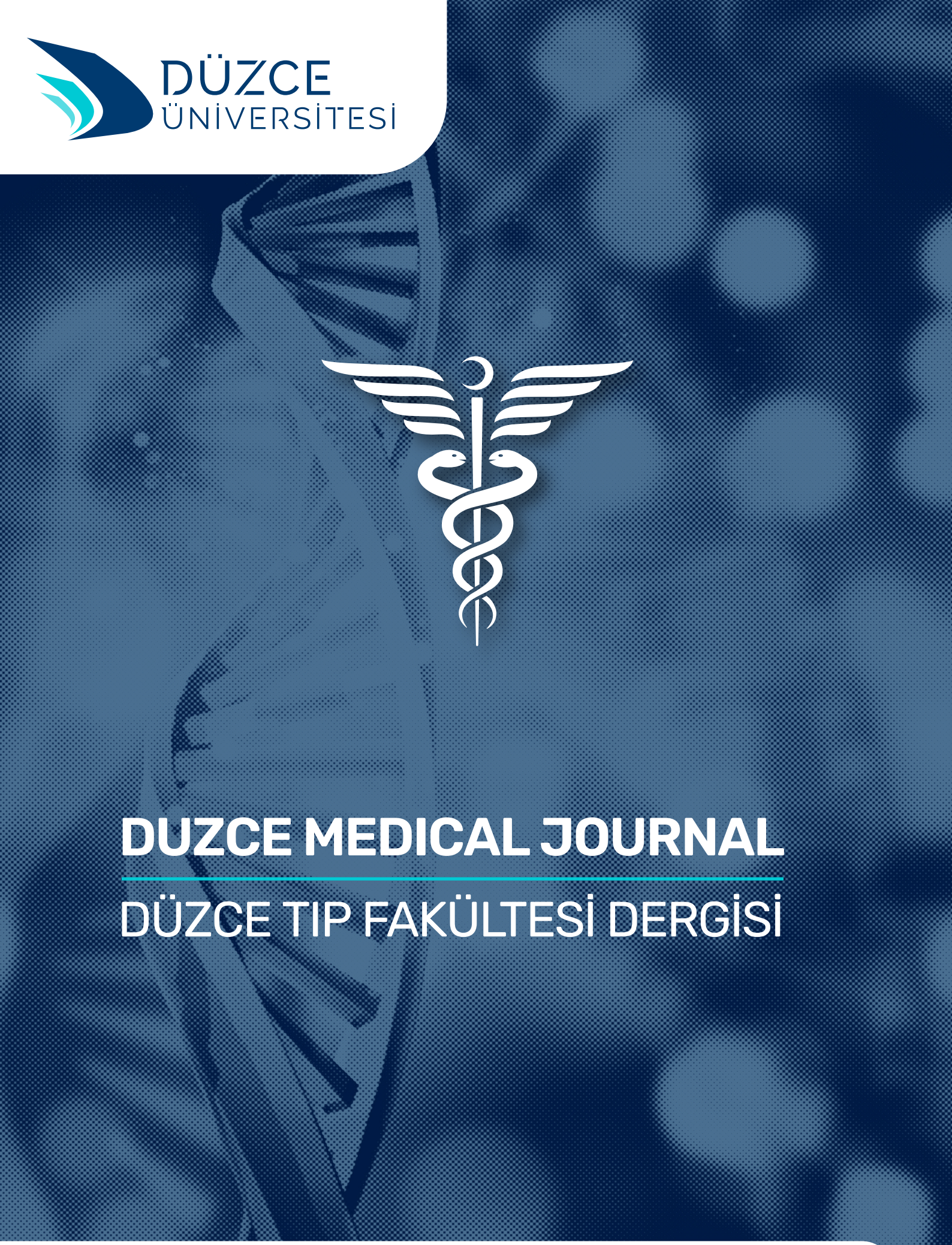

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Conscious and Correct Use of Biostatistical Methods in Medical Researches: From Planning to Reporting the Results - Part II

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

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ABSTRACT

In this part of the review, statistical tests utilized to examine hypotheses regarding population parameters on a representative sample, which forms the fundamentals of inferential statistics are discussed. The selection of an appropriate statistical test by verifying its assumptions and interpreting the results objectively is crucial for obtaining accurate conclusions. Understanding the terms related to type I and type II errors, p-value, power of the study, effect size, and confidence interval will contribute to the correct interpretation of both the results obtained from statistical tests in scientific research and the findings of articles read from the literature. In addition to univariate tests, the three most commonly employed multiple regression models are also addressed to control for the effect of potential confounding factors and other independent variables utilized in the study. Statistical computing has become much more accessible in recent times, with researchers having access to freeware packages or web applications to perform basic and advanced statistical analyses. Researchers frequently focus on the calculation of statistical tests used in data analysis, whereas, understanding the rationale behind statistical methods should be the primary goal. Therefore, this review emphasizes the logic of selecting appropriate statistical methods and interpreting the results rather than mathematical calculations. It is essential to recognize that biostatistical principles should be considered not only in the data analysis phase but also in all phases of research, from planning to report writing. It should be note that, no statistical analysis method can correct erroneous data obtained from a poorly designed study.
Keywords: Hypothesis tests; regression model; ANOVA; parametric tests; nonparametric tests; p-value.

ÖZ

Derlemenin bu bölümünde, çıkarımsal istatistiğin temelini oluşturan, popülasyonu temsil eden bir örneklem üzerinde popülasyon parametrelerine ilişkin hipotezleri incelemekte kullanılan istatistiksel testler ele alınmıştır. Varsayımlarının karşılanıp karşılanmadığı kontrol edilerek uygun istatistiksel testin seçilmesi ve bulguların yansız bir şekilde yorumlanması doğru sonuçlara ulaşılmasında çok önemlidir. Tip I ve tip II hatalar, p-değeri, çalışmanın gücü, etki büyüklüğü ve güven aralığı ile ilgili kavramların ne anlama geldiğini anlamak, hem bilimsel araştırmalarda istatistiksel testlerden elde edilen sonuçların hem de literatürden okunan makalelerin bulgularının doğru yorumlanmasına katkı sağlayacaktır. Tek değişkenli testlerin yanı sıra, olası etki karıştırıcı faktörlerin ve çalışmada ele alınan diğer bağımsız değişkenlerin etkisini kontrol etmek amacıyla en yaygın olarak kullanılan üç çoklu regresyon modeline de değinilmiştir. Araştırmacılar, temel ve ileri düzey istatistiksel analizleri yapabilecekleri ücretsiz yazılımlara ya da web uygulamalarına erişebildiklerinden, istatistiksel hesaplamaların yapılması günümüzde çok daha erişilebilir hale gelmiştir. Araştırmacılar daha çok veri analizinde kullanılan istatistiksel testlerin nasıl hesaplanacağı üzerinde durmaktadır, oysaki istatistiksel yöntemlerin arkasındaki mantığı anlamak birincil hedef olmalıdır. Bu nedenle, bu derlemede matematiksel hesaplamalardan ziyade, uygun istatistiksel yöntemlerin seçilmesi ve bulguların yorumlanması mantığı üzerinde durulmuştur. Biyoistatistik prensiplerin sadece veri analizi aşamasında değil, planlamadan rapor yazımına kadar araştırmanın tüm aşamalarında dikkate alınması gerektiğinin farkında olunması önemlidir. Unutulmamalıdır ki, iyi düzenlenmemiş bir araştırmadan elde edilen hatalı verileri düzeltecek bir istatistiksel analiz yöntemi bulunmamaktadır.
Anahtar kelimeler: Hipotez testleri; regresyon modeli; ANOVA; parametrik testler; parametrik olmayan testler; p-değeri.

INTRODUCTION

Studies that incorporate appropriate biostatistical support, from design to analysis and reporting, are the most effective experimental studies; therefore, biostatistics plays a critical role in health research. The most valuable insights into complex causal relationships can be obtained through the application of various statistical methods. As a result, as in other research fields, researchers increasingly need to use biostatistics in their study of medical sciences.

The impact of statistical sciences on medical and biological sciences has increased rapidly over the past few decades. Physicians must have knowledge of statistical principles and techniques, as the information they use in clinical decision-making is always based on the results of statistical analyses. However, conclusions drawn from statistical evidence can be incorrect or misleading, and without sufficient knowledge and understanding of statistics, physicians may not be able to make the most appropriate decisions (1-3).

When research is conducted on all subjects in the population, the population parameters are calculated without error; however, as is known, research is usually conducted on a small sample representing the population of all subjects instead of the population. When the sample is drawn to represent the population, inferences are made about the population with the help of statistics calculated from the sample, and the obtained result is generalized to the population. The sample can only be representative of the population if the sample is drawn using probability sampling methods and the sample size is sufficient.

If the sample is not representative of the population, misleading results will be obtained and this cannot be corrected by statistical procedures. On the other hand, even in a well-designed study, incorrect results may be obtained due to random variation in the sample. Thus, results from a single sample have statistical uncertainty, which is strongly related to the sample size (4).

In this part, hypothesis testing and statistical modeling will be discussed. Firstly, how to determine the appropriate test from univariate statistical tests is emphasized, and then how to select appropriate multiple analyses to eliminate the effect of other possible confounders is discussed. In addition, concepts such as standard error, confidence interval, type I error, type II error, p-value, power, etc. are also explained.

Standard Error

The standard error is a measure of the uncertainty of a test statistic and is an estimate of how much the value of the test statistic varies from sample to sample. The standard error is also defined as the difference between the population parameter (e.g. population mean μ) and the statistic calculated from the sample (e.g. sample mean $\bar{x}$) and is also referred to as sampling error. The standard error is obtained by taking the standard deviation of the sampling distribution of the statistics calculated from all possible samples of size n that can be drawn from the population.

