two multidetector CT scanners (16- slice Somatom Sensation; Siemens Healthineers or 128- slice GE Healthcare Computed Tomography Revolution EVO System). Chest CT imaging was applied in the supine position during a breath-hold.

The non-contrast scans were performed with the following parameters: tube current=70-114 automatic milliamperes; tube voltage=130 kV: helical pitch=1; slice thickness=5 mm and interval=5 mm (16- slice Somatom Sensation; Siemens Healthineers) or tube current=70-280: automatic milliamperes: tube voltage=120 kV; helical pitch=1.375; slice thickness=5 mm and interval=5 mm (128- slice GE Healthcare Computed Tomography Revolution EVO System). Images were reconstructed with a 1.25 mm slice thickness. All chest CT scans were performed at a lung window of 1200 WW and -600 WL and a mediastinal window of 400 WW and 40 WL.

Image Analysis

A radiologist with more than 14 years of experience in chest CT imaging, applied the CT image analysis in a picture archiving and diagnostic system (PACS) workstation, blinded to the clinical data and laboratory findings. Measurements of right bronchial angles (RBA), left bronchial angles (LBA), SCA, and IBA were made in coronal reformat CT images with a slice thickness of 1.5 mm or 1.25 mm. All measurements were performed by the same chest radiologist. We used a measurement model defined in previous studies (1). We calculated the range of distribution of tracheobronchial angle values in adult patients with COVID-19.

The RMB angle is defined as the angle between the vertical line passing through the lower point of the tracheal bifurcation and the line drawn on the mid-axis of the RMB. The LMB angle is defined as the angle between the vertical line passing through the lower point of the tracheal bifurcation and the line drawn on the mid-axis of the LMB. The angle formed between the intersection points of the lines drawn along the central axis of the right and left main bronchi is called the interbronchial (tracheal bifurcation) angle. Sum of RBA and LBA gives IBA. The angle measured at the intersection of the lines drawn along the right and left upper bronchi's lower borders is called the SCA (1, Figure 1).

In addition, the 25 Point CT-SS was calculated in all cases by using a visual semi-quantitative CT severity scoring system that was suggested by Pan et al. (18). In this method, scoring between 0 and 5 was made for 5 lung lobes. Zero if there is no lung involvement; if there is <5% involvement 1; if there is 5-25% involvement 2; 26-50% if

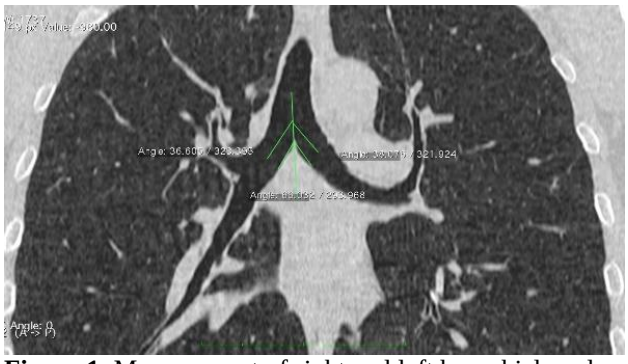

Figure 1. Measurement of right and left bronchial angles, and subcarinal angle on a coronal reformatted computed tomography image. The sum of the right and left bronchial angles gives interbronchial angle.

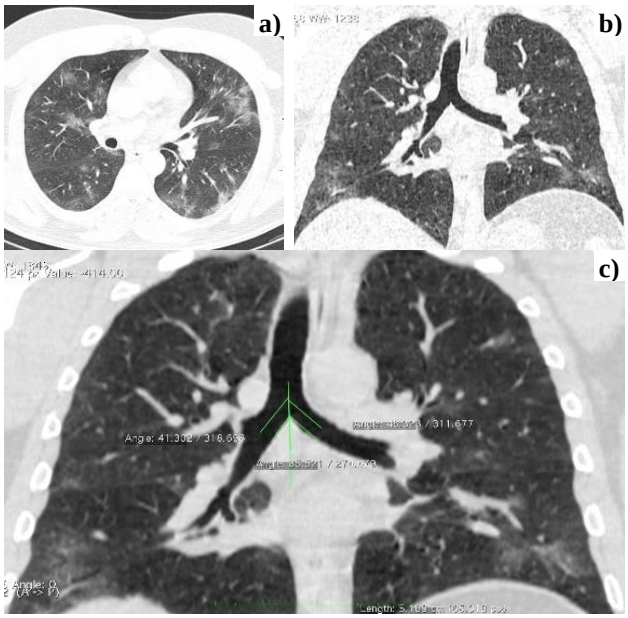

Figure 2. A 40-year-old male with COVID-19. He was discharged after medical treatment at the hospital. **a)** axial and **b)** coronal chest computed tomography scans show bilateral multifocal rounded ground-glass opacities and the computed tomography severity score was calculated 15. **c)** Measurement of right and left main bronchial angles and subcarinal angles on the coronal reformatted computed tomography image; right bronchial angle: 41.3°, left bronchial angle: 48.3°, subcarinal angle: 85.5°, and interbronchial angle: 89.6°.

any 3; if there is 51-75% involvement 4; and it is calculated as 5 if there is >75% involvement. Chest CT-SS is obtained by the sum of 5 lung lobe scores (0-25).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp. Released 2017. Armonk, NY). The conformity of the variables to the normal distribution was examined using the Kolmogorov-Smirnov test. In descriptive statistics, mean and standard deviation were used for normally distributed variables, and median and interquartile range (IQR) were used for non-normally distributed variables. In the comparison of continuous variables according to tracheobronchial angles, Student's t-test was used for those with normal distribution, and the Mann-Whitney U test for those who were not normally distributed. Spearman's rho correlation coefficient was used to evaluate the correlation between SCA, IBA, RBA, and LBA measurements in CT imaging with CT-SS. Statistical significance was established at the $p<0.05$ level.

RESULTS

A total of 92 patients, 53 (57.6%) females and 39 (42.4%) males were included in the study. The mean age of the patients was 31.48±6.55 years ranging from 18 to 40. The diagnosis of COVID-19 was confirmed by RT-PCR in 100% of these patients. Of the 92 cases, 89 (96.7%) were discharged (Figure 2), and 3 (3.3%) were died in the intensive care unit of the hospital.

Fifty-five (59.8%) COVID-19 confirmed cases had pneumonia and 37 (40.2%) COVID-19 confirmed cases had no pneumonia in their chest CT imaging at the time of admission. CT-SS were ranged from 0 to 24, with a mean value of 4.63±5.57 and a median value of 2.5 (IQR, 8). Of the 55 patients, 7 (12.7%) with only right lung involvement (mean CT-SS: 6.14±8.83), 2 (3.6%) with only left lung involvement (mean CT-SS: 1), and 46 (83.6%) with bilateral lungs involvement (mean CT-SS: 8.28±4.48).

On the coronal CT scans, the mean SCA was 77.65±15.78° (range, 43-126), the mean IBA was 81.67±15.2° (range, 31-119), the mean RBA was 39.26±7.51° (range 21-61), the mean LBA was 43.35±8.43° (range 28-77) of all patients (Table 1). In our study RBA was smaller than LBA in 72 (78.3%) patients, RBA was greater than LBA in 17 (18.5%) patients and RBA-LBA was equal in 3 (3.3%) patients.

To investigate the statistical relationship between the angles and age, we divided our patients into two age groups. The mean age of our total study patients was 31.48±6.55 years ranging from 18 to 40. To provide a numerically balanced distribution between the groups, we divided the age groups as the patients aged ≤30 years (n=45) and those aged >30 years (n=47). We could not find a statistically significant difference in SCA, IBA, RBA, and LBA between the two groups (p=0.882, p=0.459, p=0.705, and p=0.501, respectively). Also, there was no statistically significant difference between gender in terms of SCA, IBA, RBA, and LBA (p=0.826, p=0.662, p=0.994, and p=0.404, respectively).

When the patients with and without COVID-19 pneumonia were compared, results showed no statistically significant difference in SCA, IBA, RBA, and LBA in terms of occurrence of COVID-19 pneumonia (p=0.277, p=0.389, p=0.218, and p=0.227, respectively, Table 2).

There was no statistically significant correlation between the CT-SS of all patients (with and without COVID-19 pneumonia) with SCA (r_s=0.085, p=0.420), IBA (r_s=0.089, p=0.397), RBA (r_s=0.115, p=0.273), and LBA (r_s=0.105, p=0.321) measurements in CT imaging (Table 3). Also, there was no significant correlation between the CT-SS of patients with pulmonary involvement (the patients with CT-SS: 0 were excluded) with SCA (r_s=0.011, p=0.936), IBA (r_s=-0.030, p=0.828), RBA (r_s=0.005, p=0.972), LBA (r_s=-0.021, p=0.881) measurements in CT imaging.

Table 1. Descriptive statistics for tracheobronchial angles and computed tomography severity scores

	Mean±SD	Median (IQR)	Min-Max
SCA	77.65±15.78	77 (22)	43-126
IBA	81.67±15.20	82 (22)	31-119
RBA	39.26±7.51	40 (10)	21-61
LBA	43.35±8.43	43 (13)	28-77
CT-SS	4.63±5.57	2.5 (8)	0-24

SCA: subcarinal angle, IBA: interbronchial angle, RBA: right bronchial angle, LBA: left bronchial angle, CT-SS: computed tomography severity score, SD: standard deviation, IQR: interquartile range

Table 2. Comparison of tracheobronchial angles by the presence of COVID-19 pneumonia on CT images

	Absent (n=37)	Present (n=55)	p
SCA	75.46±14.56	79.13±16.52	0.277
IBA	80.00±13.43	82.8±16.30	0.389
RBA	38.08±6.58	40.05±8.04	0.218
LBA	42.03±7.14	44.24±9.15	0.227

COVID-19: coronavirus disease 2019, CT: computed tomography, SCA: subcarinal angle, IBA: interbronchial angle, RBA: right bronchial angle, LBA: left bronchial angle

Table 3. Correlations between tracheobronchial angles and computed tomography severity score

	SCA	IBA	RBA	LBA
CT-SS				
r_s	0.085	0.089	0.115	0.105
p	0.420	0.397	0.273	0.321

CT-SS: computed tomography severity score, SCA: subcarinal angle, IBA: interbronchial angle, RBA: right bronchial angle, LBA: left bronchial angle

DISCUSSION

In the current study, the SCA, IBA RBA, and LBA were measured on the reformat coronal CT images of COVID-19 patients. We investigated the correlation between SCA, IBA, RBA, and LBA measurements with the CT-SS of patients. We investigated the effects of tracheobronchial anatomy on the occurrence and severity of COVID-19 pneumonia. Our study is the first to evaluate the relationship between tracheobronchial angles and the severity of pneumonia in patients with COVID-19. Our results showed no statistically significant difference in terms of age and gender in SCA, IBA, RBA, and LBA measurements in CT imaging. We think that we gained these results because we only included adult patients older than 18 years of age in our study. Also, we did not find any statistically significant difference in SCA, IBA, RBA, and LBA in terms of the occurrence of COVID-19 pneumonia.

In the literature, tracheobronchial angle measurements were made using different techniques. Chest radiography, chest CT and cadavers were used for measurement in the studies (8). Tracheobronchial angles may differ depending on race, age, and individual and angle measurement techniques (1,8-15). In many studies on tracheobronchial angle measurements, generally, children were included in the study. Herek et al. (8) measured SCA, IBA, RBA, and LBA on coronal the reformat CT imaging of the children. They reported that SCA, IBA, and RBA values were statistically significant between children of ages younger than 10 years and older than 10 years.

In the previous studies, the IBA was found to be slightly wider than the SCA in the measurements performed on helical CT images (12). The tracheal bifurcation angle has been found an average of 65° (range, 40° to 99°) (19-22). Most authors agree in their studies that the RBA is smaller than the LBA, regardless of gender and age (4,6,7,11,20-25). In addition, according to the major clinical and anatomical texts, the RMB is wider and steeper than the left, and therefore foreign body aspiration is mostly through the right bronchus (1,4,7,19-22,24,26).

Kubota et al. (14) measured the tracheal bifurcation angle on the chest radiographs of infants and children and they found the mean tracheal bifurcation angle about 80°. We found the mean SCA was 77.65±15.78° and the mean IBA was 81.67±15.20°. In their study, they found that the RBA was always smaller than the LBA (14). RBA was smaller than LBA in 78.3% of the patients, RBA was greater than LBA in 18.5%, and RBA-LBA was equal in 3.3% of the patients, in our study, RBA was smaller than LBA in most patients. Many studies in the literature indicate that the tracheal bifurcation angle in both children and adults has a wide range with many different values (8). Mi et al. (9) included in their study 2107 subjects, ranging in age from 18 to 89 years in the Chinese population and they measured the tracheobronchial angles using multi-slice spiral CT and MPR. They found that in one-fifth of the Chinese population, RBA was larger than LBA and the left mainstem bronchus is more vertical (9). We also found that in 18.5% of the patients in our study RBA was larger than LBA.

Kamel et al. (4) found the mean SCA values were 81° in females and 76° in males without any correlation with age. These results are different from our results. We found that mean SCA values were 77.3° in females and 78° in males. We found no relationship between gender and the tracheobronchial angles in adults.

SCA and IBA may increase due to various cardiac diseases (cardiomegaly and pericardial effusion) and mediastinal lesions (11,12). Cardiac enlargement, such as cardiomegaly due to heart failure, has been reported to widen the angle between RMB and LMB (11-13). Some studies have found positive correlations between the SCA, IBA, and the size of the left atrium (1,12,27). Thus, we excluded patients with heart disease and mediastinal abnormalities.

Although the standard diagnosis of COVID-19 is the RT-PCR test, it may give false-negative results in some cases. False-negative RT-PCR results may be possibly due to insufficient viral specimens or early stages of the disease or technical problems (28,29). According to past clinical information, chest CT imaging can show abnormalities before RT-PCR testing. Therefore, recently, high-resolution CT has become one of the main screening methods, for diagnosing and assessing disease severity (30). COVID-19 pneumonia's typical imaging findings are bilateral, peripheral, and basal dominant ground-glass opacities (GGOs) with or without consolidation and bronchovascular thickening in chest CT (31). In our study, the severity of lung involvement was scored by using the visual method suggested by Pan et al. (18).

Some studies have reported that COVID-19 patients have a worse prognosis and severe clinical outcomes, especially when multiple risk factors such as diabetes, hypertension, coronary artery, and lung disease are present (32,33). To date, a wide variety of studies have been conducted on the risk factors of COVID-19 patients with pneumonia. In different studies in the literature, it has been reported that older age and comorbidities (cardiovascular and cerebrovascular diseases) are important high-risk factors that may lead to increased mortality in severe COVID-19 patients (34-36). So, we included only patients aged 18-40 years in our study. Several studies have reported a history of diabetes, hypertension, and lung and coronary artery disease as risk factors for worse prognosis and serious clinical outcomes in COVID-19 patients (32,33). Therefore, we did not include patients with comorbidities in our study.

Our study has several limitations. The first limitation is that our study is a retrospective study conducted at a single center and included a relatively limited group of patients. Therefore, a multicenter study with a large sample size is needed for further validation. Second, we used a visual semi-quantitative CT severity scoring system, so assessment of disease severity scores on CT imaging can be subjective.

CONCLUSION

In conclusion, we calculated the distribution range of tracheobronchial angle values in adult Turkish COVID-19 patients. We couldn't find a statistical relationship between the tracheobronchial angles with the severity of pneumonia in COVID-19 patients. Therefore, according to our study, the tracheobronchial angle values do not affect the severity of pneumonia and patients' clinical outcomes in COVID-19. But a large sample size study is needed for further validation.

Ethics Committee Approval: The study was approved by the Clinical Researches Ethics Committee of Kanuni Training and Research Hospital (26.11.2020, 2020/71).

Conflict of Interest: None declared by the authors.

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The corresponding author, Dr. Burcu Akman was working at Kanuni Training and Research Hospital at the time of data collection phase of the study.

Author Contributions: Idea/Concept: BA, HAAK; Design: BA; Data Collection/Processing: BA, HAAK; Analysis/Interpretation: BA, HAAK; Literature Review: BA; Drafting/Writing: BA; Critical Review: BA, HAAK.

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Evaluation of the Relationship between Simple Hemogram Indexes and Disease Severity Scores in Pediatric Familial Mediterranean Fever

Pediatrik Ailevi Akdeniz Ateşinde Basit Hemogram İndeksleri ile Hastalık Şiddet Skorları Arasındaki İlişkinin Değerlendirilmesi

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ABSTRACT

Aim: In recent years, it has been seen that simple complete blood count (CBC) parameters can be used to show subclinical inflammation in patients with familial Mediterranean fever (FMF). The aim of this study is to determine whether there is a difference in CBC parameters among FMF patient groups divided according to disease severity scores.
Material and Methods: FMF patients followed up in our clinic between 2016 and 2019, were reviewed for medical records. They were divided into three groups as those with mild, moderate, and severe diseases according to the disease severity scoring systems by Pras et al., Mor et al., and International Severity Score of FMF (ISSF). Red cell distribution width (RDW), platelet, neutrophil, lymphocyte, RDW-platelet ratio (RPR), platelet-lymphocyte ratio (PLR), and neutrophil-lymphocyte ratio (NLR) of the patients were compared among the groups.
Results: According to the scoring system of Pras et al., lymphocyte value was found significantly higher in the group with severe disease compared to the groups with mild and moderate diseases (p=0.031). PLR was significantly lower in the group with severe disease compared to moderate diseases according to the scoring system of Mor et al (p=0.008). According to ISSF, there was no difference among the groups in terms of CBC parameters.
Conclusion: Different results were obtained according to all three scoring systems. Since the ISSF is the most common and suitable system for use in FMF patients, we can conclude that there is no relationship between disease severity and RDW, RPR, NLR and PLR.
Keywords: Complete blood count; disease severity index; familial Mediterranean fever.

ÖZ

Amaç: Son yıllarda, AAA hastalarında subklinik inflamasyonu göstermek için basit tam kan sayımı (TKS) parametrelerinin kullanılabileceği görülmüştür. Bu çalışmanın amacı, hastalık şiddeti skorlarına göre ayrılan AAA hasta grupları arasında TKS parametrelerinde farklılık olup olmadığını belirlemektir.
Gereç ve Yöntemler: Kliniğimizde 2016 ve 2019 yılları arasında takip edilen AAA hastalarının tıbbi kayıtları incelendi. Hastalar, Pras ve ark., Mor ve ark. ve AAA için uluslararası şiddet skorlama sistemi (International Severity Score for FMF, ISSF) hastalık şiddeti skorlama sistemlerine göre hafif, orta ve ağır hastalığı olanlar olmak üzere üç gruba ayrıldı. Hastaların eritrosit dağılım genişliği (red cell distribution width, RDW), trombosit, nötrofil, lenfosit, RDW-trombosit oranı (RDW-platelet ratio, RPR), trombosit-lenfosit oranı (platelet-lymphocyte ratio, PLR) ve nötrofil-lenfosit oranı (neutrophil-lymphocyte ratio, NLR) gruplar arasında karşılaştırıldı.
Bulgular: Pras ve ark.'nın skorlama sistemine göre, şiddetli hastalığı olan grupta lenfosit değerinin hafif ve orta derecede hastalığı olan gruplara göre anlamlı derecede yüksek olduğu bulundu (p=0,031). Mor ve ark.'nın skorlama sistemine göre PLR, şiddetli hastalığı olan grupta orta dereceli hastalıklara göre anlamlı olarak daha düşüktü (p=0,008). ISSF skorlamasına göre, TKS parametreleri açısından gruplar arasında fark yoktu.
Sonuç: Her üç skorlama sistemine göre de farklı sonuçlar elde edildi. ISSF, AAA hastalarında en yaygın ve kullanıma uygun sistem olduğundan, hastalık şiddeti ile RDW, RPR, NLR ve PLR arasında bir ilişki olmadığı sonucuna varabiliriz.
Anahtar kelimeler: Tam kan sayımı; hastalık şiddeti indeksi; ailesel Akdeniz ateşi.

INTRODUCTION

Familial Mediterranean fever (FMF) is the most common and widely known autosomal recessively inherited monogenic autoinflammatory disease characterized by recurrent episodes of polyserositis and fever (1). FMF is mostly seen in Mediterranean cultures such as Arabs, Armenians, Jews, Greeks, Italians, and Turks (2,3). FMF patients have a mutation in the Mediterranean fever (MEFV) gene. This gene codes for a protein called pyrin. When there is a 63 gain of function mutation in the MEFV gene, pyrin protein becomes active and resulting in continuous inflammatory stimulation (4).

Infection, stress, menses, exposure to cold, some drugs, and fat-rich foods can trigger an FMF attack (5). The attacks are self-limiting and usually last between 6 hours and 3 days. (6,7). The patient's clinical condition returns to normal between attacks. However, inflammation markers do not always return to normal (7,8). Although the attacks resolve spontaneously, if attacks and inflammation are not prevented in FMF patients, amyloidosis develops and this can cause serious organ damage, especially in the kidneys. Therefore, it is vital to control inflammation in FMF patients. Studies have shown that 30% of FMF patients continue to have inflammation in the attack-free period (9). Subclinical inflammation can be demonstrated by the elevation of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fibrinogen, serum amyloid A, and various cytokines in the blood. In recent studies, it has been shown that subclinical inflammation can be measured in many diseases with very simple hemogram markers and various formulations (10-13). In particular, an increase in neutrophil and platelet counts, while a decrease in lymphocyte counts is observed. Platelets are rich in proinflammatory agents and their release plays a role in many inflammatory diseases. Circulating platelets can interact with erythrocytes, neutrophils, and lymphocytes in the vessel lumen at sites of vascular damage (14,15). Red cell distribution width (RDW) is a blood parameter that measures erythrocyte variability and size and reflects the degree of inflammation and oxidative stress. Studies have shown that there is a strong correlation between RDW and frequently used inflammation markers, CRP, and ESR (16). Simple blood parameters were investigated in terms of disease severity for many diseases (17-21).

Especially in the last 20 years, various scoring systems have been used to evaluate disease severity in many diseases including FMF. For this purpose, the first disease severity scoring system for FMF was developed in 1997 by Pras et al. (22) and it is still one of the best known scoring systems used for adult patients. In 2005, Mor et al. (23) developed a new scoring system to correct the deficiencies in the scoring system of Pras et al. (22), such as the lack of a cause and effect relationship between the severity markers and disease severity and the use of arbitrary differential values for each parameter. However, Kalkan et al. (24) showed that these two scoring systems were not congruent with each other. Thereupon, the international FMF expert group developed International Severity Score for FMF (ISSF) in 2012. The newly developed criteria are suitable for use in both clinical practice as well as drug trials in adult and pediatric FMF patents (25). These scores help clinicians to predict the severity and prognosis of the disease.

The aim of this study is to determine the relationship of these simple complete blood count parameters that can be measured in almost every laboratory, such as RDW, platelet (PLT), RDW-PLT ratio (RPR), PLT-lymphocyte ratio (PLR), and neutrophil-lymphocyte ratio (NLR), with inflammation and disease severity scores of FMF patients.

MATERIAL AND METHODS

Patients

In this study, 165 FMF patients under 18 years of age in the Pediatric Rheumatology Outpatient Clinic between March 2016 and April 2019 were evaluated. Ethical approval for the study was obtained from local ethics committee on 27.07.2020 with the approval number 2020/311. Patients who did not follow up regularly and did not use their treatment regularly were not included in the study. Patients with comorbidities (eg., autoimmune disease, acute/chronic infection, malignancy, end-stage kidney disease, liver disease, hematological disease, hypertension, diabetes mellitus, cerebrovascular disease), patients with a BMI >30, smokers, and those who received a blood transfusion within the last 4 months for any reason were excluded from the study. For these reasons, a total of 106 patients were not included in the study.

The diagnosis of the patients was made according to the Ankara 2008 pediatric FMF diagnostic criteria, where at least 2 criteria were sufficient, including; fever >38 degrees (at least 3 attacks lasting between 6 hours and 72 hours), abdominal pain (at least 3 attacks lasting between 6 hours and 72 hours), chest pain (at least 3 attacks lasting between 6 hours and 72 hours), arthritis (at least 3 attacks lasting between 6 hours and 72 hours), family history of FMF (26). Since the genetic disease carrier rate is very high in Turkey, the use of these criteria, which only have clinical criteria, has been deemed appropriate for diagnosis.

Case Definition

The disease severity scores of the patients were evaluated according to the scoring systems of Pras et al. (22), Mor et al. (23), and ISSF (Table 1).

Pras et al. (22)'s scoring included age at onset of the disease (years), number of attacks per month, presence of arthritis (acute or prolonged), presence of erysipelas-like erythema, presence of amyloidosis, and colchicine dose (mg/day) used. According to the scoring system of Pras et al. (22), 3-5 points were classified as mild disease, 6-9 points as moderate disease, and >10 as severe disease.

Mor et al. (23)'s scoring system evaluated patients according to the age of onset, the number of areas involved in attacks and throughout the course of the disease, the dose of colchicine, the number of pleural involvement, and the number of attacks with erysipelas-like erythema. According to the scoring system of Mor et al. (23), presence of ≥3 points was considered as severe disease, 2 points as moderate disease, and ≤1 points as mild disease.

The ISSF included the presence of chronic sequelae, organ dysfunction and failure, frequency of attacks, acute phase reactants, the number of sites involved in a single attack, attack types during the course of the disease, attack duration, and exertional leg pain. According to ISSF, ≤2 points was evaluated as mild disease, 3-5 points as moderate disease, and ≥6 points as severe disease.

Table 1. Disease severity scoring systems in Familial Mediterranean fever

Pras et al.		**Mor et al.**	**ISSF**	
Criteria	**Score**	**Criteria**	**Criteria**	**Score**
Age of onset (year)		1. ≥1 site in a single attack (In	1. Chronic sequela (including amyloidosis, growth	
>31	0	at least 25% of the attacks)	retardation, anemia, splenomegaly)	1
21-31	1		2. Organ dysfunction (nephrotic range proteinuria, FMF related)	1
11-20	2		3. Organ failure (heart, renal, etc., FMF related)	1
6-10	3	2. ≥2 sites in the course of the	4. A. Frequency of attacks (average number of attacks	
<6	4	disease	between 1 and 2 per month)	1
			B. Frequency of attacks (average number of attacks	
Number of attacks per month			>2 per month)	2
<1	1	3. ≥2 mg/day colchicine to	5. Increased acute-phase reactants (any of C-reactive	
1-2	2	achieve remission	protein, serum amyloid A, erythrocyte sedimentation	
>2	3		rate, fibrinogen) during the attack-free period, ≥2 weeks	
			after the last attack (at least two times 1 months apart)	1
Arthritis		4. ≥2 pleuritic attacks during	6. Involvement of more than two sites during an	
Acute	2	the course of the disease	individual acute attack (pericarditis, pleuritis, peritonitis,	
Persistant	3		synovitis, ELE, testis involvement, myalgia, and so on)	1
Erysipelas-like eritem	2		7. More than two different types of attack during the course of	
Amyloidosis	3	5. ≥2 Erysipelas-like erythema	the disease (isolated fever, pericarditis, pleuritis, peritonitis,	
Colchicine dosage (mg/day)		attacks during the courseof the	synovitis, ELE, testis involvement, myalgia, and so on)	1
1	1	disease	8. Duration of attacks (more than 72 h in at least three	
1,5	2		attacks in a year)	1
2	3		9. Exertional leg pain (pain following prolonged	
>2**	4	6. Age of onset<10 years	standings and/or exercising, excluding other causes)	1
2 mg/day unresponsive **3-5 points were classified as mild disease, 6-9 points as moderate disease, and >10 as severe disease		**≥3 points was considered as severe disease, 2 points as moderate disease, and ≤1 points as mild disease**	*Criterion 4a/4b can give 0 or 1 or 2 points altogether according to the definition **Severe disease ≥6, intermediate disease 3-5, mild disease ≤2**	

Study Design

Information on patients was collected from computer data systems and patient files. Clinical findings, family history, drug responses, gene mutations, ESR, CRP, complete blood count parameters, NLR, RPR, and PLR of all cases were recorded. All FMF patients were evaluated during the attack-free period and under colchicine treatment. In all patients, blood parameters in the attack-free period of at least 3 months were used for evaluation.

The patients were divided into three groups according to the disease severity scores as mild, moderate, and severe, and the relationship between complete blood count parameters and disease severity scores was investigated.

Data Sources and Measurement

Complete blood count analysis was performed in a tube with K3 EDTA and with the Sysmex XE-2100 hemogram device, which was regularly controlled and maintained.

Among the mild, moderate, and severe disease groups, differences in CRP (mg/L), ESR (mm/hour), RDW (%), PLT (K/uL), neutrophil (K/uL), lymphocyte (K/uL), NLR, RPR, and PLR were investigated.

The NLR was calculated by dividing the neutrophil count by the lymphocyte count, RPR was calculated by dividing RDW by PLT count, and PLR was calculated by dividing the PLT count by the lymphocyte count.

Statistical Analysis

All data were analyzed in IBM SPSS Statistics v.21.0 package program. Mean, standard deviation, median, interquartile range, and minimum -maximum values were used for the descriptive statistics. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to evaluate the normality distribution of variables. one-way ANOVA was used if the normality assumption was met, and the Kruskal-Wallis test was used if not. To examine the significant difference between groups after one-way ANOVA, the homogeneity of the variances was checked first, then the Tukey test was used in the post hoc analysis if the variances were homogeneous, and the Tamhane's T2 test was used if not. A p value of <0.05 was considered statistically significant.

RESULTS

Demographics and Clinic Characteristics of Patients

Eighty-five (51.5%) patients were male and 80 (48.5%) were female. The mean age of the patients at diagnosis was 7.4±2.2 years (median, 6; range, 10 months-17 years). The mean time between the onset of complaints and the diagnosis was calculated as 2.77±2.61 years (median, 2; range, 5 months-4 years). The mean attack duration of the patients was 2.6±1.1 (median, 0.5; range, 2-10) days. The attack frequency was 4.9±5.7 (median, 4; range, 1-52) weeks. Demographic features and laboratory parameters of the patients are summarized in Table 2.

Evaluation of Patients According to Disease Severity Scores

The distribution of patients into groups, such as mild, moderate, and severe diseases, according to disease severity scores is summarized in Table 3. While the number of patients with moderate disease was higher according to the scoring system of Pras et al. (22), the number of patients with mild disease was higher according to ISSF and the scoring system of Mor et al. (23).

Evaluation of Laboratory Parameters According to Disease Severity Scores

ESR and CRP

According to the scoring system of Pras et al. (22), CRP and ESR were different among the groups. When the source of this difference is investigated by post hoc test, CRP value was significantly lower in the group with

Table 2. Laboratory parameters of the patients

	Mean±SD	Median (IQR)	Min-Max
WBC (K/uL)	7193.5±2118.7	6800 (2500)	3800-15000
NEU (K/uL)	3816.3±1821.2	3210 (2050)	1500-11000
LYM (K/uL)	2597.7±68.1	2500 (880)	700-7000
PLT (x10^3 K/uL)	295.6±70.5	293 (83.5)	136-486
RDW (%)	14.4±1.4	14.2 (1.6)	11.9-19.5
CRP (mg/L)	4.9±8.3	1.9 (3.95)	0.12-84
ESR (mm/h)	12.2±10.0	9 (12.9)	2-49
PLR	123.1±43.2	115.7 (44.7)	49.2-347.5
RPR	0.05±0.01	0.05 (0.018)	0.01-0.10
NLR	1.66±1.17	1.42 (0.95)	0.39-8.58

SD: standard deviation, IQR: interquartile range, WBC: white blood cell, NEU: neutrophil, LYM: lymphocyte, PLT: platelet, RDW: red cell distribution width, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, PLR: platelet-lymphocyte ratio, RPR: RDW-platelet ratio, NLR: neutrophil-lymphocyte ratio

Table 3. Distribution of patients according to Familial Mediterranean fever disease severity scores (n=165)

Severity Score	Pras et al.	Mor et al.	ISSF
Mild	43 (%26.1)	107 (%64.8)	99 (%60.0)
Moderate	85 (%51.5)	30 (%18.2)	54 (%32.7)
Severe	37 (%22.4)	28 (%17.0)	12 (%7.3)

ISSF: International Severity Score for Familial Mediterranean fever

moderate disease compared to the group with severe disease (p=0.001). In addition, ESR value was significantly different between the groups with mild and severe diseases and the groups with moderate and severe diseases. Accordingly, the ESR value in the group with mild disease was significantly lower compared to the ESR value in the group with moderate disease, and that value was significantly lower compared to the group with severe disease (both p vales were <0.001).

According to the scoring system of Mor et al. (23), there was a difference among the groups in terms of CRP, and ESR values. When the source of this difference is investigated by post hoc test, CRP was significantly lower in the group with mild disease compared to the group with moderate disease (p=0.020). ESR was significantly lower in the group with mild disease compared to the groups with moderate and severe diseases (p=0.012, p=0.006, respectively).

When the patients were evaluated according to ISSF, there was a difference among the groups in terms of ESR and CRP. When this difference is investigated by post hoc test, CRP was significantly higher in the group with severe disease compared to the groups with mild and moderate diseases (p=0.011, p=0.017, respectively). Similarly, ESR was significantly higher in the group with severe disease compared to the groups with mild and moderate diseases (both p vales were <0.001). ESR increased as the severity of the disease increased.

Lymphocyte, Neutrophil, PLT, RDW, NPR, RPR and LPR

According to the scoring system of Pras et al. (22), lymphocyte levels were different among the groups. When the source of difference among the groups was investigated by post hoc test, the lymphocyte value in the group with mild disease was significantly lower than the lymphocyte value in the group with moderate disease (p=0.050). There was no difference among the groups in terms of RPR, PLR, NLR, neutrophil, PLT, and RDW values (Table 4).

According to the scoring system of Mor et al. (23), there was a difference among the groups only in terms of PLR. In paired comparisons with post hoc test, PLR was found to be significantly higher in the group with mild disease compared to the group with severe disease (p=0.037). In addition, it was significantly higher in the group with moderate disease compared to the group with severe disease (p=0.006).

According to ISSF, there was no difference between the groups in terms of RPR, PLR, NLR, neutrophil, PLT, and RDW values.

DISCUSSION

Familial Mediterranean fever is an autoinflammatory disease common all over the world, and it is estimated that there are approximately 120.000 patients in the world (27). Therefore, the clinical forms of the disease, its genetics, disease severity, pathogenesis, and indicators of inflammation have been a matter of interest. Since it is an autoinflammatory disease, the most important problem for clinicians is to control inflammation and prevent amyloidosis resulting from uncontrolled inflammation.

Measuring disease severity scores helps predict the prognosis of the disease, provides an early and effective approach to treatment, and thereby contributes to the patient's quality of life. In addition, it provides objective evaluations for various scientific research. In recent years, many studies comparing complete blood count parameters with healthy controls have shown that inexpensive and practical markers, such as NLR and PLR, can be used to evaluate disease activity and severity in autoimmune and inflammatory diseases instead of expensive tests (28-30). Uslu et al. (31) compared the NLR ratio with healthy controls and found a significantly higher NLR in FMF patients. In addition, they found a significant difference in NLR of patients with and without amyloidosis. Uluca et al. (32) measured NLR during the attack and attack-free periods and found that it was significantly higher during the attack period. Özer et al. (30) compared the NLR of patients in the attack-free period with healthy controls and suggested that NLR could be a significant marker to show subclinical inflammation in FMF patients. Yorulmaz et al. (28) evaluated NLR during the attack period, attack-free period, and in healthy controls. They found a significant difference between the attack and attack-free periods; however, they could not detect a difference between the attack-free period and healthy controls, stating that NLR was a good marker for demonstrating systemic inflammation, but they could not find such significant evidence for subclinical inflammation. In the present study, NLR showed no difference among the groups divided with respect to disease severity scores, according to all three scoring systems. In terms of PLR, there was no difference among the groups divided according to the scoring system of Pras et al. (22) and ISSF, while there was a significant difference between the groups with moderate and severe diseases and mild and severe diseases in the

Table 4. Evaluation of laboratory values according to disease severity scores

	Mild Disease		Moderate Disease		Severe Disease		
	Mean±SD	**Median (IQR) [min-max]**	**Mean±SD**	**Median (IQR) [min-max]**	**Mean±SD**	**Median (IQR) [min-max]**	**p**
WBC (K/uL)							
Pras et al.	6940.9±2178.0	6600 (2900) [3800-14900]	7111.5±2135.8	6700 (2350) [3800-15000]	7516.2±1760.3	7500 (2200) [5000-13000]	0.444
Mor et al.	7108.3±2146.2	6700 (2300) [3800-15000]	7406.5±1758.2	7400 (3075) [5000-11200]	7064.3±2127.6	7000 (2775) [3800-14900]	0.755
ISSF	7107.9±2158.3	6800 (2400) [3800-15000]	7074.6±1998.2	6700 (2650) [3800-14300]	7933.3±1523.9	7950 (800) [5100-11100]	0.402
NEU (K/uL)							
Pras et al.	3663.6±1686.8	3200 (1900) [1800-11000]	3649.4±1912.8	3000 (1990) [1500-11000]	4271.6±1579.1	4140 (2050) [1800-8200]	0.182
Mor et al.	3703.9±1775.3	3200 (1880) [1500-11000]	4109.4±1807.8	3700 (3080) [1500-9010]	3772.5±1879.3	3250 (2133) [1800-11000]	0.542
ISSF	3705.6±1789.4	3200 (1830) [1500-11000]	3770.9±1877.6	3140 (1985) [1500-11000]	4590.0±1306.7	4550 (1540) [2400-7000]	0.272
LYM (K/uL)							
Pras et al.	2389.3±779.9	2300 (1000) [990-4470]	2769.4±1003.5	2600 (1045) [700-7000]	2441.6±576.8	2400 (650) [1100-4460]	**0.031**
Mor et al.	2609.3±943.0	2500 (900) [700-7000]	2415.2±672.7	2320 (923) [1100-3800]	2754.6±833.5	2550 (680) [1400-4700]	0.330
ISSF	2569.6±911.8	2510 (900) [700-7000]	2652.4±857.3	2500 (808) [1220-6300]	2583.3±800.2	2400 (840) [1560-4460]	0.855
RDW (%)							
Pras et al.	14.5±1.6	14.0 (1.8) [12.4-19.5]	14.3±1.3	14.1 (1.4) [12.1-18]	14.6±1.4	14.6 (1.8) [11.9-18.8]	0.531
Mor et al.	14.4±1.4	14.1 (1.6) [12.1-19.5]	14.6±1.5	14.4 (1.8) [11.9-18.8]	14.5±1.1	14.5 (1.6) [12.2-16.5]	0.774
ISSF	14.5±1.5	14.2 (1.6) [12.1-19.5]	14.3±1.3	14.15 (1.3) [11.9-18]	14.7±1.1	14.65 (1.4) [12.7-16.4]	0.558
CRP (mg/L)							
Pras et al.	4.6±12.6	1.60 (1.86) [0.39-84]	3.2±3.8	1.7 (2.46) [0.12-19.7]	9.3±8.4	5.93 (13) [0.18-28]	**0.001**
Mor et al.	3.0±3.9	1.59 (1.89) [0.12-28]	6.9±7.3	3.68 (10.23) [0.18-25]	10.1±16.1	5.06 (11.37) [0.16-84]	**0.001**
ISSF	3.9±9.1	1.62 (1.99) [0.12-84]	4.7±4.6	2.09 (6.12) [0.16-17.4]	14.1±9.5	16.5 (19.7) [1.11-25.4]	**0.001**
ESR (mm/h)							
Pras et al.	8.2±5.6	7 (7) [2-22]	10.2±8.3	8 (10) [2-43]	21.0±11.6	21 (17) [2-49]	**0.001**
Mor et al.	9.6±8.1	8 (10) [2-49]	15.3±9.4	13 (16) [2-35]	18.1±12.8	15 (23) [2-43]	**0.001**
ISSF	9.7±7.6	8 (10) [2-49]	12.8±10.0	11 (14) [2-43]	28.8±9.1	29.5 (10) [6-42]	**0.001**
PLR							
Pras et al.	131.6±45.9	120.0 (47.6) [65.9-347.5]	118.7±40.4	112.6 (45.8) [49.2-252.6]	123.3 ±46.1	114.6 (37.5) [69.3-281.1]	0.286
Mor et al.	122.9±42.7	115.8 (42.1) [55.4-347.5]	140.4±50.7	135.6 (63.4) [49.2-281.1]	105.6±28.1	108.2 (51.3) [58.3-162.2]	**0.008**
ISSF	125.7±46.8	118.3 (43.8) [55.4-347.5]	116.8±36.1	110.9 (46.9) [49.2-216.5]	129.7±41.9	117.5 (77.8) [71.5-198.1]	0.410
RPR							
Pras et al.	0.05±0.01	0.05 (0.016) [0.03-0.07]	0.05±0.01	0.05 (0.018) [0.01-0.09]	0.05±0.02	0.05 (0.016) [0.03-0.10]	0.273
Mor et al.	0.05±0.01	0.05 (0.017) [0.01-0.10]	0.05±0.01	0.05 (0.016) [0.03-0.07]	0.06±0.02	0.05 (0.016) [0.04-0.10]	0.199
ISSF	0.05±0.01	0.05 (0.017) [0.01-0.10]	0.05±0.01	0.05 (0.018) [0.03-0.10]	0.05±0.01	0.05 (0.014) [0.03-0.07]	0.770
NLR							
Pras et al.	1.62±0.76	1.37 (0.81) [0.62-4.23]	1.58±1.35	1.22 (0.94) [0.39-8.58]	1.94±1.14	1.55 (1.32) [0.69-6.00]	0.285
Mor et al.	1.63±1.15	1.38 (0.75) [0.39-8.58]	2.00±1.56	1.44 (1.60) [0.42-7.39]	1.45±0.67	1.47 (1.06) [0.47-2.88]	0.174
ISSF	1.67±1.20	1.39 (0.88) [0.39-8.58]	1.61±1.20	1.24 (0.89) [0.42-7.39]	1.95±0.80	2.02 (1.26) [0.69-3.37]	0.655

SD: standard deviation, IQR: interquartile range, WBC: white blood cell, NEU: neutrophil, LYM: lymphocyte, PLT: platelet, RDW: red cell distribution width, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, PLR: platelet-lymphocyte ratio, RPR: RDW-platelet ratio, NLR: neutrophil-lymphocyte ratio

scoring system of Mor et al. (23), and PLR was significantly lower in the group with severe disease. This result was quite different from studies in the literature in which PLR in cancer and other inflammatory diseases increased as the disease severity increased (18,33,34). We can attribute this to the scarcity of studies on this subject, and perhaps to the heterogeneous division of disease severity in the scoring systems for FMF. We think that more studies are needed on PLR and disease severity scores of FMF. It should also be noted that the scoring systems of Pras et al. (22) and Mor et al. (23) are not as suitable for pediatric use as is ISSF.