The formula for estimating standard error is

$$Standard\ Error = Standard\ Deviation / \sqrt{Sample\ Size}$$

The equation demonstrates that the standard error decreases as the sample size increases. The standard error is used for hypothesis testing, confidence interval calculations, etc.

Confidence Interval

In inferential statistics, there are two different approaches to estimating population parameters: point estimation and interval estimation. In point estimation, a statistic such as mean, standard deviation, ratio, correlation coefficient, etc. calculated from the sample is used to estimate the population parameter. The probability that point estimation will precisely determine the population parameter is quite low. On the other hand, an interval estimation is a range of values that most likely contains the parameter being estimated with a given confidence level. Therefore, interval estimation is preferred in inferential statistics.

In order to calculate confidence intervals, statistics obtained from the sample, standard error, and two-tailed table statistics values obtained from the relevant theoretical distribution for the specified confidence level are needed. In statistics, a 95% confidence level is most commonly used. Confidence intervals for the population parameter are obtained using the following general notation.

$$Statistic \pm Table\ Value \times Standard\ Error$$

For example, the 95% confidence interval for an unknown population mean is given as follows:

$$\bar{x} - t_{1-\alpha/2} \times Standard\ Error \leq \mu \leq \bar{x} + t_{1-\alpha/2} \times Standard\ Error$$

Confidence intervals are directly related to hypothesis testing and sample size (or power) calculation. If, for example, the confidence intervals for the mean difference between two independent groups include 0, it indicates that there is no significant difference between the group means, whereas if they do not include 0, it indicates a significant difference between the group means. In addition, the width of the confidence intervals provides information about the sample size of the study. If the sample size in the study is small, this will cause the standard error to increase and the confidence interval to widen. Although confidence intervals are mostly symmetric, non-symmetric confidence intervals are obtained for some statistics. For example, the confidence intervals of odds ratio and relative risk used to calculate risk in health sciences are not symmetric.

HYPOTHESIS TESTING

Hypotheses are propositions concerning the population parameters from which samples are drawn. One of the objectives of inferential statistics is to test hypotheses about unknown phenomena within a population. Hypothesis testing aims to make inferences about the population parameters of interest using data obtained from a representative sample of the population.

Hypothesis testing plays a crucial role in evidence-based medicine, wherein clinical decisions are based on research findings. This contributes to increasing the accuracy of clinical decisions by ensuring that conclusions are derived objectively from the research data.

Due to the utilization of data from randomly selected samples in hypothesis testing, each result inherently carries the potential for error (type I error or type II error). Consequently, these tests cannot be employed to verify any phenomenon definitively. For instance, depending on the sample selected, one study may conclude that influenza vaccination is efficacious in disease prevention, while

another study may determine it is not. Therefore, hypothesis testing cannot be utilized to conclusively prove that influenza vaccination prevents (or does not prevent) the disease, but can be employed to support this hypothesis. However, when multiple studies on a specific topic yield similar results, they are generally considered to be valid. As a result, the findings of systematic reviews and meta-analyses are utilized in the development of diagnostic and treatment protocols, rather than relying on the results of a single study.

Parametric and Nonparametric Hypothesis Tests

Hypothesis tests are divided into parametric and nonparametric (or distribution-free) tests. Which test will be applied in the studies is decided according to whether the parametric test assumptions are met or not.

Methods that require assumptions about the distribution of variables in the population are called parametric methods. For example, in the independent samples t-test, it is assumed that the observations in the two sample groups are drawn from normally distributed populations. All parametric tests of continuous numerical variables require the assumption of a normal distribution.

Methods that do not assume the distribution of variables in the population are called nonparametric methods. Instead of using the original measurements in the data set, these methods use the rank or sign of data. When the data type is ordinal, parametric methods should not be used, considering the data as continuous numerical data.

When parametric test assumptions are satisfied, parametric tests are known to have higher power than their alternative nonparametric tests. Therefore, when the assumptions are not satisfied in studies with a large number of observations, appropriate transformations are applied to the data before using nonparametric methods. If the assumptions are satisfied after the transformation, parametric tests are applied, and if not, nonparametric methods are applied (5,6).

Formulation of Hypotheses and Steps of Hypothesis Testing

The first stage of hypothesis testing is the formulation of statistical hypotheses in accordance with the aim of the study. There are two hypotheses in hypothesis testing; the null and alternative hypotheses denoted by H_0 and H_1. The H_0 hypothesis is called the null hypothesis because it is always stated that there is no difference between groups or there is no relationship between variables, etc. The H_1 hypothesis contains inequality and can be one or two-tailed. The conclusion drawn from the hypothesis test depends on whether the hypothesis is one-tailed or two-tailed. A null hypothesis that can not be rejected in a two-tailed test can be rejected in a one-tailed test. Therefore, it is important for researchers to set up their hypotheses appropriately at the beginning of the study. If there is an expectation that the parameter related to one group will be larger or smaller than the other, a one-tailed hypothesis should be formed, and if it is only intended to examine whether there is a difference, a two-tailed hypothesis should be formed.

In the second stage of hypothesis testing, the appropriate test statistic is decided and calculations are performed. The appropriate hypothesis test is decided according to the data type of the variable of interest (quantitative or qualitative), the number of groups, whether the groups are dependent or independent, and whether the parametric test assumptions are met. Figure 1, Figure 2, and Figure 3 illustrate which statistical test is appropriate in which situation. After deciding on the appropriate test, test statistics are calculated with different formulas for each hypothesis test (5,7-9).

In the last stage, it is assessed whether the acquired result is significant or not using the predetermined significance level. At the end of each test, a test statistic is calculated that fits a theoretical statistical distribution such as z, t, F, chi-square, U, etc. Based on the value of the calculated test statistic and the corresponding critical value or p-value derived from the theoretical statistical distribution, the null hypothesis H_0 is either rejected or not rejected (9).

When statistically significant differences are observed in One-way ANOVA, Kruskal-Wallis, ANOVA in repeated measures, or Friedman tests, post hoc (pairwise or multiple comparisons) tests should be performed to determine the group(s) that cause the difference.

In univariate hypothesis testing, only the relationship or difference between the dependent variable and the independent variable is examined. The effect of potential confounding variables is not considered. Consequently, interpreting findings based solely on the results of univariate analyses may lead to erroneous conclusions. Interpreting the results of appropriate regression models that incorporate variables found to be significant in univariate analyses, along with confounding variables, will yield more accurate results.

Type I error (α) and Type II error (β)

There are two types of errors in hypothesis testing;

• *Type I error (α):* reject a null hypothesis H_0 that is actually true in the population

• *Type II error (β):* fails to reject a null hypothesis H_0 that is actually false in the population

The values α and β are probabilities and take values between 0 and 1. The difference of these probabilities from 1 is called the confidence level (1-α) and the power of the test (1-β), respectively. In general, a maximum value of 0.05 for α, and a minimum value of 0.20 for β (or 0.80 for power, 1-β) are used in research.

Although type I and type II errors can never be completely eliminated, the researcher can reduce their likelihood by increasing the sample size (10). Type II error usually occurs when the statistical power of a test is low. In the hypothesis testing process, since all analyses are performed under the assumption that the null hypothesis H_0 is true, the probability of committing a type I error is calculated. The researcher has no control over type II error (β) in the hypothesis testing process. Type II error is taken into account when calculating the sample size at the beginning of the study.

p-value

Since all hypothesis tests are performed under the assumption that the null hypothesis, H_0, is true, we are interested in the probability of committing a type I error. While the α value is determined by the researcher at the beginning of the study, the p-value is obtained based on the test statistic calculated at the end of the study. The p-value indicates the probability of falsely rejecting the true null hypothesis. In other words, the p-value indicates the probability that the observed outcomes were obtained by chance, assuming the null hypothesis H_0 is true. Since a value of 0.05 is generally considered a type I error (α), the H_0 hypothesis is rejected when the p-value obtained from the test is less than 0.05.

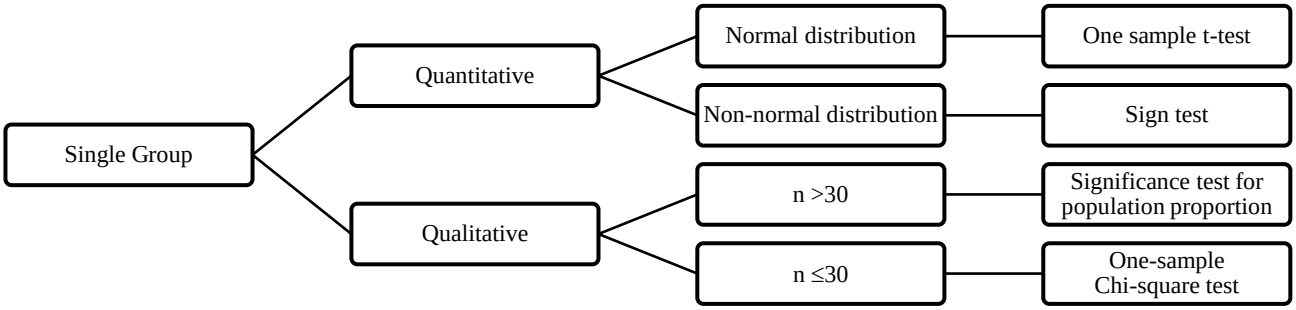

Figure 1. Flowchart for choosing appropriate statistical test for one group

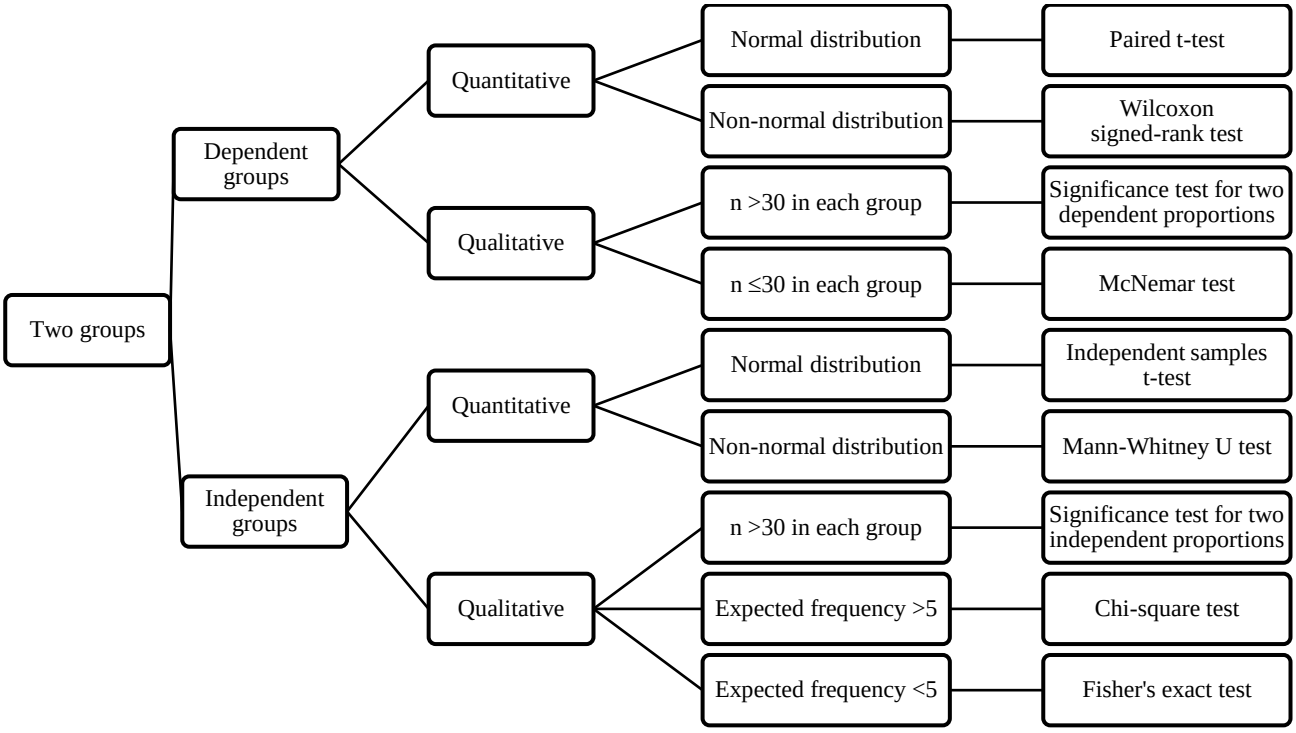

Figure 2. Flowchart for choosing appropriate statistical test for two groups

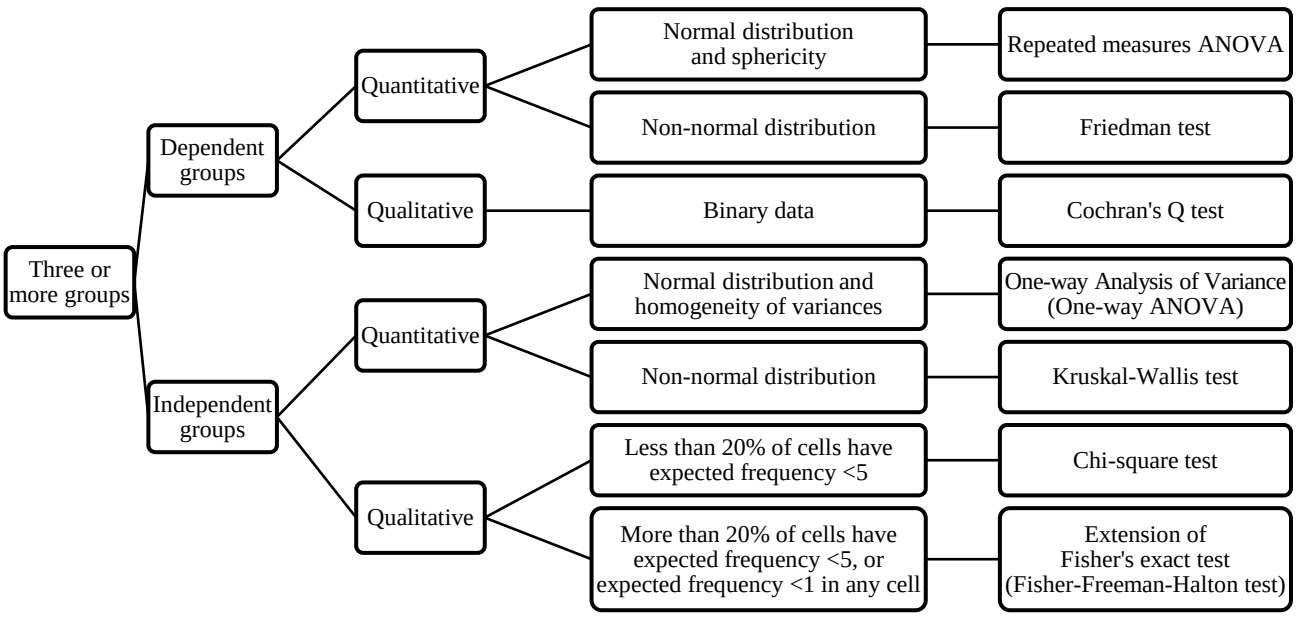

Figure 3. Flowchart for choosing appropriate statistical test for three or more groups

When reporting the p-value obtained from the test, the exact p-value (e.g. p=0.180, p=0.018, p=0.108, p=0.810) should be written in detail in the tables and the text. However, when the obtained p-value is small, it should be reported as p<0.001 instead of p=0.000.

Even small differences can be found to be significant when the number of observations in the study is too large. In this case, researchers should determine whether the statistically significant difference is also clinically significant. On the other hand, when the number of observations in the study is small, the difference or relationship that actually exists may not be determined since not enough samples are included in the study. Here, the power of the test will decrease as the probability of making a β-type error will increase (11).

Effect Size

One of the issues with hypothesis tests is that significance does not indicate the clinical or practical significance of an effect. To address this drawback, the size of the effect being investigated should be measured in a standardized method.

Effect size is a statistical measure of the magnitude of the difference between groups or association between variables observed in a study. In addition to the statistical significance decided by the p-value, it allows the clinical significance of an outcome to be assessed. While the p-value indicates whether an effect exists, the effect size indicates how large or practically important this effect is (5,9,12).

The most commonly used type of effect size in the literature are Cohen's d, Pearson correlation coefficient r, partial eta-square, and odds ratio. In recent years, it has become common to present effect size and/or confidence intervals in tables in scientific articles in the field of health sciences (12).

TYPES OF ANOVA MODELS

An ANalysis Of VAriance (ANOVA) is an appropriate statistical technique for determining the differences between independent groups on a continuous measurement. Different ANOVA models are used depending on the purpose of the study and the research design. The most commonly used ANOVA model is the one-way ANOVA (one-way between-subjects ANOVA) model, which is used to examine the effect of a factor with three or more categories on a quantitative dependent variable (13).

One-way ANOVA requires the assumption of normal distribution and homogeneity of variances. If the assumptions of normal distribution and homogeneity of variances are not met, the nonparametric Kruskal-Wallis test and Welch's ANOVA are used, respectively.

Factorial between-factor ANOVA is used to examine the effect of two or more factors on a quantitative dependent variable. When groups are created based on multiple dimensions, variations in the means can be attributed to multiple factors. For example, a three-way ANOVA can be used to analyze how treatment type, age group, and gender influence the length of hospital stay. When conducting a factorial ANOVA to examine the effects of two or more factors, it is important to include interaction terms along with the main effects. The use of one-way or two-way ANOVA models is common because the increase in number of factors makes it hard to interpret the model.

In certain designs, the tested means come from the same subjects measured at different occasions, rather than from independent groups of subjects. A within-subjects ANOVA, also known as a repeated measures ANOVA, is commonly applied in pretest-posttest designs but is not restricted to just two time points. This analysis can be used to assess differences across two or more time periods. In repeated measures analysis of variance, in the presence of independent groups, both between-group and within-group differences can be examined simultaneously. When the group × time interaction is statistically significant in this analysis, the main effects of group and time should not be interpreted directly. The change of each group over time, and the difference between groups at each time point should be examined separately and p-values are corrected for multiple comparisons (14).

An analysis of covariance (ANCOVA) is suitable for evaluating the differences in terms of a continuous dependent variable among groups while accounting for the effect of covariates. Covariates have an effect on the dependent variable. There are two ways to control their effects, either by selecting samples during the research design to make the groups similar in terms of covariates, or by statistical methods during the data analysis process. ANCOVA is a type of ANOVA that enables the effects of covariates to be controlled through the use of regression analysis. In ANCOVA, in addition to the assumptions of the analysis of variance, there are assumptions that the independent variable and the covariate are independent, the relationship between the covariate and the dependent variable is linear, and the slope of the regression lines is similar in all groups (12,13).