Another measurement that has been popular in recent years is RDW and its ratio to PLT count. RDW is a parameter that reflects the variance of the sizes of circulating erythrocytes in standard automatic complete blood count. Although traditionally used in different types of anemia, it has been found to increase during inflammation (10,30). Many studies have revealed that RDW is also associated with liver, kidney, and cardiovascular diseases. In addition, RDW is known to be a recently used inflammation marker in inflammatory diseases, such as septic shock, inflammatory bowel disease, and acute appendicitis (19,35-37). In addition, RPR has been shown to be a new and rapid laboratory index for predicting mortality in many diseases. Chen et al. (38) concluded that RPR is an inexpensive and noninvasive marker for predicting fibrosis and cirrhosis in chronic hepatitis B compared to liver biopsy. Similarly, Cetinkaya et al. (21) used RPR to determine the severity of acute pancreatitis. It was also found that RDW and PLT count correlated with disease activity in patients with rheumatoid arthritis (39). In previous studies on adult and pediatric FMF patients, markers such as RDW and PLR were used as indicators of subclinical inflammation. In another study, it was concluded that in the case of systemic lupus erythematosus (SLE), which is one of the rheumatologic diseases, RPR was positively correlated with the SLE disease activity index and other inflammatory markers; therefore, RPR was a very good prognostic indicator for evaluating SLE patients (40). As far as we know, although there are studies showing that complete blood count parameters can be used as subclinical inflammation markers in pediatric FMF patients, no study has been conducted to date in which RPR is evaluated and the relationship between complete blood count parameters with disease severity scores is investigated. In the present study, we could not detect a difference between RDW, RPR, and disease severity scores. However, in the light of other studies, we think that further studies with larger cohorts are needed on this subject. This is because ESR and CRP, which are standard acute phase reactants, increased in correlation with disease severity according to all three scoring systems. Although scoring systems are heterogeneous in themselves, the increase in these frequently used and well-known acute phase reactants in correlation with disease severity still acknowledges the reliability of the scoring systems.

In this study, we tried to make a judgment on the subject by comparing three disease severity scores based on clinical and laboratory values observed in patients during the course of the disease and hemogram parameters that can show subclinical inflammation of patients in the attack-free period. However, since disease severity scores were developed to assess the state of the disease throughout the course of the disease and none of them were sufficient to measure disease activity, we did not have a separate group for patients who had severe disease according to some of the scores but were in remission with treatment. In addition, the number of patients was limited since only patients who followed their follow-up regularly were included in the study.

CONCLUSION

In conclusion, we evaluated the complete blood count parameters that can be easily accessed and calculated in FMF according to the three disease severity scoring systems used in FMF, which are actually different from each other in content. However, we obtained different results between disease severity scores and complete blood count parameters for all three scoring systems. Since ISSF is the most common and appropriate system used in pediatric FMF patients, it can be concluded that there is no relationship between disease severity and RDW, RPR, NLR, and PLR. However, we believe that there is a need for studies with larger series by examining the current clinical status (active disease, in remission, in partial remission) and disease severity scores, which include the entire disease course, in FMF patients. There is no study in the literature comparing RPR, PLR, and NLR after dividing patients into groups according to their disease severity scores. We think that this study is valuable in this respect.

Ethics Committee Approval: The study was approved by the Ethics Committee of Selçuk University Faculty of Medicine (27.07.2020, 2020/311).

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Experience of Distance Learning of Medical Science Disciplines as a Result of the Global Pandemic COVID-19 in Ukraine and South Africa

Küresel Pandemi COVID-19'un Bir Sonucu Olarak Ukrayna ve Güney Afrika'da Tıp Bilimi Disiplinlerinin Uzaktan Eğitim Deneyimi

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ABSTRACT

Aim: This study discusses the historical background of distance learning and its pros and cons from the perspective of students and teachers. This study aimed to conduct a comparison of the effectiveness of distance learning during the quarantine period at Kharkiv National Medical University (KNMU), and the national lockdown at the University of Cape Town (UCT).
Material and Methods: A survey was used to investigate student's perspectives about distance education. An online survey consisting of 19 questions was conducted on Google Forms platform among 395 students at KNMU and among 124 students of UCT obtained from Vula site statistics. Individuals' learning progress during histological and anatomical classes using different methods such as virtual aggregators of slides and 3D programs were also observed.
Results: At KNMU, most students (72.2%, n=285) agreed that the main advantages of distance education were the extra time they had to prepare for classes and revise study materials whereas the major challenge they faced while having distance education was the lack of clinical approach to patients (69.1%, n=273). At UCT, students found that the most helpful aspect of distance learning was pre-recorded lecture videos (66.9%, n=83).
Conclusion: The pandemic has shown that distance learning is possible, but the question remains: has it proved to be efficient, and is it an inseparable part of the education system today? The role of distance education in the twenty-first century can be summarized as distance education programs catching huge popularity in the educational world with having and spreading discussable sides.
Keywords: e-learning; education; anatomy teaching; histology.

ÖZ

Amaç: Bu çalışma, öğrencilerin ve öğretmenlerin bakış açısıyla uzaktan eğitimin tarihsel arka planını ve artılarını ve eksilerini tartışmaktadır. Bu çalışmanın amacı, karantina döneminde Kharkiv Ulusal Tıp Üniversitesi (Kharkiv National Medical University, KNMU)'ndeki ve ulusal kapanma döneminde Cape Town Üniversitesi (the University of Cape Town, UCT)'ndeki uzaktan eğitimin etkinliği bakımından bir karşılaştırma yapmaktır.
Gereç ve Yöntemler: Öğrencilerin uzaktan eğitime bakış açılarını araştırmak için bir anket formu kullanıldı. Google Forms platformunda oluşturulan ve 19 sorudan oluşan çevrimiçi anket KNMU'daki 395 öğrenciye uygulandı ve 124 UCT öğrencisinde Vula site istatistiklerinden elde edilen veriler uygulandı. Bireylerin, sanal slayt toplayıcıları ve 3D programlar gibi farklı yöntemler kullanarak histoloji ve anatomi dersleri sırasında öğrenme ilerlemeleri de gözlemlendi.
Bulgular: KNMU'daki öğrenci çoğunluğu uzaktan eğitimin temel avantajlarının derslere hazırlanmak ve çalışma materyallerini gözden geçirmek için fazladan zaman ayırması olduğunu belirtirken (%72,2, n=285), uzaktan eğitim alırken karşılaştıkları en büyük zorluğun hastaya klinik yaklaşımın eksikliği olduğu konusunda hemfikirlerdi (%69,1, n=273). UCT'de öğrenciler uzaktan eğitimin en yararlı yönünün önceden kaydedilmiş ders videoları olduğunu belirtmişlerdi (%66,9, n=83).
Sonuç: Pandemi, uzaktan eğitimin mümkün olduğunu gösterdi, ancak soru şu: verimli olduğu kanıtlandı mı ve bugün eğitim sisteminin ayrılmaz bir parçası mı? Uzaktan eğitimin yirmi birinci yüzyıldaki rolü, tartışılabilir yönleri olan ve yaygınlaşan uzaktan eğitim programlarının eğitim dünyasında büyük bir popülerlik kazanması olarak özetlenebilir.
Anahtar kelimeler: e-öğrenim; eğitim; anatomi öğretimi; histoloji.

INTRODUCTION

For the first time in history, unexpectedly and abruptly, the world has been transferred to remote learning. This has caused an increased need to change the way of teaching and assessing students' knowledge in medical higher education establishments in a short time. Some universities were more prepared for it than others. Nevertheless, distance education can be as efficient as traditional indoor education when there are appropriate methods and technologies used toward its realization.

Complex unexpected events that occasionally arise in our life encourage people to search for solutions, thus, providing adaptation to new living conditions. Recently, a respiratory system-related infectious disease, coronavirus disease 2019 (COVID-19) has been declared a pandemic and has caused society to redeploy essential roles straightaway at a pace and scale without precedent. The pandemic is imposing a heavy burden on individuals and society as a whole, not only on people's health but also on the quality of education offered to students and putting the world economy under severe strain.

The COVID-19 pandemic has served as a catalyst for the shift in teaching approach at Kharkiv National Medical University (KNMU) and the University of Cape Town (UCT) Medical School because traditionally these universities have not used correspondence courses preferring classic indoor learning. In the current unforeseen situation, distance learning is basically a way of disseminating knowledge, where the educators and students are separated in distance and communicate by different technical means. As schools and universities close and people have no choice than to stay home, online learning seems to be growing exponentially. The widespread implementation of distance education aimed at the utilization of educational technologies of all sorts to provide complex remote learning opportunities for students.

Individual students have their own methods to manage study time during this current pandemic to achieve their goals. Each also has their own perspective on the effectiveness of distance learning in all terms. Understanding medical students' perspectives help teachers and medical staff to conduct a more productive teaching approach and get better results in the educational process.

Although many people believe distance education began with the invention of the internet, this is not so. Distance education is not a new concept. Its history could be classified under the stages at a macro level and under five generations at the micro level. Such classification is based on the dominant communication technologies adopted by distance education.

The earliest evidence of the origin of distance education can be traced to 1728 when Caleb Phillips advertised shorthand lessons by mail in the Boston Gazette (1). In the late 1800s, at the University of Chicago, the first major correspondence program in the United States was established in which the teacher and learner were at different locations. This way became available because of the invention of new technology - cheap and reliable postal services. In 1840, in Great Britain, Isaac Pitman implemented a system of shorthand by mailing texts transcribed into shorthand on postcards and received transcriptions from his students in return for correction. The presence of student feedback was a crucial innovation of Pitman's system. The University of London claims to be the first university to offer distance learning degrees, establishing its External Program in 1858 (2).

It should be taken into account that similar experiments in using the mail to deliver teaching occurred in other countries. During the same time, language courses were carried out in Germany via correspondence. In 1873, in the USA, Anna Ticknor created a society that provided learning opportunities to women in their homes (3).

The introduction of new technologies such as radio and television marked a new stage for the progressive development of distance education. Audio and video materials became a part of education along with printed pages. It was a significant breakthrough; the learning audience had grown enormously. All the above has improved the perception of information, thereby, increasing the degree of assimilation of knowledge. However, television and radio had a significant drawback - the student was not able to get appropriate feedback (4).

In the twenty-first century, the availability of computers and the internet makes distance learning even easier and faster. The internet has become a huge breakthrough, turning into a convenient vehicle for the rapid sharing and transfer of information on a worldwide basis, much larger than radio and television. All of that has led to a worldwide explosion of interest in distance learning and the convergence of text, audio, and video on a common virtual platform.

The COVID-19 pandemic resulted in the closure of the vast majority of educational institutions worldwide, therefore, more than 1.5 billion students in schools and universities transferred to distance learning, for the first time in history.

In this regard, the purpose of the current study is to examine the nature of distance learning, as well as to explore the possible advantages and disadvantages compared to traditional education by deliberating over the experiences of students at two universities.

MATERIAL AND METHODS

Concept and Definitions

While exploring the nature of distance learning, specific features were identified:

Flexibility: distance education provides the flexibility to complete coursework from anywhere, at any time, at its own pace. Students are able to find a healthy balance between working, studying, and family obligations.

Individuality: the teacher may choose suitable technologies individually for each specific student.

Accessibility: many students that are unable to go to a traditional school setting because of disabilities may be able to study. Distance education may help in these cases and provide opportunities for distance learners to be successful (5).

Distance education requires an alternative learning process and roles of teachers and students (6). The main roles in distance education can be classified into four subtitles:

Students: in distance education, students have a role to learn. From passive participants in the educational

process, they take a central position in distance learning. Self-control and self-motivation are crucial components of a successful process.

Teacher: the main role of the teacher is to guide the students in a virtual educational environment, and provide valuable information. Part of the supervisory obligations that a teacher usually has in traditional education is taken over by a student of a distance course.

Designer Groups: usually are represented as a team of educators, programmers, and web designers who design digital environments for beneficial teaching and learning, create content delivery systems, interaction, and evaluation.

Directors: there are people who direct the planning, implementation, and evaluation of the education process.

Data Collection

The methodology employed in the present study was based on a survey questionnaire and comparison model. The researchers at KNMU developed survey questions that were disseminated to students to complete via Google Forms. The survey included 19 questions prepared by researchers about the advantages and disadvantages of distance learning, effectiveness, activities, opinions, and time-spending. The effectiveness of distance education in terms of increasing their theoretical knowledge and clinical skills was also questioned with a Likert-type scale. Additionally, communicating with classmates, discussing with their teachers, self-studying, and their preferences about online classes, offline classes, and face-to-face education were included in the survey form. The survey was conducted among 395 international medical students at KNMU from 1st (spring uptake) to 6th academic course. The researchers at UCT had developed general course evaluation questions which were posed to 124 first-year health and rehabilitation students via the Vula (learning management system) platform.

Ethical principles were strictly adhered to, and approval was obtained from the students participating in the study. This study was approved by the Ethics Committee of Kharkiv National Medical University (10.05.2021, protocol number 5/2021).

Statistical Analysis

Data analysis was performed on a Google Sheet statistical platform. All responses from the survey were saved on docs.google.com, then the results were compared. Responses were also filtered, and students were divided into two groups according to their courses, students from the 1st to 3rd course were put into group-1 and group-2 contained students from the 4th to 6th course. The data from both cohorts were interpreted using descriptive statistics, as well as frequency and percentage.

RESULTS

Experience of Teaching Basic Sciences during the Pandemic at Kharkiv National Medical University

The introduction of quarantine restrictions and the replacement of traditional indoor learning with full distance education in March 2020 imposed a huge burden on the administration and staff of our university, which was not ready for such a sharp transition due to the lack of distance education experience earlier. The threat posed by COVID-19 is driving teachers around the world to look for the best ways to continue to support student learning outside the traditional system of learning. However, the ability to adapt to changing environmental conditions is inherent in humans, which happened this time as well. After a certain amount of time, we can conclude that the transition to distance learning was generally not as painful as it seemed at the beginning of the quarantine.

The aim of teaching a course in histology, cytology and embryology at a medical university is to form students' scientific ideas about the microscopic functional morphology of tissues, organs and systems of a healthy individual, the ways and nature of their development, and the dynamics of age-related changes in the organism. This provides a solid scientific foundation for the further study of clinical disciplines. The classic lesson in histology at KNMU is a combination of theory (discussion of the topic of the lesson, analysis of material), practice (work with a microscope and histological slides and scanning electron microphotographs) and control questions (MCQ, oral answer etc.).

The importance of anatomy and its mastering is undeniable for the formation of the future clinical thinking of the student. The classic lesson at the Human Anatomy Department of KNMU includes theoretical material (discussion of body structures and topography using anatomical terminology) and practical approach (prosections, dissections, work with a synthetic cadaver, observation of museum specimens, and virtual dissection on Anatomage table).

In accordance with the Decree of the Government of Ukraine and the Letter of the Ministry of Education and Science of Ukraine, quarantine was introduced in all the educational establishments of the country from 12 March 2020. Since then, we were compelled to hold practical classes remotely according to the existing schedule. Distance learning was carried out via the following software: powered by the Moodle platform using BigBlueButton and Zoom cloud meeting. Interaction with the departments was carried out via the monitors of the academic groups by corporative emails.

As well as before the quarantine, the first part of the online lesson consisted of a discussion of the educational material. Dynamic, interactive discussions are essential for establishing relationships between teachers and students. Such discussions took place on online platforms during the quarantine training.

As we already mentioned in our online survey above, in order to investigate students' perspectives on distance education, we got several significant figures afterward. There were 395 medical students in total at KNMU who had sent us their responses. Nine (2.3%) of responders were in 1st course, 146 (37.0%) of them were in 2nd course, 43 (10.9%) were in 3rd course, 87 (22.0%) were in 4th course, 67 (17.0%) were in 5th course while 43 (10.9%) of participants were in 6th academic course. According to their answers, 70.1% (n=277) of them had their first experience with distance education when the current pandemic started. Participants agreed that the main advantages of distance education were the extra time they had to prepare for classes and revise study materials (72.2%, n=285), more comfortable space to study at home (69.1%, n=273), more convenient access to online materials (62.0%, n=245) and the ability to watch recorded lectures repeatedly (59.2%, n=234), the higher

promotion of self-controlled study (51.1%, n=202), more time staying at home (48.1%, n=190), the higher ability to concentrate on studying (42.0%, n=166), easier to ask questions (37.2%, n=147) and the higher effectiveness of studying due to virtual explanation (32.1%, n=127).

On the other hand, students also claimed some major challenges they had faced while having distance education such as the lack of clinical approach to patients, technical problems with IT devices and internet connection, and the lack of interaction with teachers, which has a ratio of 69.1% (n=273), 45.1% (n=178), and 42.0% (n=166), respectively. Other disadvantages of distance learning were marked as the lack of group discussion (37.2%, n=147), less motivation to prepare for classes (29.1%, n=115), difficulty to concentrate (22.0%, n=87), lack of self-discipline (21.0%, n=83), difficulty to ask questions (18.2%, n=72) and the disability to afford to buy laptops or tablets to attend online lectures (9.1%, n=36).

Additionally, 61.0% (n=241) of responders stated that they considered themselves more active in online classes than in traditional face-to-face classes. 53.2% (n=210) of responders had tried online virtual platforms to practice clinical skills which were insufficient at normal online classes provided by teachers. It also showed that there were 61.0% (n=241) of total students managed their time effectively to prepare for important medical licensing examinations like KROK, IFOM, and USMLE.

Furthermore, there were 59.2% (n=234) of responders said that the lack of clinical practice was the main factor that made them frustrated, which led to the reduction in students' interest in online classes. Besides that, 54.4% (n=215) chose the lack of doctor-patient face-to-face interactions, 49.1% (n=194) picked the bad internet connection, while 23.3% (n=92) chose the teachers' lack of proper and informative explanations as factors making them distracted from the online learning process.

In every online class, 55.2% (n=218) of students usually kept their discussions going on with their groupmates, while 66.1% (n=261) stated that they often communicated with their teachers.

In addition, 64.8% (n=256) of participants said that they spent from 1 to 5 hours a day on online classes while 71.9% (n=284) of them spent the same amount of time on self-studying daily, which was a significantly high amount of time showing that students tried to manage their personal time to study or to prepare for classes by themselves apart from online academic programs (Table 1).

At the end of the survey, 30.4% (n=120) of the participants stated that distance education was extremely effective in terms of increasing their theoretical knowledge while others (9.1%, n=36) rated the effectiveness as extremely ineffective. On the other hand, in terms of improving clinical skills, there was a large number of students claiming that distance education was extremely ineffective (34.2%, n=135) compared to 12.2% (n=48) of them rating this term as extremely effective.

There were not a lot of students who felt stressed during the period of distance learning, which was proved when there were 34.2% (n=135) of participants choosing not at all to describe their stress level during this current pandemic while 14.2% (n=56) of them chose extreme.

Based on statistics that we got from our online survey, we divided responders into two main groups, group-1 included 1st to 3rd year students, and group-2 consisted of 4th to 6th academic course students. Among group-1, only 34.9% (n=69) of participants stated that they preferred the traditional form of education. This figure was higher in group-2 (38.1%, n=75, Table 2). While group-1 had 52.0% (n=103) of them who were positive about the continuation of distance education in the future, group-2 showed only 48.2% (n=95, Table 3). This result can be explained by the higher prominence of clinical knowledge and practice in the syllabus of group-2 compared to group-1 so that students from the 4th to 6th course would like to practice their skills clinically more compared to students of 1st to 3rd academic course.

The practical part of the histology classes, which includes work with histological preparations, was carried out on the basis of virtual aggregators of slides and their photographs (Figure 1). The use of high-resolution scanned

Table 1. The time students spend on online classes and self-studying every day

	Online Class	Self-study
Rarely	32 (8.1%)	20 (5.1%)
1-5 hours	256 (64.8%)	284 (71.9%)
5-7 hours	87 (22.0%)	59 (14.9%)
7+ hours	20 (5.1%)	32 (8.1%)

Table 2. Attitudes of the students in each group towards traditional education and online education

	Group-1 (n=198)	Group-2 (n=197)
Traditional Education	69 (34.9%)	75 (38.1%)
Online Education	129 (65.1%)	122 (61.9%)

Table 3. Attitudes of the students in each group towards continuation of distance learning

	Group-1 (n=198)	Group-2 (n=197)
Definitely positive	103 (52.0%)	95 (48.2%)
Definitely negative	34 (17.2%)	41 (20.8%)
Prefer combined form	61 (30.8%)	61 (31.0%)

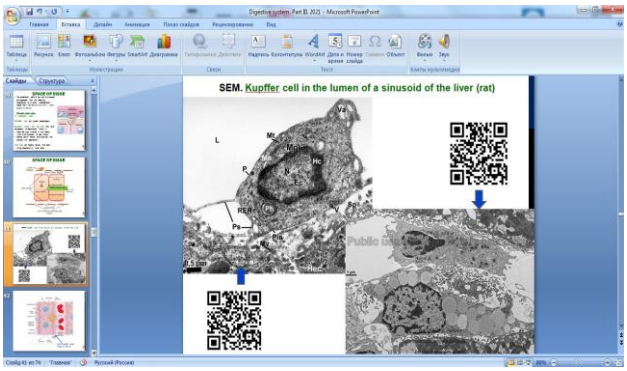

Figure 1. Online histology lecture in Moodle platform using QR code of liver ultrastructure to provide easy and comfortable access to the website with scanning electron micrograph

digital images, which can be stored in virtual archives on local or distant servers, has resulted in novel approaches to e-learning; both in the classroom and also for distance learning (4). This is leading to major changes in the way practical histology and histopathology classes are taught. There is no longer a need for individual student microscopes, technical staff for microscope maintenance or repair, or worries about the loss of valuable specimens. There is no longer a need for individual histological slide sets or to prepare new samples. It is a win-win situation that medical university administrators, teachers, and students appreciate, which has led to the widespread adoption of virtual microscopy systems in life sciences studies. View of anatomical structures was achieved via 3D programs, virtual labs, and pictures of cadaveric material (7). Virtual education may eliminate the need of preserving cadaveric material and formalin specimens, but on the other hand, the students take the opportunity to see and discover this valuable material. Moreover, the Human Anatomy Department obtained a SynDaver Anatomy model which is an ideal alternative to using human cadavers in basic anatomy classes. For some classes of Arthrology, Myology, and Splanchnology chapters were carried out while showing its structures through live sessions. Also, access to the Anatomage table data has shown enormous progress in students learning (8), and during online classes, it was possible to show structures of central nervous, peripheral nervous, and vascular systems connecting through the main department server. (Figure 2) At the World Anatomy Day symposium 2020, Prof. C Kreb's virtual anatomy lab was presented, which was successfully implemented into the educational process of our department. During the pandemic, KNMU obtained 3D Organon license keys access to access virtual anatomical atlas data. (Figure 3) Also teaching staff have developed special drawings during online teaching, to ease understanding of the material.

Assessment of knowledge was carried out with the help of oral polls, and test controls (Google forms, MCQ platforms, Kahoot). This all required a great deal of time to develop a new concept of educational approach and to make it effective without real interaction with students. The effectiveness of online assessment still remains questionable as it is quite hard to develop and achieve productive control of students' knowledge at a distance (9). In addition to lectures, practical and final classes conducted online at the Department of Human Anatomy and Department of Histology, Cytology, and Embryology, there was a need to develop and implement the technology of taking the exam under new conditions. The main task was to ensure transparency, objectivity, and efficiency of the process, taking into account the mandatory requirements and providing equal opportunities for every student. The final exams at the Morphological Sciences Departments were taken according to the same structural principle, this allowed us to achieve maximum success and effectiveness. The exam questions list was showcased in all possible electronic recourses of the department (university website, Moodle platform). The effectiveness of the process was a combination of two components. The first component included online consultations regarding the exam procedure, and the second component was the examination itself. Each academic group received invitations

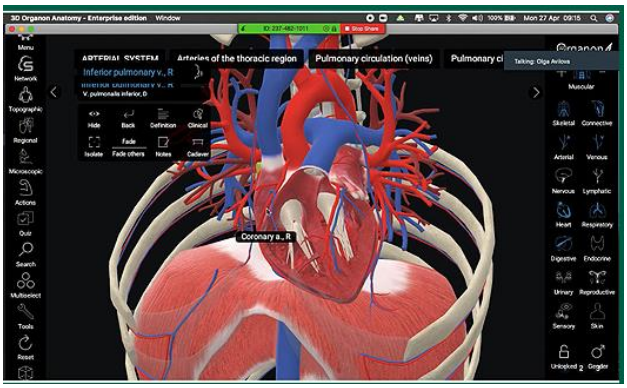

Figure 2. Practical class of human anatomy on Zoom platform. The teacher is using the 3DOrganon virtual reality platform Human Anatomy Atlas to explore the details of the human heart structure and its vasculature

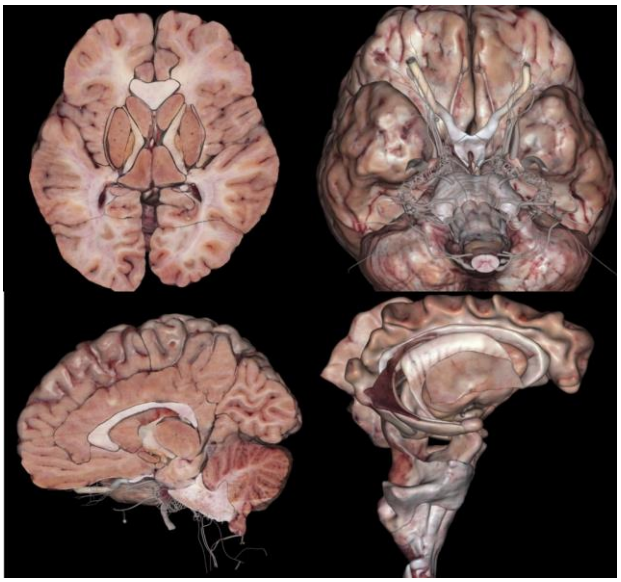

Figure 3. Set of human brain structures obtained from Anatomage table data to showcase in online class using Moodle platform

with allocated time, in which they had joined the moderator's room on Moodle platform. After the student's identification, one was given permission to proceed to the "Exam" section, where they had the opportunity to get exam questions. Four questions and the slides and diagrams with morphological structures were generated automatically by a computer program, which was also one of the main requirements for the exam. Another important point is that access to questions in the Moodle system was strictly controlled and provided to each group only at the time of the exam. After receiving the questions, the student had time to prepare, under the video surveillance of a separate moderator. Then the student was invited to the examiner's room and answered the questions by sharing his screen.

Despite the difficult conditions we all found ourselves in during the pandemic, there were quite positive results showing high knowledge acquisition during quarantine. Also, there was positive feedback from students regarding the organization of online exams, as there were no

technical failures during the session and all the examiners supported the students in this difficult time.

Experience of Teaching Human Anatomy during the Pandemic at the University of Cape Town

Teaching anatomy at the UCT includes a combination of theoretical material presented through lectures and practical sessions (anatomical prosections, body dissections, anatomical models), tutorials, and facilitated small group discussions among students (student problem-based learning (SPBL) in which students are given clinical cases to guide the sessions). Since the beginning of higher education, from the time of colonization to the era of decolonization, almost all universities in South Africa have been dependent on face-to-face learning (10).

In South Africa, the president called on all universities to shut down and find alternative ways to deliver lectures online as of 18 March 2020 as a precautionary measure (11). Following that, the country entered a national lockdown in response to the world COVID-19 pandemic from 27 March 2020 after the first confirmed case on 5 March 2020. Due to the lockdown in South Africa, all non-essential workers had to work from home, and students were also forced to be quarantined in their homes (12). The aim of the lockdown was to flatten the curve and consequently the spread of the virus by enforcing social distancing which was all over 109 countries across the world (13). These abrupt implementations led to the interruption of the academic year with all pre-clinical activities having to be moved online and with no campus access for both the academic staff members and students. The 2020 academic year had to be continued on the online platform through emergency remote teaching (ERT) by delivering the lectures, tutorials, and practical sessions remotely with new academic schedules that had to be drawn. The main goal of the ERT was to ensure that students could still access the learning material and get to catch up while staying at home. The adoption and use of online learning in universities in developing countries like South Africa show a critical need to serve students (14). Therefore, as the ERT had to be digital, all measures were taken by the university centrally to ensure a smooth transition into the process for the students by providing the data (internet bundles) and resources such as laptops and structures for students with needs i.e., interruptions at home due to the structure set up. Further to this, some students without gadgets to access the internet or who were in areas with less internet connectivity were provided with paper-based material. Both the students and academics had to undergo training in order to ensure effective readiness for the teaching modality.

The ERT was carried through the following digital platforms and software: uploading of lecture slides (which included voiceover recordings or videos), setting up of digital forums and chatrooms (for interactions between students and lecturers and also for the tutorial sessions) on the university online learning platform (Vula), and the use of software Zoom cloud meeting and the Microsoft Teams software for small group interactions.

The cadaveric specimens were electronised and included in the teaching material, the anatomy software packages (Anatomedia and Primal Pictures) were used to demonstrate the dissections and also show the anatomical structures and different body systems.

Added benefits of distance learning are that; i) teaching and learning sessions can be conducted anywhere with no demand of students and teachers coming together; ii) immediate feedback of assessments will be available to students.

In a diverse and developing country such as ours, we find a chasm between the socioeconomic backgrounds of the students in the classroom. Some arrive with Apple MacBook while some have never owned a smartphone. When the lockdown was implemented across South Africa, UCT's residences closed, and all students were forced to go home where this chasm was somewhat mirrored. Some went home to conducive study spaces while some returned to rural homesteads with poor connectivity, sharing a room with others and having to resume household chores, caring for elderly parents and children.

We had to take these factors into consideration when redesigning our courses for ERT, particularly assessments. For example, in the first year Health and Rehabilitation Science Anatomy course, the continuous assessment was traditionally in the form of weekly practical quizzes which would be completed at the end of a 3-hour practical session. However, now that these practical sessions were being held online, it is not fair nor practical to expect the students to complete the quiz at the same time as some may have to walk a distance to get to an internet café or someplace with a better network in order to complete the quiz. Therefore, the quiz was made accessible to students over 7 days and they could complete it at any time. This was the most equitable solution in a difficult circumstance. When it came to formative assessment, although we were considerate of the students' different situations, maintaining the integrity of the assessment was integral. Formative assessments were traditionally 3-hour long invigilated online exams. During ERT, for this course, these were converted to online assessments which were available to students for a period of 24 hours but timed for 3 hours, only 1 submission was allowed, and the questions could only be accessed in a linear fashion (the student could not revisit a question once they had completed it). Having the assessment available for students to take over a 24-hour period catered to the needs of vulnerable students in areas of low connectivity etc. Table 4 displays the number of students who accessed the assessment during the different time periods within the 24-hour window in which the assessment was available. It is notable that students accessed the assessment in each time period. The students had taken advantage of the flexibility that online assessments offer.

The move to ERT from traditional teaching was an unplanned but necessary action in order for the university

Table 4. Final formative assessment submission times*

Time Interval	n (%)
00:00 - 03:00	3 (2.4%)
03:00 - 06:00	3 (2.4%)
06:00 - 09:00	7 (5.6%)
09:00 - 12:00	36 (29.0%)
12:00 - 15:00	28 (22.6%)
15:00 - 18:00	22 (17.7%)
18:00 - 21:00	15 (12.1%)
21:00 - 23:59	10 (8.1%)

*: Adapted from Vula, University of Cape Town

to continue to function. There are obvious infrastructural, social, and mental disadvantages that students faced. However, there are aspects of the ERT which work well, and it is worth considering incorporating these into traditional teaching when it resumes. As a part of a course evaluation, 124 students were asked to express their opinions on the most helpful aspects of ERT (Table 5).

Lecture videos were voted the most helpful aspect of ERT by 66.9% (n=83) of the class. This may be due to the students being able to re-watch sections that they find difficult, pause the video for note-taking, and watch at the pace which is most suitable for their style of learning. Weekly quizzes on lecture content were the second most helpful aspect of ERT as students appreciated being able to consolidate their knowledge weekly. This can easily be adapted to the traditional setting in the future. Students also valued the forums in which they could ask questions, the support from lecturers, lecture slides, textbooks, and the lessons page on the learning management system which organized all the learning material. When the threat of the COVID-19 pandemic is no longer around and universities are able to resume the 'pre-COVID' way of teaching, there will be much reflection on how lecturers, course conveners, and faculties handled the situation and facilitated distance learning. By taking the opinions of the students, as in Table 5 coupled with the expertise and experiences of faculty members it is possible to create an environment that cultivates learning in an unprecedented time. Putting the student first and ensuring that they receive a quality education is key despite the challenges it poses to us as facilitators, especially in our field of anatomy which is heavily reliant on laboratory-based teaching.

DISCUSSION

In the current research, the practical experience of the two universities, in terms of the course of adaptation to distance education and students' perspectives of this, was compared. Basically, students have been divided into two camps, those who prefer online (taking into account significant advantages that have been discussed previously) and those who prefer offline education (as they are still followers of the traditional way of education and expressed a number of cons they experienced during online studies). Mostly the choice of one or another educational mode depends on the self-organization of the learner (15,16). Students who want to obtain knowledge will do it no matter what circumstances they are put in by adjusting their time to practice online on virtual platforms or to prepare for important medical exams while having extra time staying at home.

There were trace similarities in advantages and disadvantages of distance learning expressed by students of both institutions from both continents. Students enjoyed the extra time to prepare for classes (absence of necessity to commute to university at KNMU) and the flexibility given for the online submission time (access to quizzes for students over 7 days at UCT). Students found it convenient to access online material constantly (Moodle platform at KNMU and Vula Learning Management System at UCT). Students found the possibility to rewatch recorded lectures very helpful at both institutions. Also, students found it more comfortable to study at home (due to the comfortable space and ability to manage the time efficiently).

Analyzing the disadvantages of distance learning in both establishments we observed the same types of troubles students have faced and, thus, tried to find the most efficient solution. For example, the main problem was internet access and common technical problems with the devices (it was resolved by giving extra time for the assignment submission). In order to compensate for the lack of face-to-face communication with teachers and lack of group discussions in both institutions the access to online resources was constant and, thus, we were trying to provide the possibility of discussions in online forums and maximum knowledge acquisition possible in the occurred situation (17). Questioned senior students of KNMU reported a lack of clinical approach and students in 1st year of the Health and Rehabilitation course at UCT expressed a lack of laboratory-based teaching. The solution to this situation was the implementation of 3D visualization programs into educational curriculum use.

Despite the challenges of distance learning, KNMU medical students' perspectives about distance learning were positive. They adapted to the new teaching method by managing their time more productively. However, it is necessary to implement more interaction with clinical knowledge to improve the effectiveness of distance learning.

Students of UCT stated that despite the lack of proper internet access they could have completed assignments in time due to the flexibility of the submission times of online assessments. Though the lack of face-to-face interaction with educators in distance learning was a disadvantage, students could get support from lecturers in online forums. Overall, the experience of online education at UCT is positive, but students noted the lack of laboratory-based teaching as a disadvantage of distance learning.

Lecturers at higher educational institutions can deliver effective materials in both forms, the only question which remains unanswered is how to approach hands on the material in anatomy (cadaveric material, access to labs) and in histology -histological samples (peering through microscopes). Will digital programs like 3D4 Medical or virtual platforms like 3DOrganon replace the traditional work in anatomy labs? Or, maybe, we have already found the balance of blended learning -online lectures for big groups of students and offline practical classes for smaller groups of learners (18).

On the one hand, there is a need to change the paradigm of the educational process in a rapidly changing environment (19). On the other hand, distance education directly depends on the development of information and communication technologies. All in all, we suppose, distance learning will not replace traditional education models but provide a flexible alternative to many who lead hectic lives.

Table 5. Most helpful aspects of ERT according to students*

Aspects	n (%)
Lecture videos	83 (66.9%)
Quizzes	15 (12.1%
Forums	7 (5.7%)
Support from lecturers	7 (5.7%)
Lecture slides	4 (3.2%)
Textbook	4 (3.2%)
Lessons page	4 (3.2%)

ERT: emergency remote teaching, *: Adapted from Vula, University of Cape Town

CONCLUSION

COVID-19 pandemic has brought significantly major challenges to educational systems around the world. Distance learning has become one of the most effective and possible teaching methods that keep medical students and teachers on the same track of education. Sharing experiences between educational establishments in two countries helped us understand that the advantages and disadvantages of the online mode of education expressed by students during the survey are similar in both institutions. While exchanging this data we could find solutions in order to reach the main objective -efficient knowledge acquisition. We have short-term experience with distance learning in comparison with traditional indoor education, but one thing is not in dispute: it's here to stay and will continue to grow. Needless to say, the world is moving towards global changes in the educational field.

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Clinical and Radiological Evaluation of Surgically Treated Acetabulum Fractures

Cerrahi Olarak Tedavi Edilen Asetabulum Kırıklarının Klinik ve Radyolojik Değerlendirmesi

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ABSTRACT

Aim: Acetabular fractures were evaluated by Judet and Letournel in two main groups as elementary and associated fractures. There are publications in the literature that the results are worse as the complexity of the fracture increases. The aim of this study was to evaluate whether clinical outcomes are worse in associated fractured patients.

Material and Methods: The study included 48 acetabular fractures admitted to the emergency room and treated surgically between December 2011 and October 2020. Patient's trauma, additional injuries, surgical method, early or late complications, and range of motion at the last follow-up were documented. Clinical and radiological results of the patients were evaluated by modified Merle d'Aubigné, functional capacities by Harris hip scoring systems.

Results: There were 25 elementary fractures and 23 associated fractures in patients. When the complication rates were examined, the rate of those without complications was 52.0% (n=13) among elementary fractures, while the rate was 34.8% (n=8) in associated fractures. When the fracture types were evaluated according to the Harris hip score, elementary fractures have better score than associated fractures, but no statistically significant difference was observed between them (p=0.056). When evaluated according to Merle d'Aubigne score, it was observed that elementary fractures had significantly better scores (p=0.004).

Conclusion: As the complexity of the fracture increases, it can be predicted that the clinical outcomes of the patient will be worse. It is also concluded that the elementary fracture type had better clinical outcomes than the associated fracture type in our own surgically treated acetabular fractures.

Keywords: Acetabular fractures; acetabular fracture types; elementary type fractures; surgical treatment.

ÖZ

Amaç: Asetabulum kırıkları Judet ve Letournel tarafından elementer ve kompleks kırıklar olmak üzere iki ana grupta değerlendirilmiştir. Literatürde kırığın kompleksitesi arttıkça sonuçların daha da kötüleştiğine dair yayınlar bulunmaktadır. Bu çalışmanın amacı, kompleks kırıklı hastalarda klinik sonuçların daha kötü olup olmadığını değerlendirmektir.

Gereç ve Yöntemler: Çalışmaya, Aralık 2011 ve Ekim 2020 tarihleri arasında acil servise başvuran ve cerrahi olarak tedavi edilen kırk sekiz asetabulum kırığı dahil edildi. Hastaların travmaları, ek yaralanmalar, cerrahi yöntem, erken ve geç komplikasyonları ve son takipteki hareket açıklıkları kaydedildi. Hastaların klinik ve radyolojik durumları modifiye Merle d'Aubigné skoru ile ve fonksiyonel kapasiteleri ise Harris kalça skoru ile değerlendirildi.

Bulgular: Yirmi beş hastada elementer kırık ve 23 hastada kompleks kırık mevcuttu. Komplikasyon oranları incelendiğinde elementer kırıklar içerisinde komplikasyon olmayanların oranı %52,0 (n=13) iken, kompleks kırıklarda oran %34,8 (n=8) idi. Kırık tipleri Harris kalça skoruna göre değerlendirildiğinde, elementer kırıklar kompleks kırıklardan daha iyi skora sahip olmakla birlikte aralarında istatistiksel olarak anlamlı bir farklılık gözlenmemiştir (p=0,056). Merle d'Aubigné, skoruna göre değerlendirildiğinde elementer kırıkların anlamlı derecede daha iyi skorlara sahip olduğu görüldü (p=0,004).

Sonuç: Kırığın kompleksitesi arttıkça hastanın klinik sonuçlarının daha kötü olacağı tahmin edilebilir. Cerrahi olarak tedavi edilmiş asetabulum kırıklı kendi olgularımızda da, elementer kırık tipinin kompleks kırık tipine göre daha iyi klinik sonuçlara sahip olduğu sonucuna ulaşıldı.

Anahtar kelimeler: Asetabulum kırıkları; asetabulum kırık tipleri; temel kırık tipleri; cerrahi tedavi.

INTRODUCTION

Today's technological advancements and widespread use of automobiles result in high-energy traffic accidents and an increase in acetabulum fractures (1,2). Anatomical reduction and internal fixation are the current treatments for displaced acetabular fractures to decrease the incidence of later complications (3). The successful surgical treatment of acetabular fractures is the result of a steep learning curve and lengthy experience, making these fractures particularly arduous to cure (4).