Multivariate analysis of variance (MANOVA) is a statistical technique used to examine the effect of one or more factors on two or more numerical dependent variables. In other words, by comparing the mean vectors between groups, it is investigated whether there is a difference in terms of two or more dependent variables. The general assumptions of MANOVA are that the variance-covariance matrices of all groups are homogeneous and that the variables in each group have a multivariate normal distribution (13,14).

CORRELATION ANALYSIS

Correlation analysis examines whether there is a relationship between two variables, and if so, the strength and direction of the relationship. Correlation coefficients generally range between -1 and +1; as they approach -1 or +1, the strength of the relationship increases, while as they approach 0, the strength of the relationship decreases. If the sign of the coefficient is negative, it indicates that as the value of one variable increases, the value of the other variable decreases. The significance of the correlation value obtained is tested and if the result is significant, the strength of the relationship is interpreted. Otherwise, it is stated that there is no relationship between the variables.

Depending on the type of data and whether the assumptions are met, different correlation coefficients are calculated to determine the relationship between variables. Pearson and Spearman rank-order correlation coefficients are commonly used to examine the relationship between two quantitative variables. Pearson correlation coefficient is used if the relationship between variables is linear and

the assumption of normal distribution is met. There are also correlation coefficients for qualitative variables such as Phi, Cramer's V, Somer's d, etc. However, these correlation coefficients are rarely presented in studies since the chi-square test is often used to examine the relationship between qualitative variables (9).

In order to examine the relationship between numerical variables, the first step is to draw a scatter plot. The linearity of the relationship between the variables and the presence of outliers in the data that would cause misinterpretation of the relationship should be checked. A correlation coefficient does not indicate a cause-and-effect relationship between two variables. It only indicates whether the variables change together in the same direction or the opposite direction.

The relationship between more than two variables can also be examined. For example, the relationship between one dependent and two or more independent variables can be examined by multiple correlation coefficients, and the relationship between more than two sets of dependent and independent numerical variables can be examined by canonical correlation.

Due to indirect relationships, a significant correlation can be found between two variables that are not actually correlated. In this case, the partial correlation coefficient is used to examine whether there is a relationship between the variables by controlling for the effect of the covariate that has an effect on both variables.

REGRESSION MODELS

Regression analysis is a useful method for determining the relationship between a dependent variable and a group of independent variables. The overall goal of regression analysis is to predict the value of an outcome variable of interest using variables that are simple to measure, provide minimal risk to participants, can be collected in a shorter period or at a lower cost, etc. The variable whose value is being estimated in the model is called the dependent variable (response or outcome variable) and the variables used to explain the dependent variable are called independent variables (predictors or explanatory variables).

In addition to making predictions, regression analysis is also used to determine the important factors affecting the dependent variable or to determine which variables have a greater effect on the dependent variable and to make adjustments for covariates.

Depending on the type of dependent variable, the nature of the relationship between variables, the presence of censored observations, etc., many different regression analysis methods have been developed: linear regression, non-linear regression, logistic regression, Poisson regression, Cox regression, etc.

Linear Regression

Linear regression analysis is the most widely used regression analysis method in the literature. In order to use linear regression, the dependent variable must be a continuous numerical data type and the relationship between the dependent and independent variables should be linear.

The dependent variable should always be a continuous numerical variable with a normal distribution. The type of independent variables can be either quantitative (continuous numeric or discrete numeric) or qualitative (ordinal or nominal). Nominal qualitative variables are included directly in the model if they have only two categories (e.g. yes-no), but if they have more than two categories, they should be included in the model after being coded as a dummy variable. In dummy variable coding, the nominal variable is converted into a set of binary variables. Ordinal variables can be included in the model as numerical variables if there is an equal distance between categories, otherwise, they can be included as nominal variables (after coding as dummy variables).

The regression coefficients obtained for independent variables of the continuous numeric variable type indicate the average change in the dependent variable caused by a 1-unit change in the independent variable. When the independent variable is qualitative data, it shows how much the other categories differ on average from the reference category.

In articles in the field of health sciences, only the regression coefficients, confidence intervals, and their significance are commonly presented. The results on whether the assumptions of the model are met and the goodness of fit of the model are not sufficiently presented. Therefore, to evaluate the performance of the model, statistics such as mean squared error, root mean squared error, information criterion, or coefficient of determination (R^2) should also be given. Furthermore, in multiple linear regression, it is essential to assess the normality of residuals, identify influential and outlier observations, and examine for heteroscedasticity and multicollinearity.

The coefficient of determination (R^2) is often used to assess model fit. The coefficient of determination takes values between 0 and 1. It shows the percentage of the change in the dependent variable explained by the independent variables. If the regression model is to be used for prediction, a high R^2 value is required. In addition to assessing the overall fit of the model using measures such as the F test and R^2, the data should be examined for outliers and influential observations. To identify outliers, scatter plots of the standardized residuals and the estimated values (or the dependent variable) are drawn. Observations with values outside the ±2 limits in the scatter plot are usually identified as outlier observations. If the residuals are randomly scattered around 0 in this scatter plot, it indicates that the linear model is appropriate and there is no problem of heteroscedasticity. Cook's distance is used to identify influential observations that have an effect on the regression coefficients. Observations with a Cook's distance value above 1 are observations that are highly influential on the regression coefficients.

A multicollinearity problem occurs when a high degree of correlation exists among the independent variables in a multiple linear regression model. The variance inflation factor (VIF) and tolerance statistics can be utilized to determine the presence of a multicollinearity issue. A multicollinearity problem is indicated when the VIF value for the variables in the model exceeds 10 or the tolerance value is less than 0.10. This problem can be overcome by choosing one of the highly correlated variables in the model or by using special regression methods such as Ridge regression or lasso regression.

The primary objective in multiple linear regression and other multivariate analyses is to construct a model with the minimum number of variables necessary. In instances

where a substantial number of independent variables are present, univariate tests may be utilized initially to eliminate variables that do not demonstrate a significant relationship with the outcome variable. Subsequently, a multiple linear regression model can be fitted using the independent variables that have been identified as potentially relevant. In practice, variable selection is typically conducted through stepwise methods, such as stepwise, forward, or backward selection, using specific statistical criteria. The hierarchical regression method is another approach. In this method, the researcher determines the order in which variables are introduced into the model (5).

Logistic Regression

The goal of a logistic regression analysis is the same as for any other regression model used in statistics: to find the best fitting, most parsimonious, clinically interpretable model to describe the relationship between a dependent variable and a set of independent variables. Logistic regression is used when the dependent variable is a qualitative data type. Logistic regression analysis is primarily concerned with estimating the probability of the occurrence of a specified event, rather than predicting the value of the dependent variable.

The distinction between logistic and linear regression can be seen in both the form of the model and the assumptions used. In linear regression, the residuals are normally distributed, while in logistic regression the residuals follow a binomial distribution (15).

The reason why logistic regression is widely used is that it requires fewer assumptions than linear regression. Logistic regression only assumes that the relationship between the logit and the numerical independent variables is linear. In addition, when the obtained regression coefficients are taken as exponential, the interpretation of the results becomes easier since the odds ratio commonly used in health sciences is obtained.

Binary logistic regression analysis is the most commonly used model. In this model, the dependent variable should be a qualitative variable with two categories, such as "present-absent" or "diseased-healthy". Both quantitative and qualitative data types can be used as independent variables in the model.

In evaluating the model fit, statistics such as the Pearson chi-square test, Hosmer-Lemeshow test, correct classification rates, and area under the receiver operating characteristic curve are used. Correct classification rates, which show the fit between the values predicted from the model and the observed values, are widely used (14).

Cox Regression

Cox regression or Cox proportional hazards model is a regression method used in studies where the outcome of interest is not observed in all individuals in the study, in other words, in studies with censored observations.

In survival studies, individuals are monitored from a defined starting point until the occurrence of the event of interest, and factors contributing to the event are investigated. However, researchers cannot always follow all patients until the event of interest occurs. Censored observations occur because some patients are lost to follow-up, patients die from another cause, the length of follow-up is different for all patients when the study is terminated, etc.

The hazard function plays an important role in Cox regression. The hazard function gives the probability that someone who lived until the beginning of the time interval of interest will die in the next time interval.

After the Cox regression model is fitted, it is essential to examine whether the proportional risk assumption is met. For this purpose, the time × covariate interaction term is added to the model and its significance is tested. If the interaction term is significant, it indicates the proportional risk assumption is not met. Another approach to examining the assumption is plotting the ln(-ln) (survival curves). In the graph, the survival curves corresponding to the categories of the qualitative variable being parallel over time indicate that the proportional hazards assumption is satisfied (16,17).

Direct interpretation of beta coefficients in Cox regression is complicated because they correspond to changes in the log-hazard rate. The results are interpreted with hazard ratios obtained by taking the exponential of the regression coefficients. A hazard ratio quantifies how many times the hazard rate increases (or decreases) when the associated predictor variable is incremented by one unit.

CONCLUSION

The increase in the number of commercial or freeware statistical software, as well as the ability to perform analyses through web platforms, has made it easier for researchers to access these tools. Researchers who are not aware of the conditions in which statistical methods should be used and how to interpret the results are likely to make errors. Therefore, choosing the appropriate statistical methods and interpreting the results correctly is crucial when analyzing data obtained from well-designed research. Even if univariate statistical methods are used appropriately, the effects of possible confounding factors are not considered, and multiple or multivariate statistical analyses should be performed to achieve unbiased and accurate results.