Judet et al. (5) described the radiographic classification of acetabular fractures and treatment options for fracture types and a thorough understanding of the fracture by radiological imaging and selection of the best surgical strategy for the fracture type determines the success of acetabulum fracture therapy (3). Numerous studies in the literature look at the severity of acetabular fracture complications and their prognosis after surgical treatment (6). This study evaluated the influence of fracture type on the prognosis of acetabular fractures after surgical treatment.

This study aimed to evaluate the radiological and clinical outcomes of acetabular fractures treated surgically, hypothesizing that surgical treatment would result in the best radiological and clinical outcomes in cases of elementary type fractures.

MATERIAL AND METHODS

This study was conducted retrospectively in accordance with the ethical standards of the SBU Istanbul Training and Research Hospital Clinical Research Ethics Committee and the 1975 Declaration of Helsinki revised in 2013. Ethics committee approval was obtained (Decision No. 1625, 04/01/2019). Patients admitted to the emergency department and treated for acetabular fractures between December 2011 and October 2020 were screened using digital data and hospital patient files. Acetabulum fractures were classified according to Judet's classification system (5) and those with a minimum 12-month follow-up were included in the study; those that were followed conservatively, that had a follow-up period of less than 12 months, and that had inaccessible data were excluded from the study. As pediatric acetabular fractures may require different clinical approaches, this study was again excluded.

Surgical Procedure

All surgeries were performed by two experienced (more than 15 years) pelvic surgeons, and depending on the preference of the treating surgeon, each surgery was performed using 3 different surgical techniques. If the anterior approach is preferred according to the location of the fracture, ilioinguinal or modified Stoppa; if the posterior approach is to be preferred, Kocher Langenbeck or combined approach is preferred depending on the type of fracture. All patients underwent surgery under pre-op 1 gram cefazolin-sodium antibiotic prophylaxis. Antibioprophylaxis was continued for 2 days after surgery. For the prophylaxis of heterotopic ossification after surgery, patients were given 3*25 mg of indomethacin for 3 weeks.

Data and Measurements

Patients' age, gender, mechanism of trauma, concomitant injuries, co-dislocation, accompanying neurological deficits included lateral femoral cutaneous nerve status, fracture type according to Judet and Letournel (5) classification as elementary (Figure 1) or associated (Figure 2), incision preferences were obtained from patient records in this study. Hip range of motion was measured at the last visit. Clinical results were evaluated through the scoring system of Merle d'Aubigné modified by Matta (7). Harris hip score was used to evaluate the functional outcomes.

Statistical Analysis

IBM SPSS v.21.0 for Windows statistical package was used for statistical analysis. Shapiro-Wilk test was used to evaluate the normality distribution of data. The comparisons of the two independent groups were made

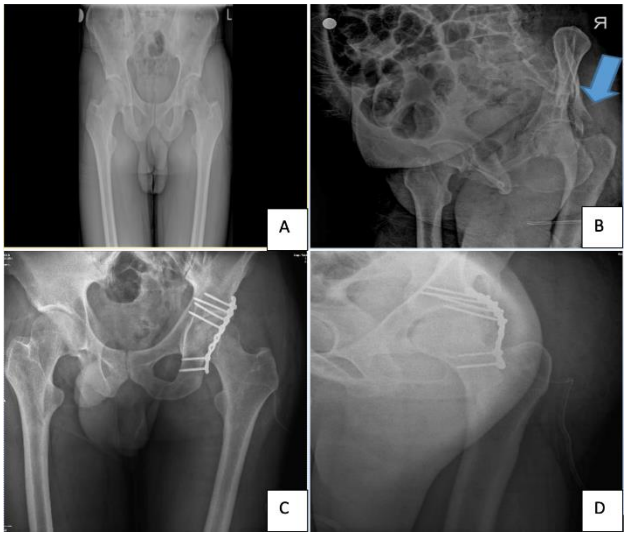

Figure 1. A) Pre-op anteroposterior view, **B)** Pre-op obturator oblique view (arrow shows "Gull Sign"), **C)** Post-op anteroposterior view, and **D)** Post-op obturator oblique view of posterior wall fracture

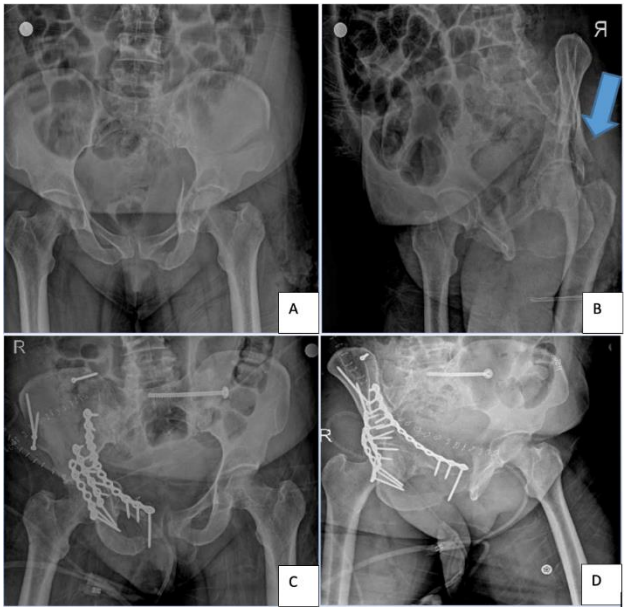

Figure 2. A) Pre-op anteroposterior view, **B)** Pre-op obturator oblique view (arrow shows "Spur Sign"), **C)** Post-op anteroposterior view, and **D)** Post-op obturator oblique view of both column acetabular fracture

with the Mann-Whitney U test, and the median, 25th and 75th percentiles, and minimum-maximum values were given. Categorical variables between groups were analyzed by Pearson chi-square, Fisher's exact, and Fisher-Freeman-Halton test, as appropriate. The statistical significance level was considered as $p<0.05$.

RESULTS

A total of 55 patients who had undergone acetabular fracture surgery were retrieved from hospital records. It was determined that 3 patients did not have up-to-date contact information and left their follow-up. It was observed that 2 patients died in the early post-op period (one with intracranial hemorrhage and the other with massive pulmonary embolism). It was observed that 1 patient was treated with the percutaneous method and 1 patient had bilateral acetabular fractures. These 7 patients were excluded from the study. A total of 48 (13 female and 35 male) patients were included in this study. There were elementary fractures in 25 patients and associated fractures in 23 patients. The median age at the time of surgery was 30 (range, 18-68) years for elementary fracture type and 39 (range, 18-73) years for associated fractures. Both groups were similar in terms of demographic and clinical characteristics (Table 1). Functional outcomes and complication rates are shown in Table 2. Fracture union was achieved in all patients.

There was no significant difference between fracture type in terms of gender, mechanism of injury, complication, and surgical approach (p=0.250, p=0.217, p=0.823, and p=0.847, respectively). While the male and female in elementary fractures was 20.0% (n=5) vs 80.0% (n=20), it was 34.8% (n=8) vs 65.2% (n=15) in associated fractures. Of the elementary fractures, 76.0% (n=19) resulted from traffic accidents, 20.0% (n=5) from falling from a height and 4.0% (n=1) from being struck by a weight. Of the associated fracture, 52.2% (n=12) resulted from traffic accidents, 34.8% (n=8) from falling from a height, 8.7% (n=2) from sports injuries and 4.3% (n=1) from being struck by a weight. A total of 10 cases were accompanied by posterior hip dislocation. While only 15 (31.3%) patients had isolated acetabulum fractures, 33 (68.8%) patients had additional injuries. As the accompanying traumas; the cases with acetabular fractures are mostly accompanied by lower extremity injuries (34%, n=19), followed by upper extremity injuries (20%, n=11) and non-acetabular pelvic ring injuries (20%, n=11).

Although the scores of elementary fractures were higher than the associated fractures according to the Harris hip score at the last follow-up of the patients, no statistical significance was found (p=0.056). When the patients were evaluated according to the modified Merle d'Aubigné score, the scores of elementary fractures were found to be statistically significantly higher than those of associated fractures (p=0.004). When the two groups were evaluated according to the range of motion of the hip joint, no statistically significant difference was found.

When the relationship between complication and fracture type was examined, the rate of those with elementary fractures with no complications was 52.0% (n=13), with osteoarthritis was 16.0% (n=4), with infection was 4.0% (n=1), with heterotopic ossification was 12.0% (n=3), with neurological problems (including lateral femoral cutaneous nerve palsy) was 12.0% (n=3), and with AVN was 4.0% (n=1). The rate of those with associated fractures with no complications was 34.8% (n=8), with osteoarthritis was 26.1% (n=6), with infection was 8.7% (n=2), with heterotopic ossification was 8.7% (n=2), with neurological deficit (including lateral femoral cutaneous nerve palsy) was 17.4% (n=4), and with AVN was 4.3% (n=1).

DISCUSSION

In 1964, Judet and Letournel (5) proposed a classification for surgical treatment plan based on the anatomy of the pelvis and the biomechanics of the fracture. This classification is the most used classification today and we opted to use it in this study. This study grouped surgically treated acetabular fractures according to fracture type, elementary and associated, investigating the effects of fracture type on functional outcomes and complication rates after surgical treatment. We found that there is no significant range of motion difference between fracture types. In addition, the modified Merle d'Aubigné score is significantly better in the elementary group. The results of this study were compatible with the literature (8,9).

Acetabulum fractures are often accompanied by additional injuries. In one study, extremity fractures were associated

Table 1. Demographic and clinical characteristics of the patients in both fracture type

	Elementary Fracture (n=25)	Associated Fracture (n=23)	p
Age (years), median (25th -75th) [min-max]	30 (26-39) [18-68]	39 (24-56) [18-73]	0.219
Gender, n (%)			
Female	5 (20.0%)	8 (34.8%)	0.250
Male	20 (80.0%)	15 (65.2%)	
Mechanism of injury, n (%)			
Traffic accident	19 (76.0%)	12 (52.2%)	
Falling from high	5 (20.0%)	8 (34.8%)	0.217
Weight drop on	1 (4.0%)	1 (4.3%)	
Sport accident	0 (0.0%)	2 (8.7%)	
Time from injury to surgery (days)	5 (4-7) [1-16]	7 (4-10) [1-16]	0.285
Surgical incision preference, n (%)			
İlioinguinal	7 (28.0%)	5 (21.8%)	
Modified Stoppa	2 (8.0%)	4 (17.4%)	0.847
Kocher Langenbeck	15 (60.0%)	13 (56.5%)	
İlioinguinal + Kocher Langenbeck	1 (4.0%)	1 (4.3%)	

Descriptive statistics were presented as median, 25th and 75th percentiles, and minimum-maximum

Table 2. Comparison of postoperative results in both fracture type

	Elementary fracture (n=25)	Associated fracture (n=23)	p
Hip Joint extension	0° (0-0) [-10°-0°]	0° (0-0) [-10°-0°]	0.506
Hip Joint flexion	110° (110-120) [90°-120°]	110° (100-120) [90°-120°]	0.188
Hip Joint adduction	20° (15-30) [0°-40°]	15° (15-20) [0°-40°]	0.110
Hip Joint abduction	30° (20-40) [0°-30°]	25° (20-30) [0°-30°]	0.132
Hip Joint external rotation	20° (20-30) [0°-40°]	30° (20-35) [0°-35°]	0.372
Hip Joint internal rotation	20° (15-20) [0°-30°]	15° (10-20) [0°-25°]	0.055
Harris score	90 (88-91) [39-96]	82 (65-91) [22-96]	0.056
Merle d'Aubigné score	17 (16-17) [6-18]	15 (13-16) [7-18]	**0.004**
Complications, n (%)			
None	13 (52.0%)	8 (34.8%)	0.823
Osteoarthritis	4 (16.0%)	6 (26.1%)	
Infection	1 (4.0%)	2 (8.7%)	
Heterotopic ossification	3 (12.0%)	2 (8.7%)	
Neurological deficit	3 (12.0%)	4 (17.4%)	
Avascular necrosis	1 (4.0%)	1 (4.3%)	

Descriptive statistics were presented as median, 25th and 75th percentiles, and minimum-maximum

with acetabulum fractures at a rate of 42.7% (1). In this study, this rate was found to be 54%. The most common accompanying injury was fractures associated with the lower extremity, consistent with this study.

When we started to treat acetabular fractures surgically, we routinely preferred the ilioinguinal approach. As our experience increased, we started to prefer the modified Stoppa approach, where we had a better chance of approaching the fracture. In most of the fractures requiring an anterior approach, we use the modified Stoppa approach in our recent cases. In this study, we could not evaluate the success of the modified Stoppa approach, as we did not have the number of cases to be compared with the ilioinguinal approach. However, there are increasing numbers of studies in the literature stating that the modified Stoppa approach is a good alternative to the ilioinguinal approach (10-12).

One of the controversial issues is heterotopic ossification prophylaxis (13). We routinely administered indomethacin prophylaxis in our cases. Despite indomethacin, our heterotopic ossification rate is around 10%.

In general, acetabular fracture surgery is not emergent, and performing the surgery after taking 3-5 days to treat additional medical problems and minimize the possibility of increased bleeding (14) is appropriate. Madhu et al. (15) reported anatomical fracture reduction is easier if it occurs within 15 days for elementary fractures and within 5 days for associated fractures. Johnson et al. reported that the results of surgical treatment performed after 3 weeks were dramatically poor (16). In our series, the mean duration of surgery for patients was 6.4 days (1-16 days).

The factors affecting the prognosis of patients have been discussed in the literature. In many studies, it has been reported that the clinical outcomes of patients treated surgically in acetabular fractures depend on the quality of reduction and that the results are better than patients treated conservatively (6,17,18). Matta (19) reported in a study that the results were worse in cases where the complexity of the fracture increased. In another study, Johnson et al. (16) showed that good reduction positively affects clinical outcomes. In a recent study, the type of fracture, surgical time, and reduction quality were stated as the most important parameters (9). In this study, it could not be evaluated because the duration of the operation could not be reached from the records of the patients. Likewise, the step-off amount required to evaluate the reduction quality could not be evaluated because it could not be measured due to the lack of calibration. The increase in the complexity of the fracture affects the results negatively, which is consistent with this study.

This study has strengths and limitations. The strongest aspect of this study is the comparison of fracture types in the surgical treatment of acetabular fractures with similar patient distributions in the two groups; the weakest aspect is the limited sample size and retrospective study design.

CONCLUSION

Acetabular fractures are less common than other extremity injuries. Therefore, it is important that each treated case is well documented and followed up prospectively. In conclusion, we achieved better functional results after surgical treatment of acetabular fractures in cases of elementary type fractures; however, we believe that functional results are also good after surgical treatment of associated fractures.

Ethics Committee Approval: The study was approved by the Ethics Committee of İstanbul Training and Research Hospital (04.01.2019, 1625).

Conflict of Interest: None declared by the authors.

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The Effects of Nasal Septum Deviation on Ocular Examination Findings: Does Deviated Nasal Septum Cause Impaired Vision?

Nazal Septum Deviasyonunun Göz Muayenesi Bulgularına Etkileri: Nazal Septum Deviasyonu Görme Bozukluğuna Sebep Olur mu?

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ABSTRACT

Aim: Nasal obstruction due to nasal septum deviation is associated with systemic diseases such as cardiopulmonary disease, neurological and vascular problems. But the effect of pure nasal deviation on the ocular system has not been precisely investigated. The aim of this study was to analyze the association of nasal septal deviation with ocular examination findings.
Material and Methods: Twenty-seven adult patients underwent septoplasty and 31 controls were included in the study. The study group was conducted on patients with pure nasal septum deviation which is significantly obstructing the nasal airway (>50%). In ophthalmological examination; peripapillary retinal nerve fiber layer, macular and choroidal thickness measurements were obtained. The examination findings were compared between the study and control groups.
Results: The mean macular thicknesses at nasal-500µm were 305.89±32.79 and 287.87±25.00 in the study and control groups, respectively (p=0.021). The mean macular thicknesses at nasal-1000µm were 353.04±21.28 and 341.16±17.97 in the study and control groups, respectively (p=0.025). The mean thickness of choroid was statistically significantly different at central (p=0.036) and peripheral measurements; nasal-500µm (p=0.020); nasal-1000µm (p=0.001); nasal-1500µm (p<0.001); temporal-500µm (p=0.023) and temporal-1000µm (p=0.045). No statistically significant difference was found between the two groups according to ocular tension, thickness of cornea, keratometry, anterior chamber depth, axial length of cornea, and retinal nerve fiber layer thickness.
Conclusion: This is one of the pioneer studies evaluating the ocular examination findings in patients with nasal septum deviation. Our results indicate the increased thickness of both macula and choroid in patients with nasal septum deviation.
Keywords: Nasal septum deviation, macular thickness, choroid thickness, ocular asymmetry.

ÖZ

Amaç: Nazal septum deviasyonuna bağlı burun tıkanıklığı, kardiyopulmoner hastalık, nörolojik ve vasküler problemler gibi sistemik hastalıklar ile ilişkilidir. Ancak saf nazal deviasyonun oküler sistem üzerindeki etkisi tam olarak araştırılmamıştır. Bu çalışmanın amacı nazal septal deviasyonun oküler muayene bulguları ile ilişkisini incelemektir.
Gereç ve Yöntemler: Bu çalışmaya septoplasti yapılmış olan 27 yetişkin hasta ve 31 kontrol dahil edildi. Çalışma grubu nazal hava yolunda ciddi bir şekilde tıkanıklık (>50%) oluşturan sadece nazal septum deviasyonlu hastalar ile oluşturuldu. Göz muayenesinde; perifer retina sinir lifi tabakası, makuler ve koroid kalınlık ölçümleri elde edildi. Muayene bulguları çalışma ve kontrol grupları arasında karşılaştırıldı.
Bulgular: Çalışma ve kontrol gruplarında nazal-500µm'deki ortalama makula kalınlıkları sırasıyla 305,89±32,79 ve 287,87±25,00 idi (p=0,021). Nazal-1000µm'deki ortalama makula kalınlıkları çalışma ve kontrol gruplarında sırasıyla 353,04±21,28 ve 341,16±17,97 idi (p=0,025). Ortalama koroid kalınlığı santral (p=0,036) ve periferal ölçümlerde; nasal-500µm (p=0,020), nazal-1000µm (p=0,001), nasal-1500µm (p<0,001), temporal-500µm (p=0,023) ve temporal-1000µm (p=0,045) istatistiksel olarak anlamlı şekilde farklıydı. Oküler tansiyon, kornea kalınlığı, keratometri, ön kamara derinliği, kornea aksiyel uzunluğu ve retina sinir lifi tabakası kalınlığı açısından iki grup arasında istatistiksel olarak anlamlı bir farklılık bulunmadı.
Sonuç: Bu çalışma nazal septum deviasyonu olan hastalarda oküler muayene bulgularını değerlendiren öncü çalışmalardan biridir. Sonuçlarımız, nazal septum deviasyonu olan hastalarda hem makula hem de koroid kalınlığının arttığını göstermektedir.
Anahtar kelimeler: Nazal septum deviasyonu, makuler kalınlık, koroid kalınlık, oküler asimetri.

INTRODUCTION

Nasal obstruction is one of the most common complaints in otolaryngology practice. Nasal obstruction might be associated with many different factors such as; nasal septum deviation (NSD), turbinate hypertrophy, nasal valve problems, nasal polyposis, etc. (1-4). NSD is responsible for a significant proportion of nasal obstruction problems (2,3). The literature supports the association of nasal obstruction with other system diseases such as cardiopulmonary disease, neurologic, and vascular problems. The increase of upper airway pressure secondary to upper airway obstruction (UAO) is highly associated with pulmonary hypertension and right ventricular dysfunction in both children and adult population (5). The nasal vaults account for more than 50% of total airway resistance (6). Nasal obstruction due to NSD is associated with increased resistance of the upper respiratory track, increased CO_2 concentration, and decreased O_2 saturation in arterial blood (7,8). Hypoxia and response to hypoxia may cause vasoconstriction and increase arterial resistance (4). The ocular system has a very sensitive and vulnerable blood supply and can be adversely affected by many chronic hypoxic and inflammatory processes. In the literature, the effects of chronic hypoxic conditions such as obstructive sleep apnea syndrome (OSAS) and obstructive pulmonary disease on the ocular system have been studied (9,10). But, the effect of pure nasal deviation on the ocular system has not been precisely investigated. Şahin et al. (11) in their study investigating the effects of NSD on choroidal thickness showed that the choroidal thickness decreased in patients with NSD and there was a significant increase after surgery.

In this study, we aimed to assess whole other ocular examination findings besides the choroidal thickness in patients with NSD.

MATERIAL AND METHODS

This study was conducted at the Department of Otolaryngology between June 2018 and September 2018. The study was approved by the local ethics committee (Ethics Committee of Düzce University Faculty of Medicine, dated: 18.06.2018, and numbered: 77) and all participants provided written informed consent according to the Helsinki declaration.

Twenty-seven adult patients underwent septoplasty and 31 controls were included in the study. The study group was conducted on patients with pure NSD which is significantly obstructing the nasal airway (>50%). Patients with other abnormal exam findings such as nasal turbinate hypertrophy, allergic rhinitis, or nasal polyposis were excluded from the study. The control group was randomly conducted among the patients in the otorhinolaryngology clinic who had neither nasal obstruction symptoms nor any significant nasal obstruction findings in physical examination. All participants were evaluated about comorbid diseases and the followings were accepted as exclusion criteria: 1) diabetes mellitus, 2) coronary heart disease, 3) hypertension, 4) chronic kidney disease, 5) chronic obstructive pulmonary disease (COPD), 6) diagnosed ocular diseases (such as ocular surgery, ocular trauma, anterior or posterior segment disease, glaucoma, etc.), and 7) history of snoring or witnessed apnea. A life quality questionnaire (nasal obstruction symptom evaluation, NOSE) was applied to the study group (1). We categorized the study group as does not have any significant difficulty, very mild, moderate, fairly, and severe difficulties on the NOSE index (Table 1). All participants were evaluated by experienced otolaryngology and ophthalmology physicians. The findings were recorded by the otolaryngologist: Age, gender, comorbid diseases, main complaint, duration of the main complaint, the severity of nasal obstruction, history of allergy, nasal examination, and flexible nasopharyngoscopy findings. Patients with nasal obstruction other than NSD were excluded from the study.

Oftalmological Examination

All participants underwent a complete ocular examination, including assessment of best corrected visual acuity, ocular motility, pupillary reflexes, slit-lamp biomicroscopy, intraocular pressure (IOP) measurement with Goldmann applanation tonometry, gonioscopy with three-mirror contact lenses, ultrasound central corneal thickness (CCT) measurement (Echoscan US 500; Nidek Co. Ltd, Aichi, Japan), and dilated fundus examination. Peripapillary retinal nerve fiber layer (RNFL), macular, and choroidal thickness measurements were obtained using EDI-OCT (SD-OCT; Heidelberg Engineering, Heidelberg, Germany). Ophthalmological examinations and optical coherence tomography (OCT) scans were performed by an experienced ophthalmologist unaware of the clinical information of the participants. We performed all measurements three times and used the average of the measurements for the statistical analyses.

The examination findings were compared between the study and control groups. Also, examination findings were compared according to the NOSE index.

Statistical Analyses

Normality assumption was examined with Shapiro-Wilk test. Comparison of study and control groups for continuous data were done with Independent sample t-test.

Table 1. Nasal obstruction symptom evaluation index (1)

	Not a problem	Very mild problem	Moderate problem	Fairly bad problem	Severe problem
Nasal congestion or stuffiness	0	1	2	3	4
Nasal blockage or obstruction	0	1	2	3	4
Trouble breathing through my nose	0	1	2	3	4
Trouble sleeping	0	1	2	3	4
Unable to get enough air through my nose during exercise or exertion	0	1	2	3	4

Pearson chi-square test was used to analyze categorical variables. Descriptive statistics were given with mean and standard deviation for continuous data, and with frequency and percentage for categorical data. IBM SPSS v.22 was used for statistical analyses and the statistical significance level was considered as 0.05.

RESULTS

A total of 58 participants were included in the study. There were 27 and 31 patients in the study and control groups, respectively. There were 16 (59.3%) males and 11 (40.7%) females in study group; 21 (67.7%) males and 10 (32.3%) females in control group. The mean ages of the study and control groups were 33.22±13.51 and 34.42±12.78 years, respectively (Table 2). There was no statistically significant difference between the two groups according to age (p=0.730) and gender (p=0.503). The mean duration of complaints of the patients was 3.92 years.

The mean macular thicknesses at nasal-500μm were 305.89±32.79 and 287.87±25.00 in the study and controls, respectively (p=0.021). The mean macular thicknesses at nasal-1000μm were 353.04±21.28 and 341.16±17.97 in the study and controls, respectively (p=0.025). The sub-macular choroidal thickness at all locations was thicker in the NSD group compared to the control group. The mean thickness of choroid was statistically significantly different at central (p=0.036) and peripheral measurements, nasal-500μm (p=0.020); nasal-1000μm (p=0.001); nasal-1500μm (p<0.001); temporal-500μm (p=0.023), and temporal-1000μm (p=0.045). Table 3 summarizes the ocular examination findings.

There was no statistically significant difference was found between the two groups according to ocular tension, thickness of cornea, keratometry (K1, K2), anterior chamber depth (ACD), axial length of cornea (Table 4), and RNFL thickness (Table 5).

There was no correlation between ocular examination findings and the NOSE index.

DISCUSSION

The nasal septum is an anatomic structure located in the anteroposterior direction in the nose and divides the nasal passage as left and right nares. The nasal septum has bony and cartilage components. The deviation of the nasal septum is usually associated with trauma or congenital abnormalities. The deviated nasal septum leads to breathing difficulties, headache, sleep disturbance, coronary heart diseases, and many neurologic diseases (12,13).

It is known that the nasal airway forms 50% of total airway resistance and nasal obstruction has a critical role in physiologic pulmonary ventilation. People start breathing through their mouths when nasal obstruction occurs. This situation is called “upper airway resistance” which is associated with impaired pulmonary ventilation (14). Many studies have shown that chronic nasal obstruction causes impaired pulmonary ventilation (15,16). Upper airway resistance is caused to decreased pulmonary oxygenation and increased heart/pulmonary rates. Increased breath rate doesn’t allow optimal gas exchange. Thus, deviation of the nasal septum causes upper airway resistance, hypoxia, hypercapnia, and increased intrathoracic pressure. All these changes may affect sympathetic and parasympathetic balance (17). The deviated nasal septum is strongly associated with many comorbid diseases owing to hypoventilation (4,18). The effects of chronic nasal obstruction due to NSD on the pulmonary system are similar to other chronic hypoxic situations such as OSAS and COPD.

Table 2. Demographics of study and control groups

	NSD (n=27)	Control (n=31)	p
Age (year)	33.22±13.51	34.42±12.78	0.730
Gender, (n %)			
Male	16 (59.3)	21 (67.7)	0.503
Female	11 (40.7)	10 (32.3)	

NSD: nasal septum deviation

Table 3. Thickness of macula and choroid

	NSD (n=27)	Control (n=31)	p
CM	236.85±54.02	224.87±25.44	0.298
NM-500	305.89±32.79	287.87±25.00	**0.021**
NM-1000	353.04±21.28	341.16±17.97	**0.025**
NM-1500	356.89±16.82	349.03±17.46	0.088
TM-500	297.67±24.28	291.00±19.31	0.249
TM-100	336.81±21.54	329.32±12.72	0.121
TM-1500	328.26±21.68	322.42±12.82	0.227
CC	398.15±70.74	351.32±91.76	**0.036**
NC-500	395.41±72.46	345.35±84.64	**0.020**
NC-1000	392.59±73.74	318.48±79.73	**0.001**
NC-1500	386.07±74.73	294.81±79.24	**<0.001**
TC-500	400.22±62.08	353.58±85.80	**0.023**
TC-1000	392.59±65.32	351.26±85.44	**0.045**
TC-1500	380.37±64.29	339.90±89.79	0.057

NSD: nasal septum deviation, CM: central macula, NM: nasal macula, TM: temporal macula, CC: central choroid, NC: nasal choroid, TC: temporal choroid

Table 4. Ocular examination findings

	NSD (n=27)	Control (n=31)	p
OT-Right	13.93±2.68	13.40±2.61	0.453
TC-Right	553.89±41.33	525.61±91.63	0.145
K1	43.25±1.31	43.55±1.05	0.339
K2	44.03±1.10	43.40±3.61	0.385
ACD	3.02±0.39	3.20±0.35	0.062
Axial length	22.66±0.66	22.94±0.63	0.096

NSD: nasal septum deviation, OT: ocular tension, TK: thickness of cornea. K1-K2: keratometry, ACD: anterior chamber depth

Table 5. Retinal nerve fiber layer thickness

	NSD (n=27)	Control (n=31)	p
T	71.93±8.15	70.39±11.82	0.572
TS	140.89±22.62	133.00±25.78	0.224
NS	112.07±29.97	107.77±21.98	0.532
N	82.81±16.00	81.90±24.92	0.871
NI	113.96±21.18	119.23±27.20	0.420
TI	149.81±19.57	143.61±23.22	0.280
G	103.11±10.18	101.00±9.59	0.420

NSD: nasal septum deviation, T: temporal, TS: temporal superior, NS: nasal superior, N: nasal, NI: nasal inferior, TI: temporal inferior, G: global

The association between NSD and the ocular system is understudied. Unfortunately, there is not enough data analyzing the effects of NSD on the ocular system. In our study, we found that macular and choroidal thickness was increased in the NSD group in comparison with healthy controls.

This result is very interesting and one of the pioneer studies analyzing the ocular system findings of NSD. The pathophysiology of changes in the ocular system is still unclear. However, this association might be related to increased upper airway resistance and impaired vascular compliance secondary to increased pulmonary vascular pressure. Similar pathophysiologic changes and vascular impairment were shown in OSAS (9,19). The impaired blood supply is considered as a risk factor for optic nerve functions (11). Furthermore, studies showed that the increased vascular resistance secondary to triggered renine-angiotensine arch in patients with upper airway resistance syndrome (20,21). This mechanism is also another independent pathophysiologic pathway that may play role in impaired ocular systems in patients with NSD. Bayhan et al. (22) and Xin et al. (23) reported decreased macular thickness in patients with OSAS. In contrast, Ozge et al. (24) reported increased sub-macular choroid thickness in patients with OSAS. They linked the increased macular thickness contrary to the other studies, to the younger population of their study. Microvascular change after a chronic disease is a process and takes time. Continuing vascular adaptation may change choroid blood supply and choroid structure.

The reported variable results for macular thickness might be associated with different stages of microvascular change in patients with OSAS. The mean age of our study population was 33.22±13.51 which is significantly lower than in similar studies. The mean duration of complaints of the patients was 3.92 years. The increased macular and choroid thickness in the present study may be associated with the short duration of the complaint in the younger study population and the early stage of microvascular change. We believe that our results indicate macular changes in the early stage of tissue hypoxia and hypercapnia owing to the microvascular changes. Although the mean age of the study group was low in the report by Şahin et al. (11), they found the choroid thickness decreased in the study group, but they did not give any information about the duration of the patients' complaints.

The main limitation of the current study is the lack of objective data about chronic hypoxia, hypercapnia, and systemic inflammation. We excluded participants if they have any diagnosed OSAS or potential comorbid diseased but we did not perform polysomnography tests for included patients.

CONCLUSION

This is one of the pioneer studies evaluating ocular examination findings in patients with NSD. Our results showed that macular and choroid thickness increases in patients with NSD, but the duration of symptoms may be effective on these findings. It is obvious that other studies with large numbers of patients are needed to obtain more information on this subject.

Ethics Committee Approval: The study was approved by the Ethics Committee of Düzce University Faculty of Medicine (18.06.2018, 77).

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Evaluating the Results of Retrograde Intramedullary Nailing for Distal Femur Fractures: A Level 3 Trauma Center Retrospective Study

Distal Femur Kırıkları için Retrograd Intramedüller Çivileme Sonuçlarının Değerlendirilmesi: 3. Basamak Travma Merkezi Retrospektif Çalışması

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ABSTRACT

Aim: Distal femur fractures can be treated with modern anatomic plates and nails. This study aimed to examine the clinical and radiological results of displaced distal femur fractures in adult people who had been treated with a retrograde intramedullary nail (RIN).
Material and Methods: The study included all patients who underwent RIN surgery for distal femur fracture between January 2013 and April 2018 in a level 3 trauma center. Patients were divided into two groups based on fracture pattern: open fracture and closed fracture. The preoperative and postoperative clinical, radiological and functional characteristics of patients who had RIN for a distal femur fracture were analyzed.
Results: Thirty patients were included in the study. 21 (70%) patients were male. The median age of the patients was 39 (range, 18-58) years. 17 (56.7%) of the affected femur were left-sided. The etiology of the fractures was traffic accident and fall in 19 (63.3%) patients and gunshot injury in 11 (36.7%) patients. Of the patients, 17 (56.7%) were closed fractures and 13 (43.3%) were open fractures. There were no significant differences between two groups related to the surgery time (p=0.086), fluoroscopy time (p=0.805), blood loss (p=0.967), and hospitalization time (p=0.967), clinical pain and function scores (p=0.341, p=0.902), and union time (p=0.385) at the postoperative period.
Conclusion: RIN is a minimally invasive method that may prevent excessive blood loss and decrease the duration of surgery time. It is an effective and reliable surgical intervention that should be considered for the treatment of distal femur fractures.
Keywords: Distal femur fractures; trauma; intramedullary nail; retrograde.

ÖZ

Amaç: Distal femur kırıkları modern anatomik plak ve çiviler ile tedavi edilebilmektedir. Bu çalışmanın amacı retrograd intramedüller çivi (RİÇ) ile tedavi edilen erişkin kişilerde deplase distal femur kırıklarının klinik ve radyolojik sonuçlarının incelenmesidir.
Gereç ve Yöntemler: Çalışmaya, Ocak 2013 ile Nisan 2018 arasında 3. seviye bir travma merkezinde distal femur kırığı nedeniyle RİÇ ameliyatı geçiren tüm hastalar dahil edildi. Hastalar kırık paternine göre açık kırık ve kapalı kırık olmak üzere iki gruba ayrıldı. Distal femur kırığı nedeniyle RİÇ tedavisi yapılan hastaların ameliyat süreci ve ameliyat sonrası dönemdeki klinik, radyolojik ve fonksiyonel özellikleri analiz edildi.
Bulgular: Çalışmaya 30 hasta dahil edildi. 21 (%70) hasta erkekti. Hastaların ortanca yaşı 39 (aralık, 18-58) yıl idi. Etkilenen femurların 17 (%56,7)'si sol taraflıydı. Kırık etiyolojisi 19 (%63,3) hastada trafik kazası ve düşme, 11 (%36,7) hastada ise ateşli silah yaralanmasıydı. Hastaların 17 (%56,7)'si kapalı kırık, 13 (%43,3)'ü açık kırıktı. İki grup arasında ameliyat süresi (p=0,086), floroskopi süresi (p=0,805), kan kaybı (p=0,967) açısından ve ameliyat sonrası dönemde hastanede yatış süresi (p=0,967), klinik ağrı ve fonksiyon skoru (p=0,341; p=0,902) ve kaynama zamanı (p=0,385) açısından istatistiksel olarak anlamlı fark bulunmadı.
Sonuç: RIN fazla kan kaybını engelleyen ve ameliyat süresini de kısaltabilen minimal invaziv bir cerrahi yöntemdir. Distal femur kırıklarının tedavisinde akla getirilmesi gereken etkili ve güvenilir bir cerrahi seçenektir.
Anahtar kelimeler: Distal femur kırıkları; travma; intramedüller çivi; retrograd.

Presented as an oral presentation at the International Congress of Future Medical Pioneers (May 8-9, 2021; Online).

INTRODUCTION

Distal femur fractures are rare fractures (1). They occur in approximately 3-5% of all femur fractures (1,2). In young patients, high-energy trauma causes distal femur fractures; in older patients, low-energy trauma causes distal femur fractures (2,3). These fractures are associated with high mortality and morbidity (4). They are difficult to treat (2). They can lead to long-term permanent disabilities and serious injuries, such as nonunion, delayed union, and implant failure (5,6). Surgical treatment of distal femur fractures is more effective than conservative treatment (2). These fractures can be treated with external fixation, fixed-angle blade plates, dynamic condylar screws (DCS), locking plates, and intramedullary nails (7). Nails cause the least amount of damage to the fracture site, provide a possible mechanical advantage from a device near to the femur's axis, and promote blood supply by reaming the intramedullary canal (8,9). However, antegrade intramedullary nails are inadequate to fix small and osteoporotic fragments located distal femur (10).

The major goal of this study was to look back on the clinical and radiological results of displaced distal femur fractures in adult people who had been treated with a retrograde intramedullary nail (RIN).

MATERIAL AND METHODS

This study was approved by the local ethics committee (Prof. Dr. Cemil Taşçıoğlu City Hospital ethics committee, dated 17/04/2018, and numbered 883). Patients' informed consents were obtained. The study included patients who underwent RIN surgery between January 2013 and April 2018 in a level 3 trauma center. We excluded patients with pathological fractures, vascular injuries, neurological disease, and unfollowed patients. Two groups of patients were formed as close fractures and open fractures according to Gustilo-Anderson's classification (11). Patient data including surgical time, perioperative hemorrhage, preoperative and postoperative hospitalization period, time to union, and knee function after complete fracture healing according to the Knee Society Score (KSS) were recorded from the Prof. Dr. Cemil Taşçıoğlu City Hospital data center. Patient characteristics between the two groups were compared.

Surgical Technique

The patients underwent surgery in the supine position under general anesthesia or spinal anesthesia as per the decision of the anesthesia team. To ensure the reduction of the distal fragment, a sterile operating sheet was placed under the knee to maintain the knee at 30 degrees of flexion (Figure 1a). An incision from the lower pole of the patella to the tuberous tibia is made to expose the patellar tendon. The patellar tendon is splitted longitudinally and the knee joint was observed between the tendon while preserving the paratenon (Figure 1b). To determine the femur entry point, a single Kirchner (K) wire was advanced from the incision line. All surgeries were performed under C-arm fluoroscopy to determine the appropriate entry point (Figure 2a, 2b). Afterward, the entrance was engraved with the thickest (16 mm) cannula with simultaneous closed reduction of the fracture. An intramedullary guidewire was advanced from the center of the medulla. The femur was reamed through the guidewire with a flexible reamer. A nail (Tasarım Medical branded locking retrograde femoral nail) one millimeter smaller than the medullary thickness measured for fracture fixation was prepared using the height measurement guide and placed on the femur. The distal screws were statically locked using the distal screw guide. Proximal screws were

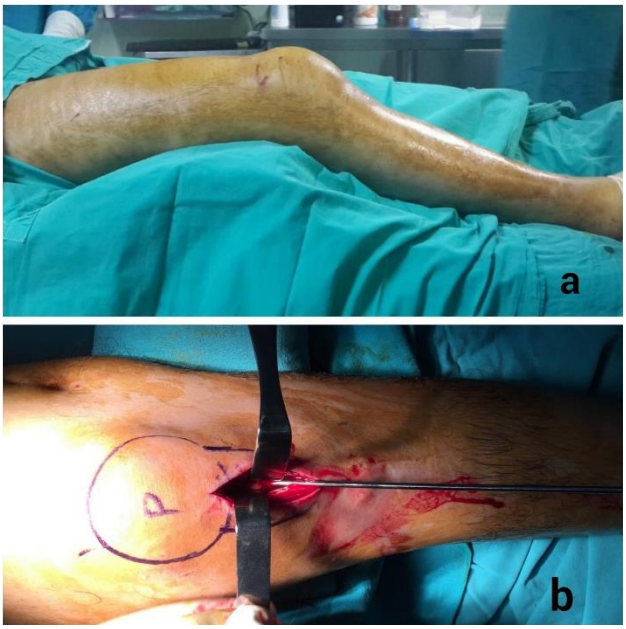

Figure 1. Surgical preparation and incision appearance; **a)** retrograde nail application position; **b)** surgical skin incision

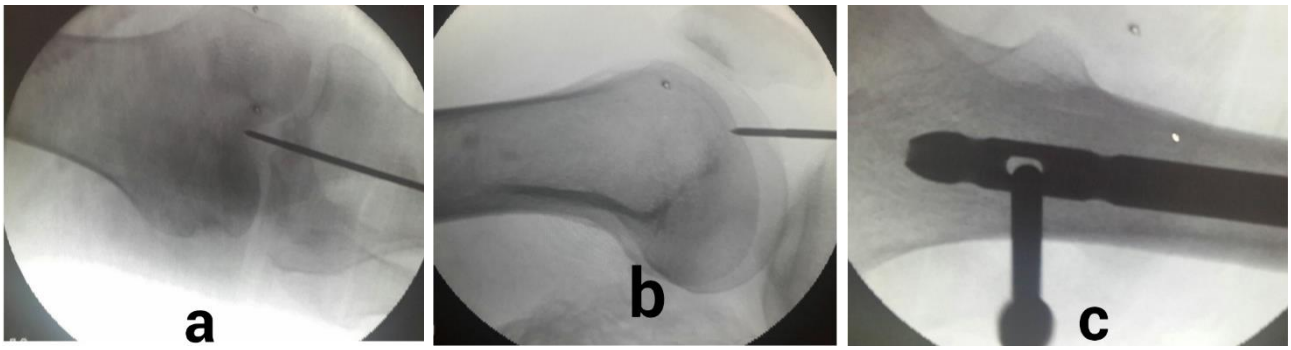

Figure 2. X-ray shown of retrograde nail placement; **a)** appropriate entry site for retrograde intramedullary nail on x-ray table anterior-posterior; **b)** appropriate entry site for retrograde intramedullary nail on x-ray table lateral; **c)** screw placement to proximal hole of retrograde nail by free hand

applied in the anteroposterior plane using the free-hand technique so most of the time spent under fluoroscopy was during the proximal screw fixation (Figure 2c) The fracture line and nail were checked under fluoroscopy and the end cup was adapted to the nail. The incision layers were closed in a standard fashion. No hemovac drainage was used in the patients. If there is an open fracture, firstly the wound was debrided and washed with 5000 cc saline. The skin defects were repaired primarily without excessive tension. No patient required a secondary reconstruction for skin coverage.

Statistical Analysis

The normality test for continuous variables was performed using the Shapiro-Wilk test. Descriptive statistics for continuous variables were expressed as median (interquartile range, Q3-Q1) [minimum-maximum] and as numbers and percentages for categorical variables. The Mann-Whitney U test was used to compare the variables of two independent groups. The Pearson chi-square test and the Fisher-Freeman-Halton test were used to compare categorical variables. A p value of <0.050 was accepted as the statistical significance level. Data were analyzed using IBM SPSS v.22.0 (IBM Corporation, New York, USA).

RESULTS

The records of 35 patients were evaluated retrospectively. Five patients were excluded (four for not followed up, and one for vascular complication). The study group consisted of 30 patients. Twenty-one (70%) of the patients were male, and 9 (30%) were female. The median age of the patients was 39 (range, 18-58) years. Seventeen (56.7%) of the affected femur was left-sided, while 13 (43.3%) were right-sided. The etiology of the fractures was gunshot injury in 11 (36.7%) patients and traffic accident and fall in 19 (63.3%) patients. The median follow-up period was 18 (range, 12-48) months.

The median preoperative fluoroscopy usage time was 81 (range, 68-116) seconds. The median perioperative blood loss was 129.5 (range, 114-150) ml. The median duration of surgery was 59.5 (range, 51-74) minutes. The median postoperative length of hospital stay was 3.5 (range, 2-6) days. The median time of union was 25 (range, 21-30) weeks.

Neither drain placement nor postoperative blood transfusion was required in any of the patients. No infection or neurovascular complication was observed in the patients. The KSS results were excellent in the 6th month, with a median functional and pain KSS scores of 95 (range, 85-100) points and 90 (range, 86-93) points, respectively.

According to Gustilo-Anderson classification, 11 (36.7%) of the patients were type 3A and 2 (6.7%) of the patients were type 1 open fractures. Of the patients, 17 (56.7%) were closed fractures and 13 (43.3%) were open fractures. There was no statistically significant difference in terms of the demographic characteristics; age (p=0.530), gender (p=0.229), and side (p=0.225) of the groups (Table 1). Also, no statistically significant differences were found between the groups related to the surgical characteristics; surgery time (p=0.086), fluoroscopy time (p=0.805), and blood loss (p=0.967), and their clinical and radiological follow-ups; hospitalization time (p=0.967), clinical score (KSS) of pain (p=0.341) and function (p=0.902), and union time (p=0.385) in the postoperative (Table 2).

DISCUSSION

In femur fractures, different orthopedic implants and different surgical techniques can be preferred by paying attention to the location of the fracture and the characteristics of the patient. In this study, we examined the clinical results of retrograde intramedullary nail treatment, which is rarely applied to distal femur fractures. Due to increased high-energy injuries, femur fracture incidence increases, and more complex fractures occur. High-energy traumas are responsible for distal femur fracture etiologies in young patients. It is most common after a traffic accident (12). Distal femur fractures seen in advanced age develop after simple falls (13). Our sample consisted of young patients, and as reported in the literature, there are high-energy traumas in their etiology.

Table 1. Demographic characteristics of the patients

	Close Fracture (n=17)	Open Fracture (n=13)	p
Age (years)	39 (26) [18-58]	39 (21) [22-55]	0.530
Gender, n (%)			
Male	11 (84.6)	10 (58.8)	0.229
Female	2 (15.4)	7 (41.2)	
Side, n (%)			
Right	4 (30.8)	9 (52.9)	0.225
Left	9 (69.2)	8 (47.1)	

Descriptive statistics were presented as median (interquartile range) [min-max]

Table 2. Clinical follow-up characteristics of the patients

	Close Fracture (n=17)	Open Fracture (n=13)	p
Union time (week)	25 (2.5) [21-29]	26 (3.5) [21-30]	0.385
Surgery time (minute)	58 (6) [52-66]	64 (15) [51-74]	0.086
Fluoroscopy time (second)	82 (13) [72-103]	80 (19.5) [68-116]	0.805
Blood loss (ml)	129 (15.5) [114-150]	130 (12) [117-145]	0.967
Hospitalization (day)	3 (2) [2-6]	4 (1) [3-5]	0.967
Clinical score (KSS)			
Pain score	92 (5) [86-93]	88 (4.5) [86-93]	0.341
Function score	95 (7.5) [90-100]	95 (7.5) [85-100]	0.902

KSS: knee society score, descriptive statistics were presented as median (interquartile range) [min-max]

The range of injuries associated with femur shaft fractures, as well as its relationship to mortality, is poorly understood. The most prevalent concomitant bone injury in the research population was to the tibia/fibula (20.5%), ribs/sternum (19.1%), and non-shaft femur (18.9%), with 5.8% of them being femur neck fractures (14). In our study, the most common type of concomitant trauma was contralateral distal radius fracture. The most common comorbid visceral organ injury was lung injury (18.9%), followed by intracranial (13.5%) and hepatic (6.2%) injuries (14). We obtained that the most common comorbid visceral organ injury was lung injury as high as 40%.

It was reported that long bone injuries are associated with substantial blood loss requiring blood transfusion in the previous studies. The amount of blood transfusion was low in patients with extra-capsular femur fractures, which were usually required in the post-operative period (15). In our study, for hemoglobin values below 10 g/dl blood transfusion was performed and the operation was performed. However perioperative blood loss was low so postoperative transfusion was not required; thus, the risks of blood transfusion were overcome.

According to recent research, periarticular knee surgery site infection (SSI) rates might range from 2% to 88%, depending on the fracture location (16). According to the study by O'Toole et al. (17), the risk of septic arthritis as a result of retrograde nailing of an open femur fracture is negligible (1.1%). In our study, 43.3% of patients had open fractures; no septic arthritis or infection developed in any of our patients.

In treatment with distal RIN, factors playing a role in surgery time include anesthesia time, positioning of the patient, the duration for reducing the fracture, and locking of the nail with the freehand technique. Prolonged surgery favors complications such as increased bleeding and risk of infection and non-union. The method of anesthesia is usually not dependent on the surgeon's preference, depends on the anesthesia team, and is dictated by comorbid factors in the patient. However, problems can be prevented by shortening the time of surgery with appropriate techniques and methods.

A common occupational danger in orthopedic trauma surgery is radiation exposure through intra-operative fluoroscopy (18,19). Fluoroscopy is used particularly intensively to confirm the reduction of the fractures, so the patient and surgical team are exposed to radiation (20). One study showed that the median dose-area product (DAP) meter for dynamic hip screws in extra-capsular femur neck fractures was 668 mGy/cm^2 (ST 36 s); for short proximal femur nail was 1040 mGy/cm^2 (ST 49 s); for long femur nail for diaphyseal fractures was 1720 mGy/cm^2 (ST 2 m 36 s); for manipulation and Kirschner wire fixation in distal radius fractures was 25 mGy/cm^2 (ST 25 s), and for volar-locking plate fixation in the distal radius, fractures was 27 mGy/cm^2 (ST 23 s). In comparison to junior surgeons, more experienced surgeons used less radiation in the operating room (18). No study specifies the time of fluoroscopy in cases with retrograde femur nail, but the median time was 81 (range, 68-116) seconds in our study.

One complication encountered in retrograde intramedullary nailing is damaging the neurovascular structures by the proximal screw. Riina et al. (21) stated that placement of proximal anteroposterior locking screw to the level of trochanter minor and above would reduce the risk of femur artery injury. In our study, all proximal locking screws were placed at the level of the minor trochanter minor or above, with no neurovascular complications.

The reamerization of the bone has many benefits in terms of accelerating the fracture union. The disadvantages of reamerization include risk of fat embolism and impaired endosteal blood supply; and weakening of the bone caused by the thinning of the cortex (22). However, reamerization also has some advantages, increasing the stability of the fracture by increasing the contact surface between the nail and the bone cortex and contributing to recovery through internal autologous bone grafting to the fracture site (23). Our study aimed to accelerate the union by allowing autologous grafting and using larger diameter nails to increase the stability by reamerizing.

Antegrade intramedullary nails are inadequate to fix small and osteoporotic fragments located distal femur, as a result, plate osteosynthesis is the treatment of choice for distal femur fractures (10). However, excessive soft tissue dissection may impair vascular circulation such that nonunion and infection rates increase (25).

Since the fixation of these fractures with the plate-screw causes changes in the axial load transfer of the femur, osteopenia develops at the proximal end of the plate. In this case, re-fractures may be observed (2). Because the intramedullary nail is located in the medullary cavity, it leads to less stress than plates and external fixators and therefore we see less failure of fixation (2,7).

Also, the RIN is usually performed by minimally invasive techniques. Thus, the damage to the soft tissues is less (10). This is perhaps why RIN has historically been preferred in the intramedullary treatment of distal metaphyseal fractures. The duration of surgery was shorter for plate osteosynthesis than for RIN because of performing the minimally invasive technique, as well as the amount of bleeding was higher (2). RIN leads to less bleeding and our patient group required no blood transfusions. Although the operation time is relatively long, we suggest performing RIN for distal femur fractures rather than plate osteosynthesis, for lowering the complications.

Limitation: Since we conducted our study retrospectively, we do not know how many days the patients in our study were operated on and whether additional injuries were made at the same time.

CONCLUSION

Open fracture treatments have difficulties in the surgical and postoperative period compared to closed fractures due to both the trauma mechanism and the deterioration of the soft tissue cover. In our study, RIN treatment was applied to open fractures immediately after necessary debridement. However, no clinical difference was observed in the perioperative and postoperative period between closed fractures that were also treated with RIN.

RIN is a minimally invasive method that may prevent excessive blood loss and decrease the duration of surgery time. It is an effective and reliable surgical intervention that should be considered for the treatment of the distal femur fractures.

Ethics Committee Approval: The study was approved by the Ethics Committee of Prof. Dr. Cemil Taşçıoğlu City Hospital (17.04.2018, 883).

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Psychosocial Risk Factors for Depression in Pregnant Adolescents

Adölesan Gebelerde Depresyonun Psikososyal Risk Faktörleri

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ABSTRACT

Aim: Pregnancy in adolescence can negatively affect psychological, physical, and social development, and depression is more common in these pregnancies. This study aimed to investigate frequency of antenatal depression, and its psychological and social antecedents, in adolescent pregnancies.
Material and Methods: This cross-sectional survey study included 272 pregnant adolescents admitted to the antenatal outpatient clinic in a tertiary hospital between May and August 2019. The Edinburgh Postpartum Depression Scale (EPDS) and a structured questionnaire about demographic and psychosocial characteristics, and obstetric history, were used.
Results: The mean age of the pregnant adolescents was 17.2±0.8 (range, 14-19) years, 232 (85.3%) of them were married, and their mean age at first marriage was 16.6±1.3 years. The mean total EPDS score was 8.7±6.2, and 58 (21.3%) of them had depressive symptoms. As education level decreased, the frequency of depressive symptoms increased (p=0.001). In total, 36 (62.1%) of the 58 pregnant adolescents with an EPDS score ≥13 had a history of depression; the other 22 (37.9%) had no history. The prevalence of depression symptoms was significantly higher in pregnant adolescents with a history of depression (p=0.001). In total, 17 pregnant adolescents with a depression risk score ≥13 were diagnosed with depression, and psychiatric support and counseling were provided.
Conclusion: In pregnant adolescents, low educational status and a previous history of depression were closely associated with antenatal depression. Effective antenatal screening should be performed in all pregnant adolescents, especially those with risk factors, to check for antenatal depression and identify those who need psychological support.
Keywords: Adolescent pregnancy; antenatal depression; Edinburgh Postpartum Depression Scale.

ÖZ

Amaç: Adölesan gebelikleri bireyin psikolojik, bedensel ve sosyal gelişimini olumsuz etkiler ve bu gebeliklerde depresyona daha sık rastlanır. Bu çalışmada, adölesan gebelerde antenatal depresyonun yaygınlığı ile psikolojik ve sosyal nedenlerinin araştırılması amaçlanmıştır.
Gereç ve Yöntemler: Kesitsel tipteki anket çalışması, Mayıs 2019 ve Ağustos 2019 tarihleri arasında üçüncü basamak bir hastanenin antenatal polikliniğine başvuran 272 adölesan gebe ile yapıldı. Gebelere, demografik ve psikososyal özellikler ile obstetrik öykünün sorgulandığı yapılandırılmış anket formu ve Edinburgh Postpartum Depresyon Ölçeği (EPDÖ) uygulandı.
Bulgular: Adölesan gebelerin yaş ortalaması 17,2±0,8 (aralık, 14-19) yıl olup 232 (%85,3)'si evli ve ortalama evlilik yaşı 16,6±1,3 yıl idi. EPDÖ toplam puan ortalaması 8,7±6,2 bulundu ve gebelerin 58 (%21,3)'inde depresif semptomlar tespit edildi. Eğitim düzeyi azaldıkça, depresif semptomların görülme sıklığı artmaktaydı (p=0,001). EPDÖ skoru ≥13 olan 58 adolesan gebenin, 36 (%62,1)'sında depresyon öyküsü bulunurken, diğer 22 (%37,9)'sinde depresyon öyküsü yoktu. Geçirilmiş depresyon öyküsü veren gebelerde depresyon semptomlarının görülme sıklığı anlamlı şekilde daha yüksek bulundu (p=0,001). Depresyon riski puanı ≥13 olan toplam 17 gebeye depresyon tanısı konularak, psikiyatrik yardım ve danışmanlık verildi.
Sonuç: Adölesan gebelerde, düşük eğitim seviyesi ve geçirilmiş depresyon öyküsü antenatal depresyon ile yakından ilişkilidir. Antenatal depresyonun tanınması ve psikolojik desteğe ihtiyacı olan adölesanların belirlenmesi için etkin antenatal tarama tüm adölesan gebelere, öncelikle risk faktörü taşıyan gebelere yapılmalıdır.
Anahtar kelimeler: Adölesan gebelik; antenatal depresyon; Edinburgh Postpartum Depresyon Ölçeği.

INTRODUCTION

Depression during pregnancy is an important health problem because it adversely affects maternal and child health, and leads to postpartum depression (1,2). In one-third of women with postpartum depression, depressive symptoms begin during pregnancy; when left untreated, approximately 20% of cases progress to chronic depression (3). Because untreated perinatal depression and other mood disorders may have devastating effects on the mother, newborn, and family, recognition, and treatment of depression in pregnant women is extremely important (4,5).

Adolescent pregnancies adversely affect psychological, physical, and social development, and pose a serious threat to mental well-being. Depression symptoms are also common in adolescent pregnant women, who frequently experience emotional stress and social problems (6,7). Previous studies reported an increased risk of perinatal depression in adolescent pregnant women and many factors that increase the risk of depression in these pregnancies have been reported (8-11). Young maternal age, maternal anxiety, previous history of depression, insufficient social support, low income and education levels, marital incompatibility, domestic violence, unintended pregnancy, inadequate antenatal care, and substance abuse have all been identified as risk factors for the development of depression in adolescent pregnant women (12,13).

It is important to screen for psychological and social risk factors for the development of antenatal depression in adolescent pregnant women, and to identify pregnant adolescents at risk of developing depression, to allow for early psychological support and prevention of the development of postpartum depression. In this study, we investigated the frequency and psychosocial causes of antenatal depression in adolescent pregnant women.

MATERIAL AND METHODS

This cross-sectional study was conducted in the antenatal outpatient clinic at the University of Health Sciences Zekai Tahir Burak Women's Health Care Training and Research Hospital, Ankara between May and August 2019. The study protocol was approved by the local ethics committee (ethics committee number: 36/2018), and all subjects provided written informed consent before enrollment.

A questionnaire consisting of 20 questions, which was developed by the study researchers based on the literature, was completed by adolescent pregnant women. The questionnaire collected data on sociodemographic characteristics (age, body mass index (BMI), marital status, marriage duration, family type, work status, income, education, planning for pregnancy, and smoking status) and obstetric history (gestational week, gravidity, parity, and history of depression in the last 5 years). A history of depression in pregnant women was defined as a diagnosis of depression following psychiatric evaluation, use of antidepressants, or receiving psychotherapy within the last 5 years. We excluded adolescent pregnant women who were currently being followed up with a diagnosis of depression or use of antidepressant medication, multiple pregnancies, or a history of any chronic disease. After obtaining informed consent from all of the adolescent pregnant women who agreed to participate in the study, the questionnaire data and the Turkish versions of the Edinburgh Postnatal Depression Scale (EPDS) were administered by the researcher.

Edinburgh Postpartum Depression Scale (EPDS)

The EPDS is the most commonly used questionnaire for screening for depression during pregnancy and the postnatal period. This scale was developed in Edinburgh in 1987 by Cox et al. (14). It is a self-report scale consisting of 10 items, in a 4-point Likert format. The answers consisting of four options are scored between 0-3, the lowest score that can be obtained from the scale is 0 and the highest score is 30. Each item has a different scoring. The total score of the scale is obtained by summing the scores for each item; a high total score indicates more severe depressive symptoms. The validity and reliability study for the Turkish version of the scale was performed by Engindeniz et al. (15).

In this study, the Turkish version of the EPDS has been used and, a total score of ≥13 was taken as the cut-off point for the presence of depressive symptoms. The participants were classified into two groups according to the scores on the EPDS, i.e., a group with depressive symptoms (EPDS≥13) and a group without depressive symptoms (EPDS<13). The groups were compared in terms of age, BMI, gravidity, parity, gestational age, marital status, family type, employment status, income, education, previous history of depression, and smoking status. Those with an EPDS score ≥13 were referred for detailed psychiatric evaluation.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows software (version 21.0; SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to assess the normality of the distribution of the data. Continuous and normally distributed variables are expressed as mean±standard deviation, and intra-group differences were investigated using Student's t-test. Continuous variables with non-normal distributions are expressed as median (minimum-maximum), and differences between variables were analyzed using the Mann-Whitney U test. Categorical variables are expressed as numbers (percentages) and were analyzed using the chi-square test. Two-sided p values were considered statistically significant at <0.05.

RESULTS

We enrolled 272 adolescent pregnant women who presented at the antenatal clinic of our hospital. Their mean age was 17.2±0.8 (range, 14-19) years. Their mean age at first marriage was 16.6±1.3 years and 232 (85.3%) of them were married; 126 (46.3%) of them had a nuclear family. Consanguineous marriage was identified in 46 (16.9%) of the participants. The mean EPDS score was 8.7±6.2, and 58 (21.3%) of the women had an EPDS score ≥13 (indicating antenatal depression).

The pregnant adolescent women with EPDS scores ≥13 and <13 are compared in Table 1. The groups were similar in terms of age, BMI, gravidity, parity, gestational age, marital status, work status, family type, income, and smoking status. Whereas 32 (55.2%) participants who had only graduated from primary school had an EPDS score ≥13, only 4 (6.9%) who were educated to a high school

level or above had such a score. As education level decreased, the presence of depressive mood became more common (p=0.001), and 48 (17.6%) of the participants had a history of depression. In total, 36 (62.1%) of the 58 adolescent pregnant women with an EPDS score ≥13 had a history of depression; the other 22 (37.9%) had no such history. The incidence of depressive symptoms was significantly higher in participants who were previously diagnosed with depression (p=0.001). Psychiatric evaluations of the 58 (21.3%) pregnant women with an EPDS score≥13 were conducted, and 17 (29.3%) of them were diagnosed with depression and given psychiatric support and counseling.

DISCUSSION

Depression is the most common psychiatric disorder in pregnancy, affecting more than 13% of pregnant women (16). In this study, the incidence of depressive symptoms in adolescent pregnant women was 21.3% (n=58), and 6.3% (n=17) of the participants were diagnosed with depression. Also, we identified two sociodemographic and psychological risk factors for antenatal depression: a low education level and previous history of depression.

Psychosocial health is poorer in adolescent pregnant women compared to older age groups. Many studies have concluded that the risk of depression is increased in adolescent pregnant women (17,18). Osok et al. (17) reported that 32.9% of such individuals showed clinical symptoms of depression and 15.9% of them had features of severe depression. In a study comparing the development of depression between pregnant women aged ≥18 and <18 years, the risk of depression was 18.2 times higher in the adolescent group (18). In another study, depressive symptom scores were significantly higher in 40% of pregnancies under the age of 20 years compared to those in women aged ≥20 years (19). In a study comparing pregnant women aged ≥20 years with adolescent pregnant women, the rate of depression requiring medical treatment was 9.8% in the adolescent group (20). The results varied among these studies due to the inclusion of different age groups, and the use of different scales and cut-offs therefor.

Table 1. Sociodemographic and clinical characteristics of pregnant adolescents according to EPDS scores

	EPDS ≥13 (n=58)	EPDS <13 (n=214)	p
Age (years)	17.3±0.6	17.1±0.9	0.111
BMI	25.3±4.2	26.4±5.7	0.171
Gravidity	1 (0-3)	1(0-2)	0.356
Parity	1 (0-2)	0 (0-1)	0.187
Gestational age			
1st trimester	20 (34.5)	64 (30.0)	
2nd trimester	16 (27.6)	72 (33.6)	0.652
3rd trimester	22 (37.9)	78 (36.4)	
Marital status			
Married	48 (82.8)	184 (86.0)	0.539
Single	10 (17.2)	30 (14.0)	
Family type			
Nuclear family	24 (41.4)	102 (47.7)	0.395
Extended family	34 (58.6)	112 (52.3)	
Work status			
Employed	8 (13.8)	22 (10.3)	0.449
Unemployed	50 (86.2)	192 (89.7)	
Income			
More than expense	20 (34.5)	70 (32.7)	
Equal to expense	28 (48.3)	110 (51.4)	0.913
Less than expense	10 (17.2)	34 (15.9)	
Education status			
Primary school	32 (55.2)	46 (21.5)	
Middle school	22 (37.9)	146 (68.2)	**0.001**
High school and above	4 (6.9)	22 (10.3)	
History of depression			
Yes	36 (62.1)	12 (5.6)	**0.001**
No	22 (37.9)	202 (94.4)	
Smoking			
Yes	12 (20.7)	34 (15.9)	0.387
No	46 (79.3)	180 (84.1)	

EPDS: Edinburgh Postpartum Depression Scale, the values were presented as mean±standard deviation, median (minimum-maximum), or number (percentage)

The EPDS is the most commonly used depression screening tool in perinatal care. Although the optimal cut-off value for screening is unknown, cut-off values of 10 or higher and 13 or higher are most often used to identify women who might have depression, in all periods of pregnancy (21). A cut-off point of 13 for the risk of depression was used in this study.

Antenatal depression is influenced by many factors, most of which are detectable during pregnancy, such as psychological factors. Many studies have reported that risk factors such as a lack of social support, previous history of depression, perceived stress, presence of a mental disorder before pregnancy, low economic and educational status, and physical and sexual violence often cause perinatal depression in pregnancy. Other risk factors include attitude to pregnancy, substance use, parental stress, low self-esteem, low self-efficacy, and social isolation (17,20,22).

A history of depression, discontinuation of treatment in an individual with a history of depression, a history of postpartum depression, and a family history of depression are among the risk factors for antenatal depression in adolescents. In this study, adolescents with a previous history of depression had higher EPDS scores and a higher risk of depression than those who had no such history. A previous history of depression is an important risk factor for antenatal depression; therefore, it is crucial to question adolescent pregnant women to determine if there is a history of depression.

Adolescent pregnancies are more common in individuals with low income and educational levels. While there is an inverse relationship between the level of education and the likelihood of having children at an early age, the risk of depression increases as the level of education decreases. Lancaster et al. (13) identified a low education level as a risk factor for depression symptoms during perinatal screening. In our study, the risk of antenatal depression increased significantly as the education level of adolescent pregnant women decreased, and we found no relationship between income level and depression risk.

While adolescent pregnancies in developed countries typically occur in unmarried young people, and due to inadequate contraception methods, they mostly occur in association with marriage under social pressure, cultural reasons, and traditional family structures in developing countries. In this study, the rate of marriage was found to be 232 (85.3%), and 244 (89.7%) of the adolescent pregnant women stated that they wanted their pregnancy.

The incidence of depressive symptoms during pregnancy varies by trimester, and anxiety and depression are seen more often in the first trimester. Low or no social support during pregnancy, inadequate nutrition and self-care, a negative attitude during antenatal follow-up, fear of childbirth, and concerns about the baby's health all increase the risk of depression during the last trimester of pregnancy. In a study conducted by Miguez et al. (23), the prevalence of depression was 23.4%, 17.0%, and 21.4% in the first, second, and third trimesters of pregnancy, respectively. In this study, the risk of depression was similar among all three trimesters, and no significant difference in depression score was observed according to gestational age.

There were some limitations to our study. Depression is a multifactorial psychiatric disease, i.e., many factors may affect its development. However, we only investigated demographic and psychosocial factors. Also, we did not investigate the relationship between anxiety and depression risk in this study. Another limitation was the small sample size. Strengths of our study included the use of the EPDS, which is widely applied for depression screening, and the collection of data by a physician in a face-to-face interview setting.

Diagnosing depression is difficult in adolescent pregnant women because depressive signs and symptoms of depression are similar to physiological changes and common complaints during pregnancy. Effective routine screening for antenatal depression is essential for the early identification of depressive symptoms in pregnant adolescent women. To recognize antenatal depression, identify adolescents who need psychological support, and provide counseling, effective screening of all adolescent pregnant women should be performed using standard tests with established validity and reliability. Starting from the first pregnancy visit, screening with the EPDS at least once during the second and third trimesters will help identify adolescent pregnant women at risk of depression.

CONCLUSION

A low education level and previous history of depression are closely associated with antenatal depression in adolescent pregnant women. Such women should be closely followed throughout their pregnancy, with psychological support provided when necessary. In this way, chronic depression continuing throughout the postpartum period can be prevented.

Ethics Committee Approval: The study was approved by the Ethics Committee of Zekai Tahir Burak Women's Health Training and Research Hospital (17.07.2018, 36).

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Evaluation of Visceral Adiposity Indexes Associated with Atherogenic Plasma Index in Individuals with Type 2 Diabetes

Tip 2 Diyabetli Bireylerde Aterojenik Plazma İndeksi ile İlişkili Visseral Adipozite İndekslerinin Değerlendirilmesi

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ABSTRACT

Aim: This study was aimed to investigate visceral adiposity indicators and the atherogenic index of plasma (AIP) in type 2 diabetes mellitus (T2DM) patients.
Material and Methods: A total of 353 adults aged between 18 and 74 years were included in this study. Bodyweight, height, waist, and hip circumference were measured; fasting blood glucose, HbA1c, and lipid profile (total cholesterol, triglyceride, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol) values were analyzed. Visceral adiposity index (VAI), lipid accumulation product (LAP), body adiposity index (BAI), body shape index (ABSI), body roundness index (BRI), conicity index (CI), and AIP levels were calculated.
Results: The study included 116 (32.9%) males and 237 (67.1%) females, with a mean age of 57.8±11.5 years. AIP z-scores were found to be directly related to T2DM (OR, 5.03; 95% CI: 1.95-13.01), while VAI z-scores were less associated with T2DM (OR, 1.10; 95% CI: 1.03-1.18). According to the ROC curve analysis, although the area under the curve (AUC) is weak to distinguish diabetic patients with VAI, LAP, and AIP, it is statistically significant ($p<0.001$, AUC: 0.619, cut-off= 5.1, 95% CI: 0.561-0.677; $p=0.007$, AUC: 0.583, cut-off= 63.2, 95% CI: 0.523-0.642; and $p=0.001$, AUC: 0.606, cut-off= 0.4, 95% CI: 0.547-0.665, respectively).
Conclusion: VAI, LAP, and AIP are strong predictors of diabetes, AIP is a better predictor of predicting diabetes than VAI and LAP.
Keywords: Atherogenic plasma index; visceral adiposity index; lipid accumulation product; type 2 diabetes.

ÖZ

Amaç: Bu çalışmanın amacı tip 2 diyabet (T2DM) hastalarında visseral yağlanma göstergelerini ve aterojenik plazma indeksini (atherogenic index of plasma, AIP) araştırmaktır.
Gereç ve Yöntemler: Bu çalışmaya 18 ve 74 yaşları arası toplam 353 yetişkin birey dahil edildi. Vücut ağırlığı, boy uzunluğu, bel ve kalça çevresi ölçüldü; açlık kan şekeri, HbA1c ve lipid profil (total kolesterol, trigliserit, düşük dansiteli lipoprotein kolesterol ve yüksek dansiteli lipoprotein kolesterol) değerleri analiz edildi. Visseral adipozite indeksi (visceral adiposity index, VAI), lipit birikim ürünü (lipid accumulation product, LAP), vücut adipozite indeksi (body adiposity index, BAI), vücut şekli indeksi (body shape index, ABSI), vücut yuvarlaklık indeksi (body roundness index, BRI), koniklik indeksi (conicity index, CI) ve AIP seviyeleri hesaplandı.
Bulgular: Çalışmaya, yaş ortalaması 57,8±11,5 yıl olan 116 (%32,9) erkek ve 237 (%67,1) kadın dahil edildi. AIP z-skorlarının doğrudan T2DM ile ilişkili olduğu (OR, 5,03; %95 GA: 1,95-13,01), VAI z-skorlarının T2DM ile daha az ilişkili olduğu (OR, 1,10; %95 GA: 1,03-1,18) belirlendi. ROC eğrisi analizine göre, eğri altında kalan alan (area under the curve, AUC) VAI, LAP ve AIP ile diyabetik hastaları ayırt etmekte zayıf olsa da istatistiksel olarak anlamlıdır (sırasıyla $p<0,001$; AUC: 0,619; kesme değeri= 5,1; %95 GA: 0,561-0,677; $p=0,007$; AUC: 0,583; kesme değeri= 63,2; %95 GA: 0,523-0,642 ve $p=0,001$; AUC: 0,606; kesme değeri= 0,4; % 95 GA: 0,547-0,665).
Sonuç: VAI, LAP ve AIP diyabetin güçlü prediktörleridir, AIP diyabeti öngörmede VAI ve LAP'den daha iyi bir prediktördür.
Anahtar kelimeler: Aterojenik plazma indeksi; visseral adipozite indeksi; lipid birikim ürünü; tip 2 diyabet.

INTRODUCTION

Diabetes mellitus (DM) is the ninth leading cause of death globally. One out of every eleven adults worldwide is diagnosed with DM, and Type 2 diabetes mellitus (T2DM) accounts for about 90% of cases (1). According to the Turkey Nutrition and Health Survey (TBSA) findings, conducted in 2017, the prevalence of the disease was found to be 9.1% in adults over 19 years old in our country (2).

It has been proven that excess body fat has been linked to an increased risk of cardiometabolic disorders, inflammatory diseases, and metabolic diseases such as DM (3-5). Obesity's harmful effects are caused by adipose tissue distribution in the body, especially central obesity, which is closely linked to T2DM patients' morbidity and mortality (6,7). Assessment of obesity, abdominal obesity, and adiposity as diabetes determinants who people with diabetes are becoming more significant (8). Obesity is assessed using a globally agreed body mass index (BMI), but BMI is inadequate for determining "body fat mass" and "lean body mass", which determine abdominal (central) or visceral obesity (9).

Dual-energy X-ray absorptiometry (DEXA), computed tomography (CT), magnetic resonance imaging (MRI), and dual bioelectrical impedance analysis (BIA) may all be used to reliably calculate adipose tissue. However, due to factors such as the need for specialized medical personnel, time limitations, radiation exposure, and high costs, they are not appropriate for regular clinical use (10,11).

Waist circumference, visceral adiposity index (VAI), body adiposity index (BAI), body shape index (ABSI), body roundness index (BRI), conicity index (CI), and lipid accumulation product (LAP) are all used to identify visceral adiposity (8,12,13). Obesity and insulin resistance are factors that lead to diabetic dyslipidemia and cardiovascular disease risk in people with T2DM, which is characterized by hypertriglyceridemia, low serum "high-density lipoprotein cholesterol (HDL-C)", and high serum "low-density lipoprotein cholesterol (LDL-C)" levels. In recent years, the atherogenic index, a newly developed parameter, has been used to measure plasma atherogenicity in T2DM patients, and it has been proposed that it is related to T2DM (14).

In people with type 2 diabetes, it's crucial to look at obesity, visceral adiposity, and cardiovascular disease risk factors all at once (15). Waist circumference, VAI, BAI, LAP, BRI, ABSI, and CI are all used to estimate the risk of T2DM from cardiometabolic diseases, but it's unclear which index is a better predictor (15-18). The aim of this study was to determine visceral adiposity indicators and the atherogenic plasma index in T2DM patients.

MATERIAL AND METHODS

This study is designed as descriptive, and cross-sectional. Individuals who applied to the Internal Medicine Polyclinic of Erzincan Mengücek Gazi Training and Research Hospital between December 2019 and May 2020 comprise the study's population. By not using any sample selection method in the study, 353 adults who voluntarily agreed to participate with the full count method were included. The study included individuals of ages 18 to 74 with a BMI of 18.5 to 35 kg/m^2 and no other chronic conditions (cardiovascular diseases, polycystic ovary syndrome, thyroid dysfunction, asthma, etc.) other than T2DM, those not on hormone therapy, and those not using lipid-lowering agents; while excluding pregnant and lactating women and those with any malignant and inflammatory diseases or acute infections. Individuals who volunteered to take part in the study were enrolled after reading and signing an informed consent form. Descriptive characteristics, anthropometric measurements, and biochemical parameters were used to collect data for the analysis.

The ethics committee approval dated 03.12.2019 and numbered 12-10 was received by Erzincan Binali Yıldırım University Human Research Ethics Committee for this research. The researchers' information form contains questions about the individuals' socio-demographic characteristics, introductory characteristics, and disease states. With a precision scale (sensitive to ±0.1 kg) that was adjusted at regular intervals, body weight was measured without shoes and in light clothing. Height was measured with a wall-mounted stadiometer with an accuracy of 0.1 cm. BMI was evaluated according to the classification of the World Health Organization (WHO) by calculating kg/m^2 with the body weight / height formula (19). A non-stretch tape measure was used to measure the circumference of the waist (WC), passing across the midpoint between the lower costal border and the iliac crest. The hip circumference was determined with a non-stretch tape measure from the highest point on the hip on the right side of the individual. A doctor assessed the blood pressure in the right arm in a sitting position using an accurate sphygmomanometer after 15 minutes of rest.

Blood samples were collected from individuals in the morning hours following a 12-hour fast to test serum biochemical parameters. The results of biochemical parameters demanded by the patients' physicians (fasting blood glucose (FBG), glycated hemoglobin (HbA1c), total cholesterol, triglyceride (TG), LDL-C, HDL-C) were used. The spectrophotometric method was used to calculate FBG and lipid profile from serum using a Beckman Coulter Olympus AU2700 Plus Chemistry Analyzer (Beckman Coulter, Tokyo, Japan) unit. HbA1c was analyzed by an HPLC analyzer (G8 Tosoh, Japan). Individuals with HbA1c ≥6.5% were diagnosed with T2DM. Values of VAI, LAP, BAI, ABSI, BRI, CI, and atherogenic index of plasma (AIP) were calculated with the formulas given below:

VAI (male) = [WC (cm) / (39.68 + (1.88xBMI)] x (TG/1.03) x (1.31/HDL-C)

VAI (female) = [WC (cm) / (36.58 + (1.89xBMI)] x (TG/0.81) x (1.52/HDL-C)

LAP (male) = [WC (cm)-65] x [TG (mmol/L)]

LAP (female) = [WC (cm)-58] x [TG (mmol/L)]

BAI = [Hip circumference (cm) / Height (m)$^{1.5}$]-18

ABSI = [WC (cm) / (BMI$^{2/3}$ x Height$^{1/2}$)]

BRI = 364.2-365.5 x [1 - (WC/2π)^2 / (0.5 × Height^2)$^{1/2}$]

CI = WC (m) / [0.109 x (√Body weight (kg) / Height (m)]

AIP = Log10 [TG/HDL-C]

Statistical Analysis

In the analysis of data, the IBM SPSS v.22.0 (IBM Corp. Armonk, N.Y., USA) package program was used. The normality of the distribution of numerical variables was evaluated using the Kolmogorov-Smirnov test. Descriptive statistics for continuous variables were shown

as mean, standard deviation, median, minimum-maximum. Mann-Whitney U test or Independent samples t-test was used for continuous variables. When testing the diagnostic value of VAI, LAP, and AIP; the receiver operator characteristics (ROC) curve analysis was used. The association between different obesity measures (VAI, LAP, AIP) and DM was evaluated by logistic regression analysis, followed by the calculation of the odds ratio (OR) with a corresponding 95% confidence interval (95% CI). The model was adjusted for age and gender. Hosmer-Lemeshow goodness-of-fit test was used for evaluating the model fit. Additionally, chi-square test statistics of models and Nagelkerke R^2 were presented. The significance level was accepted as $p<0.05$ for statistical tests.

RESULTS

This research included 116 (32.9%) males and 237 (67.1%) females, with a mean age of 57.8±11.5 years. Table 1 displays the characteristics of individuals based on their diabetes status. VAI (p<0.001), LAP (p=0.007), AIP (p=0.001), systolic blood pressure (SBP, p=0.002), diastolic blood pressure (DBP, p=0.011), FBG (p<0.001), HbA1c (p<0.001), total cholesterol (p=0.002), triglyceride (p=0.001), and LDL-C (p=0.009) values were all higher in people with diabetes than in those without diabetes, while HLD-C (p=0.010) values were lower.

Table 2 was shown the relationship between VAI, LAP, AIP, and DM in adult individuals evaluated using an adjusted logistic regression model. After adjusting for age and gender, AIP z-scores were found to be directly related to DM (OR, 5.03; 95% CI: 1.95-13.01), while VAI z-scores were less associated with DM (OR, 1.10; 95% CI: 1.03-1.18).

Compared to other indices, VAI has the highest area under the curve (AUC) in the ROC analysis (Figure 1). When the ROC curves are examined, the AUC is weak to differentiate patients with VAI, LAP, and AIP diabetes, but it is statistically significant (p<0.001, cut off= 5.1, AUC: 0.619, 95% CI: 0.561-0.677; p=0.007, cut off= 63.2, AUC: 0.583, 95% CI: 0.523-0.642; p=0.001, cut off= 0.4, AUC: 0.606, 95% CI: 0.547-0.665), respectively).

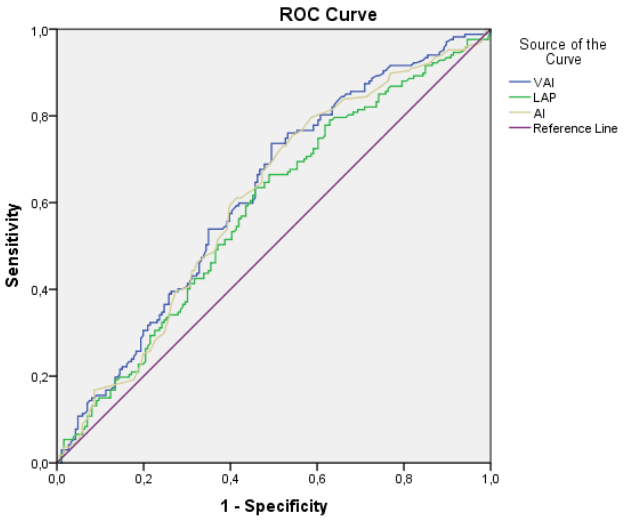

Figure 1. ROC curves for VAI, LAP, and AIP as predictors for diabetes

ROC: receiver operator characteristics, VAI: visceral adiposity index, LAP: lipid accumulation product, AIP: atherogenic index of plasma

Table 1. Characteristics of individuals with and without diabetes

	No (n=186)	Yes (n=167)	p
Age (year)	56.7±11.7	59.1±11.1	0.051
Body mass index (kg/m^2)	30.1±4.5	29.7±4.5	0.556
Waist circumference (cm)	100.7±12.0	101.4±12.3	0.455
Hip circumference (cm)	110.1±10.4	109.9±11.2	0.825
Waist/hip	0.9±0.1	0.9±0.1	0.482
VAI	3.9 (2.6-7.2) [1.4-23.9]	5.8 (3.5-8.1) [1.3-22.1]	**<0.001**
LAP	53.9 (35.3-92.5) [14.2-489.3]	71.5 (44.3-98.5) [14.0-336.0]	**0.007**
Body adiposity index	34.5±6.5	34.7±7.1	0.764
Body shape index	0.8±0.1	0.8±0.1	0.064
Body roundness index	5.8±1.8	6.0±1.9	0.391
Conicity index	1.3±0.1	1.3±0.1	0.082
AIP	0.3 (0.2-0.5) [0.1-1.2]	0.4 (0.3-0.6) [0.0-1.0]	**0.001**
SBP (mmHg)	13 (12-14) [9-16]	14 (12-15) [9-16]	**0.002**
DBP (mmHg)	7 (6-8) [5-10]	8 (6-8) [5-10]	**0.011**
FBG (mg/dL)	113 (98-169) [74-566]	156 (112-219) [74-591]	**<0.001**
HbA1c (%)	6.1 (5.5-7.1) [5.2-11.9]	7.1 (6.3-9.2) [4.8-14.2]	**<0.001**
Total cholesterol (mg/dL)	209.4±50.1	226.9±53.2	**0.002**
Triglyceride (mg/dL)	123 (90-184) [45-487]	158 (109-191) [45-525]	**0.001**
LDL-C (mg/dL)	129.9±39.5	141.1±41.3	**0.009**
HDL-C (mg/dL)	55 (45-59) [28-84]	50 (45-56) [28-94]	**0.010**

VAI: visceral adiposity index, LAP: lipid accumulation product, AIP: atherogenic index of plasma, SBP: systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, descriptive statistics were shown as mean±standard deviation or median (Q1-Q3) [min-max]

Table 2. The relationship of VAI, LAP, and AIP indices with diabetes

	VAI z-score (Model 1)		LAP z-score (Model 2)		AIP z-score (Model 3)	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Model	1.10 (1.03-1.18)	**0.005**	1.01 (1.00-1.09)	0.056	5.03 (1.95-13.01)	**0.001**
Hosmer-Lemeshow	χ^2:10.297 p=0.225		χ^2:3.880 p=0.868		χ^2:7.246 p=0.510	
Model χ^2 and p	χ^2:15.541 p=0.001		χ^2:10.812 p=0.013		χ^2:18.450 p<0.001	
Nagelkerke R^2	0.057		0.040		0.068	

VAI: visceral adiposity index, LAP: lipid accumulation product, AIP: atherogenic index of plasma, OR: odds ratio, CI: confidence interval, model adjusted for age and gender

DISCUSSION

Obesity is a worldwide epidemic and leads to a 20% increased risk of acute myocardial infarction and obesity plays a major role in the development of T2DM. T2DM is a major risk factor for cardiovascular disease (20). Visceral obesity has been proven to be a more critical risk factor for T2DM (21). VAI, and LAP are indicators that express central fat accumulation and have been developed to be independently associated with impaired fasting glucose, type 2 diabetes, and coronary heart disease (22). AIP could predict the size of lipoprotein particles, subsequently showing a positive correlation with the risk of cardiovascular disease. Furthermore, AIP can provide information on the severity of insulin resistance, which is associated with impaired glucose metabolism (23).