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REFERENCES

1. Sungur MA. Importance of biostatistics in medical researches. Duzce Med J. 2021;23(3):232-3.
2. Karahan S, Karaağaoğlu AE. Development of biostatistics: from past to future. Duzce Med J. 2021;23(3):234-8.
3. Karabulut E. Conscious and correct use of biostatistical methods in medical researches: from planning to reporting the results-I. Duzce Med J. 2022;24(1):1-6.
4. Altman DG, Machin D, Bryant TN, Gardner MJ. Statistics with confidence: Confidence intervals and statistical guidelines. 2nd ed. London: BMJ Books; 2000.
5. Alpar R. Spor, sağlık ve eğitim bilimlerinden örneklerle uygulamalı istatistik ve geçerlik-güvenirlik. 6th ed. Ankara: Detay; 2020. Turkish.
6. Grech V, Calleja N. WASP (Write a Scientific Paper): Parametric vs. non-parametric tests. Early Hum Dev. 2018;123:48-9.
7. Dawson B, Trapp RG. Basic & clinical biostatistics. 4th ed. New York: McGraw-Hill; 2004.
8. Daniel WW, Cross CL. Biostatistics: A foundation for analysis in the health sciences. 11th ed. Hoboken, NJ: Wiley; 2019.
9. Özdemir P, Karabulut E, Alpar R. Biyoistatistik. In: Günalp S, editor. Temel Kadın Hastalıkları ve Doğum Bilgisi. 4th ed. Ankara: Güneş Tıp Kitapevleri; 2024. Turkish.
10. Banerjee A, Chitnis UB, Jadhav SL, Bhawalkar JS, Chaudhury S. Hypothesis testing, type I and type II errors. Ind Psychiatry J. 2009;18(2):127-31.
11. Cohen HW. P values: use and misuse in medical literature. Am J Hypertens. 2011;24(1):18-23.
12. Field A. Discovering statistics using IBM SPSS statistics. 6th ed. London: SAGE; 2024.
13. Tabachnick BG, Fidell LS. Using multivariate statistics. 6th ed. Boston: Pearson; 2013.
14. Alpar R. Uygulamalı çok değişkenli istatistiksel yöntemler. 6th ed. Ankara: Detay; 2021. Turkish.
15. Hosmer DW, Lemeshow S, Sturdivant RX. Applied logistic regression. 3rd ed. Hoboken, NJ: Wiley; 2013.
16. Hosmer DW, Lemeshow S. Applied survival analysis: Regression modeling of time-to-event data. New York: John Wiley & Sons; 1999.
17. Kleinbaum DG, Klein M. Survival analysis: A self-learning text, 2nd ed. New York: Springer; 2005.

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Can Inflammatory Indexes Predict the Severity of Mucosal Inflammation in Ulcerative Colitis?

İnflamatuar İndeksler Ülseratif Kolitte Mukozal İnflamasyonun Şiddetini Tahmin Edebilir mi?

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ABSTRACT

Aim: Inflammatory indexes can relate to disease activity in ulcerative colitis (UC). This study aimed to evaluate whether the inflammatory indexes of neutrophil-lymphocyte ratio (NLR), C-reactive protein (CRP)-albumin ratio (CAR), CRP-lymphocyte ratio (CLR), and systemic immune-inflammation index (SIII) might predict the severity of mucosal inflammation in UC.
Material and Methods: This retrospective case-control study included 184 UC patients and 101 healthy controls. The Mayo clinical score and Mayo endoscopic score were used for the clinical and endoscopic features of UC. Truelove and Richards's method for the severity of mucosal inflammation determined the histological activity index (HAI).
Results: The inflammatory index values, were higher in UC patients compared to the control group ($p=0.007$ for NLR, and $p<0.001$ for the others). The patients having endoscopic, clinic, and histologically active disease had higher inflammatory index values than those in remission ($p<0.001$ for all). UC patients with extensive disease had higher inflammatory index values than the patients who had limited disease ($p<0.001$ for all). The HAI values were positively correlated to all inflammatory indexes, and the correlation was the strongest between the HAI and CLR ($rho=0.737$, $p<0.001$). Regarding HAI, the diagnostic accuracy of all inflammatory indexes for detecting the clinically active disease was statistically significant, and there was no significant difference between them in terms of diagnostic accuracy.
Conclusion: The inflammatory indexes of NLR, CAR, CLR, and SII might predict the severity of histological inflammation in UC.
Keywords: Ulcerative colitis; inflammatory indexes; mucosal inflammation.

ÖZ

Amaç: İnflamatuar indekslerin ülseratif kolitte (ÜK) hastalık aktivitesi ile ilişkili oldukları bilinmektedir. Bu çalışma nötrofil-lenfosit oranı (NLR), C-reaktif protein (CRP)-albümin oranı (CAR), CRP-lenfosit oranı (CLR) ve sistemik immün-inflamasyon indeksini (SIII) içeren inflamatuar indekslerin ÜK'de mukozal inflamasyonun şiddetini tahmin edip edemeyeceğinin değerlendirilmesi amaçlanmıştır.
Gereç ve Yöntemler: Bu retrospektif vaka kontrol çalışmasına 184 ÜK hastası ve 101 sağlıklı kontrol dahil edilmiştir. ÜK'nin klinik ve endoskopik özellikleri için Mayo klinik skoru ve Mayo endoskopik skoru kullanılmıştır. Mukozal inflamasyon şiddeti, Truelove ve Richards yöntemine göre histolojik aktivite indeksi (HAI) ile belirlenmiştir.
Bulgular: ÜK hastalarında, inflamatuar indeks değerleri kontrol grubuna göre daha yüksek bulunmuştur (NLR için $p=0,007$ ve diğerleri için $p<0,001$). Endoskopik, klinik ve histolojik olarak aktif hastalığı olan vakaların inflamatuar indeks değerleri remisyonda olan vakalardan daha yüksek idi (tümü için $p<0,001$). Yaygın hastalığı olan ÜK hastalarının sınırlı hastalığı olan hastalardan daha yüksek inflamatuar indeks değerleri vardı (tümü için $p<0,001$). HAI değerleri tüm inflamatuar indekslerle pozitif korelasyon göstermekteydi ve en güçlü korelasyon HAI ile CLR arasında idi ($rho=0,737$; $p<0,001$). HAI ile ilişkili olarak, klinik olarak aktif hastalığın tespiti için tüm inflamatuvar indekslerin tanısal doğruluğu istatistiksel olarak anlamlıydı ve tanısal doğruluk açısından aralarında anlamlı bir fark yoktu.
Sonuç: NLR, CAR, CLR ve SIII'i içeren inflamatuar indeskler, ÜK'de histolojik inflamasyonun şiddetini tahmin edebilir.
Anahtar kelimeler: Ülseratif kolit; inflamatuar indeksler; mukozal inflamasyon.

INTRODUCTION

Inflammatory bowel diseases (IBDs) have relapsing and remitting courses. Exact pathogenic mechanisms in IBDs remain elusive, and the pathogenesis is multifactorial. The prevalence of IBDs is increasing, especially in developed countries. Inflammation is confined to the colonic mucosa in ulcerative colitis (UC). Bloody diarrhea is a common symptom of UC, which causes a psychosocial burden (1).

For the choice of therapeutic modalities, assessment of the disease activity is mandatory in UC. In the follow-up period, patients usually undergo colonoscopy, which can cause discomfort. Clinical, endoscopic, and laboratory features do not always correlate in UC patients. Easily applicable and inexpensive markers estimating the clinical, endoscopic, biochemical, and histological features of the disease are needed in daily clinical practice (2).

The neutrophil-lymphocyte ratio (NLR), the C-reactive protein (CRP)-albumin ratio (CAR), the CRP-lymphocyte ratio (CLR), and the systemic immune-inflammation index (SIII) are overlooked parameters in inflammatory conditions, including IBDs (3-12). Inflammatory indexes are cheap and easily interpreted tests by practitioners and have been attractive to clinicians in chronic inflammatory diseases so far. Many studies evaluated the effectiveness of inflammatory indexes in IBDs. It is worth noting that while not all studies have found a correlation, the majority have identified positive associations between disease activity and inflammatory indexes in UC (4,7-13).

Inflammation in the gut wall is related to worse clinical outcomes and relapse in IBDs, and therefore, mucosal healing was declared as an ideal therapeutic target (2,14,15). The severity of mucosal inflammation represents a target distinct from endoscopic mucosal healing (15). Mucosal inflammation in UC can be evaluated quantitatively, and there are different histological scoring systems in UC (15-19).

Although inflammatory indexes have been reported to correlate with different disease activity parameters, there is currently no data regarding the relationship between inflammatory indexes and the severity of histopathological activity in UC. With this regard, this study aimed to delineate whether inflammatory indexes might predict the inflammatory activity in the colonic mucosa of patients with UC, along with the clinical and laboratory characteristics of the disease.

MATERIAL AND METHODS

Subjects

This is a single-center retrospective, cross-sectional study. Patients with UC admitted to the gastroenterology department of our institute and underwent colonoscopy between January 2012 and July 2023 were retrospectively evaluated in the study. This group included both newly and previously diagnosed patients. A second cohort of healthy subjects served as controls. The local ethics committee approved the study (05.12.2023, 2021/147).

Subjects with clinical conditions that can alter inflammatory indexes, such as sepsis, any malignancies, any hematological diseases, severe organ failure, including cirrhosis, acute or chronic infections, autoimmune and other chronic inflammatory diseases, and patients with gut resection, were excluded from the study. In addition, patients with active disease due to infectious causes (amebiasis and cytomegalovirus colitis) were also excluded from the study. The healthy control group included participants who underwent a colonoscopy for indications other than IBD and whose colonoscopy results were normal. Medical records of all patients were reviewed, and 184 eligible patient files and 101 healthy controls were evaluated.