When age and gender were included in the model, VAI, LAP, and AIP were found to be statistically significant in identifying patients with diabetes, and AIP was found to be better predictive of T2DM than VAI and LAP.

Concentrations of TG and HDL-C, as well as BMI and WC, are used to calculate the visceral adiposity index and should be an easy tool to assess the risk of metabolic disorders associated with insulin resistance (24). VAI has been shown in research to be a method for distinguishing patients with T2DM (16,25-27). This research supports the previous studies, as well.

Lipid accumulation product is uncomplicated, cheap, and could be a useful index combining WC and TG (28). LAP was found to be effective in distinguishing people with diabetes in a study (26). In research by Tian et al. (18) LAP was found to be linked to diabetes. It was determined in this study that LAP can be used to distinguish patients with diabetes, but that once age and gender are included in the model, it is no longer useful in defining diabetes.

Visceral adiposity index and LAP levels are very efficient in predicting cardiovascular disease risk factors in various clinical studies (20-22,29-31). In people with T2DM and visceral obesity, lipid and lipoprotein metabolism problems are common, resulting in diabetic dyslipidemia and a high risk of cardiovascular disease. The lipid profile of a person with diabetes who is obese and has impaired glycemic control is negatively affected (14). AIP, a new atherogenicity indicator, is easily measured as "the logarithm of the ratio of TG/HDL-K" levels, and it rises as the risk of atherosclerosis rises, outperforming conventional lipid profiles in predicting atherosclerosis and cardiovascular disease. AIP levels grow much faster as obesity, especially abdominal obesity, grows (32,33). While TG and HDL-K levels are appropriate when calculating AIP levels, BMI and WC levels must be used in conjunction with these parameters when calculating VAI levels, and WC and TG must be used when calculating LAP levels (30). When examining the literature, it has been proposed that AIP is a strong predictor of increased cardiovascular risk in people with T2DM (23,34-38).

Improved glycemic control and lifestyle changes in diabetic patients (increasing physical activity, adopting healthier eating patterns, and so on), obesity, visceral adiposity, and lipid control may lower cardiovascular disease risk.

One of the limitations of the study is as a cross-sectional study, and thus a causal association between VAI, LAP, AIP, and diabetes cannot be determined. Second, a small patient population was observed, and data was obtained from a single center. There is a need for research with a large patient group from different centers.

CONCLUSION

Visceral adiposity index, LAP, and AIP are both efficient predictors of diabetes, with AIP outperforming VAI, and LAP in terms of predicting diabetes.

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Relationship between Histopathological Stages of Liver and Albumin-Bilirubin Score in Hepatitis B Infection

Hepatit B Enfeksiyonunda Karaciğer Histopatolojik Evreleri ile Albumin-Bilirubin Skoru Arasındaki İlişki

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ABSTRACT

Aim: In this study, sensitivity and specificity of the albumin-bilirubin (ALBI) score were investigated to detect significant liver fibrosis, and these findings were then compared to fibrosis-4 (FIB-4) and aspartate aminotransferase to platelet ratio index (APRI) scores.

Material and Methods: A total of 69 patients were included in the study. Of these patients, 54 (78.3%) were male and 15 (21.7%) were female. Serology, molecular analysis, biochemical parameters, and pathology results of the patients who underwent a liver biopsy due to a chronic hepatitis B virus (HBV) infection, were retrospectively evaluated. ALBI, APRI, and FIB-4 scores were calculated. To predict the fibrosis stage, F≥2 and F≥4, ALBI, APRI, and FIB-4 scores were investigated using the receiver operator characteristic (ROC) curve analysis.

Results: The area under the ROC curve with 95% confidence interval (CI) for the ALBI, APRI, and FIB-4 scores were 0.613 (95% CI: 0.463-0.762, p=0.160), 0.658 (95% CI: 0.513-0.803, p=0.040), and 0.731 (95% CI: 0.570-0.891, p=0.004), respectively, to predict the F≥2, and 0.758 (95% CI: 0.544-0.971, p=0.090), 0.604 (95% CI:0.451-0.757, p=0.490), and 0.923 (95% CI: 0.856-0.990, p=0.005), respectively, in prediction of F≥4. The sensitivity and specificity rates of the ALBI score were 61.1% and 64.7%, respectively, for the cut-off value of -2.81 in predicting F≥2, and 75.0% and 70.8% for the cut-off value of -2.78 in predicting F≥4.

Conclusion: ALBI scores can be used to detect F≥2 in patients with chronic HBV. However, it is not yet clear whether this approach is superior to other non-invasive methods for detecting F≥4.

Keywords: Hepatitis B; chronic; biopsy; fibrosis.

ÖZ

Amaç: Bu çalışmada albümin-bilirubin (ALBI) skorunun belirgin karaciğer fibrozisini saptamada duyarlılık ve özgüllüğü araştırıldı ve bu bulgular fibrozis-4 (FIB-4) ve aspartat aminotransferaz/trombosit oranı indeksi (aspartate aminotransferase to platelet ratio index, APRI) skorları ile karşılaştırıldı.

Gereç ve Yöntemler: Bu çalışmaya toplam 69 hasta dahil edildi. Hastaların 54'ü (%78,3) erkek ve 15'i (%21,7) kadın idi. Kronik hepatit B virüs (HBV) enfeksiyonu nedeniyle karaciğer biyopsi yapılan hastaların seroloji, moleküler analiz, biyokimyasal parametreler ve patoloji sonuçları retrospektif olarak değerlendirildi. ALBI, APRI ve FIB-4 skorları hesaplandı. Fibrozis evresi F≥2 ve F≥4'ü tahmin etmek için ALBI, APRI ve FIB-4 skorları, alıcı işlem karakteristiği (receiver operator characteristic, ROC) eğrisi analizi kullanılarak araştırıldı.

Bulgular: ALBI, APRI ve FIB-4 skorları için F≥2 tahmininde %95 güven aralığı (GA) ile ROC eğrisi altında kalan alan, sırasıyla, 0,613 (%95 GA: 0,463-0,762; p=0,160), 0,658 (%95 GA: 0,513-0,803; p=0.040) ve 0,731(%95 GA: 0,570-0,891; p=0,004) iken F≥4 tahmininde ise sırasıyla, 0,758 (%95 GA: 0,544-0,971; p=0,090), 0,604 (%95 GA: 0,451-0,757; p=0,490) ve 0,923 (%95 GA: 0,856-0,990; p=0,005) idi. ALBI skoru için duyarlılık ve özgüllük değerleri F≥2 tahmininde -2.81 kesim değeri için sırasıyla, %61,1 ve %64,7 iken F≥4 tahmininde -2.78 kesim değeri için sırasıyla, %75,0 ve %70,8 idi.

Sonuç: Kronik HBV'li hastalarda F≥2'yi belirlemek için ALBI skoru kullanılabilir. Ancak, bu yaklaşımın F≥4'ü saptamak için diğer invaziv olmayan yöntemlerden üstün olup olmadığı henüz açık değildir.

Anahtar kelimeler: Hepatit B; kronik; biyopsi; fibrozis.

INTRODUCTION

Hepatitis B virus (HBV) infection remains a global common health problem, despite the effective vaccine and antiviral treatments that are available (1). It is predicted that approximately 257 million people have been chronically infected with HBV worldwide (2). Eastern Asia, Sub-Saharan Africa, and the Pacific Islands have the highest prevalence rates (3,4). The seroprevalence of the global hepatitis B surface antigen (HBsAg) is predicted to be 3.6% (3).

The estimated number of HBV carriers in Turkey is approximately 3.3 million, and the general prevalence of HBV is 4.57% (5). The prevalence ranges from two percent to three percent in the west of Turkey, whereas it ranges from seven percent to eight percent in the east of Turkey (6).

The risk of developing liver cirrhosis, hepatocellular carcinoma (HCC), and hepatic decompensation is high in chronic HBV (7). Cirrhosis develops in more than 40% of patients with chronic HBV if it is left untreated (7,8). The rate of HCC development within ten years in patients with cirrhosis is 30% (9). Monitoring severe fibrosis and/or inflammation in a hepatitis B infection determines both the treatment and the antiviral treatment strategy (10). The stage (≥2) of liver fibrosis in a chronic HBV infection is the main parameter when deciding whether to initiate treatment (11).

The liver biopsy procedure is accepted as the gold-standard method for diagnosing fibrosis and determining its stage (12). However, an insufficient volume of the biopsy sample may decrease the accuracy of the diagnosis (13,14). As well as being an invasive procedure that may harbor several complications, a liver biopsy may result in an incorrect diagnosis in cases of heterogeneous/dyshomogeneous pathology (15).

As many patients object to repeated biopsies during the follow-up to their disease, and there are serious complication problems, such as bleeding due to the biopsy, non-invasive methods have been developed to detect the stage of liver fibrosis (16,17).

The first method is the aspartate aminotransferase to platelet ratio index (APRI), which was used in 2003 by Wai et al. (18) to detect hepatitis C virus (HCV)-related hepatic fibrosis in patients. The second method is the fibrosis-4 (FIB-4) index, which was developed for chronic HCV/HIV coinfections and then validated for other liver diseases (19,20).

The albumin-bilirubin (ALBI) score, which is the subject of this study, is a new method that was developed to predict the severity of poor liver function, as well as the results of patients with acute liver failure (21). Moreover, the prognostic importance of the ALBI scores in patients with primary biliary cirrhosis was also investigated (22). There is a limited amount of studies that evaluate the ALBI score in predicting fibrosis in chronic HBV infections.

In this study, we analyzed the treatment-naive patients who were diagnosed with chronic HBV infections and underwent a liver biopsy at our hospital. We aimed to determine the diagnostic and threshold values of the ALBI score, which is among the non-invasive biochemical markers, in evaluating liver fibrosis in chronic HBV infections and compare it with other non-invasive markers, such as the APRI and FIB-4 scores.

MATERIAL AND METHODS

Study Design

In this study, the serology, molecular analysis, biochemical parameters, and pathology results of the patients who underwent a liver biopsy due to a chronic HBV infection at Gülhane Training and Research Hospital between October 2016 and September 2019 were retrospectively evaluated. The inclusion and exclusion criteria were as follows: 1) HBsAg and HBV-DNA positivity (≥2000 IU/mL) for more than six months; 2) not having received any antiviral treatment; 3) laboratory analyses allowing ALBI, APRI, and FIB-4 scoring on either the same day as the liver biopsy or the day before; 4) not having any other comorbid diseases that affect the liver; and 5) no immunosuppression. Every patient who met the above criteria was included in the study.

Ethical approval for the study was obtained from the Ethics Committee of Non-Interventional Studies at Health Sciences University, Gülhane Training and Research Hospital (Ethics committee number: 2019/19/339).

Serology and Molecular Testing

The serological and molecular analysis of the serum and plasma samples was performed in our microbiology virology laboratory. HBsAg was qualitatively analyzed using the chemiluminescent enzyme immunoassay (CLIA) technique with the Architect HBsAg Reactive Kits (Abbott, Germany) on the Architect i2000SR system (Abbott, Germany) according to the manufacturer's instructions. To detect HBV-DNA, an isolation device (Magnesia 2448 Anatolia Geneworks, Turkey) and HBV-DNA isolation kit (Viral DNA isolation kit, Anatolia Geneworks, Turkey) were used. The PCR mixture, which was prepared with a Real-Time PCR kit (Bosphore HBV Quantification Kit v2, Turkey), was amplified on a Real-Time PCR device (Montania 4896 Anatolia Geneworks, Turkey).

Non-Invasive Markers of Liver Fibrosis

The demographic and biochemical data of the patients included in the study, such as age, platelet count, serum albumin, total bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels were used. These markers, including the ALBI, APRI, and FIB-4 scores, were calculated based on the following formulas:

ALBI score =

log (bilirubin (mmol/L) x 0.66) - (albumin (g/L) x 0.085).

The ALBI scores one, two, and three were defined as values less than -2.60, between -2.60 and -1.39, and more than -1.39, respectively (21).

APRI score =

AST (normal upper limit) / platelet (10^9/L) x 100 (18).

FIB-4 Index =

age x AST (U/L) / (platelet (10^9/L) x ALT (U/L)$^{1/2}$) (23).

Histopathological Examination

The patients' liver needle biopsy pathology results, which were obtained from the Department of Pathology's archives, were re-evaluated. The chronic hepatitis activity level and fibrosis stage that were detected in the microscopic examination, performed with preparations with histochemical Hematoxylin Eosin and Masson Trichrome stains, and were stated in the pathology report, were recorded as histopathological data. Ishak modified hepatitis activity index (mHAI) grading and staging system was used to grade chronic hepatitis and stage fibrosis. In this scoring system, the activity level range is

between 0-18, and the fibrosis stages are between 0-6. The mHAI (0-18), which represents necroinflammatory activity, includes the piecemeal necrosis score (0-4), confluent necrosis score (0-6), focal lytic necrosis, apoptosis, and focal inflammation score (0-4), and portal inflammation score (0-4). F0-F1 is accepted as the absence of fibrosis or mild fibrosis, F4-F6 is accepted as severe or significant fibrosis, and F5-F6 is accepted as cirrhosis (24). In this study, the pathological fibrosis scores were defined as follows: F≥2=F2-F6 and F≥4=F4-F6.

Statistical Analysis

Data that was obtained in the study was statistically evaluated using the IBM SPSS v.25 (SPSS Inc, Chicago, IL, USA) package software. To examine whether the variables were normally distributed, they were analyzed using visual methods (histogram and probability plots) and the Kolmogorov-Smirnov test. During the statistical evaluation of the data, descriptive statistics, median, interquartile range (IQR), minimum-maximum, number, and percentage were used. The numerical variables were compared with the Mann-Whitney U or Kruskal-Wallis tests, and categorical variables were analyzed using the chi-square test. To predict the F≥2 and F≥4, the ALBI score, APRI, and FIB-4 indices were investigated using the receiver operator characteristic (ROC) curve analysis. Histology of the liver biopsy was accepted as the gold-standard diagnostic method, and the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR), negative likelihood ratio (-LR), and the accuracy rates of the optimal cut-off values in fibrosis prediction of ALBI, APRI, and FIB-4 were calculated according to the standard formulas. The confidence interval (CI) was determined as 95% in all the statistical evaluations. A p value of less than 0.05 was accepted as statistically significant.

RESULTS

A total of 69 patients between the ages of 13 and 68 years were included in the study. Out of 69 patients, 54 (78.3%) were male and 15 (21.7%) were female. There were only two patients under the age of 18: two female patients aged 13 and 15 years. The median age of the patients was 30 (IQR, 21-48; range, 13-68) years, and the male patients were younger than the female patients, with the median age of 23 (IQR, 21-45; range, 16-64) years vs. 45 (IQR, 39-55; range, 13-68) years (p=0.006). Other demographic and clinical data during the liver biopsy, such as gender, age, albumin, AST, ALT, total bilirubin, and platelet counts were shown in Table 1.

Liver biopsy scores of 26.1% (n=18) of the patients were F≥2. The rates of patients with a histopathological activity index (HAI) score of <5, 5-9 and >9 were 53.6% (n=37), 40.6% (n=28), and 5.8% (n=4), respectively.

The area under the ROC curve (AUROC) values of ALBI, APRI, and FIB-4 for the diagnosis of F≥2 and F≥4 were 0.613 (95% CI: 0.463-0.762, p=0.160) and 0.758 (95% CI: 0.544-0.971, p=0.090); 0.658 (95% CI: 0.513-0.803, p=0.040) and 0.604 (95% CI: 0.451-0.757, p=0.490); and 0.731 (95% CI: 0.570-0.891, p=0.004) and 0.923 (95% CI: 0.856-0.990, p=0.005), respectively. The optimal cut-off values of ALBI, APRI, and FIB-4 for the diagnosis of F≥2 and F≥4 were -2.81 and -2.78; 0.35 and 0.37; and 1.11 and 1.18, respectively. The ROC curves of the ALBI, APRI, and FIB-4 performances in predicting F≥2 and F≥4 were shown in Figure 1, and the optimal cut-off values, sensitivity, specificity, PPV and NPV for the cut-off values in predicting F≥2 and F≥4 were shown in Table 2.

The AUROC values of ALBI, APRI, and FIB-4 for the diagnosis of HAI≥5 were 0.513 (95% CI: 0.375-0.651, p=0.857), 0.721 (95% CI: 0.601-0.841, p=0.002), and 0.598 (95% CI: 0.457-0.739, p=0.163), respectively. The optimal cut-off values of ALBI, APRI, and FIB-4 for the diagnosis of HAI≥5 were -2.98, 0.44, and 1.00, respectively. The ROC curves of the ALBI, APRI, and FIB-4 performances in predicting HAI≥5 were shown in Figure 2, and the optimal cut-off values, sensitivity, specificity, PPV, and NPV for the cut-off values in predicting HAI≥5 were shown in Table 3.

The median value of the HBV-DNA viral load in the 69 patients who were included in the study was $1.5x10^4$ IU/ml (IQR, $2.5x10^3$ - $2.8x10^7$, range, 8 - $5x10^9$). The median value of the HBV-DNA viral load was $1.2x10^4$ IU/ml (IQR, $2.5x10^3$ - $4x10^6$, range, $1x10^2$ - $6.9x10^9$) in F<2 and $1.7x10^6$ IU/ml (IQR, $1.5x10^4$ - $2x10^8$, range, 8 - $5x10^9$) in F≥2 (p=0.020).

The median value of the APRI was 0.35 (IQR, 0.24-0.57, range, 0.1-3.1) in F<2, and 0.51 (IQR, 0.34-0.90, range, 0.2-4.6) in F≥2 (p=0.040). The median value of APRI was 0.29 (IQR, 0.22-0.56, range, 0.1-0.8) in HAI<5, 0.49 (IQR, 0.32-0.59, range, 0.2-3.1) in HAI:5-9, and 1.73 (IQR, 0.83-3.97, range, 0.6-4.6) in HAI>9 (p=0.001).

Table 1. Basic characteristics of the patients who underwent liver biopsy due to hepatitis B infection

Fibrosis Staging	F0	F1	F2	F3	F4	F6	Total
Patient number, n (%)	30 (43.5)	21 (30.4)	6 (8.7)	8 (11.6)	3 (4.3)	1 (1.4)	69 (100)
Gender, n (%)							
Male	23 (76.7)	16 (76.2)	5 (83.3)	7 (87.5)	2 (66.7)	1 (100)	54 (78.3)
Female	7 (23.3)	5 (23.8)	1 (16.7)	1 (12.5)	1 (33.3)	0 (0.0)	15 (21.7)
Age (year)	27 (22-44)	28 (20-47)	32.5 (20-57)	34.5 (21-58)	48 (42-56)	53	30 (21-48)
Albumin (g/L)	42 (38-45)	43 (41-44)	41 (37-43)	41 (38-43)	41 (34-43)	37	42 (39-42)
AST (U/L)	26 (22-49)	38 (24-60)	54 (22-213)	48 (32-128)	35 (31-35)	40	35 (23-55)
ALT (U/L)	33 (22-65)	66 (28-105)	145 (21-218)	66 (26-218)	28 (22-37)	46	46 (24-103)
Total bilirubin (µmol/L)	10.3 (6.9-15.5)	10.3 (8.6-16.3)	8.6 (4.6-17.2)	9.5 (5.2-17.6)	15.5 (10.3-18.1)	29.2	10.3 (6.9-15.5)
Platelet count (10^9/L)	228 (201-272)	232 (194-258)	253 (202-294)	229 (186-311)	174 (135-190)	263	229 (197-271)

AST: aspartate aminotransferase, ALT: alanine aminotransferase, descriptive statistics were presented as the median (25^{th}-75^{th} percentile)

Table 2. ROC analysis results of ALBI score, APRI, and FIB-4 index in predicting Ishak fibrosis stage in patients with HBV

	Cut-off	AUROC	95%CI	p	Sn	Sp	PPV	NPV	+LR	-LR	Accuracy
ALBI											
F≥2	-2.81	0.613	0.463-0.762	0.160	61.1	64.7	37.9	82.5	1.73	0.60	63.8
F≥4	-2.78	0.758	0.544-0.971	0.090	75.0	70.8	13.6	97.9	2.57	0.35	71.0
APRI											
F≥2	0.35	0.658	0.513-0.803	**0.040**	77.8	51.0	35.9	86.7	1.59	0.44	58.0
F≥4	0.37	0.604	0.451-0.757	0.490	100	49.2	10.8	100	1.97	0	52.2
FIB-4											
F≥2	1.11	0.731	0.570-0.891	**0.004**	61.1	90.2	68.8	86.8	6.23	0.43	82.6
F≥4	1.18	0.923	0.856-0.990	**0.005**	100	87.7	33.3	100	8.13	0	88.4

ROC: receiver operator characteristics, ALBI: albumin-bilirubin, APRI: aspartate aminotransferase to platelet ratio index, FIB-4: fibrosis-4, HBV: hepatitis B virus, AUROC: area under the ROC curve, CI: confidence interval, Sn: sensitivity, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value; +LR: positive likelihood ratio, -LR: negative likelihood ratio

Table 3. ROC analysis results of ALBI score, APRI, and FIB-4 index in predicting HAI≥5 grade in patients with HBV

	Cut-off	AUROC	95%CI	p	Sn	Sp	PPV	NPV	+LR	-LR	Accuracy
ALBI	-2.98	0.513	0.375-0.651	0.857	65.6	37.8	47.7	56.0	1.06	0.91	50.7
APRI	0.44	0.721	0.601-0.841	**0.002**	62.5	70.3	64.5	68.4	2.10	0.53	66.7
FIB-4	1.00	0.598	0.457-0.739	0.163	46.9	81.1	68.2	63.8	2.48	0.66	65.2

ROC: receiver operator characteristics, ALBI: albumin-bilirubin, APRI: aspartate aminotransferase to platelet ratio index, FIB-4: fibrosis-4, HBV: hepatitis B virus, AUROC: area under the ROC curve, CI: confidence interval, Sn: sensitivity, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value; +LR: positive likelihood ratio, -LR: negative likelihood ratio

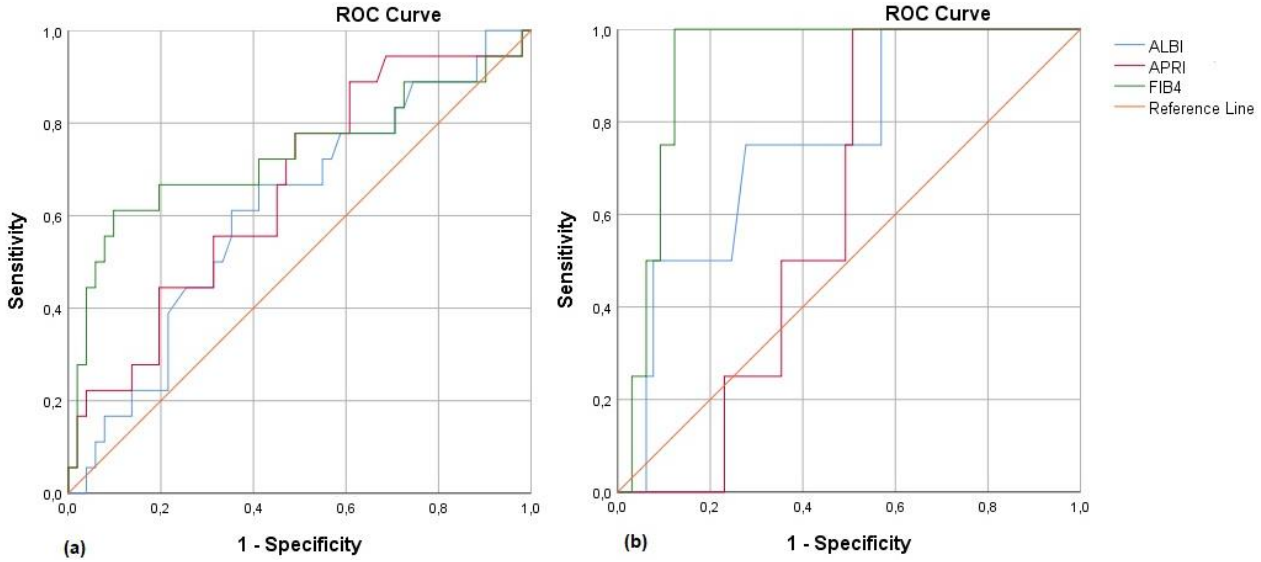

Figure 1. Evaluation of ALBI score, APRI, and FIB-4 index performances in prediction of fibrosis stage **a)** F≥2, **b)** F≥4
ROC: receiver operator characteristics, ALBI: albumin-bilirubin, APRI: aspartate aminotransferase to platelet ratio index, FIB-4: fibrosis-4

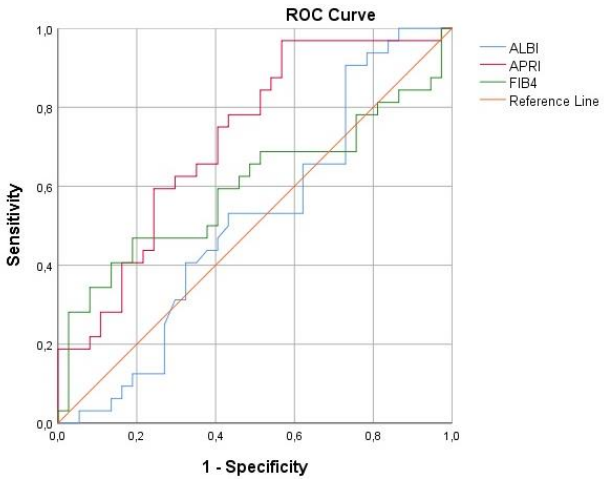

Figure 2. Evaluation of ALBI score, APRI, and FIB-4 index performances in prediction of HAI≥5
ROC: receiver operator characteristics, ALBI: albumin-bilirubin, APRI: aspartate aminotransferase to platelet ratio index, FIB-4: fibrosis-4, HAI: hepatitis activity index

The median value of FIB-4 was 0.65 (IQR, 0.44-0.99, range, 0.0-3.4) in F<2 and 1.18 (IQR, 0.70-1.63, range, 0.2-4.4) in F≥2. The median value of FIB-4 was higher in patients with F≥2, and the difference was statistically significant (p=0.004). The median value of FIB-4 was 0.55 (IQR, 0.44-0.99, range, 0.0-3.4) in HAI<5, 0.80 (IQR, 0.43-1.23, range, 0.2-2.8) in HAI:5-9, and 1.39 (IQR, 0.98-3.71, range, 0.8-4.4) in HAI>9. The FIB-4 index was significantly different in patients with HAI>9 (p=0.040).

The median value of ALBI was -2.91 (IQR, -3.17 to -2.61, range, -3.5 to 0.6) in HAI<5, -2.96 (IQR, -3.10 to -2.71, range, -3.3 to 0.5) in HAI:5-9, and -2.66 (IQR, -2.83 to -2.33, range, -2.9 to -2.2) in HAI>9. No significant difference was found in terms of the ALBI median values among the HAI groups (p=0.210). The median value of ALBI was -2.93 (IQR, -3.10 to -2.70, range, -3.5 to 0.6) in F<2 and -2.77 (IQR, -3.0 to -2.63, range, -3.3 to 0.5) in F≥2 (p=0.160).

DISCUSSION

Detecting the stages of liver fibrosis is important for determining the prognosis of chronic liver disease, choosing the specific treatment for the patient, and to follow-up on the success of the treatment (25). For the last 50 years, liver biopsy as an invasive method has been accepted as the gold standard in liver fibrosis staging (26). Non-invasive alternative methods have been investigated as an alternative to invasive procedures in detecting liver fibrosis (27). Serum tests, proteomic profiles/genetic tests, and imaging techniques are invasive methods that are used in the assessment of fibrosis. The advantages of these techniques are that they are less invasive, there is a low risk of sampling fault and relatively high inter-observer variability/variation, and the measurements can be repeated. Therefore, they allow fibrosis to be continuously followed-up (28).

In this study, ALBI, APRI, and FIB-4 scores had similar diagnostic values for the patients with F≥2 of chronic HBV. However, we found that FIB-4 was better for detection in patients with severe fibrosis or cirrhosis. APRI had a higher diagnostic value for detection in patients with HAI≥5 compared to ALBI and FIB-4.

The first non-invasive method is the APRI score, which was identified in patients with chronic hepatitis C by Wai et al. (18). It is calculated using the AST value and platelet count. It is known that AST levels increase and platelet counts decrease in advanced liver fibrosis. Although platelet production reduces due to the reduced thrombopoietin production by hepatocytes, the platelets are sequestrated in the spleen due to the liver fibrosis progression and the developing portal hypertension. Although the release of AST from mitochondrion increases with liver injury, it reduces fibrosis clearance (30). In this study, the median platelet count was determined as $229x10^9$/L, whereas it was detected as $253x10^9$/L for F2, $229x10^9$/L for F3, and $174x10^9$/L for F4. The platelet count for F4 was below the mean platelet count. The median value of the serum AST levels was recorded as 35 U/L. Although the AST levels for F2 and F3 were 54 and 48 U/L, respectively, the level for F4 was recorded as 35 U/L. This was lower than the others but had the same median value. This was due to the low number of patients with advanced fibrosis, as there were 14 patients for F2 and F3, but only three patients for F4 in the study.

APRI score can be used for diagnostic purposes for both significant fibrosis and cirrhosis. In our study, there was a significant difference in terms of the APRI score according to the presence of fibrosis. The AUROC values in patients with chronic HBV were 0.658 and 0.604 in F≥2 and F≥4, respectively. The cut-off value with the highest rates of sensitivity and specificity was 0.35 for F≥2 (sensitivity, 77.8%; specificity, 51.0%) and 0.37 for F≥4 (sensitivity, 100%; specificity, 49.2%). The AUROC value of the APRI scores in this study was lower than the value, 0.80, that was found by Wai et al. (18). In other studies on patients with chronic HBV, the AUROC values ranged from 0.639 to 0.878 (31-33). Although our results were consistent with the studies in the literature, the low AUROC value that we found may be associated with the low number of patients that were included in the study, as well as the patient group, which consisted of young patients without significant fibrosis. In this study, although the sensitivity of the APRI score increased in advanced stages of fibrosis, the specificity was low, which reveals that APRI cannot replace the gold-standard method of a liver biopsy in assessing the presence and stage of liver fibrosis.

FIB-4 is another non-invasive marker. It is formulated with age, ALT and AST levels, and the platelet count. Like APRI, the FIB-4 values differ due to the increased AST/ALT ratio and reduced platelet count, which is caused by liver fibrosis. In our study, the AUROC value was 0.731 for F≥2 and 0.923 for F≥4. A statistically significant difference was found in the FIB-4 score according to the presence of fibrosis. The cut-off value with the highest rates of sensitivity and specificity was 1.11 for F≥2 (sensitivity, 61.1%; specificity, 90.2%) and 1.18 for F≥4 (sensitivity, 100%; specificity, 87.7%). Our results were similar to the results of other studies in the literature. In the other studies, the AUROC value of FIB-4 was ranged from 0.646 to 0.812 for F≥2, and from 0.715 to 0.818 for F≥4 (31-34). In our study, the FIB-4 score was more successful than the other non-invasive methods, APRI and ALBI, in terms of detecting the presence of significant fibrosis in patients with chronic HBV.

ALBI score was first developed to predict the prognosis of patients with cirrhosis with or without liver cancer (16). Therefore, it is a new model that uses serum albumin and bilirubin to assess the severity of liver function (35). It has a more significant performance compared to the Child-Pugh and MELD scores when predicting the long-term survival rate for patients with HBV-related cirrhosis (33). The number of studies that show a correlation between fibrosis and ALBI in patients with chronic HBV is quite low. In one study, which included 217 patients with chronic HBV, the AUROC value of the ALBI score was 0.698 for F≥2, and 0.843 for F≥4. The sensitivity and specificity rates in the cut-off value of >-2.7 were 42.6% and 91% for F≥2, and 70.45% and 86.13% for F≥4, respectively (36). In another study, which included 91 patients with chronic HBV, although the AUROC value of the ALBI score for fibrosis prediction was 0.849, the sensitivity and specificity rates in the cut-off value of -2.19 in distinguishing cirrhotic and non-cirrhotic cases were 85.7% and 74%, respectively (16). In this study, the AUROC values for F≥2 and F≥4 were 0.613 and 0.758, respectively. Although sensitivity was 61.1% and specificity was 64.7% for F≥2 in the cut-off value of -2.81, sensitivity was 75.0% and specificity was 72.3% for F≥4 in the cut-off value of -2.78. The results were similar to the results of other studies in the literature (16,36). Our study is important as it is one of the rare studies where the ALBI score results are assessed to predict fibrosis in patients with chronic HBV. However, this study has several limitations. The major limitation is that the number of patients is not high enough to obtain general data; either the data or the studies should be multicenter, in addition to including a high number of patients, in order to represent measured the general population. The second limitation is that the ALBI scores could not be dynamically. For this reason, a relationship between the dynamic scores and the transition between the liver function and fibrosis stages and ALBI scores could not be established.

CONCLUSION

In conclusion, it was found that the ALBI score was a prediction index that can be subjectively evaluated, easily reached, and calculated using a non-invasive blood test. Additionally, the ALBI score can be used to detect the absence or presence of fibrosis, especially in patients with chronic HBV. However, although non-invasive methods, such as the ALBI score, cannot replace a liver biopsy, multicenter cohort studies with a high number of samples and studies where more reliable results can be obtained using standardization are needed.

Ethics Committee Approval: The study was approved by the Ethics Committee of Gülhane Training and Research Hospital (22.10.2019, 19/339).

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Evaluation of Preoperative Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio for their Predictive Value in Determining Short-Term Mortality in Patients with Operable Colorectal Cancers

Opere Edilebilir Kolorektal Kanserli Hastalarda Kısa Dönem Mortalitenin Belirlenmesinde Preoperatif Nötrofil-Lenfosit Oranı ve Trombosit-Lenfosit Oranının Öngörü Değerlerinin Değerlendirilmesi

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ABSTRACT

Aim: The aim of this study was to investigate whether preoperative neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have a predictive value in short-term mortality in patients with operable colorectal cancer (CRC).
Material and Methods: A total of 231 (93 female, 138 male) patients with operated CRC between 2016 and 2021 in a university hospital were analyzed retrospectively. Median age was 68 (range, 26-92) years and patients had been under follow-up for a median of 25 (range, 0-54) months. Patients were grouped with respect to survival, those who were alive (n=175) and those who died (n=56) during the follow-up.
Results: The area under the curve for NLR was 0.649 (95% CI: 0.563-0.734, p=0.001), optimal cut-off was 5.08 and demonstrated a sensitivity of 48.2% and a specificity of 81.7% for predicting mortality. The area under the curve for PLR was 0.635 (95% CI: 0.546-0.723, p=0.002), optimal cut-off was 221.5 and demonstrated a sensitivity of 55.4% and a specificity of 72.0%. Multiple regression analysis revealed that recurrence (OR: 60.910, 95% CI: 9.807-378.319, p=<0.001), leakage (OR: 10.724, 95% CI: 1.281-89.747, p=0.029), high NLR (OR: 3.735, 95% CI: 1.602-8.711, p=0.002) and higher age (OR: 1.136, 95% CI: 1.081-1.193, p<0.001) were independently associated with mortality.
Conclusion: The results of this study support studies indicating that preoperative NLR and PLR are effective in predicting short-term mortality in CRC patients who underwent surgical resection. Although further studies are necessary, these biomarkers are promising for future use as prognostic tools in CRC patients.
Keywords: Colorectal cancer; mortality; neutrophil lymphocyte ratio; platelet lymphocyte ratio.

ÖZ

Amaç: Bu çalışmanın amacı, opere edilebilir kolorektal kanserli (KRK) hastalarda ameliyat öncesi nötrofil-lenfosit oranı (NLO) ve trombosit-lenfosit oranının (PLO) kısa dönem mortalitede prediktif bir değeri olup olmadığının araştırılmasıdır.
Gereç ve Yöntemler: 2016 ve 2021 yılları arasında bir üniversite hastanesinde KRK ameliyatı yapılmış olan toplam 231 (93 kadın, 138 erkek) hasta geriye dönük olarak incelendi. Ortanca yaş 68 (aralık, 26-92) yıl ve takip süresi ortancası 25 (aralık, 0-54) ay idi. Hastalar sağ kalım durumuna göre, sağ kalanlar (n=175) ve takipler sırasında ölenler (n=56) olmak üzere gruplandırıldı.
Bulgular: NLO için eğri altında kalan alan 0,649 (%95 GA: 0,563-0,734; p=0,001), optimal kesim noktası 5,08 idi ve bu kesim noktası mortaliteyi tahmin etmek için %48,2 duyarlılık ve %81,7 özgüllük gösterdi. PLO için eğri altında kalan alan 0,635 (%95 GA: 0,546-0,723; p=0,002), optimal kesim noktası 221,5 idi ve bu kesim noktası %55,4 duyarlılık ve %72,0 özgüllük gösterdi. Çoklu regresyon analizi, nüks (OR: 60,910; 95% GA: 9,807-378,319; p=<0,001), sızıntı (OR: 10,724; 95% GA: 1,281-89,747; p=0,029), yüksek NLO (OR: 3,735; 95% GA: 1,602-8,711; p=0,002) ve yaşın artışının (OR: 1,136; 95% GA: 1,081-1,193; p<0,001) bağımsız olarak mortalite ile ilişkili olduğunu ortaya koydu.
Sonuç: Bu çalışmanın sonuçları, cerrahi rezeksiyon uygulanan KRK hastalarında preoperatif NLO ve PLO'nun kısa dönem mortaliteyi öngörmede etkili olduğunu gösteren çalışmaları desteklemektedir. Bu konuda daha fazla çalışma gerekli olmasına rağmen, bu biyobelirteçler, KRK hastalarında prognostik araçlar olarak gelecekte kullanım açısından umut vericidir.
Anahtar kelimeler: Kolorektal kanser; mortalite; nötrofil lenfosit oranı; trombosit lenfosit oranı.

INTRODUCTION

Colorectal cancer (CRC) is the third most frequently diagnosed and the second most deadly malignancy in the world for both sexes (1). Even though mortality rates have decreased with advances in diagnosis and treatment, the clinical outcomes of CRCs remain largely unpredictable (2).

The TNM and Dukes classifications, which are used to predict the clinical course of CRCs and treatment decisions, have considerable limitations (3,4). Patients with the same clinical and pathological stage at diagnosis may show different prognoses, possibly due to differences in tumor biology (2). Although various parameters, including those evaluating the systemic inflammatory response, were found to have prognostic value independent of TNM classification in various diseases, data is limited (5,6). For these reasons, there is an ongoing need to identify optimal biomarkers that may be useful in predicting relapse, prognosis, and patients that can benefit from different treatment strategies (7).

The relationship between inflammation and cancer has been better defined and understood in recent years (8). Neutrophils and platelets, which are the main cells contributing to inflammatory response, have been established to promote tumor growth through the effects of cytokines and chemokines, while lymphocytes show antitumor activity (7,9). In addition, it has been shown that cancer-related inflammation affects different stages of cancer development and progression, such as proliferation, metastasis, etc. (8,10,11). Supporting evidence for the contributing role of chronic inflammation in CRC development has also been shown by the identification of chronic inflammatory bowel disease as a risk factor for CRC (12,13).

The effect of some inflammatory biomarkers calculated from simple hemogram parameters on the prognosis of various cancers, especially CRC, is an interesting topic. In addition to the increasing need for prognostic biomarkers, there is a high value in identifying markers that are easy to obtain, common, and inexpensive (14). Although there are various studies exploring the predictive value of inflammatory markers such as neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) in the prognosis of CRC, some points on this subject are still controversial.

The aim of this study was to evaluate whether preoperatively measured NLR and PLR have predictive value for the assessment of short-term mortality in patients operated for CRC.

MATERIAL AND METHODS

Patients

The medical records of 231 patients who underwent surgical treatment (curative resection) for Stage I-IV colon cancer between 2016 and 2021 at the General Surgery Department of Osmangazi University Medical Faculty Hospital, Eskisehir, Turkey were retrospectively reviewed. The definitive diagnosis of the patients was confirmed by routine pathology, and the study included patients with CRC of all stages. The exclusion criteria of the study were as follows: patients with synchronous and metachronous cancer, patients undergoing emergency surgery, those receiving neoadjuvant therapy, patients with cirrhosis, those with autoimmune diseases, those with hematological malignancies, individuals who received systemic corticosteroids in the last 6 months, subjects with active infection, and patients with incomplete clinicopathological data. This study was approved by the Non-Interventional Clinical Research Ethics Committee of Eskisehir Osmangazi University (Date: 01.06.2021, No: 05).