Assessment of the Clinical and Endoscopic Activities

The Mayo clinical score (MCS) was applied for the clinical activity of patients with UC, and it was scored between 0-12. Scores of ≤2 were classified as clinical remission, whereas scores of >2 indicated an activation. The disease extent of the patients with UC was defined in agreement with the Montreal classification (20). Proctitis and left-sided colitis were recorded as limited diseases, whereas extensive colitis and pancolitis were recorded as extensive diseases. Mayo endoscopic score (MES) index was used for the endoscopic activity of UC and was classified as remission (0), mild (1), moderate (2), and severe (3) colitis. Scores of (0) and (1) were recorded as inactive diseases, whereas (2) and (3) were recorded as active diseases.

Determination of the Inflammatory Indexes

Laboratory data were noted from the subject files prior to colonoscopy, one week before the procedure. Inflammatory indexes, including the NLR, SIII, CAR, and CLR, were calculated as defined in the literature (8). The CAR and CLR values were obtained by the division of the CRP levels (mg/L) by the albumin (gr/dl) and lymphocyte values ($10^3/mm^3$). The NLR was calculated as the neutrophil counts ($10^3/mm^3$) divided by the lymphocyte counts ($10^3/mm^3$). For SIII, neutrophil counts ($10^3/mm^3$) were multiplied by the platelet counts ($10^3/mm^3$) and divided by the lymphocyte counts ($10^3/mm^3$).

Histopathologic Evaluation in Ulcerative Colitis

The same pathologist who was blind to the participants evaluated the formalin-fixed, paraffin-embedded, and hematoxylin and eosin (H&E)-stained colonic biopsies of the UC patients and performed grading through a scale similar to that developed by Truelove and Richards (16). According to the severity of inflammatory mucosal inflammation, each component of the scale was scored. Active inflammation (0-3), chronic inflammation (0-2), and crypt distortion (0-3) were the components of the scale. The histologic activity index (HAI) was defined as the sum of the scores of these components. The highest mucosal inflammation score was 8, and scores of <5 were recorded as histological remission, whereas ≥5 was recorded as activation (16).

Statistical Analysis

Statistical analyses were performed using the MedicReS E-PICOS Version 21.3. Descriptive statistics were presented by frequencies and percentages for categorical variables, and the median was presented with its interquartile ranges for continuous ones. Kolmogorov-Smirnov and Shapiro-Wilk tests were used for testing normality. Any continuous variables did not fit the criteria for normality, so comparisons were performed using the Mann-Whitney U test. Associations were checked by using Spearman's rank correlation due to at least one of the variables being ordinal. A chi-squared test was used for comparison of categorical variables. Fisher's exact test and Yates's correction for continuity were used according to the range of expected

values. Diagnostic accuracy was evaluated using receiver operating characteristics (ROC) curve analysis, and cut-off levels were estimated by Youden's index. The confidence level for statistical significance was defined as 95 percent.

RESULTS

In total, 184 (140 males and 44 females) UC patients and 101 (78 males and 23 females) healthy controls eligible for the study were evaluated. The groups were similar with respect to age and gender. Demographic, clinical, and laboratory characteristics of the subjects are presented in Table 1. Erythrocyte sedimentation rate (ESR), CRP, leucocyte, neutrophil, and platelet values were higher, whereas albumin value was lower in UC patients, as expected. Although the median lymphocyte value was numerically lower in the patients with UC compared to the control group, the difference was not statistically significant (p=0.299). Most patients with UC had limited and active disease in the colonoscopy, and the number of patients in activation was higher than those in remission, according to MCS. The median HAI was 5 in the patient group. The number of patients under treatment was also higher than the number of patients without any treatment. CLR, CAR, NLR, and SIII values were higher in the UC patients compared to healthy controls (p=0.007 for NLR, and p<0.001 for the others).

With respect to disease activity parameters, UC patients having endoscopic, clinic, and histologically active disease had higher CLR, CAR, NLR, and SIII values than those in remission (p<0.001 for all). There was no statistically significant difference between the patients who were under treatment and those without any treatment. In colonoscopy, UC patients with extensive disease had higher inflammatory index values than the patients who had limited disease (p<0.001 for all, Table 2).

HAI was positively correlated to inflammatory indexes, and the correlation was the strongest between HAI and CLR (rho=0.737, p<0.001, Table 3).

Table 1. Demographic, clinical, and histopathologic characteristics of the study groups

	Ulcerative Colitis Patients (n=184)	Healthy Controls (n=101)	p
Gender, n (%)			
Female	44 (23.9)	23 (22.8)	0.828
Male	140 (76.1)	78 (77.2)	
Age (years)	32 (24.2-46.7) [18-83]	35 (25.5-46) [19-65]	0.596
CRP (mg/L)	10.3 (3-23.4) [0.15-138]	2.8 (1.2-4.2) [0.20-22.39]	**<0.001**
ESR (mm/h)	19 (9-44.7) [1-120]	8 (3-15) [1-42]	**<0.001**
Leucocyte ($x10^3/\mu L$)	7.7 (6.4-9.6) [3.5-8.7]	7.1 (5.9-8.7) [3.2-15.7]	**0.024**
Neutrophil ($x10^3/\mu L$)	5 (3.6-6.7) [1.64-14.75]	4.5 (3.4-5.6) [1.6-481]	**0.036**
Lymphocyte ($x10^3/\mu L$)	1.9 (1.6-2.5) [0.63-5.03]	2.1 (1.6-2.6) [0.76-168]	0.299
Platelet ($x10^3/\mu L$)	305 (245.2-377.7) [108-1039]	251 (220.5-279) [130-389]	**<0.001**
Albumin (gr/dl)	4.3 (3.9-4.6) [2.1-5.4]	4.5 (4.3-4.7) [3.40-5.30]	**<0.001**
Disease duration (years)	3 (1-7) [0-35]	-	-
Location of UC, n (%)			
Remission	12 (6.5)		
Limited disease	128 (69.6)	-	-
Extensive colitis	44 (23.9)		
MES of UC, n (%)			
Remission (MES 0-1)	89 (48.4)	-	-
Activation (MES 2-3)	95 (51.6)		
MCS of UC, n (%)			
Remission (MCS ≤2)	53 (20.8)	-	-
Activation (MCS >2)	131 (79.2)		
HAI of UC	5 (2-7) [0-8]	-	-
HAI of UC, n (%)			
Remission (HAI <5)	76 (41.3)	-	-
Activation (HAI ≥5)	108 (58.7)		
Treatment, n (%)			
No treatment	39 (28.8)	-	-
Under treatment	145 (71.2)		
CLR	5.1 (1.5-13.3) [0.08-115]	1.2 (0.6-1.9) [0.01-9.78]	**<0.001**
CAR	2.4 (0.6-5.9) [0.03-54.20]	0.58 (0.2-0.9) [0.04-4.57]	**<0.001**
NLR	2.3 (1.7-3.6) [0.86-9.32]	2.1 (1.5-2.8) [0.03-188.63]	**0.007**
SIII	761.5 (474.2-1206.3) [178.29-4994.16]	518.5 (362-706.8) [7.28 -69980.78]	**<0.001**

CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, UC: ulcerative colitis, MES: Mayo endoscopic score, MCS: Mayo clinical score, HAI: histologic activity index, CLR: C-reactive protein-lymphocyte ratio, CAR: C-reactive protein-albumin ratio, NLR: neutrophil-lymphocyte ratio, SII: systemic immune-inflammation index, descriptive statistics for numerical variables were presented as median (interquartile range, 25^{th}-75^{th} percentile) [minimum-maximum]

Table 2. Inflammatory indexes of the patients according to the clinical activity, location, and treatment status

	Location of Ulcerative Colitis			p
	Remission (n=12)	**Limited (n=128)**	**Extensive (n=44)**	
CLR	2.2 (1.3-2.5) [0.1-5.4]	2.3 (1.8-3.5) [0.08-77.2]	3 (1.8-14.4) [0.3-115]	**<0.001**
CAR	0.4 (0.2-0.9) [0.06-4.4]	2 (0.5-4) [0.03-34.2]	7.1 (2.9-19) [0.1-54.2]	**<0.001**
NLR	2.2 (1.3-2.5) [0.9-5.7]	2.3 (1.8-3.5) [0.8-9.3]	3 (1.8-4.4) [1.09-7.79]	**<0.001**
SII	535 (305-742) [257-1529]	699 (461-988) [178.3-4994]	1231 (684-1807) [347-4645]	**<0.001**

	MES of Ulcerative Colitis		p
	Remission, MES 0-1 (n=89)	**Activation, MES 2-3 (n=95)**	
CLR	1.75 (0.8-5.46) [0.08-43.82]	10 (4.3-19.6) [0.33-115]	**<0.001**
CAR	0.9 (0.4-2.8) [0.09-25.23]	4.2 (2.2-9.8) [0.22-54.2]	**<0.001**
NLR	2.08 (1.5-3) [0.8-6.2]	2.7 (2-3.8) [0.9-9.32]	**<0.001**
SII	606 (404-888.5) [178.9-1971]	932 (622-1445) [178.3-4994]	**<0.001**

	MCS of Ulcerative Colitis		p
	Remission, MCS ≤2 (n=53)	**Activation, MCS >2 (n=131)**	
CLR	1.3 (0.5-2.9) [0.1-16.8]	7.8 (3.6-16) [0.08-115]	**<0.001**
CAR	0.6 (0.3-1.2) [0.05-15.7]	3.5 (1.7-7.9) [0.03-54.2]	**<0.001**
NLR	2 (1.4-2.6) [0.8-5.9]	2.6 (1.9-3.7) [0.9-9.3]	**<0.001**
SII	539 (375-815) [178.9-2030]	876 (566-1314) [178.3-4994]	**<0.001**