Procedure and Follow-up

The TNM classification of malignant tumors (8^{th} edition), described by the Union for International Cancer Control (UICC), was used to determine the TNM stage (15). Surgeries were performed according to the TNM stage of each patient with a laparoscopic or open approach. After the surgery, patients were routinely examined at 3-month intervals for the first 2 years, and every 6 months thereafter. The median follow-up of patients included in the study was 25 (range, 0-54) months. During the follow-up period, 56 patients died and 175 patients were alive.

Groups and Variables

Two groups were formed from the survivors and the deceased. Clinical data including age, gender, clinicopathological features (histopathology, primary tumor site, staging, type of surgery, extent of resection, postoperative complications, metastasis location), and preoperative laboratory data were obtained from the medical records of the patients. Neutrophil, lymphocyte, monocyte, and thrombocyte counts were measured by routine blood tests. The NLR value was determined by dividing the absolute neutrophil count by the absolute lymphocyte count; PLR was determined by dividing the absolute platelet count by the absolute lymphocyte count. In general, patients were admitted to the hospital two days before the operation and all laboratory values, including NLR and PLR, were obtained on the day of hospitalization.

Statistical Analysis

All analyses were performed on SPSS v.25 (SPSS Inc., Chicago, IL, USA) with a significance threshold set as a two-tailed p value of <0.05. The Kolmogorov-Smirnov test was used to determine whether continuous variables were normally distributed. Continuous data are given as mean±standard deviation (SD) or median (interquartile range, IQR) [min-max] according to the normality of distribution, and as frequency (percentage) for categorical variables. Between-group analyses were performed with the independent samples t-test or the Mann Whitney U test depending on the normality of distribution. Categorical variables were analyzed with the Pearson chi-square test or the Fisher's exact test. Receiver operating characteristics (ROC) curve analysis was performed to evaluate the predictive performance of variables in terms of mortality. Multiple logistic regression analysis (forward conditional method) was performed for the identification of variables that were significantly associated with mortality.

RESULTS

Among the patients included in the study, 138 (59.7%) were males, and the median (IQR) [minimum-maximum] age was 68 (16) [26-92] years. Median follow-up duration was 25 (18) [0-54] months. During the follow-up period 56 patients died, 175 patients were alive. Demographic, clinical, pathological and laboratory data of the patients were given in Table 1. In the deceased group, significantly

Table 1. Summary of patients and tumor characteristics and laboratory measurements with regard to mortality

	Total (n=231)	Exitus (n=56)	Alive (n=175)	p
Age (years)	68 (16) [26-92]	75 (11.5) [37-92]	65 (16) [26-87]	**<0.001**
Gender				
Female	93 (40.26%)	25 (44.64%)	68 (38.86%)	0.442
Male	138 (59.74%)	31 (55.36%)	107 (61.14%)	
Location				
Right colon	92 (39.83%)	24 (42.86%)	68 (38.86%)	
Transverse colon	29 (12.55%)	12 (21.43%)	17 (9.71%)	
Descending colon	24 (10.39%)	5 (8.93%)	19 (10.86%)	0.107
Sigmoid colon&rectosigmoid region	27 (11.69%)	6 (10.71%)	21 (12.00%)	
Rectum	59 (25.54%)	9 (16.07%)	50 (28.57%)	
Pathological diagnosis				
Non-mucinous adenocarcinoma	162 (70.13%)	38 (67.86%)	124 (70.86%)	0.795
Mucinous adenocarcinoma	69 (29.87%)	18 (32.14%)	51 (29.14%)	
Tumor size (mm)	43 (30) [1-130]	45 (27.5) [6-130]	40 (30) [1-122]	0.281
Number of lymph nodes	23 (19) [3-66]	20 (15) [4-66]	26 (19) [3-66]	**0.003**
Number of metastatic lymph nodes	0 (2) [0-25]	1 (2) [0-12]	0 (2) [0-25]	**0.013**
Differentiation				
Poor	29 (12.55%)	8 (14.29%)	21 (12.00%)	
Moderate	170 (73.59%)	42 (75.00%)	128 (73.14%)	0.697
Well	32 (13.85%)	6 (10.71%)	26 (14.86%)	
Radial surgical margin positivity	2 (0.87%)	2 (3.57%)	0 (0.00%)	0.058
Distal surgical margin positivity	2 (0.87%)	1 (1.79%)	1 (0.57%)	0.427
Perineural invasion	65 (28.14%)	20 (35.71%)	45 (25.71%)	0.201
Lymphovascular invasion	96 (41.56%)	23 (41.07%)	73 (41.71%)	1.000
T stage				
T1	9 (3.90%)	0 (0.00%)	9 (5.14%)	
T2	31 (13.42%)	1 (1.79%)	30 (17.14%)	**<0.001**
T3	142 (61.47%)	33 (58.93%)	109 (62.29%)	
T4	49 (21.21%)	22 (39.29%)	27 (15.43%)	
N stage				
N0	134 (58.01%)	24 (42.86%)	110 (62.86%)	
N1	66 (28.57%)	22 (39.29%)	44 (25.14%)	**0.031**
N2	31 (13.42%)	10 (17.86%)	21 (12.00%)	
Stage				
Stage 1	30 (12.99%)	1 (1.79%)	29 (16.57%)	
Stage 2	90 (38.96%)	21 (37.50%)	69 (39.43%)	**0.009**
Stage 3	81 (35.06%)	22 (39.29%)	59 (33.71%)	
Stage 4	30 (12.99%)	12 (21.43%)	18 (10.29%)	
Liver metastasis	26 (11.26%)	10 (17.86%)	16 (9.14%)	0.120
Type of surgery				
Laparoscopy	41 (17.75%)	3 (5.36%)	38 (21.71%)	**0.010**
Open surgery	190 (82.25%)	53 (94.64%)	137 (78.29%)	
Operation				
Right hemicolectomy	81 (35.06%)	19 (33.93%)	62 (35.43%)	
Transverse hemicolectomy	17 (7.36%)	5 (8.93%)	12 (6.86%)	
Left hemicolectomy	33 (14.29%)	11 (19.64%)	22 (12.57%)	
Anterior resection	34 (14.72%)	8 (14.29%)	26 (14.86%)	0.620
Low anterior resection	56 (24.24%)	10 (17.86%)	46 (26.29%)	
Abdominoperineal resection	8 (3.46%)	3 (5.36%)	5 (2.86%)	
Other	2 (0.87%)	0 (0.00%)	2 (1.14%)	
Ostomy	62 (26.84%)	21 (37.50%)	41 (23.43%)	0.058
Hemoglobin (g/dl)	12.08±2.20	10.95±1.95	12.44±2.16	**<0.001**
Hematocrit (%)	37.30±5.78	34.30±5.19	38.26±5.65	**<0.001**
White blood cell ($x10^3$)	7.69 (3.55) [3.12-23.89]	8.42 (4.16) [3.60-19.71]	7.50 (3.46) [3.12-23.89]	0.062
Neutrophil ($x10^3$)	5.10 (3.32) [1.77-20.64]	6.16 (4.12) [2.10-17.39]	4.90 (2.76) [1.77-20.64]	0.057
Lymphocyte ($x10^3$)	1.50 (1) [0.18-5.70]	1.21 (0.71) [0.47-4.30]	1.60 (0.92) [0.18-5.70]	**0.001**
Platelet ($x10^3$)	284 (142) [75-781]	314 (182.5) [75-636]	276 (121) [109-781]	0.123
Mean platelet volume (fl)	9.42±1.17	9.13±1.11	9.51±1.18	**0.033**
Neutrophil / lymphocyte ratio	3.09 (2.84) [1.05-27.94]	4.58 (4.69) [1.34-21.33]	3.00 (2.04) [1.05-27.94]	**0.001**
Platelet / lymphocyte ratio	177.50 (135.92) [29.41-824.53]	230.44 (198.39) [29.41-824.00]	171.88 (119.51) [54.62-824.53]	**0.002**
Length of stay in hospital (day)	6 (3) [3-40]	7 (4) [3-40]	6 (3) [3-21]	**0.032**
Follow-up time (months)	25 (18) [0-54]	11 (18.5) [0-37]	28 (18) [7-54]	**<0.001**
Leakage	10 (4.33%)	7 (12.50%)	3 (1.71%)	**0.002**
Infection	36 (15.58%)	11 (19.64%)	25 (14.29%)	0.453
Recurrence	11 (5.26%)	8 (17.78%)	3 (1.83%)	**<0.001**
Early (≤30 days) mortality	8 (3.46%)	8 (14.29%)	-	N/A

Data were given as mean±standard deviation or median (interquartile range) [minimum-maximum] for continuous variables, and as frequency (percentage) for categorical variables

higher values were determined for age (p<0.001), number of metastatic lymph nodes (p=0.013), frequency of T4 (p<0.001) and N1 disease (p=0.031), frequency of stage 4 disease (p=0.009), open surgery (p=0.010), length of hospital stay (p=0.032), leakage (p=0.002), recurrence (p<0.001), NLR (p=0.001) and PLR (p=0.002) compared to survivors. In the survivors, significantly higher values were found for regional lymph node count (p=0.003), frequency of T2 (p<0.001) and N0 disease (p=0.031), frequency of stage 1 disease (p=0.009), laparoscopic surgery (p=0.010), hemoglobin (p<0.001), hematocrit (p<0.001) and lymphocyte counts (p=0.001), mean platelet volume (MPV, p=0.033) and follow-up times (p<0.001) compared to the deceased group.

ROC analysis was used to identify optimal cut-off values for NLR and PLR in predicting mortality. The area under the curve (AUC) for NLR was 0.649 (95% CI: 0.563-0.734) and the optimal cut-off value was 5.08, showing a sensitivity value of 48.2% and a specificity value of 81.7%. The AUC for PLR was 0.635 (95% CI: 0.546-0.723) and the optimal cut-off was 221.5, showing a sensitivity of 55.4% and a specificity of 72.0% (Table 2, Figure 1).

We performed multiple logistic regression analysis to determine the risk factors of mortality. Patients with high NLR (≥5.08) were found to have a 3.735-fold higher risk of death than those with lower values (OR: 3.735, 95% CI: 1.602-8.711, p=0.002). Patients with leakage had a 10.724-fold higher risk of death than those without (OR: 10.724, 95% CI: 1.281-89.747, p=0.029). Patients with recurrence had a 60.910-fold higher risk of death than those without (OR: 60.910, 95% CI: 9.807-378.319, p<0.001). In addition, we found higher age (p<0.001) was associated with an increased risk of death (Table 3). Other variables included in the model, gender (p=0.059), number of metastatic lymph nodes (p=0.236), T stage (p=0.126), N stage (p=0.296), tumor stage (p=0.282), type of surgery (p=0.172), hemoglobin (p=0.194), MPV (p=0.133) and PLR (p=0.143) were found to be non-significant.

Table 2. Performance of NLR and PLR to predict mortality

	NLR	PLR
Cut-off	≥5.08	≥221.5
Sensitivity	48.21%	55.36%
Specificity	81.71%	72.00%
Accuracy	73.59%	67.97%
PPV	45.76%	38.75%
NPV	83.14%	83.44%
AUC (95.0% CI)	0.649 (0.563-0.734)	0.635 (0.546-0.723)
p	**0.001**	**0.002**

NLR: neutrophil to lymphocyte ratio, PLR: platelet to lymphocyte ratio, PPV: positive predictive value, NPV: negative predictive value, AUC: area under ROC curve, CI: confidence interval

Table 3. Significant risk factors of the mortality, multiple logistic regression analysis

	β	SE	p	OR	95% CI
Age	0.127	0.025	**<0.001**	1.136	1.081-1.193
NLR (≥5.08)	1.318	0.432	**0.002**	3.735	1.602-8.711
Leakage	2.373	1.084	**0.029**	10.724	1.281-89.747
Recurrence	4.109	0.932	**<0.001**	60.910	9.807-378.319

SE: standard error, OR: odds ratio, CI: confidence interval, NLR: neutrophil to lymphocyte ratio, Nagelkerke R^2=0.444; correct prediction=85.17%, p<0.001

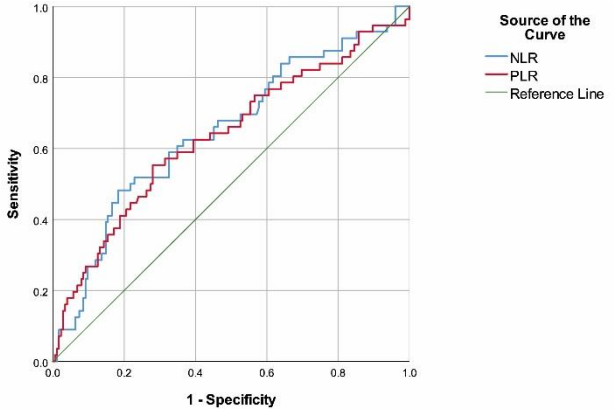

Figure 1. ROC curve of NLR and PLR to predict mortality
ROC: receiver operating characteristics, NLR: neutrophil/lymphocyte ratio, PLR: platelet/lymphocyte ratio

DISCUSSION

Studies assessing prognostic factors in CRC have gained interest in recent years due to the importance of prognosis prediction in individualized treatment planning in cancers which often demonstrate heterogeneous characteristics. For this purpose, we investigated preoperative NLR and PLR values in predicting short-term mortality in patients with resectable CRC and found that both NLR and PLR may be somewhat beneficial in this regard. In addition, we found that the factors independently associated with short-term CRC mortality were recurrence, leakage, NLR, and age.

The relationship of inflammatory markers obtained from hemogram parameters with the prognosis of various diseases has been a topic of interest for the last two decades. Numerous studies have been conducted on the prognostic value of both NLR and PLR in CRC patients, as they are common, inexpensive, and readily available (14).

NLR is one of the most investigated inflammation-related biomarkers in CRC, as the systemic inflammatory response associated with cancer is usually associated with increased circulating neutrophil counts (7). In the study by Xia et al. (9), NLR was found to be a valuable factor in predicting postoperative complications in early-stage (T1-T2) rectal cancers. In addition, a preoperative NLR greater than 2.8 was found to be an independent prognostic factor for poor disease-free survival. In our study, we included patients with CRC at all stages and the mortality-predicting cut-off for NLR was found to be 5.08, and having an NLR value exceeding this threshold was independently associated with mortality. In the study of Inamato et al. (16) in which a group of inflammatory markers was evaluated (including NLR) in CRC patients, the authors found NLR to be associated with overall survival and disease-free survival. The NLR cut-off value in this study was found to be 2.05; however, it has been established by various studies that cut-off values for NLR vary in a wide range from 2 to 5. The relatively high cut-off value in our study may be due to the low number of patients and heterogeneity of our groups, but it is also possible that the inclusion of patients with any CRC stage was associated with this finding (7,17). On the other hand, in our study, evaluations such as overall survival or disease-free survival were not performed because we did not have sufficient data.

In their study evaluating only stage III CRC patients, Yasui et al. (18) found that postoperative inflammation-based prognostic markers (including NLR) accurately predicted

overall survival and relapse-free survival; whereas, preoperative values had relatively lower value in this context. Interestingly, the mentioned study had a different design than many studies in the literature. The groups were classified according to elevated or normal inflammatory status at preoperative and postoperative assessment. For all markers evaluated, the group with normal inflammatory parameters had a significantly better prognosis than those with consistently elevated values. In our study, similar to many studies in the literature, the groups were evaluated only with preoperative NLR.

In addition to these studies, numerous reviews and meta-analyses have shown that various inflammatory biomarkers, including NLR, can be utilized as inexpensive biomarkers that can be used to predict the prognosis of patients with CRC at various stages, and may be useful in identifying high-risk patients who may benefit from adjuvant treatments (7,17,19-22). However, it has been reported that multicenter prospective studies are required to find the optimal cut-off values, as the characteristics of the enrolled patients and the cut-off values for each marker vary greatly from study to study (23). The cut-off value of NLR in our study was also consistent with this diversity in the literature. In relation, the literature shows that the prognostic value of NLR in patients with CRC is limited and inconsistent (16,23,24). In the review of Rossi et al. (23), it was stated that NLR can only be used as a prognostic marker in unresectable metastatic CRCs, and the authors emphasized the inconsistency of data in early-stage resectable CRC and resectable metastatic CRC. In addition, almost all studies focusing on the prognostic role of inflammatory markers have been reported to suffer from various forms of bias due to many problems, including their retrospective nature, heterogeneity of patient characteristics, and the lack of a common platform of exclusion criteria, mostly regarding comorbidities that may affect blood cell count (23).

Since platelets considerably contribute to inflammation, platelet-derived biomarkers have also been studied extensively for their role in CRC (7,9). PLR is one of these biomarkers. In the literature, studies exploring the relationships between CRC prognosis and blood count indices have mostly evaluated PLR together with NLR (7). Similar to NLR, PLR seems to be somewhat associated with the prognosis of CRC in many studies; however, a number of studies have claimed that it is not effective for prognostic evaluations (7,17,23-27). A study by Xia et al. (9) demonstrated that PLR was associated with both postoperative complications and poor overall survival and disease-free survival in patients with early-stage rectal cancer. In the study by Inamato et al. (16), which included all CRC stages and identified a PLR threshold value of 195, it was determined that PLR was not associated with overall survival or disease-free survival. The PLR cut-off in our study was 221.5, which was consistent with the PLR values varying in a wide range between 140-300 in the literature (7,17). In the review of Rossi et al. (23), it was stated that data were insufficient to support a prognostic role for PLR in CRC. In our study, it was found that PLR had a predictive value in predicting short-term mortality of CRC, consistent with the majority of literature.

The present study also identified recurrence, leakage, preoperative NLR, and age were independently associated with short-term mortality in patients who had undergone surgery for CRC. Leakage is one of the most serious early complications of CRC surgery and there are many publications in the literature showing that it increases mortality and morbidity in the short term, although its effects on long-term mortality are not clear (28-31). On the other hand, recurrence remains one of the most important clinical problems of curative CRC resection and there are publications in the literature that it is an indicator of poor prognosis (31-34). Finally, although few studies have shown that age does not affect prognosis, there are more studies reporting increased mortality rates in CRC patients over 75 years of age (32,33,35-37). Our findings in our study showing that recurrence, leakage, and age have predictive effects on mortality are consistent with the literature. Since determining prognosis gains importance in the planning of CRC treatment, research continues on both established prognostic factors and new prognostic factors in line with new biological, genetic and molecular information (4).

The most important limitation of our study is that it is a retrospective study. Other limitations of our study are that it is a single-center study, the limited number of patients, and heterogeneous patient characteristics due to the inclusion of patients at all CRC stages. In addition, due to insufficient data and a short follow-up period, survival assessments such as overall survival, disease-free survival, and relapse-free survival could not be performed. There are still unclear points in the literature regarding the utility of these inexpensive and common biomarkers that can be beneficial in predicting the prognosis of CRC patients, such as optimal cut-off values and validation. For this purpose, there is a need for prospective studies that include better categorized, homogeneous, and larger patient groups.

CONCLUSION

The results of our study support that preoperative NLR and PLR are effective in predicting short-term mortality in patients with operable CRC. Although the results were found to be significant, it can be said that the sensitivity and specificity values are relatively low. Therefore, more studies are needed to use these parameters in clinical practice. In addition, age, high NLR (≥5.08), leakage, and recurrence were determined as independent risk factors associated with early mortality in patients who underwent surgery for CRC. NLR and PLR are promising for future use as prognostic biomarkers in CRC patients, given that larger studies confirm the validity of these findings.

Ethics Committee Approval: The study was approved by the Non-Interventional Clinical Research Ethics Committee of Eskişehir Osmangazi University (01.06.2021, 05).

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Lower Levels of Vitamin B12 Among Patients with Viral Warts Compared with Control Subjects: A Retrospective Study

Viral Siğili Olan Hastalarda Kontrol Grubuna Kıyasla Daha Düşük Vitamin B12 Seviyeleri: Retrospektif Bir Çalışma

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ABSTRACT

Aim: Viral wart is a common benign infectious dermatosis. It remains unclear why not everyone exposed to HPV develops warts. It is known that vitamin and mineral deficiencies can affect impair natural and acquired immunity and lead to a tendency to get more infections. This study aimed to determine the potential utility of evaluating vitamin B12, folic acid, and ferritin levels in patients with viral warts.

Material and Methods: In this retrospective study, 70 patients who were diagnosed with viral warts in the dermatology outpatient clinic of Karabük University Training and Research Hospital between January 2018 and December 2019 were included. 70 healthy individuals matched with viral wart patients in terms of gender and age were included as the control group. Serum vitamin B12, folic acid, and ferritin levels of both groups were detected and compared from the hospital automation system.

Results: Vitamin B12 level was detected statistically significantly lower in the viral wart group as compared to the control group (p=0.046). Although serum ferritin levels were found lower in the viral wart group, this difference was not statistically significant (p=0.677). No statistically significant difference was detected between the viral wart and control groups in terms of folate levels (p=0.879).

Conclusion: In patients with viral warts with low serum vitamin B12 levels, replacement therapy could be considered. Also, the evaluation of serum ferritin level, an iron storage parameter in the body, is important in patients with viral warts infection.

Keywords: Wart; vitamin B12; folic acid; ferritin; viral warts.

ÖZ

Amaç: Viral siğil, yaygın olarak görülen benign enfeksiyöz bir dermatozdur. HPV'ye maruz kalan herkesin neden siğil geliştirmediği belirsizliğini korumaktadır. Vitamin ve mineral eksikliklerinin doğal ve kazanılmış bağışıklığı zayıflatabileceği ve daha fazla enfeksiyon kapma eğilimine yol açabileceği bilinmektedir. Bu çalışma, viral siğili olan hastalarda vitamin B12, folik asit ve ferritin düzeylerinin değerlendirilmesinin potansiyel faydasını değerlendirmeyi amaçlamıştır.

Gereç ve Yöntemler: Bu geriye dönük çalışmaya, Ocak 2018 ve Aralık 2019 tarihleri arasında Karabük Üniversitesi Eğitim ve Araştırma Hastanesi dermatoloji polikliniğinde viral siğil tanısı almış olan 70 hasta dahil edildi. Kontrol grubu olarak cinsiyet ve yaş açısından viral siğil hastaları ile uyumlu olan 70 sağlıklı birey dahil edildi. Hastane otomasyon sisteminden her iki grubun serum vitamin B12, folik asit ve ferritin düzeyleri tespit edildi ve karşılaştırıldı.

Bulgular: Serum vitamin B12 düzeyi, viral siğili olan hasta grubunda kontrol grubuna göre istatistiksel olarak anlamlı derecede daha düşük tespit edildi (p=0,046). Serum ferritin düzeyleri viral siğil grubunda daha düşük bulunmakla birlikte, bu fark istatistiksel olarak anlamlı değildi (p=0,677). Folat seviyeleri açısından, viral siğil ve kontrol grupları arasında istatistiksel olarak anlamlı bir farklılık tespit edilmemiştir (p=0,879).

Sonuç: Serum vitamin B12 düzeyi düşük viral siğili olan hastalarda vitamin B12 replasman tedavisi düşünülebilir. Ayrıca viral siğil enfeksiyonu olan hastalarda vücutta demir depolama parametresi olan serum ferritin düzeyinin değerlendirilmesi önemlidir.

Anahtar kelimeler: Siğil; B12 vitamini; folik asit; ferritin; viral siğil.

INTRODUCTION

Viral wart is benign infectious dermatosis manifested mainly by hyperkeratotic papules on the skin and mucous membranes. The causative human papilloma virus (HPV) often enters the body through areas in the skin where the epidermis is damaged and forms viral wart lesions. Complete recovery is expected in months or years based on the age and immune status of the patient, subtype of the causative HPV, and the region where the lesion develops (1). Viral warts are mostly cosmetic disorders, but they can sometimes be painful or cause secondary bacterial infection. Viral warts can also negatively impact the quality of life of patients (2) The treatment of warts generally includes local destructive methods and, local or systemic immunotherapy (3).

Vitamin B12 plays an important role in lipid, carbohydrate, and protein metabolism in the body (4). Inadequate intake or malabsorption of vitamin B12 could lead to vitamin B12 deficiency. This deficiency can cause multi-organic and systemic dysfunctions, especially in hematological, neurological, and neuropsychiatric systems (5). Changes in cellular metabolism affect the functions of cells involved in immunity (6). Vitamin B12 facilitates the production of T-cells, such as cytotoxic T-cells (7). In the case of vitamin B12 deficiency, B cell diversity and natural killer (NK) cell activity decrease, and antibody response is also impaired (7). Vitamin B12 has immunomodulatory effects, especially on cellular immunity. Vitamin B12 replacement decreases the CD+4/CD+8 ratio, and increases NK cell activity, in cases with vitamin B12 deficiency (8).

Iron is a mineral that has many biological functions in the body, such as oxygen transport to cells, destruction of pathogens, regulation of cytokine production and action, and IFNγ production (7,9,10). Iron is important in the differentiation and proliferation of T cells and helps to regulate the ratio of T helper cells and cytotoxic T cells (7). Iron homeostasis is tightly regulated in healthy individuals, as iron is required in the immune response of T-lymphocytes and antimicrobial mechanisms of macrophages (11,12). Serum ferritin level is an accessible and important parameter to evaluate the body's iron storage in the absence of systemic infection and inflammation (10).

Folate (vitamin B9) is involved in DNA synthesis and methylation reactions (13,14). Folate maintains or increases the cytotoxic activity of the NK cell, supports Th 1-mediated immune responses, and provides adequate antibody response to antigens (7). Cell culture studies have shown that folate deficiency favors a decrease in T-lymphocyte proliferation, an increase in CD4+ cells, a decrease in CD8+ cells, and consequently, a greater tendency to develop infections (15).

Antimicrobial substances, phagocytes, and NK cells in the serum constitute the body's natural immunity (7). Vitamins and trace elements in the body contribute to natural and acquired immunity; thus, vitamin and mineral deficiencies lead to weakness in immunity and a greater tendency to be infected (16). There is limited information in the literature about the relationship between viral, bacterial, and parasitic diseases of the skin and vitamin and mineral deficiencies.

In this study, we aimed to examine vitamin B12, folate, and ferritin levels in patients with viral warts.

MATERIAL AND METHODS

This retrospective study was conducted between January 2018 and December 2019 in the department of dermatology at a tertiary care hospital in Karabük. The study was performed in accordance with the principles of the Declaration of Helsinki and approved by our Institutional Ethical Committee (2021/609). A total of 70 patients with viral warts were enrolled in this study. All types of viral warts including common warts, verruca plana, genital warts, and plantar warts were included in the study. Cases that have a pregnancy, breastfeeding, malignancy, active infection, metabolic and endocrine disorders, liver disease, and also receive any replacement or drug therapy for vitamin B12, folic acid, and iron were excluded from the study. The control group consisted of 70 people that applied to the hospital for non-wart reasons, had similar age and gender characteristics with the patient group, and met the exclusion criteria. The age and gender of patients and control group were noted. The serum vitamin B12, folic acid, and ferritin levels of all patients were obtained from the hospital automation system. The min-max values of the laboratory parameters were considered normal for vitamin B12 (211-911 pg/mL), folic acid (5-17 ng/mL), and ferritin (22-322 ng/mL), respectively. Results below the minimum values for all parameters were considered deficient.

Statistical Analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS) version 21 (SPSS software, Chicago, IL, USA), and p values less than 0.05 were considered statistically significant. The normality distributions of numerical variables were evaluated with the Kolmogorov-Smirnov test. In the comparison of paired groups, the Independent samples t-test was used for normally distributed variables while the Mann-Whitney U test was used for variables that were not normally distributed. Gender distribution was analyzed with the Pearson chi-square test. Normally distributed data were presented as mean±standard deviation while non-normally distributed data were presented as median (interquartile range) [minimum-maximum], and categorical data were presented with number and percentage.

RESULTS

The study group included 70 patients (52 females, 18 males), while the control group had 70 patients (46 females, 24 males). There was no significant difference in terms of gender between the groups (p=0.268). The mean age of the study group was 28.61±11.73 (range, 14-69) years, while the mean age of the control group was 30.02±11.23 (range, 15-56) years. There was no significant difference across the groups (p=0.370). The baseline demographics of the groups were shown in Table 1.

Serum vitamin B12 level was significantly lower in the viral wart group compared to the control group (302.54±91.81 vs 331.49±91.49, p=0.046). While serum ferritin level was found to be lower in the viral wart group than in the control group (median 17 vs 20), the difference was not statistically significant (p=0.677). Also, there was no significant difference was found between folic acid levels of the wart group and the control group (p=0.879). Serum vitamin B12, folic acid, and ferritin levels of patient and control groups were given in Table 2.

Table 1. Baseline demographics of the participants

	Wart Group	Control Group	p
Age, mean±SD (min-max)	28.61±11.73 (14-69)	30.02±11.23 (15-56)	0.370
Gender, n (%)			
Male	18 (25.7%)	24 (34.3%)	0.268
Female	52 (74.3%)	46 (65.7%)	

Table 2. Serum vitamin B12, folic acid, and ferritin levels of the wart group and control group

	Wart Group	Control group	p
Vitamin B12	302.54±91.81	331.49±91.49	**0.046**
Ferritin	17 (25) [3-170]	20 (32.8) [4-200]	0.677
Folic acid	7.5 (3) [5-16]	7 (3) [4-19]	0.879

Descriptive statistics were presented as mean±standard deviation or median (interquartile range) [minimum-maximum]

DISCUSSION

The skin and mucous membranes are the first lines of defense, covering the outer and inner surfaces of the body against viruses, bacteria, and all pathogens. These physical barriers need maintenance for their integrity and optimal functioning. Micronutrients, including vitamin B12, iron, and folate, have vital roles in ensuring this maintenance (7).

In our study, serum vitamin B12 level was found to be significantly lower in the viral wart group. In their study including all wart types, Tamer et al. (17) found lower vitamin B12 levels in the patient group compared to healthy individuals, similar to the results of our study. Demir et al. (18) examined vitamin B12, ferritin, and some blood parameters in patients with genital warts. In their study, they did not find a relationship between genital warts and vitamin B12, but they found high levels of ferritin in patients with genital warts. The differences between our study and Demir et al. (18) study may be due to the wart types, age and gender distribution of the patients included in the study.

Another study again from Turkey investigated the presence of HPV in the cervical samples of women by a polymerase chain reaction and demonstrated that vitamin B12 and folate deficiencies were significantly higher in HPV (+) cases than in HPV (-) cases (19). In a Brazilian cohort study, it was shown that dietary intake of vitamin B12 is associated with a reduced risk of non-oncogenic HPV persistence in males (20). Similarly, in a study conducted on 201 female patients with persistent or intermittent cervical HPV infection, an inverse correlation was found between serum vitamin B12 values and HPV persistence (21). There is one more study suggesting lower vitamin B12 levels may be associated with high-grade cervical lesions that could be HPV-related (22). Although the viral subtypes are different in common warts and genital warts, all findings from these studies suggest that there may be an association between lower vitamin B12 levels and HPV infections or HPV virulence.

In this study, we found lower serum ferritin levels in the viral wart group, though not significant. Lactoferrin contributes to the body's natural immunity and chelates iron and is widely found in body fluids and specific neutrophil granules of polymorphonuclear leukocytes (23). It can both prevent the entry of the virus into the cell and possess antiviral effects in the cell by being localized in the nuclear region of the cell. Antiviral effects of lactoferrin have been demonstrated against many viruses, including human papillomavirus (24). Therefore, the evaluation of serum ferritin level, an iron storage parameter in the body, is important in patients with viral warts infection.

Folate levels were not different between the groups in the presented study. Tamer et al. (17) also found similar folate values in the viral wart and control group in their retrospective case-control study. However, vitamin B12 deficiency leads to a metabolic block in the use of folate at the cellular level. Therefore the evaluation of folate levels in individuals with vitamin B12 deficiency may not be accurate. In the literature, it is recommended to reevaluate serum metabolites such as methylmalonic acid, homocysteine, and folate levels after vitamin B12 replacement therapy in individuals with vitamin B12 deficiency. Since serum homocysteine level increases in serum folate and vitamin B12 deficiencies, it has been reported that if the serum homocysteine level does not decrease after vitamin B12 replacement therapy, folate deficiency and replacement should be considered as well (25).

If the serum vitamin B12 level is <200 μg/mL, it is referred to as vitamin B12 deficiency. However, levels between 200-400 μg/mL are borderline, and reevaluation with methylmalonic acid and homocysteine levels is required to confirm the diagnosis (5). The methylmalonic acid level is considered more specific in the evaluation of vitamin B12 deficiency due to the high homocysteine level in the case of folate deficiency (26). When vitamin B12 deficiency is suspected from laboratory examination, further evaluation may be required along with the routine laboratory levels of the patients. Therefore, we think that if the vitamin B12 levels of patients with viral warts are at the borderline limit, they should be re-evaluated with these additional tests and the result should be confirmed.

This study has several limitations worth noting, firstly the retrospective design. It would be better if we could evaluate the response in viral warts after replacement in patients with low vitamin B12. Moreover, since serum folic acid level is affected by low vitamin B12 levels it would be logical to use rechecked folic acid levels after vitamin B12 replacement.

There are few studies currently available addressing the link between vitamin B12 levels and viral warts. In this study, we found significantly lower vitamin B12 levels in patients with viral warts compared to controls. Further studies are needed to confirm our results, and also to reveal the effect of vitamin B12 replacement in wart patients with low vitamin B12 levels.

CONCLUSION

Serum vitamin B12 levels should be checked in patients with viral warts, and replacement therapy could be considered in patients with low levels. Also, it is important to evaluate the serum ferritin level, an iron storage parameter in the body, in patients with viral warts infection.

Ethics Committee Approval: The study was approved by the Non-Interventional Clinical Research Ethics Committee of Karabük University (06.08.2021, 609).

Conflict of Interest: None declared by the authors.

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The Effects of Cryopreserved Human Amniotic Membrane and Platelet-Rich Plasma on Seroma Development after Mastectomy and Axillary Dissection in Rats

Sıçanlarda Mastektomi ve Aksiller Diseksiyon Sonrası Seroma Gelişimi Üzerine Kriyoprezerve İnsan Amniyotik Membran ve Trombositten Zengin Plazmanın Etkileri

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ABSTRACT

Aim: Seroma is one of the most common complications after mastectomy and axillary dissection. It occurs as a result of prolongation of the exudative-inflammatory phase of wound healing. The aim of this study is to evaluate the effects of human amniotic membrane (HAM) and platelet-rich plasma (PRP) on seroma formation.
Material and Methods: A total of 24 rats were grouped as control, PRP, and HAM groups. All rats underwent radical mastectomy and axillary lymph node dissection. Saline in the control group, PRP in the second group, and HAM in the third group were applied to the dissection area. The groups were compared in terms of the condition of the surgical field, seroma volume, and histopathological changes.
Results: Seroma volume decreased in the PRP group, but not in the HAM group. Lymphocyte, eosinophil, histiocyte, and fibroblast levels were significantly lower both in the PRP and HAM groups compared to the control group. E-cadherin and TGF-β1 immunoreactivities of PRP and HAM groups were higher than in the control group.
Conclusion: In this study, it was observed that PRP and cryopreserved HAM increased tissue healing and decreased the inflammatory process. However, although local PRP application significantly reduced seroma formation, it was determined that HAM application did not reduce seroma formation. It was thought that this might be due to the fact that the smooth surface of HAM mechanically prevents the adhesion of the tissues. More beneficial results will be obtained with the modification to be made in the preparation and application of HAM.
Keywords: Axillary dissection; human amniotic membrane; mastectomy; platelet-rich plasma; seroma.

ÖZ

Amaç: Seroma, mastektomi ve aksiller lenf nodu diseksiyonu sonrası en sık görülen komplikasyonlardan bir tanesidir. Yara iyileşmesinin eksüdatif ve inflamatuar fazının uzamasının bir sonucu olarak ortaya çıkar. Bu çalışmanın amacı, insan amniyotik membranı (human amniotic membrane, HAM) ve trombositten zengin plazma (platelet-rich plasma, PRP)'nın seroma oluşumu üzerindeki etkilerini değerlendirmektir.
Gereç ve Yöntemler: Toplam 24 adet sıçan kontrol, PRP ve HAM grubu olarak gruplara ayrıldı. Tüm sıçanlara radikal mastektomi ve aksiller lenf nodu diseksiyonu yapıldı. Diseksiyon bölgesine kontrol grubunda salin, ikinci grupta PRP ve üçüncü grupta ise HAM uygulandı. Tüm gruplar cerrahi alanın durumu, seroma hacmi ve histopatolojik değişiklikler açısından karşılaştırıldı.
Bulgular: PRP grubunda seroma hacminde azalma görüldü, ancak HAM grubunda azalma gözlenmedi. Hem PRP hem de HAM grubunda lenfosit, eozinofil, histiyosit ve fibroblast seviyeleri kontrol grubuna göre anlamlı derecede daha düşük olduğu tespit edildi. PRP ve HAM gruplarının E-cadherin ve TGF-β1 immünreaktivitelerinin de kontrol grubuna göre daha yüksek olduğu tespit edildi.
Sonuç: Bu çalışmada PRP ve kriyoprezerve HAM'ın doku iyileşmesini arttırdığı ve inflamatuar süreci azalttığı gözlendi. Ancak lokal PRP uygulanması seroma oluşumunu önemli ölçüde azaltsa da lokal HAM uygulamasının seroma oluşumunu azaltmadığı belirlendi. Bunun HAM'ın pürüzsüz yüzeyinin mekanik olarak dokuların yapışmasını engellemesinden kaynaklanabileceği düşünüldü. HAM'ın hazırlanmasında ve uygulanmasında yapılacak olan modifikasyon ile daha faydalı sonuçlar elde edilecektir.
Anahtar kelimeler: Aksiller diseksiyon; insan amniyotik membran; mastektomi; trombositten zengin plazma; seroma.

INTRODUCTION

Breast cancer is the most common malignancy among women worldwide and a leading cause of cancer-related death (1). Since the first radical mastectomy (RM) by William Halsted in 1894, surgical techniques have improved significantly, providing patients with exceptional survival rates. Seroma is one of the most common complications after breast cancer surgery (2). Its incidence varies between 10% to 52% (3). It usually regresses within a few weeks. However, in some patient groups, this process may take several months (2-4). In addition to RM and modified radical mastectomy (MRM) surgical procedures, performing axillary lymph node dissection (ALND) increases the likelihood of seroma formation. It has also been reported in more minor surgical techniques such as sentinel lymph node biopsy (SLNB), breast-conserving surgery, and subcutaneous mastectomy/nipple-sparing surgery (5).

The RM experimental animal model is a suitable model to evaluate the acute response and wound healing after the surgical procedure (6). During the mastectomy and ALND procedure, a large surgical area is formed, resulting in damage to many blood vessels and lymphatic structures. As a result of these damages, seroma is formed accompanied by blood and lymphatic leakage. It is stated that this fluid is an exudate containing cellular components of acute inflammation (7). In the study by McCaul et al. (3), it was reported that the fluid accumulated after breast cancer surgery was caused by the prolongation of the exudative-inflammatory phase of wound healing. In a study by Watt-Boolsen et al. (7), it was reported that seroma may be an indicator of prolongation of the first phase of wound healing.

Human amniotic membrane (HAM) is a medical biomaterial that has been used for a long time in corneal reconstruction, ocular surface injuries, skin burns, tissue fillings, and neural tube defect operations (8,9). It has been reported that HAM is valuable in clinical applications due to its beneficial functional properties that can facilitate biological activities without causing ethical debates regarding the use of human tissue (10). HAM has non-tumorigenic, low immunogenicity, antibacterial, antiviral and anti-inflammatory effects. It also secretes various active factors such as transforming growth factor-1β (TGF-1β), epidermal growth factor, stromal cell-derived factor, vascular endothelial growth factor, and polymorphic collagen (11).

Platelet-rich plasma (PRP), which is a plasma portion 3-5 times richer than normal plasma levels in terms of platelets, at high concentrations; growth factors such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), insulin-like growth factor (IGF), TGF-1β and fibrinogen, fibronectin, osteonectin, osteocalcin, vitronectin, thrombospondin certain proteins and peptides such as (12). For this reason, it has been used in many studies in terms of wound healing.

Tissue healing is a complex process involving many factors such as cytokines and growth factors (11). Exogenous cytokines are used in clinics to promote healing. These products usually use some type of growth factor, thus contributing to one stage of wound healing. However, it is known that more than one factor combination may be more effective in wound healing.

It is known that HAM and PRP contribute to wound healing by many factors and many pathways. Therefore, it is predicted that it may have positive effects on the healing of the flap created during mastectomy and the seroma, which is considered as a complication. The aim of this study is to examine the effects of local application of HAM and PRP as a biological material on seroma and wound healing after mastectomy and ALND.

MATERIAL AND METHODS

Animals

This experimental study was approved by Sivas Cumhuriyet University Animal Experiments Local Ethics Committee on 14.11.2020 with the number 367. All institutional and national guidelines for the care and use of laboratory animals were followed.

A total of 24 rats were divided into three groups; Control group (n=8), PRP application group (n=8), and HAM application group (n=8). In addition, 4 rats were used to obtain PRP.

Supply and Preparation of Cryopreserved Human Amniotic Membrane (HAM)

Placenta was obtained after elective cesarean section (38 weeks) from the patient who had negative HBV, HCV, HIV, and syphilis tests and had no history of premature rupture of membranes, endometritis, or meconium ileus. The placenta was transferred to the laboratory at +4°C in a container under sterile conditions. The placenta with adherent fetal membranes was washed with Phosphate Buffer Saline (PBS) containing 50 µg/ml penicillin, 50 µg/ml streptomycin and 2.5 µg/ml amphotericin B. The amnion was separated from the chorion by blunt and sharp dissection under sterile conditions in the processing area. HAM was washed several times with PBS. It was then laid on a nitrocellulose membrane (Whatman, Schleicher, and Schuell optitaran BA-S 85) with the epithelial surface facing up. Cryo tubes in which Dulbecco's Modified Eagle's Medium (DMEM) solution and glycerol solution were prepared in equal proportions were prepared. Prepared nitrocellulose papers were washed for the last time with PBS, cut into required sizes, and placed in cryo tubes. The tubes were placed in a -80°C cabinet. It was removed 24 hours before the procedure and kept at room temperature. It was removed from the tube during the operation and placed in physiological saline. The HAM was separated from the nitrocellulose paper with the help of forceps in SF and made ready for use in the appropriate size for the dissection area.

Platelet Rich Plasma (PRP) Supply and Preparation

Venous blood was collected from donor rats (n=4) prepared to obtain PRP, placed in tubes containing 3.2% sodium citrate, and the supernatant was obtained by centrifugation at 400 G for 10 minutes. The collected supernatant was re-centrifuged at 800 G for 10 minutes. After centrifugation, the upper two-thirds of plasma was discarded, and the lower third was laboratory-confirmed as PRP. The obtained PRP was used in the study without waiting.

Study Design and Surgical Procedure

The experimental study was carried out in the Experimental Animals Laboratory of the same center with the approval of the Animal Experiments Local Ethics

Committee of Sivas Cumhuriyet University, Faculty of Medicine, Experimental Medicine Research and Application Center. Before and after the experiment, the subjects were housed in detached cages of equal size and fed with standard feed and tap water. Rats were kept in a sound-insulated room with a 12-hour light/dark cycle at a room temperature of 22±1°C and in a 55±6% humidity environment and fed at an appropriate rate. The experiment was carried out between 9:00 and 16:00, and the light and sound levels of the experimental environment were kept under constant control. Rats were fasted at least 6 hours before the procedure. Anesthesia was administered with Xylazine HCL 3 mg/kg and Ketamine 90 mg/kg intraperitoneally. A total of 24 rats were randomly divided into 3 groups of 8 each. ***Control Group:*** After RM and ALND, primary closure was performed by washing with 1 cc saline. ***PRP Group:*** Before primary closure after RM and ALND, 0.5 ml of PRP prepared locally under the flap and in the axillary space was applied and the skin was closed by primary suturing. ***HAM Group:*** After RM and ALND, the HAM prepared under the flap was laid as a single layer before primary closure and the skin was closed with a primary suture (Figure 1). The mean dissection area was 3 cm^2. Rats were not treated with antibiotics. Experimental animals were kept in the laboratory for 7 days after (13) surgery and were followed during the observation period to record the presence of infection, seroma, or abscess. On the 7^{th} postoperative day after anesthesia, the seroma formed in the wounds of the patients was aspirated with injectors and the amount of seroma obtained was recorded as milliliters. Wound sites were evaluated in terms of wound infection and wound healing amount. In the evaluation of wound infection, it was investigated whether there was hyperemia, necrosis, temperature increase, and discharge at the wound site. After the evaluation of the subjects was completed, the subjects were sacrificed with 200 mg/kg pentothal. Tissue samples were taken for pathological examination.

Histopathological Method

Necropsies of the rats were made and the tissues taken were fixed in a 10% neutral formalin solution. Tissues were taken into paraffin blocks after routine alcohol-xylol follow-up procedures. Sections of 4 µm taken from the tissues were stained with hematoxylin-eosin. The studies of Calisir et al. (14) were analyzed according to the modification of histopathological scoring (Table 1).

Immunohistochemical Study

4 µm sections taken on slides containing polylysine were passed through the xylol and alcohol series, after washing with PBS, they were kept in 3% H_2O_2 for 10 minutes to inactivate endogenous peroxidase. In order to reveal the antigen in the tissues, they were treated with antigen retrieval solution for 2x5 minutes at 500 watts. It was then incubated with primary antibodies of E-cadherin (Santa Cruz, Cat. No. sc-8426) and TGFβ1 (Santa Cruz, Cat. No. sc-130348) (dilution 1/200) at room temperature for 45 min. left for incubation. Secondarily; Large Volume Detection System: anti-Polyvalent, HRP (Thermofischer, Catalog no: TP-125-HL) was applied as recommended by the manufacturer. DAB (3,3′-Diaminobenzidine) was used as chromogen. After counterstaining with Mayer's Hematoxylin, it was covered with entellan and examined under a light microscope. In the examination, it was evaluated as no immunoreactivity (0), mild (1), moderate (2), severe (3), and very severe (4).

Statistical Analysis

All of the data obtained as a result of the experiments were converted into numerical values. SPSS program (SPSS v.20.0 for windows) was used for statistical analysis. All experimental results were expressed as median, interquartile range, and minimum-maximum. In the histopathological examination, the difference between the groups of the data obtained semi-quantitatively was determined by the Kruskal-Wallis H test, and the determination of the groups forming the difference was determined by the Mann-Whitney U test with Bonferroni correction ($p \cong 0.017$). Statistical significance was defined at the $p<0.05$ level.

RESULTS

Subjects were followed up for 7 days for complications after RM and ALND. At the end of the seventh day, all subjects were reevaluated and all groups were sacrificed. The macroscopic appearance of the surgical field and seroma volume were recorded and histopathological samples were taken from the tissues.

Macroscopic Assessment

The rats were followed throughout the experiment and the final evaluation was made on the 7^{th} day. Wound infection was detected macroscopically in 2 subjects in the control

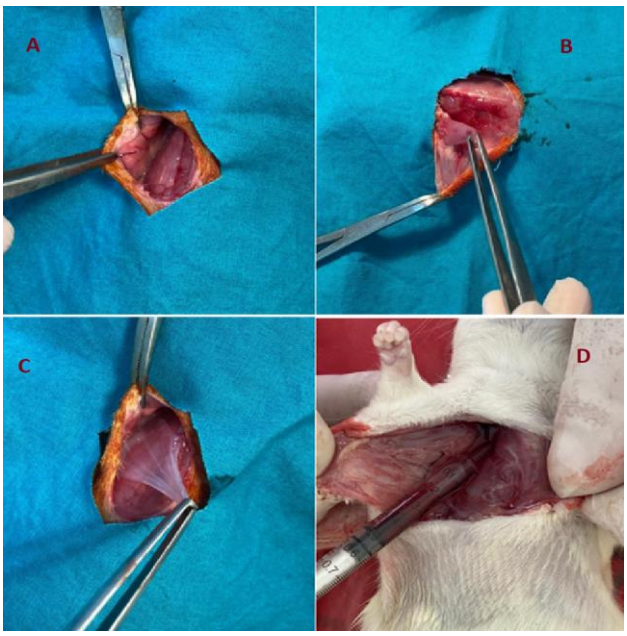

Figure 1. A) tissue dissection in mastectomy; **B)** pectoralis major muscle dissection for radical mastectomy; **C)** applying human amniotic membrane to the dissection area; **D)** seroma measurement 1 week after radical mastectomy

Table 1. Histological scoring criteria

Pathological State	Score	Definition
Lymphocytes, Eosinophils, Histiocytes, Fibroblasts	0	Intact
	1	A small amount and scattered
	2	A small amount and all areas
	3	There are a lot and scattered
	4	There are a lot and all area

group and in 1 subject in the PRP group. However, wound infection was not observed in any of the subjects in the HAM group. No flap necrosis or wound dehiscence was observed in any rat. There were changes in the surgical field consistent with seroma deposition.

Seroma Volume

Measurements of the amount of seroma formed were made by the same author. The amount of seroma formed was measured. An average of 3.5 ml of seroma was accumulated in the control group, 0.5 ml in the PRP group, and 4 ml in the HAM group (p=0.021, Figure 2). It was observed that less seroma occurred in the PRP group compared to the control group (p=0.015). The seroma formation in the HAM group was higher than in the control group (p=0.023).

Histopathologic Assessment

A statistically significant difference was found between the groups (Table 2, Figure 3). While lymphocyte, eosinophil, histiocyte, and fibroblast activity were moderate and severe in the control group, it was observed that the number of these cells decreased in the PRP and HAM groups (p<0.001, p=0.002, p<0.001, p<0.001, respectively).

Immunohistochemical Findings

Statistically significant differences were detected between the groups in immunohistochemical examinations (Table 3, Figure 4). While E-cadherin immunoreactivity was mild in the control group, it was severe in the PRP and HAM groups. TGFβ1 immunoreactivity, on the other hand, could not be detected at a significant level in the control group, but it was detected at a moderate level in the PRP and HAM groups (both p<0.001).

DISCUSSION

Dead spaces may occur in tissues where skin flaps are created and surgical dissection is performed, such as radical mastectomy, modified radical mastectomy, breast conserving surgery, ALND, sentinel lymph node biopsy, and breast biopsies. Seroma occurs as a result of leakage of lymphatic and vascular fluid into these dead spaces (15). It is one of the most common wound complications in the early postoperative period. Although the pathogenesis of seroma formation has not been clearly clarified, it has been reported that damage to the axillary lymph channels is one of the important causes (16). It is also known that the prolonged healing process is effective in the formation of this complication (17). With the creation of the skin flap during surgery, local inflammatory events and chemoreactant

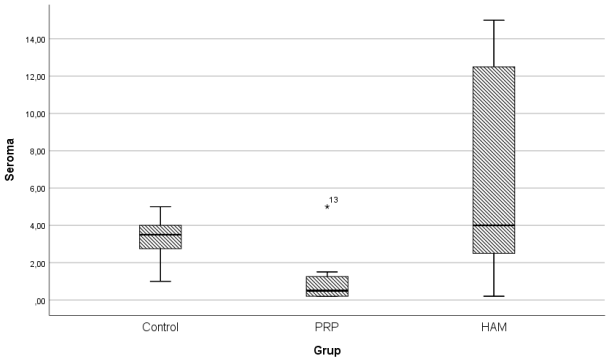

Figure 2. Median seroma volumes in each group

Table 2. Lymphocyte, eosinophil, histiocyte, and fibroblast activity by groups

	Control	PRP	HAM	p
Lymphocytes	4 (0) [3-4]	2 (0) [2-3]	2 (0) [2-2]	**<0.001**[a]
Eosinophils	2 (0.5) [1-2]	1 (0) [1-1]	1 (0) [1-2]	**0.002**[b]
Histiocytes	3 (0) [3-4]	2 (0) [2-3]	2 (0) [2-2]	**<0.001**[c]
Fibroblasts	3 (0) [3-4]	2 (0) [1-2]	2 (0) [2-3]	**<0.001**[d]

PRP: platelet-rich plasma, HAM: human amniotic membrane, [a]: $p_{Control-PRP}<0.001$; $p_{Control-HAM}<0.001$; $p_{PRP-HAM}=0.317$; [b]: $p_{Control-PRP}=0.003$; $p_{Control-HAM}=0.015$; $p_{PRP-HAM}=0.317$; [c]: $p_{Control-PRP}=0.001$; $p_{Control-HAM}<0.001$; $p_{PRP-HAM}=0.317$; [d]: $p_{Control-PRP}<0.001$; $p_{Control-HAM}=0.001$; $p_{PRP-HAM}=0.171$

Table 3. E-Cadherin and TGFβ1 immunoreactivity

	Control	PRP	HAM	p
E-Cadherin	1 (0) [0-1]	3 (0) [3-3]	3 (0) [2-3]	**<0.001**[a]
TGFβ1	1 (0.5) [2-3]	2 (0) [1-2]	2 (0) [2-2]	**<0.001**[b]

TGFβ1: Transforming growth factor-1β, PRP: platelet-rich plasma, HAM: human amniotic membrane, [a]: $p_{Control-PRP}<0.001$; $p_{Control-HAM}<0.001$; $p_{PRP-HAM}=0.317$; [b]: $p_{Control-PRP}<0.001$; $p_{Control-HAM}<0.001$; $p_{PRP-HAM}=0.317$

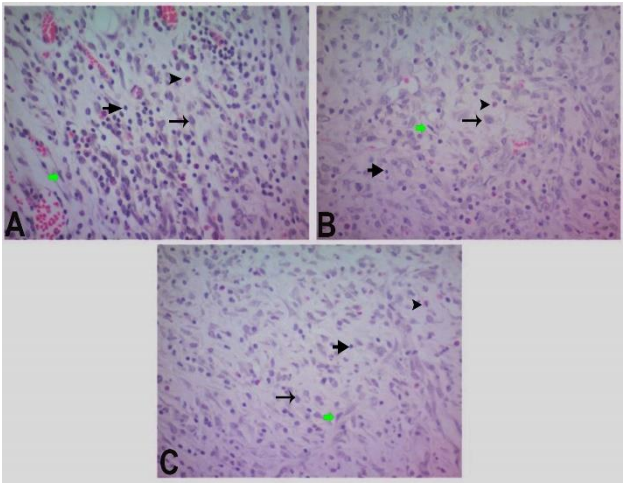

Figure 3. A) very severe lymphocyte infiltration (arrow), severe histiocyte (thin arrow), and fibroblast activity (green arrow) with moderate eosinophil infiltration (arrowhead) in the control group; **B)** mild eosinophil infiltration (arrowhead) with moderate lymphocyte (arrow), histiocyte (thin arrow), and fibroblast activity (green arrow) in platelet-rich plasma group; **C)** moderate lymphocyte (arrow), histiocyte (thin arrow), fibroblast activity (green arrow), and mild eosinophil infiltration (arrowhead) in human amniotic membrane group; (x40, hematoxylin&eosin)

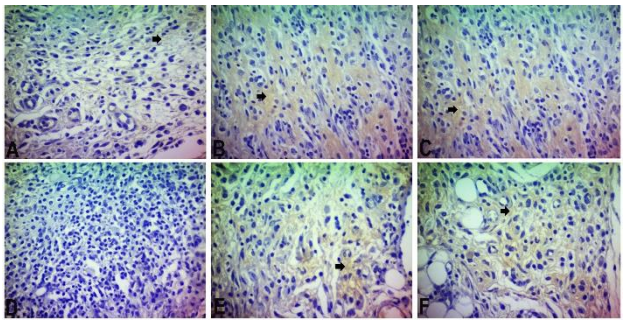

Figure 4. A) mild E-cadherin immunopositivity, **D)** TGFβ1 immunonegativity in the control group; **B)** severe levels of E-cadherin, **E)** moderate TGFβ1 immunopositivity in platelet-rich plasma group; **C)** severe levels of E-cadherin, **F)** moderate TGFβ1 immunopositivity in human amniotic membrane group; (arrows, x40, hematoxylin&eosin)

agents such as histamine, adenosine, and prostaglandin increase. As a result of increased vascular permeability due to these agents, serous fluid flows into the surgical area and contributes to seroma formation (18). One of the factors that increase this local inflammation is foreign bodies that are delayed in penetrating the tissue and ischemic tissue pieces left in the dissection area during surgery.

There are many studies in the literature that are thought to be effective in preventing seroma formation. The majority of these studies; it is designed on the basis of reducing the surgical cavity area, shortening the inflammatory process, and accelerating healing. In this context, different surgical techniques, pressure dressings, techniques that provide immobilization, tissue adhesives, and different agents have been involved in studies to prevent this complication (19,20). Platelets have an important role in hemostasis, tissue regeneration, and host defense. PRP is rich in various growth factors. Today, it is a biological material that is used in many surgical methods, chronic ulcers, and different clinical requirements. Its usage areas are increasing day by day (21). In the scope of the study, HAM with different biological properties such as PRP was examined. HAM, which is the avascular collagen matrix in the innermost layer of the placenta, is a biomaterial that is trying to find its place in current use. These properties differ depending on the preparation techniques of HAM and there is no consensus on the preparation techniques (22). HAM has anti-inflammatory, anti-fibrotic, and anti-adhesive properties. Known to accelerate wound healing (23). The extracellular matrix contains cytokines and growth factors. It has excellent biocompatibility as it shows low immunogenicity after decellularization (24). For all these reasons, in our study, the effects of PRP and cryopreserved HAM, which have extraordinary biological activity, on seroma formed after RM and ALND were examined.

There are many factors affecting the wound healing rate in surgeries with large flap areas. It has been reported that prolongation of the inflammatory process is effective in seroma formation (3). Therefore, the status of inflammatory cells is very important. Anti-inflammatory properties of PRP and HAM have been reported in studies (24,25). In our study, the levels of lymphocytes, eosinophils, and histiocytes were measured in the surgical field on the postoperative 7th day (13). Inflammatory cells were observed to be lower in the PRP and HAM applied groups compared to the control group. These results showed us that PRP and HAM have anti-inflammatory properties in line with the literature.

The activity of growth factors such as TGF-β decreases in chronic wounds. However, after the application of PRP and HAM, which have specific biological activity, these growth factors are released and have an important role in supporting wound healing. TGF-β accelerates epithelialization and healing by stimulating apoptosis of inflammatory cells, cell proliferation and collagen production (26). After wound formation, E-cadherin expression has an important role in cell maturation and regulation of cell layers (27). It has been reported that e-cadherin excretion increases as a result of amniotic membrane application, especially at the edges of wounds (28). In our study, E-cadherin and TGF-β1 levels, which are positive indicators of wound healing, were examined in tissue. It was observed that it was significantly higher in both PRP and HAM groups compared to the control group. These results showed us that HAM and PRP have positive effects on wound healing.

When evaluated in terms of seroma volume, it was observed that there was a serious decrease in seroma formation in the subjects who underwent PRP, in line with the literature (14). We think that this positive effect is due to both the anti-inflammatory properties of PRP and its enhancing properties in local growth factors. However, it is predicted that the application of PRP to the surgical area after cancer operations may have negative effects in terms of tumor treatment (29). Because of this negativity, in our study, the effect of HAM, a biological material reported to have a non-tumorigenic effect, which can be an alternative to PRP, on seroma was investigated (30). When the group in which local cryopreserved HAM was applied to the flap area after RM and ALND was compared with the control group, it was observed that there was no decrease in seroma formation. Despite the decrease in inflammatory cells and the increase in factors such as TGF-β1 and E-cadherin, no decrease in seroma formation was observed. Although the reason for this is not fully elucidated, we think that the smooth surface of the HAM, which prevents adhesion, may have prevented the reduction of the dead space under the flap (31).

Cryopreserved HAM is a biological material with high biocompatibility. However, it may cause adverse effects in seroma formation due to reasons such as being a xenograft, being a foreign tissue, and slower adaptation by the host tissue than PRP. Therefore, autograft or allograft applications may be beneficial. In addition, we think that it may be beneficial to apply different techniques in HAM preparation. We think that the use of HAM as a whole, especially in practice, increases its anti-adhesion effectiveness. We think that changing the structure of its smooth surface or applying it in smaller particles may reduce the anti-adhesive effect. In this way, we predict that it may be beneficial in the formation of seroma by taking advantage of its anti-inflammatory properties and positive effects on wound healing.

There were some limitations of the present study. In experimental studies on seroma formation, many scoring systems that were not included in our study, such as adhesion score, were used. Not using these scoring systems can be considered a limitation of this study. In addition, the number of groups was expanded in different studies. This can be considered a limitation

CONCLUSION

In our study, it was observed that PRP had positive effects on seroma formation after breast surgeries and ALND. However, its use is limited due to limitations in its use after cancer surgery. Therefore, HAM, a different biomaterial with non-tumorigenic properties, has been investigated. When examined in terms of infection and slow tissue healing, which are the mechanisms of action of seroma formation, it was seen that HAM had positive effects. However, cryopreserved HAM did not decrease seroma formation. We think this is due to the anti-adhesive properties of HAM. We think that the preparation and application of HAM have the potential to be beneficial against seroma formation if modifications are made.

Ethics Committee Approval: The study was approved by the Animal Experiments Local Ethics Committee of Sivas Cumhuriyet University (14.11.2020, 367). All institutional and national guidelines for the care and use of laboratory animals were followed.

Conflict of Interest: None declared by the authors.

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The Relation Between Serum Alpha Defensin-1 Levels with Clinical Course and Prognosis in Crimean-Congo Hemorrhagic Fever

Kırım-Kongo Kanamalı Ateşinde Serum Alfa Defensin-1 Düzeylerinin Klinik Seyir ve Prognoz ile İlişkisi

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ABSTRACT

Aim: Crimean-Congo Hemorrhagic Fever (CCHF) is a viral zoonotic infection characterized by fever and bleeding. Alpha-defensin-1 (AD-1) is an antimicrobial peptide. The aim of this study was to investigate the relationship between the clinical course and prognosis of CCHF and AD-1 serum levels, and also to examine the role of AD-1 in the pathogenesis of the disease.
Material and Methods: Fifty patients diagnosed with CCHF and hospitalized at the Atatürk University Faculty of Medicine Department of Infectious Diseases and Clinical Microbiology, and 38 healthy control were included in this study. Serum AD-1 levels were measured using ELISA methods and compared between the groups.
Results: Serum AD-1 levels in the patients were significantly higher than those in the control group (p=0.017). Of the patients, 18 (36%) were classified as severe clinical course, 16 (32%) as moderate clinical course, and 16 (32%) as mild clinical course. There was no statistically significant difference among the three groups in terms of serum AD-1 levels (p=0.729). Median serum AD-1 levels were 171.0 (range, 126.8-221.2) ng/ml in the fatal cases, and 118.7 (range, 91.9-183.3) ng/ml in the surviving patients, and the difference between these two groups was statistically significant (p=0.014).
Conclusion: As a result, the increased serum AD-1 levels in CCHF patients, remained higher in severe course patients and in the fatal cases. On the basis of these results, AD-1 appears to indicate the clinical course and provide useful information about mortality. More extensive research should be performed to make generalizations on this subject.
Keywords: Alpha defensin-1; antimicrobial peptide; Crimean-Congo Hemorrhagic Fever.

ÖZ

Amaç: Kırım-Kongo Kanamalı Ateşi (KKKA), ateş ve kanama ile seyreden zoonotik viral bir enfeksiyondur. Alfa defensin-1 (AD-1) antimikrobiyal bir proteindir. Bu çalışmanın amacı AD-1 düzeyleri ile KKKA'da klinik seyir ve prognoz arasındaki ilişkiyi araştırmak, aynı zamanda AD-1'in hastalığın patogenezindeki rolünü de incelemektir.
Gereç ve Yöntemler: Bu çalışmaya KKKA tanısı almış olan ve Atatürk Üniversitesi Tıp Fakültesi Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Bölümünde yatarak takip edilen 50 hasta ve 38 sağlıklı kontrol dahil edildi. Serum AD-1 düzeyleri ELISA yöntemi ile ölçüldü ve gruplar arasında karşılaştırıldı.
Bulgular: Hastalardaki serum AD-1 düzeyleri kontrol grubundan anlamlı şekilde daha yüksekti (p=0,017). Hastaların 18 (%36)'i ağır klinik seyir, 16 (%32)'sı orta düzeyde klinik seyir ve 16 (%32)'sı hafif klinik seyir olarak sınıflandırıldı. Serum AD-1 düzeyleri açısından bu üç grup arasında istatistiksel olarak anlamlı bir farklılık yoktu (p=0,729). Ortanca serum AD-1 seviyeleri, ölümcül vakalarda 171,0 (aralık, 126,8-221,2) ng/ml ve hayatta kalan hastalarda 118,7 (aralık, 91,9-183,3) ng/ml idi ve bu iki grup arasındaki bu farklılık istatistiksel olarak anlamlı idi (p=0,014).
Sonuç: Sonuç olarak, KKKA hastalarında artan serum AD-1 düzeyleri ağır seyirli hastalarda ve ölümcül vakalarda daha da yüksek seyretmektedir. Bu sonuçlara dayalı olarak, AD-1'in klinik seyri gösterdiği ve mortalite hakkında faydalı bilgiler sağladığı görülmektedir. Bu konuda genellemeler yapabilmek için daha geniş çaplı araştırmalar yapılmalıdır.
Anahtar kelimeler: Alfa defensin-1; antimikrobiyal peptidler; Kırım-Kongo Kanamalı Ateşi.

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INTRODUCTION

The agent involved in Crimean-Congo Hemorrhagic Fever (CCHF) is an enveloped RNA virus belonging to the *orthonairovirus* genus from the family *Nairoviridae* (1). The virus is particularly borne by *Hyalomma marginatum marginatum* ticks. The infection is transmitted due to infected ticks adhering to humans, nosocomial transmission, and through body fluids such as blood from animals in the viremic period. The infection has a high mortality. CCHF is the most common viral hemorrhagic fever worldwide. The incubation period is 3-7 days. Initial symptoms are characterized by sudden onset fever, headache, muscle pain, and dizziness. Hemorrhage is most commonly seen from the nose and gums, while hematemesis, melena, hematuria, hemoptysis, and intra-abdominal and vaginal bleeding may also be seen in patients with a clinically severe course (2,3).

The defensins, which are arginine-rich molecules, are the most important antimicrobial peptide group in mammals (4). They consist of 30-40 amino acids with molecular weights between an average of 3 and 6 kDa (5). The defensins are expressed by monocyte/macrophages, some T lymphocyte cells, immature dendritic cells, and natural killer (NK) cells in humans (6).

Defensins are closely related to protein-protein and protein-DNA interactions. This interaction contributes to binding to enveloped and non-enveloped (HPV and human adenovirus, HAdV) viruses. Since defensins are cationic and amphipathic, they can react in a charge-charge manner with ligands and also hydrophobically. For α-defensin in particular, defensin oligomerization and structural stability deriving from the disulfide bond may have a greater effect on binding (7).

Enveloped viruses have to create a fusion between the lipid bilayer and the host cell membrane, in order to introduce their own genes into the host cell. Alpha defensin-1 (AD-1) binding thus directly alters HIV-1 fusion through the interaction with gp 41. AD 1-4 directly prevents binding and adhesion to HIV-1 (8). Defensins' ability to serve as lectin and their selective sugar-binding capacities contribute to their antiviral properties (7). AD 1-3 have been shown to be effective against HIV, to whose receptors they bind with high affinity with their lectin-like properties (9).

The pathogenesis of CCHF is not fully understood. The immune system is important, but the response is insufficient in patients with severe disease. Defensin production begins as a result of viral infections. Defensins are known to be able to play direct and indirect roles in viral pathogenesis. Studies have investigated AD-1 levels in some viral diseases, but none have yet been considered AD-1 in CCHF. The purpose of this study was to determine AD-1 levels in patients with CCHF and to evaluate its relationship with clinical severity.

MATERIAL AND METHODS

Patients

Fifty patients definitively diagnosed with CCHF through investigation of samples using RT-PCR and/or ELISA and hospitalized for monitoring at the Atatürk University Infectious Diseases and Clinical Microbiology Clinical were included in the study. The study protocol was approved by the local ethics committee (24.05.2012, 21-5), and written informed consent was obtained from all patients included in the study. The study was designed and conducted in accordance with the ethical guidelines in the Declaration of Helsinki. Patients were divided into mild/moderate and severe on the basis of clinical severity. The defined criteria for patients were the presence of at least one; leukocyte count ≥10,000/mm^3, thrombocyte count ≤20,000/mm^3, aspartate aminotransferase (AST) ≥200 IU/L, alanine aminotransferase (ALT) ≥150 IU/L, activated partial thromboplastin time (aPTT) ≥60 sec, or fibrinogen ≤110 µg/dl in the first 5 days after onset of clinical symptoms as a severe case and absence of any of these as a mild/moderate case (10). Thirty-eight healthy volunteers were enrolled as the control group.

Biochemical Evaluation

Patient blood specimens were collected on a voluntary basis. Written informed consent was obtained from all patients before the examination. Five-milliliter blood specimens were collected after hospitalization. After 30 min these were then centrifuged for 5 min at 2000 rpm for sera separation. One milliliter was placed into one Eppendorf tube and 2 ml into another one. Two mL serum samples were sent to the Refik Saydam Hygiene Center reference laboratory in accordance with the appropriate transportation norms. Patients included in this study were those identified with specific IgM antibody positivity as a result of tests performed by that reference laboratory or with the presence of CCHF virus in sera confirmed using PCR. Thirty-eight healthy individuals were enrolled as a control group. Patient and control group sera (in 1 ml tubes) were stored at -80 °C until the study.

Serum AST, ALT, creatine phosphokinase (CK), lactate dehydrogenase (LDH) levels were measured using original kits on a Roche Diagnostics device (Roche Diagnostics, Mannheim, Germany), while hemogram parameters as white blood cell (WBC) and platelet (PLT) were determined with the Beckman Coulter LH 780 (Beckman Coulter Ireland Inc. Mervue, Galway, Ireland) device in the laboratory. Prothrombin time (PT-INR) and aPTT were analyzed in the ACL Top 700 ® (Instrumentation Laboratory, Bedford, MA, USA).

Serum AD-1 Level Measurement

Serum AD-1 levels were measured using a commercial ELISA kit (USCN, P.R. China) (SEB705Hu for Defensin Alpha 1, Neutrophil DEF a1, Human). In line with the test procedure, 100 µL of serum sample was added to each ELISA plaque well and was then incubated for 2 h at 37 ºC. The wells were then emptied and 100 µL of previously prepared detection reagent A was placed in each well and incubated for 1 h at 37 ºC. The wells were then emptied and washed three times. Next, 100 µL detection reagent B was placed in the wells and incubated for 30 min at 37 ºC. The wells were then emptied and washed five times; 90 µL substrate solution was then added and incubated at 37 ºC for 15-20 min. Finally, a 50 µL stop solution was added with no emptying and washing and the results were expressed with 450 nanometric absorbance values. The linear range of the test was measured as 0.312-20 ng/ml.

Statistical Analysis

Statistical Package for Social Sciences (IBM-SPSS v.20.0) software was used for statistical analysis. Normality of distribution was evaluated using the Kolmogorov-Smirnov test. Numerical data were expressed as mean and standard

deviation or median, interquartile range, minimum, and maximum values, while categorical data were expressed as numbers and percentages. The Mann-Whitney U test and Kruskal-Wallis test were used for data analysis. Relationships between results were evaluated using Spearman correlation analysis. A p value of <0.05 was regarded as significant for all tests.

RESULTS

The study group involved 50 patients, 26 (52%) male, and 24 (48%) female. The healthy control group consisted of 38 individuals, 20 male (52.6%) and 18 female (47.4%). Gender distributions in the patient group (p=0.883) and in the control group (p=0.872) were similar. The mean age of patients was 49.12±18.33 years, compared to 40.92±16.51 in the control group.

Laboratory values of patients were shown in Table 1. Mean AD-1 levels were 126.0±30.5 ng/ml in the patient group and 112.2±24.5 ng/ml in the control group, the difference being statistically significant (p=0.017).

In terms of patients' clinical courses, 18 (36%) were classified as severe, 16 (32%) as moderate, and 16 (32%) as mild. Four (8%) of the 50 patients enrolled in the study died, while 46 (92%) were discharged in a healthy condition.

Serum AD-1 levels were compared among the patients with CCHF according to their clinical severity. Median AD-1 levels were 116.8 (range, 91.9-191.2) ng/ml in the mild group, 124.7 (range, 94.9-177.7) ng/ml in the moderate group, and 123.4 (range, 97.6-221.2) ng/ml in the severe group (Table 2, Figure 1). No statistically significant difference was determined among the three groups in terms of serum AD-1 levels (p=0.729). Also, AD-1 levels were significantly higher in the non-surviving patients compared to those that survived (p=0.014). The surviving patient group and the control group were compared separately in terms of AD-1 levels, the difference being threshold significant (p=0.015, Table 3).

The correlation coefficient between serum AD-1 levels and laboratory tests frequently employed in the diagnosis, treatment, and prognosis of CCHF are shown in Table 4. However, no significant correlation was determined between AD-1 level with hematological and biochemical parameters.

Table 1. Patients' non-specific laboratory findings (n=50)

Parameter	Median (Q_1-Q_3) [Min-Max]	Reference interval
WBC ($10^3/\mu$)	2 (1.5-2.6) [0.9-11.7]	4.3-10.3
PLT ($10^3/\mu L$)	36 (19-54.8) [6-128]	150-450
AST (U/L)	343 (149-521) [48-11150]	1-50
ALT (U/L)	163 (83-336) [26-2944]	1-50
CK (U/L)	634 (168-1506) [48-7641]	1-171
LDH (U/L)	636 (424-994) [186-9458]	1-247
PT (sec)	11 (10-12) [9.0-18.9]	10.0-15.9
aPTT (sec)	37 (31-40) [23-64]	26.5-36
INR	1 (0.9-1.3) [0.8-94.0]	0.9-1.3

Q_1-Q_3: 25th-75th percentile, WBC: white blood cell, PLT: platelet, AST: aspartate aminotransferase, ALT: alanine aminotransferase, CK: creatine kinase, LDH: lactate dehydrogenase, PT: prothrombin time, aPTT: activated partial thromboplastin time, INR: international normalized ratio

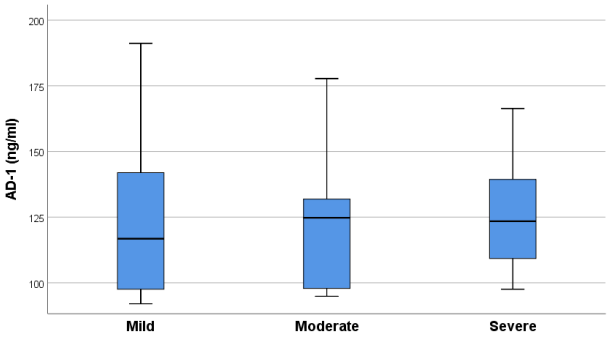

Figure 1. Demonstration of serum AD-1 levels in groups divided by severity of CCHF disease

Table 2. Disease severity and serum AD-1 levels

	Mild (n=16)	Moderate (n=16)	Severe (n=18)	p
AD-1 (ng/mL)	116.8 (97.5-142.4) [91.9-191.2]	124.7 (97.8-131.9) [94.9-177.7]	123.4 (108.0-140.6) [97.6-221.2]	0.729

AD: alpha defensin, data were expressed as median (25th - 75th percentile) [minimum-maximum]

Table 3. Comparison of AD-1 levels between surviving and non-surviving patients and the control group

	Patients (n=50)		p*	Control Group (n=38)	p#
	Surviving (n=46)	Non-surviving (n=4)			
AD-1 (ng/mL)	118.7 (98.2-133.5) [91.9-183.3]	171.0 (132.8-213.7) [126.8-221.2]	**0.014**	110.2 (92.7-129.5) [65.7-178.2]	**0.015**

AD: alpha defensin, p*: non-surviving vs. surviving patients, p#: surviving patients vs. control group, data were expressed as median (25th - 75th percentile) [minimum-maximum]

Table 4. Correlation between serum AD-1 levels and laboratory parameters

		WBC	PLT	AST	ALT	LDH	PT	aPTT	INR
AD-1 (ng/mL)	r_s	0.253	-0.027	0.063	0.116	0.029	-0.313	0.084	-0.112
	p	0.077	0.850	0.664	0.421	0.842	0.001	0.564	0.437

AD: alpha defensin, WBC: white blood cell, PLT: platelet, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, PT: prothrombin time, aPTT: activated partial thromboplastin time, INR: international normalized ratio

DISCUSSION

The principal findings of this prospective study were as follows; i) the CCHF patients exhibited higher AD-1 levels than the control group; ii) AD-1 levels were higher in fatal cases than in surviving patients; iii) WBC and PLT as the routine laboratory tests were lower in the patient group, while AST, ALT, LDH, and CK increased. Finally, AD-1 level exhibited a significant relation with clinical severity in CCHF patients. The study findings emphasize the role of AD-1 in demonstrating clinical course and prognosis in CCHF. There are no studies in the literature concerning serum AD-1 in viral hemorrhagic fevers.

The most abundant cationic peptides, an important component of the immune system with antimicrobial effects, are the defensins. These are able to inactivate several bacteria, protozoa, and viruses. They are also chemotactic agents for macrophages, T cells, and immature dendritic cells (9).

AD-1 has proven efficacy against bacteria such as *Staphylococcus aureus*, *Enterobacter aerogenes*, *Escherichia coli*, *Mycobacterium avium*, and *Mycobacterium intracellulare*. AD 1-3 have been reported to be effective against *Mycobacterium tuberculosis* (6). AD 1-3 have also been shown to inhibit enzymatic activity by binding with high affinity to anthrax lethal factor.

Direct interaction of defensins with the lipid double layer of enveloped viruses can destroy or destabilize the virus (7). They can inhibit the majority of enveloped viruses through neutralization of the lipid double membrane. The sensitivity of enveloped viruses to α-defensins varies (7). Studies investigating defensin levels in various viral diseases have confirmed their antiviral effects. AD-1 has been reported to inhibit viral replication and viral proteins in cell cultures. AD 1-4 have been shown to be effective against HSV1, HSV2, influenza virus, adenovirus, and CMV (6,11,12). AD-1 has been proved to bind directly to HSV-1 and to membranes containing phosphatidylcholine (7). Another study showed that AD 1-6 inhibited HSV infection for protection against HSV infection (9). Generally, the defensins block the host cell receptor and binding to viral glycoproteins, so they prevent HSV-1 and HSV-2 infection (13). Buck et al. (14) described AD 1-3 and AD-5 as powerful antagonists against papillomavirus infection, a non-enveloped virus. Also, other studies have shown that ADs are necessary for preventing HAV, HIV-1, HSV-1, and influenza A virus (7,8,12,14-16).

These studies support the idea that AD-1 stimulation is not specific to CCHF. The natural immune system is important in the resolution of the disease in CCHF. Inflammatory mediators play an important role in patients that die due to CCHF. Four (8%) of the 50 patients in this study died. Our study suggests that CCHF increases the release of serum AD-1 and supports previous studies. We think that the increasing AD-1 levels we identified in CCHF may contribute to host defense. This may suggest that patients dying due to CCHF have insufficient activity or levels for eradication. AD may be less sensitive in some viral strains or in a high viral load.

Defensins also are important molecules in the generation of immune response with their immunomodulatory peptide properties. They act as regulators in some diseases as a response to various inflammatory stimuli. α-defensins have recently been reported to be involved in the pathogenesis of autoimmune diseases. In a study of patients with Behçet's disease, Mumcu et al. (17) determined significantly higher AD 1-3 levels in patients with severe disease. Serum AD-1 levels were significantly higher in the fatal cases and the surviving patients in our study. So, we think that AD-1 can be used as an immunomodulator, particularly in severe and mortal patients in which viremia is high.

Defensins are known to serve as tumor cell regulators. Gunes et al. (18) reported statistically significantly high AD 1-3 in patients with bladder cancer. A correlation has been determined between AD 1-3 expression and colorectal adenoma and carcinoma (6). CCHF is also a lymphovascular disease. The virus is first replicated in the lymph glands and produces characteristic endothelial infection. AD-1 levels were high in our patients through a similar mechanism.

Alpha defensins facilitate thromboembolic events in chronic heart failure and pro-thrombosis. They increase coagulation by inhibiting tissue plasminogen activators. The relation between α-defensin and mortality has also been investigated in these patients and has been described as prognostic (19). In CCHF, a plasminogen activator inhibitor is released for the inhibition of fibrinolysis with IL-1 and TNF, and coagulation mechanisms are activated. AD-1 levels in patients with the severe and mortal diseases may have facilitated coagulation cascade activation and progression toward disseminated intravascular coagulation. It exhibited no antiviral effectiveness in these patients.

There are a number of limitations to this study. These include the fact that α-defensin only was investigated. In addition, the effect of the viral load and gene polymorphism were not investigated. Finally, we did not examine the entire cascade.

CONCLUSION

AD-1 levels increasing with the severity of the disease also play an important role in the pathogenesis of the CCHF. Higher AD-1 levels have been measured in mortal patients and those with a clinically severe course. Future studies on this subject can help illuminate the pathogenesis of CCHF infection and even direct treatment. Defensins can be used in the future in the prevention and treatment of infectious diseases. Studies with wider case series and different gene polymorphism regions are needed to shed light on treatment.

Ethics Committee Approval: The study was approved by the Non-Drug Clinical Research Ethics Committee of Atatürk University (24.05.2012, 21-5).

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The Relationship of Body Mass Index with Platelet Counts and Donation Frequency of Platelet Apheresis Donors

Vücut Kitle İndeksinin Trombosit Aferez Donörlerinin Trombosit Sayıları ve Donasyon Sıklığı ile İlişkisi

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ABSTRACT

Aim: The aim of this study was to investigate the demographic characteristics of platelet apheresis donors during the coronavirus disease 2019 (COVID-19) pandemic, to examine the association of platelet counts with blood groups and body mass index (BMI), and to characterize the effects of BMI on platelet apheresis donation.
Material and Methods: In this retrospective study, the demographic characteristics of platelet apheresis donors admitted to our center between January 2020 and January 2021 were examined, and hemogram parameters, previous platelet donation frequencies, and blood groups information were recorded. The relationship between donors' platelet counts, blood groups, and BMI were analyzed.
Results: There was no statistically significant relationship between the platelet counts and blood groups. Although platelet counts were higher in Rh positive donors, no statistically significant relationship was found between platelet counts and the Rh group (p=0.675). Even though the platelet counts were lower in low-weight donors, there was no statistically significant relationship between platelet count and BMI groups (p=0.134). The lower collected platelet counts of the normal group, compared to the obese group, was found to be statistically significant (p=0.001). The number of donations for the overweight group being higher than the normal and obese groups was found to be statistically significant (p=0.002).
Conclusion: Even though the platelet count increases with obesity, it does not have any statistical significance in healthy platelet donors. In conclusion, obesity does not have an inverse relationship with platelet apheresis donation and high BMI should not be considered as a prognostic factor for donation.
Keywords: Platelet; apheresis; donors; body mass index.