	HAI of Ulcerative Colitis		p
	Remission, HAI <5 (n=76)	**Activation, HAI ≥5 (n=108)**	
CLR	1.4 (0.7-3) [0.1-12.4]	10.3 (5-19) [0.08-115]	**<0.001**
CAR	0.6 (0.3-1.3) [0.05-4.4]	5 (2.5-10.3) [0.03-54.2]	**<0.001**
NLR	1.9 (1.4-2.5) [0.8-5.7]	2.9 (2.1-3.9) [1.1-9.3]	**<0.001**
SII	533 (364-769) [178-1627]	1014 (672-1443) [307-4994]	**<0.001**

	Treatment		p
	No Treatment (n=39)	**Under Treatment (n=145)**	
CLR	4.8 (1.4-10) [0.08-50.4]	5 (1.6-13.5) [0.1-115]	0.573
CAR	2.2 (0.5-4.5) [0.03-47.7]	2.7 (0.6-6) [0.05-54.2]	0.796
NLR	2.3 (1.7-4.4) [1.04-9.3]	2.4 (1.8-3.6) [0.8-7.8]	0.907
SII	763.05 (476.46-1384.16) [232.83-4994.16]	760 (473.8769-1095.93) [178.29-4645.9]	0.314

MES: Mayo endoscopic score, MCS: Mayo clinical score, HAI: histologic activity index, CLR: C-reactive protein-lymphocyte ratio, CAR: C-reactive protein-albumin ratio, NLR: neutrophil-lymphocyte ratio, SII: systemic immune-inflammation index, descriptive statistics for numerical variables were presented as median (interquartile range, 25th-75th percentile) [minimum-maximum]

Table 3. Correlations between the histological activity index and the inflammatory indexes

	CLR		CAR		NLR		SII	
	rho	**p**	**rho**	**p**	**rho**	**p**	**rho**	**p**
HAI	0.737	0.001	0.725	0.001	0.444	<0.001	0.501	0.001

HAI: histologic activity index, CLR: C-reactive protein-lymphocyte ratio, CAR: C-reactive protein-albumin ratio, NLR: neutrophil-lymphocyte ratio, SII: systemic immune-inflammation index

The ROC curve analysis revealed that significant predictive values of CLR, CAR, NLR, and SIII for the clinically active disease according to HAI (Figure 1). Regarding prediction for the clinically active disease according to HAI, none of these parameters was superior to the other (Table 4).

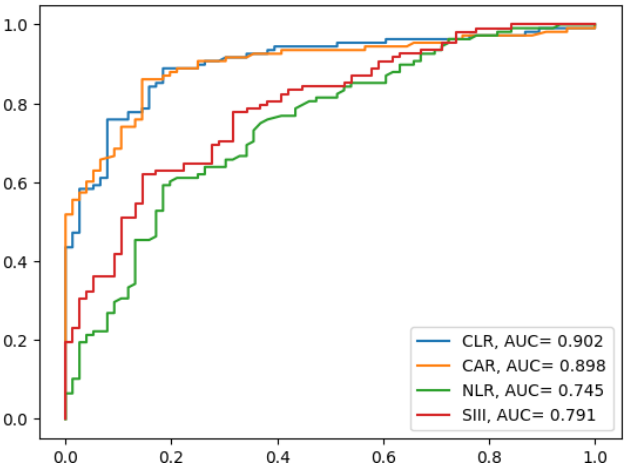

Figure 1. Receiver operating characteristics (ROC) curve of the inflammatory indexes in predicting clinically active ulcerative colitis

DISCUSSION

We reported higher CRP, CAR, CLR, and SIII values in the UC group compared to healthy controls, and the results were consistent with the previous reports. According to endoscopic, clinical, and histological parameters, our results revealed higher inflammatory index values in active UC patients than in remission. Extensive location of the disease also resulted in higher values of inflammatory indexes compared to limited location.

In a previous report, the clinical activity of UC was assessed according to Truelove and Witt's criteria (TWC).

Table 4. Predictive values of the inflammatory indexes for clinically active disease according to histologic activity index

	AUC	95% CI	p	Cut-off	Sensitivity	Specificity	PPV	NPV	LR+	LR-
CLR	0.902	0.858 - 0.945	**<0.001**	>3.389	0.888	0.816	0.872	0.838	4.820	0.137
CAR	0.898	0.853 - 0.943	**<0.001**	>2.011	0.860	0.855	0.893	0.813	5.942	0.164
NLR	0.745	0.675 - 0.815	**<0.001**	>2.561	0.608	0.803	0.813	0.592	3.078	0.489
SIII	0.791	0.727 - 0.855	**<0.001**	>866.983	0.617	0.855	0.857	0.613	4.263	0.448

CLR: C-reactive protein-lymphocyte ratio, CAR: C-reactive protein-albumin ratio, NLR: neutrophil-lymphocyte ratio, SIII: systemic immune-inflammation index, AUC: area under the curve, CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value, LR: likelihood ratio

CAR and interleukin-6 (IL-6) values were higher in active UC patients (10). In a Japanese study, UC patients (n=273) with long disease duration (>7 years) were evaluated, and CAR was positively associated with moderate to severe endoscopic activity (MES 2 and 3) but not with mucosal healing (MES 0). In the mentioned study, clinical remission was determined according to the stool frequency (<3 stool per day), and there was no association between CAR and clinical remission (11). In the current study, we also used the MES method. Patients with endoscopic activation (MES 2 and 3) had higher CAR, CLR, NLR, and SIII values than those in remission (MES 0 and 1). We reported higher values of the inflammatory indexes in patients who were in clinical activation (MCS >2). Inconsistent results in these studies may partly be due to the methodological differences. Steroids, immunosuppressive, and other agents can alter the serum neutrophil, and lymphocyte counts, and resolution of the inflammation may also inhibit CRP secretion (4). Not only the correlation with the disease activity parameters but also inflammatory indexes were declared to be in association with response to treatment in UC (8,9).

In the liver, CRP is produced via the secretion of IL-6, tumor necrosis factor-alpha, and rapidly and it normalizes with the termination of the inflammation. IL-6 is known to be the most potent pro-inflammatory cytokine for CRP secretion (4). Although CRP is widely used in daily practice, it can be affected by gender, age, smoking, and body weight. Additionally, due to some genetic polymorphisms, CRP may not show an adequate acute phase response (21). Environmental and demographic factors (age, sex, smoking, obesity) and diabetes account for only 22 percent to 30 percent of the inter-individual variability in CRP. Family studies revealed that CRP levels have a heritable trait (22). Hypoalbuminemia at disease diagnosis of UC is also associated with poorer clinical outcomes (23).

Neutrophilic infiltration leads to mucosal damage and increased epithelial permeability (24). Neutrophil counts were higher in UC patients compared to the control group in the current study. Feng et al. (10) retrospectively evaluated UC patients (n=306). They reported higher NLR and platelet lymphocyte ratio values in clinically active patients than those in remission, according to TWC. It was noteworthy that there were strong correlations between NLR and fecal calprotectin, pointing to the diagnostic accuracy of NLR. In another study, after adjusting statistically significant traditional inflammatory markers, including CRP, ESR, and WBC counts, the NLR was not reported as a useful predictor of clinical activity in UC patients, according to TWC (25).

Lin et al. (8) found higher SIII values correlating to disease activity, and they pointed to the better diagnostic accuracy of SIII among the other indexes in UC patients (n=187). Similarly, a recent report declared higher SIII values compared to healthy controls and positive correlations between MCS and SIII values in 185 UC patients (13). Reactive thrombocytosis, neutrophilia, and lymphopenia are well-known laboratory results in IBDs, and they can be attributed to the ongoing inflammation leading to higher SIII values in UC (8). Our results about SIII values were similar to the previous reports.

Although not statistically significant, lymphocyte counts were lower in UC patients compared to healthy controls in our results. Lower lymphocyte counts in the circulation can be due to the recruitment of the lymphocytes into the inflammation site (8,26). CLR was reported to be in association with the disease activity in UC previously, as in our study. Unlike the other inflammatory indexes, CAR and CLR values were also found to be predictive for the treatment response, and these indexes might be important prognostic markers if they can be proven in further prospective research (8,9).

The body mentioned above highlights the diagnostic and prognostic utilities of the inflammatory indexes (7,8,27). The most important findings in our study were the associations between the inflammatory indexes and the severity of mucosal inflammation, and quantifying the different phenotypes of UC provided an objective approach. The severity of mucosal inflammation (HAI) in UC patients positively correlated to endoscopic and clinical activity indexes. These results point out the higher mucosal inflammatory activity in the different activity phases of UC and its possible relation to worse clinical outcomes. Besides the other positive correlations between HAI and inflammatory indexes, the strongest correlation between HAI and CLR may be due to lower lymphocyte counts and relatively higher CRP values, as mentioned above (8,26).

Persistent inflammation in the colonic mucosa of UC patients is related to poorer long-term clinical outcomes. Ongoing colonic inflammation is also a good predictor of the disease recurrence and the unresponsiveness to treatment (14,15,28). Ardizzone et al. (14) prospectively evaluated the newly diagnosed moderate-to-severely active UC patients (n=157) who had received steroids initially. After five years of follow-up, they reported that UC patients who had mucosal inflammation despite the steroid treatment had a high rate of colectomy and the need for immunosuppressive agents. This study points out the importance of the resolution of mucosal inflammation for better clinical outcomes (14).