ÖZ

Amaç: Bu çalışmanın amacı, koronavirüs hastalığı 2019 (coronavirus disease 2019, COVID-19) pandemi döneminde trombosit aferez donörlerinin demografik özelliklerinin araştırılması, trombosit sayılarının kan grupları ve vücut kitle indeksi (VKİ) ile ilişkisinin gözden geçirilmesi ve VKİ'nin trombosit aferez bağışı üzerindeki etkisini karakterize etmektir.
Gereç ve Yöntemler: Bu geriye dönük çalışmada Ocak 2020 ve Ocak 2021 tarihleri arasında merkezimize başvuran trombosit aferez donörlerinin demografik özellikleri incelendi ve hemogram parametreleri, daha önceki trombosit bağış sıklığı ve kan grubu bilgileri kaydedildi. Bağışçıların trombosit sayıları, kan grupları ve VKİ arasındaki ilişki analiz edildi.
Bulgular: Trombosit sayıları ile kan grupları arasında istatistiksel olarak anlamlı bir ilişki bulunmamıştır. Trombosit sayıları Rh pozitif bağışçılarda daha yüksek olmakla birlikte trombosit sayıları ile Rh grubu arasında istatistiksel olarak anlamlı bir ilişki bulunmamıştır (p=0,675). Düşük kilolu donörlerde trombosit sayıları daha düşük olsa da trombosit sayısı ile VKİ grupları arasında istatistiksel olarak anlamlı bir ilişki yoktu (p=0,134). Obez grup ile karşılaştırıldığında, normal kilolu gruptaki daha düşük trombosit torba sayısı istatiksel olarak anlamlı olarak bulundu (p=0,001). Normal kilolu ve obez gruplara göre daha fazla sayıda olan, fazla kilolu gruba yapılan bağış sayısı istatistiksel olarak anlamlı olduğu bulundu (p=0,002).
Sonuç: Trombosit sayısı obezite ile birlikte artsa da bu durum sağlıklı trombosit donörlerde istatiksel olarak bir anlamlılığa sahip değildir. Sonuç olarak, obezitenin trombosit aferez bağışı ile ters bir ilişkisi yoktur ve yüksek VKİ bağış için prognostik bir faktör olarak kabul edilmemelidir.
Anahtar kelimeler: Trombosit; aferez; donör; vücut kitle indeksi.

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INTRODUCTION

The increasing demand for platelet transfusions for patients with different medical diagnoses has led to the acceleration in the use of apheresis for platelet concentrates carried out in a dedicated area under the supervision of a transfusion practitioner (1). Platelets play an important role in primary hemostasis and are used in patients for various reasons, primarily to stop or prevent life-threatening bleeding (2).

Many authors have reported that apheresis is a safer procedure due to its less frequent side effects compared to whole blood donations, and collecting platelets also prove advantageous in that up to three adult doses of platelets can be given per donation (3). Platelet apheresis is expensive and a huge financial burden to procure (4). In addition, the significant decrease in the number of donors during the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic in 2020 led to the disruption of supply from regional blood centers, and the platelet supply in the hospitals' blood banks reached critically low levels.

Obesity definition and grading are evaluated based on body mass index (BMI) by the weight/height (kg/m^2) formula. In the obesity guideline by the Turkish Endocrinology Association, the obesity classification recommended by the World Health Organization (5) is defined as underweight, BMI <18.5; normal, BMI: 18.5-24.9; overweight, BMI: 25-29.9, and obese, BMI ≥30 (obesity is also subdivided into categories as class 1, BMI: 30-34.9; class 2, BMI: 35-39.9; and class 3, BMI: ≥40).

Lifestyle changes such as high-calorie foods and increased inactivity due to the SARS-CoV-2 pandemic are responsible for the development of obesity, one of the major global health problems. It has also been documented in publications that obesity is characterized by the presence of a prothrombic condition resulting from the combination of increased thrombin production (6), platelet hyperactivity, and decreased fibrinolysis (7), so the increased platelet reactivity is considered to play a role among the different events that accelerate the risk of atherothrombosis.

During the pandemic period, our hospital continued to actively prepare platelet apheresis from active platelet donors. The present study aimed to investigate the demographic characteristics of platelet apheresis donors and to review the relationship of platelet counts with BMI. Therefore, the main objective of the present study was to characterize the effect of BMI on platelet apheresis donation.

MATERIAL AND METHODS

In the present retrospective study, the platelet apheresis donors were selected among the platelet apheresis donors from January 2020 to January 2021 in line with the standard operation procedure (SOP). A total of 1198 participants were outpatients of the clinic aged between 18 and 65 years.

The body height of the participants was measured on a flat surface without shoes. Bodyweight, BMI, fat percentage, fat mass, and fat-free mass of the patients were determined through a bio-impedance device [Tanita-BC418]. Patients were excluded from the study if they had any disease other than regulated hypertension, chronic inflammatory process, hematological disorders, infectious disease, ischemic heart disease, and history of corticosteroid use, alcohol use, and smoking. Demographic features, family history, chronic diseases, and hematological parameters (hemoglobin and platelet) were obtained. All values in the study were calculated from patients' complete blood count (CBC) analysis. A routine electronic CBC device was used (XN-CBC, Sysmex, Bornbarch 1, 22848 Norderstedt, Germany) for this purpose.

Platelet aphereses were collected using Terumo or Beckman Coulter Haemonetics. The demographic characteristics of the platelet apheresis donors (weight, height, BMI) were calculated, and the information regarding hemogram parameters, previous platelet donation frequency, blood group, and viral serology were recorded.

In this study, patients were divided into three groups according to BMI; the underweight and normal group (BMI <24.9), the overweight group (BMI: 25-29.9), and the obese group (BMI ≥30), and then these groups were compared. The relationship between the donors' platelet counts, blood groups, and BMI values was analyzed.

The study was approved by the Non-Interventional Clinical Researches Ethics Committee of İstanbul Medipol University (decision date: 01.04.2021 and number: 401).

Statistical Analysis

NCSS 2007 (Number Cruncher Statistical System, Kaysville, Utah, USA) program was used for statistical analysis. While evaluating the data, descriptive statistical methods (mean, standard deviation, median, interquartile range, minimum, maximum, frequency, and percentage) were used, as well as the Shapiro-Wilk test to assess data distribution. The Kruskal-Wallis test (post hoc Dunn) was used for the comparison of three or more groups, and the Mann-Whitney U test was used for two-group comparisons that did not exhibit a normal distribution. Pearson or Fisher's exact chi-square tests were used to identify the relationship among qualitative data. Significance was evaluated at a level of $p<0.05$.

RESULTS

Of the 1198 donors enrolled in the study, 55 (4.5%) were female and 1134 (95.5%) were male. The age of the patients in relation to their gender was not statistically significant (p=0.355). There was a statistically significant difference between males and females of heights (p=0.001). The weight of the males was greater than that of the females (p=0.001). Also, the BMI of the males was greater than that of the females and the difference was found to be statistically significant (p=0.001, Table 1).

Since the number of female donors is low, the following study statistics were obtained solely from the male participants. The median age was 35 (range, 18-65) years, and the median BMI was 26.3 (range, 20.4-41.5) kg/m^2. Hemoglobin levels were between 12.1 and 18.8, and the median hemoglobin level was 15.2 g/dl. The median platelet count was 245 with a range of 170-493 ($x10^3$). The number of collected platelet bags varied from 1 to 3, and the median bag count was 2. The number of donations to our hospital ranged from 0 to 18. Each patient's blood pressure was measured before the procedure. The systolic blood pressure of the patients ranged from 110 to 160 with

a median of 123, while diastolic blood pressure ranged from 10 to 110 with a median of 79 mmHg (Table 2).

The blood types of donors from in order of frequency were 45.1% (n=512) A positive, 22.3% (n=253) 0 positive, 12.1% (n=137) B positive, 11.2% (n=127) AB positive, 5.8% (n=66) A negative, 2.4% (n=27) 0 negative, 0.6% (n=7) B negative, and 0.4% (n=5) AB negative. The collection procedure for 35.1% (n=398) of the donors was performed in the Haemonectics machine, while the procedure for 64.9% (n=736) of the donors was performed in the Terumo machine. 23.9% (n=271) of the donors had previously been platelet donors in our hospital, while 76.1% (n=863) applied for donation for the first time. Of the donors, 0.4% (n=4) were HBS antigen positive, 0.3% (n=3) were anti-HCV positive and 0.1% (n=1) was syphilis positive, thus their platelet aphereses were destroyed.

The donors' platelet value of 54.3% (n=616) was less than 250, while the platelet value of 45.7% (n=518) of the donors was 250 and above. Even though platelet counts were higher in Rh positive donors, no statistically significant relationship was found between the platelet count and the Rh group (p=0.675, Table 3).

Table 1. Comparison of the donors by gender

	Female (n=55)		Male (n=1134)		p
	Mean±SD	**Median (IQR) [min-max]**	**Mean±SD**	**Median (IQR) [min-max]**	
Age (years)	33.9±8.5	34 (16) [20-52]	35.4±9.3	35 (13) [18-65]	0.355
Weight (kg)	67.3±11.7	65 (16) [50-99]	83.5±12.4	83 (15) [54-130]	**0.001**
Height (cm)	163.6±6.7	163 (8) [151-185]	175.9±6.8	176 (10) [150-195]	**0.001**
BMI (kg/m^2)	25.4±4.4	24.1 (6.2) [19.7-41.2]	26.8±3.7	26.3 (4.9) [20.4-41.5]	**0.001**

SD: standard deviation, IQR: interquartile range, BMI: body mass index

No statistically significant relationship was found between the BMI group of the donors and the platelet count of $<250x10^3$ and $\geq250x10^3$. Even though the platelet counts were observed to be lower in low-weight donors, there was no statistically significant difference between platelet count and BMI groups (p=0.134, Table 4). However, high platelet counts in overweight and obese patients, although not statistically significant in the present study, are believed to be associated with platelet count and obesity.

The platelet value of the first-time donor group was 249.88±48.06, which was higher than that of the previous donor group 243.55±44.12, and was found to be statistically significantly different (p=0.043).

The number of platelet bags collected showed a statistically significant difference by the BMI groups (p=0.001). The lower collected platelet counts of the normal group, compared to the obese group, was found to be statistically significant. The number of donations showed a statistically significant difference by the BMI groups (p=0.002). The number of donations for the overweight group was higher than the normal and obese groups, and found to be statistically significant (Table 5).

DISCUSSION

The results of the present study showed that, although the platelet count was high in obese patients, no significant difference was found between BMI and platelet count in the comparison of the platelet donor subgroups; however,

Table 2. Clinical characteristics of the study group

	Mean±SD	Median (IQR) [min-max]
Hemoglobin (g/dl)	15.4±1.0	15.2 (1.3) [12.1-18.8]
Hematocrit (%)	44.2±17.8	45 (2.3) [35-40.7]
MCV (fl)	83.2±31.9	81.9 (4.3) [57.4-85.7]
Platelet (x10^3)	248.4±47.2	245 (61) [170-493]
Number of platelet bag	2.3±0.7	2 (1) [1-3]
Number of donation	0.6±1.6	0 (0) [0-18]
SBP (mmHg)	124.8±7.5	123 (2) [110-160]
DBP (mmHg)	78.8±8.9	79 (2.4) [10-110]

SD: standard deviation, IQR: interquartile range, BMI: body mass index, MCV: mean corpuscular volume, SBP: systolic blood pressure, DBP: diastolic blood pressure

Table 3. Relationship between platelet and blood groups

Blood Groups	Platelet<250	Platelet≥250	p
Rh Negative	55 (8.9%)	50 (9.7%)	0.675
Rh Positive	561 (91.1%)	468 (90.3%)	

Table 4. Relationship between platelet and body mass index

BMI Groups	Platelet<250	Platelet≥250	p
Normal	209 (33.9%)	150 (29.0%)	
Overweight	295 (47.9%)	256 (49.4%)	0.134
Obese	112 (18.2%)	112 (21.6%)	

Table 5. Comparison of the groups by body mass index

	Normal Weight (n=359)		Overweight (n=551)		Obese (n=224)		p
	Mean±SD	**Median (IQR) [min-max]**	**Mean±SD**	**Median (IQR) [min-max]**	**Mean±SD**	**Median (IQR) [min-max]**	
Number of platelet bag	2.2±0.7	2 (1) [1-3]	2.3±0.7	2 (1) [1-3]	2.4±0.7	2 (1) [1-3]	**0.001**
Number of donation	0.4±1.0	0 (0) [0-7]	0.8±1.9	0 (1) [0-18]	0.5±1.2	0 (0) [0-9]	**0.002**

SD: standard deviation, IQR: interquartile range

although the high platelet counts in overweight and obese patients were not statistically significant in the present study, it is believed that platelet count and obesity are related.

The relationship between increased platelet count and platelet activation is unclear. Platelet count and activation are associated with essential thrombocytosis (8) with chronic inflammation, and inflammatory bowel diseases (9). In the present study, even though the platelet counts were lower in underweight donors, this was not found to be statistically significant.

In the present study, the most common blood type was A Rh positive (45.1%) followed by 0 Rh positive (22.3%). The blood group distribution of apheresis donors is substantially similar to the blood group distribution rates in Turkey (10).

Fantuzzi et al. (11) reported that the increase in adipose tissue in obesity triggers inflammation, while a study conducted by Farhangi et al. (12) showed that the platelet count increases with inflammation. In the present study, the platelet count was higher in obese patients with high BMI compared to underweight ones, but this was not found to be statistically significant. However, the number of platelet bags collected by the BMI group shows a statistically significant difference. The lower collected platelet count of the normal group, compared to the overweight and obese group, was found to be statistically significant.

In a study by Kutluturk et al. (13), the platelet count was found to be significantly higher in obese individuals than in non-obese individuals, and this was established as the finding that evaluates the relationship between platelet count and cardiovascular disease metabolic risk factors in obesity. Also in the present study, the lowest platelet count was found in underweight donors.

Eren et al. (14) showed that BMI is higher in the Rh positive group, supporting that Rh immunization may be indirectly related to body mass index and platelet count. In the present study, platelet counts were observed to be higher in Rh positive donors, but no statistically significant difference was found.

In the present study, males donated more platelets than females. Doğu et al. (15) reported that more males donated platelet apheresis than females, and Guo et al. (16) reported that the proportion of males was five times higher than females in a study conducted in 5 centers in China. The authors of the present study believe that this lower donor rate in females is due to the low hemoglobin values of females due to menstruation, pregnancy, breastfeeding periods, and thin vascular structures.

CONCLUSIONS

Due to the increase in the number of overweight and obese platelet donors compared to normal weight and underweight donors, the number of platelet bags collected is statistically significantly higher. Even though the platelet count increases with obesity, it does not have any statistical significance in healthy platelet donors. There is no statistical relationship between blood group, Rh group, and BMI. In conclusion, obesity does not have an inverse relationship with platelet apheresis donation and it is not suitable to count high BMI as a prognostic factor for donation.

Ethics Committee Approval: The study was approved by the Non-Interventional Clinical Researches Ethics Committee of İstanbul Medipol University (01.04.2021, 401)

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Acute Onset Chronic Inflammatory Demyelinating Polyneuropathy Following COVID-19

COVID-19 Sonrası Akut Başlangıçlı Kronik İnflamatuar Demiyelinizan Polinöropati

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ABSTRACT

The cases of Guillain Barre Syndrome (GBS) have been reported following the coronavirus disease 2019 (COVID-19). Here, we describe a case that evolved from GBS to chronic inflammatory demyelinating polyneuropathy (CIDP) after COVID-19 in terms of contributing to the literature due to its different aspects. In the cerebrospinal fluid examination of the acute onset mixed type polyneuropathy case, albuminocytological dissociation was not detected. The patient was given a loading dose and monthly maintenance intravenous immunoglobulin (IVIG) for six months. Blood ferritin levels gradually decreased in parallel with clinical improvement. Four months after the IVIG treatment was terminated, the findings recurred and the CIDP was developed and IVIG treatment was continued. Long-term follow-up of post-COVID-19 GBS patients is important in terms of recurrence and chronicity. Ferritin level may be a biochemical marker in the clinical follow-up of these cases.
Keywords: COVID-19; GBS; ferritin; axonal; recurrence; prognosis.

ÖZ

Koronavirüs hastalığı 2019'u (coronavirus disease 2019, COVID-19) takiben Guillain Barre Sendromu (GBS) vakaları bildirilmiştir. Burada farklı yönleri nedeniyle literatüre katkı sağlaması açısından COVID-19 sonrası GBS'den kronik inflamatuar demiyelinizan polinöropatiye (chronic inflammatory demyelinating polyneuropathy, CIDP) evrilen bir olguyu tanımladık. Akut başlayan mikst tipte polinöropati olgusunun beyin omurilik sıvısı incelemesinde albüminositolojik dissosiasyon saptanmadı. Hastaya yükleme dozu ve altı ay boyunca aylık idame intravenöz immünglobulin (IVIG) verildi. Kan ferritin düzeyleri klinik iyileşme ile paralel tedricen azaldı. IVIG tedavisi sonlandırıldıktan 4 ay sonra bulgular tekrarladı, CIDP gelişti ve IVIG tedavisine devam edildi. COVID-19 sonrası GBS hastalarının uzun süreli takibi nüks ve kroniklik açısından önemlidir. Bu olguların klinik takibinde ferritin düzeyi biyokimyasal bir belirteç olabilir.
Anahtar kelimeler: COVID-19; GBS; ferritin; aksonal; nüks; prognoz.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) primarily involves the respiratory system but also causes central and peripheral nervous system findings. Acute polyradiculopathy is among the rare neurological complications of COVID-19 infection (1). Guillain Barre syndrome (GBS) develops following many viral diseases such as *campylobacter jejuni*, Epstein-Barr virus, influenza or cytomegalovirus (2). It has been reported recently that COVID-19 can cause GBS, recurrent GBS, and the worsening of chronic inflammatory demyelinating polyradiculopathy (CIDP) (3). It has been reported GBS associated with COVID-19 did not show classical and electrophysiological features and it had a lot of variations (4).

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Here, we present a case of acute motor-sensory axonal neuropathy (AMSAN) as a variant of GBS after COVID-19 and relapsed ten months later.

CASE REPORT

A 39-year-old female, hospitalized with dry cough, dyspnea, and fever. A positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was found in the RT-PCR assay at the nasopharyngeal swab and ground-glass opacities in the lung at computed tomography (CT) scan were seen. One week after her discharge, she was admitted to the neurology outpatient clinic with difficulty in walking and generalized pain. Neurological examination revealed for 3/5 weakness in the distal of the upper and lower extremities. Cranial nerve, cerebellar system, and sensory examination was normal. Deep tendon reflexes could not be detected in the lower extremities; the Babinski reflex was negative.

Nerve conduction studies showed prolonged distal motor latencies (DML), small compound muscle action potentials (CMAPs) and decreased nerve conduction velocities (NCVs) in the tibial and median motor nerves bilaterally. Sensory nerve conduction studies showed prolonged sensory latencies, small sensory nerve action potentials (SNAPs) and decreased NCVs in the sural nerves bilaterally. The latencies of the tibial F-waves were prolonged bilaterally. Abnormal spontaneous potentials were recorded in the lower extremity muscles in needle electromyography (EMG). These EMG findings were found compatible with the AMSAN variant of GBS.

A mild elevated white blood cells count (70 /mm^3), normal protein level (10 mg/dl), and normal sugar level (65 mg/dl) was detected in the cerebrospinal fluid (CSF) analysis. PCRs for Herpes simplex virus 1-2, SARS-CoV-2, serologic antibody tests for Epstein-Barr virus, cytomegalovirus, and *Borrelia burgdorferi* were found negative in CSF. The patient's had normal IgG index (0.1) without oligoclonal bands of the CSF. Anti-ganglio side antibody, including anti-GM1, GM2, GM3, GD1a, GD1b, Gt1b, and GQ1b was negative.

Intravenous immunoglobulin (IVIG) treatment was given 0.4 gr/kg/day for 5 days as starting dose. Since the neurological findings showed partial improvement, maintenance IVIG (1 gr/kg monthly) was continued. Treatment was terminated because nerve conduction studies returned to normal in the repeated EMG at 6 months. Modified Erasmus GBS Outcome score (mEGOS) reduced from 6 to 3, gradually. Ferritin levels were also gradually got normal limits in the six months (monthly ferritin values: 1259, 946, 488, 282, 271, and 170 ng/ml, respectively, normal range: 10-204 ng/mL). However, difficulty in walking and sensory complaints in the hands developed again after 10 months. Repeated EMG showed slowing in tibial motor NCVs, small CMAPs in median and tibial motor nerves. Decreased NCVs in sural sensory nerves was detected. The IVIG treatment was restarted as 1gr/kg monthly.

DISCUSSION

SARS-CoV-2, like other viruses, can trigger GBS in the post-infectious period. The first COVID-19 related GBS case has been reported by Zhao et al. (5) in 2020. Acute polyneuropathies have been reported with severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East Respiratory Syndrome coronavirus (MERS-CoV), which have been previously caused epidemics (6).

A large Italian study reported an incidence of GBS as 0.42% after COVID-19 (7).

GBS symptoms typically start several days to a few weeks after acute viral illnesses. In recent studies, the mean time interval from COVID-19 to GBS symptoms was reported 11±6 days (4). GBS cases related to COVID-19 have a wide spectrum. The most common electrophysiological pattern is acute inflammatory demyelinating polyneuropathy (AIDP), less frequently acute motor axonal neuropathy (AMAN), and AMSAN (4). It has been reported abnormalities in the central nervous system together with GBS symptoms. Albuminocytological dissociation is reported in the CSF in 75% of the cases. The absence of typical areflexia in some cases has been reported as an interesting finding. It was reported that 73-75% of the patients showed varying degrees of improvement in 5 days to 8 weeks. CSF protein elevation is known as an important biomarker determining the severity and extent of the disease (8). In our patient, the CSF protein level was low, and the clinical course showed a slow recovery. We detected serum ferritin level as an important biomarker.

mEGOS has been shown to be a significant predictive parameter in GBS patients. The Brighton criteria are helpful to confirm the diagnosis of GBS variants, evaluating different features (10). The patient's clinical presentation, CSF findings, nerve conduction studies evaluate and score between 1 and 4 (level 1: the highest certainty). Srivastava et al. (11) determined 66% of the COVID-19-related GBS patients as level 1, 24% as level 2, 6% as level 3, and 3% as level 4 according to the Brighton criteria. Our patient shows level 3 diagnostic acuity according to the Brighton criteria.

The mortality rate was reported as 5.8% in GBS cases after COVID-19. Partial and complete recovery was reported in 72% of patients (3).

Ferritin is one of the acute phase reactants as such C-reactive protein, haptoglobin, fibrinogen and it has a critical role in inflammation (12). Lino et al. (13) observed a strong relationship between serum ferritin level at the first days of hospital admission and mortality in COVID-19 patients. Some research focused that ferritin levels in the first seven days were the sign of early hyper inflammation secondary to cytokine storm (14). A meta-analysis showed that ferritin is a marker of progression to critical illness (15). So far, it has been emphasized that hyperferritinemia syndrome in the early days may help identify high-risk patients. In our case, hyperferritinemia persisted although other acute phase reactants returned to normal in the first month. It may be important in following the course of the disease in GBS cases after COVID-19.

Recurrent GBS (rGBS) is defined as 2 or more episodes of GBS that recur with ≥4 months intervals in incompletely healed cases and ≥2 months intervals in patients who fully recover. rGBS develops in 2-5% of patients with previous GBS (16,17). McDonnel et al. (18) reported a case of rGBS triggered by COVID-19, who had two GBS attacks before. GBS treatment-related fluctuation is defined as at least one grade worsening in disability scores within 2 months after discontinuation of immunotherapy (17). CIDP is characterized by a slow

progressive relapsing course that gradually worsens over 8 weeks (19). Suri et al. (20) defined acute onset CIDP as worsening of GBS symptoms after 8 weeks from onset or worsening attacks of the neurological findings at least 3 times. It is difficult to distinguish rGBS, treatment-related fluctuations, from CIDP. There may be spectrum-like transitions between rGBS and CIDP. Partial worsening of neurological findings developed after 4 months from the cessation of immune therapy in our patient. This is differing from treatment-associated fluctuation.
Although relapses reported in 2-5% of GBS patients (21), repeated clinical relapses may suggest a more chronic disease process or the diagnosis is an acute CIDP initially in this patient.

CONCLUSION

Post-COVID-19 GBS may occur in different patterns and it may progress to CIDP. GBS cases should be followed up in terms of recurrence and chronicity. Ferritin level may be a biochemical marker in the clinical follow-up of these cases.

Informed Consent: Written informed consent was obtained from the patient for publication.

Conflict of Interest: None declared by the authors.

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An Abnormality of Medial Plantar Nerve: A Rare Case Report

Mediyal Plantar Sinir Anormalliği: Nadir Bir Olgu Sunumu

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ABSTRACT

There are not many articles about the anatomical knowledge of the variable conditions of the sole of the foot, but normally knowledge of this area is essential for surgeries such as reconstructive or peripheral nerve compression syndrome. In this region, medial plantar nerve (nervus plantaris medialis) innervates the abductor and flexor muscles of the thumb. In addition, there is a region where receives the skin sensation in the foot. When it is damaged or exposed to pressure by any muscle due to an abnormal course, it can't stimulate the relevant muscles and the person may experience numbness and loss of muscle function on the sole of the foot. During a routine cadaver dissection, an abnormal course of nervus plantaris medialis was observed on the left side of a male cadaver. In this case, we presented the abnormal course of some branches of medial plantar nerve and discussed the clinical significance.
Keywords: Medial plantar nerve; tibial nerve; flexor digitorum brevis; abnormality; anatomy.

ÖZ

Ayak tabanının değişken koşullarının anatomik bilgisi hakkında pek fazla makale yoktur, ancak normalde bu bölgenin bilgisi, rekonstrüktif veya periferik sinir sıkışma sendromu gibi ameliyatlar için çok önemlidir. Mediyal plantar sinir (nervus plantaris medialis), bu bölgede başparmağa ait abdüktör kası ve fleksör kasları inerve eder. Ayrıca ayakta deri duyusunu aldığı bir bölge de mevcuttur. Hasarlandığında ya da anormal bir seyir sebebiyle herhangi bir kas tarafından basıya maruz bırakıldığında ilgili kasları uyaramaz ve kişi için ayak tabanında bir uyuşukluk ve kas fonksiyon kaybı söz konusu olabilir. Rutin bir kadavra diseksiyonu sırasında, bir erkek kadavranın sol tarafında anormal bir nervus plantaris medialis seyri gözlemlendi. Bu vakada mediyal plantar sinirin bazı dallarının anormal seyri gösterilmiş ve klinik önemi tartışılmıştır.
Anahtar kelimeler: Mediyal plantar sinir; tibial sinir; flexor digitorum brevis; anomali; anatomi.

INTRODUCTION

Lateral plantar nerve (LPN) and medial plantar nerve (MPN) arise from tibial nerve. Generally tibial nerve divides proximal to the tarsal tunnel as two branches (1). After the bifurcation, MPN travels parallel to the medial plantar artery that is one of the terminal branches of the posterior tibial artery (2). MPN is a larger one and after the flexor retinaculum, before appears between abductor hallucis muscle and flexor digitorum brevis muscle, it passes deep to adductor hallucis muscle (3). After that, it divides a proper digital plantar nerve and separates as three common digital plantar nerves. Flexor hallucis brevis, abductor hallucis and the first lumbrical are innervated by MPN (4). After the MPN gives its proper digital branches, it courses towards the inner half of the thumb. It is a nerve with a long course and is located more superficially, so a clinical picture called Joplin's neuroma can be seen. If the nerves are examined with ultrasound in pain that does not go away under the feet, it is noticed that the trunk and rafts of the MPN can be seen very clearly (5).

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There are few articles about the MPN. Even though there are some articles about the variation of the LPN (6).
In this case report, we presented the abnormal course of some branches of MPN and discuss the clinical significance of this variation.

CASE REPORT

During educational dissection for the medical students, the MPN was observed with its abnormal course on the left side of a Caucasian male cadaver. The superficial fascia and the plantar fascia were cautiously lifted. The bifurcation for MPN and LPN was observed under the distal part of the flexor retinaculum. The MPN and the artery which lies with MPN were located superficial to the first layer of the sole. They were found among the plantar fascia and abductor hallucis muscle in Figure 1. Although we know that the MPN lies below the flexor digitorum brevis muscle and then branches towards the phalanges, the branches to phalanges I-IV were described above the muscle as shown in Figure 2. We could not observe any different abnormality in the other parts.

DISCUSSION

We presented the abnormal course and some branches of MPN. Until flexor digitorum brevis, the course was normal. MPN was observed to behave like cutaneous branches. Similar cases like this have been seen to be rare (5,6). Knowledge of the variant conditions of the MPN can prevent from undesirable situations during surgery such as reconstructive surgery which is for island pedicle flap (7). On the other hand, the position course of MPN is very important for peripheral nerve entrapment treatment and peripheral neuropathies affecting the peripheral nervous system like a distal sensory polyneuropathy (8,9). One of the most common problems associated with the MPN is neurofibroma, and one of the most common causes is hypertrophy of the nerve. It presents with difficulty in walking, difficulty in standing, and a painful period. Therefore, it can be confused with compression caused by a variation of the MPN (10). MPN tumors are most common in peripheral nerve sheaths, and therefore, conditions that present with pain under the foot should be emphasized if there is an abnormal course and therefore compression. Swelling and edema developing on the MPN trace can sometimes be a sign of a tumor with a variation (11).

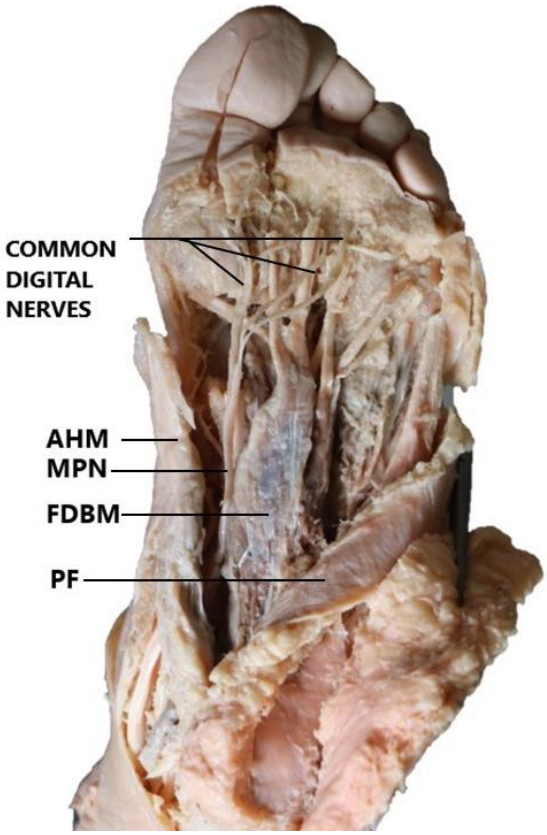

Figure 1. Abnormal course of the medial plantar nerve
MPN: medial plantar nerve (nervus plantaris medialis), AHM: abductor hallucis muscle, FDBM: flexor digitorum brevis muscle, PF: plantar fascia

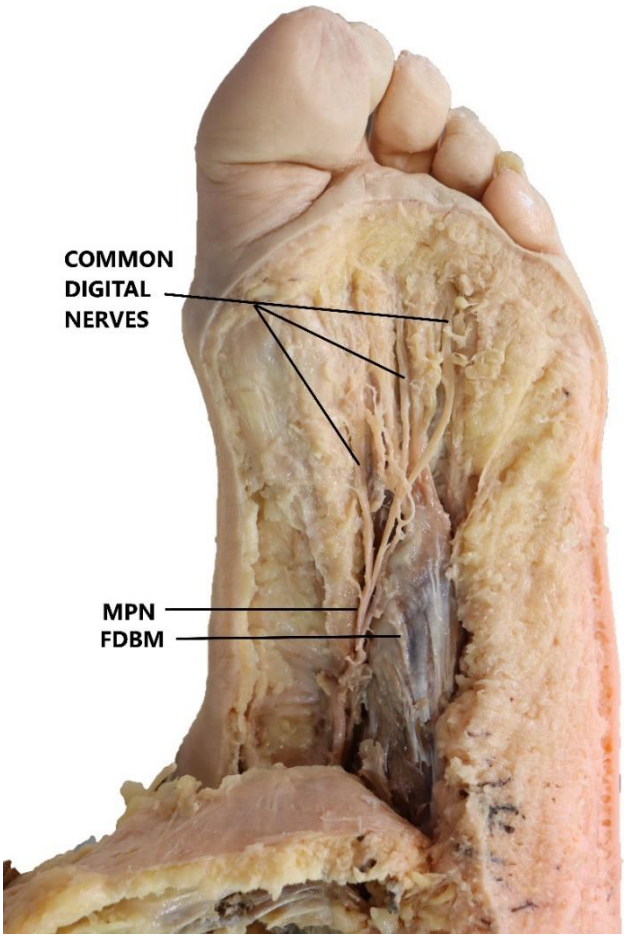

Figure 2. Abnormal course of the medial plantar nerve
MPN: medial plantar nerve (nervus plantaris medialis), FDBM: flexor digitorum brevis muscle

Informed Consent: Since our study was a case report that includes a cadaveric study, there was no consent form.

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ETİK SORUMLULUK

- "İnsan" öğesini içeren tüm çalışmalarda Helsinki Deklerasyonu Prensipleri'ne (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) uygunluk aranır. Bu tip çalışmalarda yazarların, GEREÇ VE YÖNTEMLER bölümünde çalışmayı bu prensiplere uygun olarak yaptıklarını, kurumlarının etik kurullarından onay ve çalışmaya katılmış insanlardan "bilgilendirilmiş olur" (informed consent) aldıklarını belirtmeleri gerekmektedir.
- Çalışmada "Hayvan" öğesi kullanılmış ise yazarların, GEREÇ VE YÖNTEMLER bölümünde Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) prensipleri doğrultusunda çalışmalarında hayvan haklarını koruduklarını ve kurumlarının etik kurullarından onay aldıklarını belirtmeleri gerekmektedir.
- Olgu sunumlarında hastalardan "bilgilendirilmiş olur" (informed consent) alınmalıdır.
- Etik kurul onay bilgisi GEREÇ ve YÖNTEMLER bölümünde kurul adı, onay tarihi ve sayısı ile birlikte belirtilmelidir.
- Eğer çalışmada direkt-indirekt ticari bağlantı veya maddi destek veren kurum mevcut ise yazarlar; kullanılan ticari ürün, ilaç, firma vb. ile ticari hiçbir ilişkisinin olmadığını veya varsa nasıl bir ilişkisinin olduğunu (konsültan, diğer anlaşmalar), editöre sunum sayfasında belirtmelidirler.
- Yazarlar çalışma ile ilgili kişisel ve finansal tüm ilişkilerin bildirilmesinden sorumludur. Makalenin başvurusu ve/veya değerlendirmesi ile ilişkili herhangi bir çıkar çatışması olup olmadığının açıkça beyan edilmesi gerekmektedir.
- Makalelerin bilimsel ve etik kurallara uygunluğu yazarların sorumluluğundadır.

BAŞVURU DOSYALARI

Makaleler aşağıda belirtilen şekilde ayrı dosyalar halinde sisteme yüklenmelidir.

Telif Hakkı Devir Formu: Başvuru sırasında sistemden alınacak Telif Hakkı Devir Formu tüm yazarlar tarafından makaledeki yazar sıralamasına uygun şekilde imzalanmış olmalıdır.

Başvuru Mektubu: Makalenin türü, daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmadığı, varsa çalışmayı maddi olarak destekleyen kişi ve kuruluşlar ve bu kuruluşların yazarlarla olan ilişkileri (yoksa olmadığı) belirtilmelidir. Makalenin konusuyla ilgili olarak önerilen, yazarlarla ve kurumlarıyla ilgisi olmayan en az iki hakemin adları, akademik unvanları, kurumları, iletişim bilgileri ve e-posta adresleri yazılmalıdır. Editörlerin hakemleri seçme hakkı saklıdır.

Başlık Sayfası: Makalenin başlığını (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, tüm yazarların adlarını, akademik unvanlarını, ORCID® numaralarını, kurumlarını, e-posta adreslerini ve ayrıca sorumlu yazarın adını, yazışma adresini, telefon numarasını, e-posta adresini içermelidir. Makale daha önce bilimsel bir toplantıda sunulmuş ise toplantı adı, tarihi ve yeri (yoksa sunulmadığı) belirtilmelidir.

Ana Metin: Makalenin başlığı (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, Öz (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), Ana Metin (gönderilen makalenin türüne uygun olarak bölümlere ayrılmış), Kaynaklar, Tablolar ve Şekil açıklamaları yer almalıdır.

Etik Kurul Onay Belgesi: Tüm araştırma makaleleri için Etik Kurul Onay Belgesi ayrı bir dosya olarak yüklenmelidir.

Not: Makalede şekil, resim veya fotoğraf varsa bunların da her biri ayrı birer dosya olarak yüklenmelidir.

MAKALE TÜRÜNE GÖRE KULLANILMASI GEREKEN BÖLÜMLER

Araştırma Makalesi

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, GEREÇ VE YÖNTEMLER, BULGULAR, TARTIŞMA, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 200-250 kelime arasında olmalıdır.

ABSTRACT, "Aim, Material and Methods, Results, Conclusion" şeklinde yapılandırılmalıdır.

ÖZ, "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç" şeklinde yapılandırılmalıdır.

Derleme (Sadece Davetli)

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, Konu ile İlgili Alt Başlıklar, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 150-200 kelime arasında olmalıdır.

Olgu Sunumu

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, OLGU SUNUMU, TARTIŞMA, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 100-150 kelime arasında olmalıdır.

Diğer

Bu üç temel makale türü dışındaki (editöre mektup, editöryel yorum/tartışma vb.) yazıların hazırlanmasında da genel yazım kuralları geçerlidir. Bu tür yazılarda başlık ve öz bölümleri yoktur. Kaynak sayısı 5 ile sınırlıdır. İthaf olunan makale sayı ve tarih verilerek belirtilmelidir. Yazının sonunda yazarın ismi, kurumu ve adresi yer almalıdır. Mektuba cevap, editör veya makalenin yazarları tarafından, yine dergide yayınlanarak verilir.

YAZARLARA BİLGİLENDİRME

YAZIM KURALLARI
- Makaleler Microsoft Word® belgesi olarak hazırlanmalıdır.
- Sayfa kenarlarında 2,5 cm boşluk bırakılmalıdır.
- Sayfa numaraları sayfanın sağ alt köşesine yerleştirilmelidir.
- Tüm metinler 12 punto Times New Roman karakteri kullanılarak çift satır aralığı ile sola hizalanmış olarak yazılmalıdır.

ANAHTAR KELİMELER
- Anahtar kelime sayısı en az 2 olmalı, kelimeler birbirlerinden noktalı virgül (;) ile ayrılmalıdır.
- Türkçe anahtar kelimeler Türkiye Bilim Terimleri (TBT)'ne (http://www.bilimterimleri.com), İngilizce anahtar kelimeler Medical Subject Headings (MESH)'e (http://www.nlm.nih.gov/mesh/MBrowser.html) uygun olarak verilmelidir.

İSTATİSTİKSEL YÖNTEMLER
- Tüm araştırma makaleleri biyoistatistik açıdan değerlendirilmeli ve uygun plan, analiz ve raporlama ile belirtilmelidir. Bu makalelerde, GEREÇ VE YÖNTEMLER bölümünün son alt başlığı "İstatistiksel Analiz" olmalıdır.
- Bu bölümde çalışmada kullanılan istatistiksel yöntemler ne amaçla kullanıldığı belirtilerek yazılmalı, istatistiksel analiz için kullanılan paket programlar ve sürümleri belirtilmelidir.
- p değerleri ondalık üç basamaklı (p=0,038; p=0,810 vb.) olarak verilmelidir.
- Makalelerin biyoistatistik açıdan uygunluğunun kontrolü için ek bilgi www.icmje.org adresinden temin edilebilir.

KISALTMALAR
- Terim ilk kullanıldığında parantez içinde kısaltmayla birlikte açık olarak yazılmalı ve tüm metin boyunca aynı kısaltma kullanılmalıdır.
- Uluslararası kullanılan kısaltmalar Bilimsel Yazım Kurallarına uygun şekilde kullanılmalıdır.

TABLOLAR VE ŞEKİLLER
- Metinde ilgili cümlenin sonunda (Tablo 1) ve/veya (Şekil 1) şeklinde belirtilmelidir.
- Tablolar (başlıklarıyla birlikte) ve şekiller (açıklamalarıyla birlikte) kaynaklardan sonra ve her biri ayrı bir sayfada olacak şekilde metnin sonuna eklenmelidir.
- Tablo başlıkları tablo üstünde (Tablo 1. Tablo başlığı), şekil açıklamaları ise şeklin altında (Şekil 1. Şekil açıklaması), ilk harfleri büyük olacak şekilde yazılmalıdır.
- Tablolarda ve şekillerde kısaltma veya sembol kullanılmış ise altında dipnot olarak açıklanmalıdır.
- Şekiller ve fotoğraflar, .png, .jpg vb. formatta ve en az 300 dpi çözünürlükte ayrı dosyalar halinde yüklenmelidir.
- Şekil ve fotoğraf alt yazıları, son tablonun olduğu sayfadan sonra, ayrı bir sayfada sırasıyla verilmelidir.
- Daha önce basılmış şekil, resim, tablo, grafik vb. kullanılmış ise yazılı izin alınmalı ve açıklama olarak belirtilmelidir. Bu konudaki hukuki sorumluluk yazarlara aittir.

TEŞEKKÜR
- Eğer çıkar çatışması/çakışması, finansal destek, bağış ve diğer bütün editöryel (İngilizce/Türkçe değerlendirme) ve/veya teknik yardım varsa, bu bölümde, KAYNAKLAR bölümünden önce belirtilmelidir.

KAYNAKLAR
- Kaynaklar, kullanım sırasına göre numaralandırılmalı ve metin içinde ilgili cümlenin sonunda parantez içinde numaralarla (1) veya (1,2) veya (3-5) şeklinde verilmelidir.
- Kaynaklar dizini, metin içinde kaynakların kullanıldığı sıraya göre oluşturulmalıdır.
- Yazar sayısı 6 veya daha az ise tüm yazarlar belirtilmeli, 7 veya daha fazla ise ilk 6 yazar belirtildikten sonra "et al." eklenmelidir.
- Kongre bildirileri, kişisel deneyimler, basılmamış yayınlar, tezler ve internet adresleri kaynak olarak gösterilmemelidir.
- DOI tek kabul edilebilir online referanstır.

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