Mucosal inflammation in UC can progress to dysplasia and cancer. It is a potential risk for colorectal cancer. The risk of malignity depends on the duration, extent, and inflammatory activity in UC. It was estimated that the risk

of colorectal cancer is increasing within the range of 0.5 percent to 1.0 percent per year after 8 to 10 years of disease in UC (1). The higher inflammatory index values in patients with extensive location may be a clue for the progression to malignity if further studies can prove it. The median disease duration was calculated as three years in this study, and the duration did not correlate to indexes. The anti-inflammatory effect of the medications may be a possible reason for this result.

Today, the best treatment goal of UC patients focuses on mucosal healing besides the control of symptoms (29). The colonoscopic biopsy is an invasive procedure, and even in clinically quiescent disease showing no endoscopic activity, the colonic mucosa can have the features of microscopic inflammation in 16-100 percent of the cases (15). Discrepancies are also common between the histologic and endoscopic results (17). If the diagnostic accuracies of the inflammatory indexes for predicting mucosal inflammation in UC can be proven prospectively, inflammatory indexes can be taken into consideration as non-invasive and inexpensive tests.

There are many histological activity scoring methods in UC. The Truelove and Richards method is the first developed one (16). Riley, Geboes, Rutter, and Rubin's methods are extensively used methods in clinical trials. The prognostic efficiencies of these scores were also evaluated during or after treatment in UC, and they quantified the severity of mucosal inflammation along with acute and chronic inflammatory activity (15-19). Although these scores are routinely used, they are partially or not validated, and inter-observer variabilities are also possible. The diagnostic accuracy of the serum inflammatory indexes and the histopathological scoring systems in UC can be evaluated prospectively.

In UC patients, CAR, CLR, NLR, and SIII values can predict the severity of mucosal inflammation (HAI) along with the other phenotypic features of the disease. Regarding clinical activity, ROC curves of inflammatory indexes for HAI in predicting the clinically active UC were not different. However, larger sample-sized cohorts might reveal significant results for inflammatory indexes to predict the clinical activity of UC.

We first examined the accuracy of serum inflammatory indexes for predicting the severity of mucosal inflammation according to Truelove and Richards's method. This study has several limitations. It was a single-centered retrospective research. Additional clinical features, including extra-intestinal manifestations, family history, types of medications, and smoking habits, were not evaluated in the current study. In the histopathological examination, only one pathologist evaluated the colonic mucosal biopsies, but the evaluation with more than one independent pathologist could expose the inter-observer variabilities.

CONCLUSION

Inflammatory indexes, as cheap and easily applicable tests, can predict the severity of mucosal inflammation in UC. Inflammatory indexes can be examined for the prediction of mucosal inflammation severity with different inflammatory scoring methods prospectively, and the different traits of UC can be evaluated. This study should be considered a preliminary and the first step for future research.

Ethics Committee Approval: The study was approved by the Ethics Committee of Şehit Prof. Dr. İlhan Varank Training and Research Hospital (05.12.2023, 2021/147).

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REFERENCES

1. Osterman MT, Lichtenstein GR. Ulcerative colitis. In: Feldman M, Friedman SL, Brandt JL, editors. Sleisenger and Fordtran's gastrointestinal and liver disease, vol 2. Philadelphia: Elsevier Saunders; 2016. p.2023-61.
2. Chen F, Hu Y, Fan YH, Lv B. Clinical value of fecal calprotectin in predicting mucosal healing in patients with ulcerative colitis. Front Med (Lausanne). 2021;8:679264.
3. Słowińska-Solnica K, Pawlica-Gosiewska D, Gawlik K, Owczarek D, Cibor D, Pocztar H, et al. Serum inflammatory markers in the diagnosis and assessment of Crohn's disease activity. Arch Med Sci. 2021;17(1):252-7.
4. Feng W, Zhu L, Liu Y, Xu L, Shen H. C-reactive protein/albumin ratio and IL-6 are associated with disease activity in patients with ulcerative colitis. J Clin Lab Anal. 2023;37(3):e24843.
5. Zhang MH, Wang H, Wang HG, Wen X, Yang XZ. Effective immune-inflammation index for ulcerative colitis and activity assessments. World J Clin Cases. 2021;9(2):334-43.
6. Qin G, Tu J, Liu L, Luo L, Wu J, Tao L, et al. Serum albumin and C-reactive protein/albumin ratio are useful biomarkers of Crohn's disease activity. Med Sci Monit. 2016;22:4393-400.
7. Chen YH, Wang L, Feng SY, Cai WM, Chen XF, Huang ZM. The relationship between C-reactive protein/albumin ratio and disease activity in patients with inflammatory bowel disease. Gastroenterol Res Pract. 2020;2020:3467419.
8. Lin H, Bai Z, Wu Q, Chu G, Zhang Y, Guo X, et al. Inflammatory indexes for assessing the severity and disease progression of ulcerative colitis: a single-center retrospective study. Front Public Health. 2022;10:851295.
9. Con D, Andrew B, Nicolaides S, van Langenberg DR, Vasudevan A. Biomarker dynamics during infliximab salvage for acute severe ulcerative colitis: C-reactive protein (CRP)-lymphocyte ratio and CRP-albumin ratio are useful in predicting colectomy. Intest Res. 2022;20(1):101-13.
10. Feng W, Liu Y, Zhu L, Xu L, Shen H. Evaluation of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio as potential markers for ulcerative colitis: a retrospective study. BMC Gastroenterol. 2022;22(1):485.

11. Furukawa S, Yagi S, Shiraishi K, Miyake T, Tange K, Hashimoto Y, et al. Effect of disease duration on the association between C-reactive protein-albumin ratio and endoscopic activity in ulcerative colitis. BMC Gastroenterol. 2022;22(1):39.
12. Header DA, Aboelwafa RA, Elkeleny MR, Bedewy ES, Ellakany AI. C-reactive protein/albumin ratio (CAR) as a marker for detecting acute severe ulcerative colitis in Egyptian patients. Rev Gastroenterol Mex (Engl Ed). 2022;87(4):447-54.
13. Xie Y, Zhuang T, Ping Y, Zhang Y, Wang X, Yu P, et al. Elevated systemic immune inflammation index level is associated with disease activity in ulcerative colitis patients. Clin Chim Acta. 2021;517:122-6.
14. Ardizzone S, Cassinotti A, Duca P, Mazzali C, Penati C, Manes G, et al. Mucosal healing predicts late outcomes after the first course of corticosteroids for newly diagnosed ulcerative colitis. Clin Gastroenterol Hepatol. 2011;9(6):483-9.e3.
15. Bryant RV, Winer S, Travis SP, Riddell RH. Systematic review: histological remission in inflammatory bowel disease. Is 'complete' remission the new treatment paradigm? An IOIBD initiative. J Crohns Colitis. 2014;8(12):1582-97.
16. Truelove SC, Richards WC. Biopsy studies in ulcerative colitis. Br Med J. 1956;1(4979):1315-8.
17. Riley SA, Mani V, Goodman MJ, Dutt S, Herd ME. Microscopic activity in ulcerative colitis: what does it mean? Gut. 1991;32(2):174-8.
18. Geboes K, Riddell R, Ost A, Jensfelt B, Persson T, Löfberg R. A reproducible grading scale for histological assessment of inflammation in ulcerative colitis. Gut. 2000;47(3):404-9.
19. Rutter M, Saunders B, Wilkinson K, Rumbles S, Schofield G, Kamm M, et al. Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. Gastroenterology. 2004;126(2):451-9.
20. Silverberg MS, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a working party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol Hepatol 2005;19 Suppl A:5A-36A.
21. Brull DJ, Serrano N, Zito F, Jones L, Montgomery HE, Rumley A, et al. Human CRP gene polymorphism influences CRP levels: implications for the prediction and pathogenesis of coronary heart disease. Arterioscler Thromb Vasc Biol. 2003;23(11):2063-9.
22. Margaglione M, Cappucci G, Colaizzo D, Vecchione G, Grandone E, Di Minno G. C-reactive protein in offspring is associated with the occurrence of myocardial infarction in first-degree relatives. Arterioscler Thromb Vasc Biol. 2000;20(1):198-203.
23. Khan N, Patel D, Shah Y, Trivedi C, Yang YX. Albumin as a prognostic marker for ulcerative colitis. World J Gastroenterol. 2017;23(45):8008-16.
24. Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature. 2011;474(7351):298-306.
25. Demir AK, Demirtas A, Kaya SU, Tastan I, Butun I, Sagcan M, et al. The relationship between the neutrophil-lymphocyte ratio and disease activity in patients with ulcerative colitis. Kaohsiung J Med Sci. 2015;31(11):585-90.
26. Giuffrida P, Corazza GR, Di Sabatino A. Old and new lymphocyte players in inflammatory bowel disease. Dig Dis Sci. 2018;63(2):277-88.
27. Keleş A, Dagdeviren G, Yücel Celik O, Öztürk AC, Obut M, Çelen Ş, et al. Can inflammatory indices be used to predict adverse pregnancy outcomes in pregnant women with recurrent urinary tract infection? Duzce Med J. 2022;24(3):215-20.
28. Mosli MH, Feagan BG, Sandborn WJ, D'haens G, Behling C, Kaplan K, et al. Histologic evaluation of ulcerative colitis: a systematic review of disease activity indices. Inflamm Bowel Dis. 2014;20(3):564-75.
29. Magro F, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. J Crohns Colitis, 2017;11(6):649-70.

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Efficacy and Tolerability of Topical Dapsone vs Benzoyl Peroxide in Mild to Moderate Acne Vulgaris Treatment: A Retrospective Study

Hafif-Orta Şiddetli Akne Vulgaris Tedavisinde Topikal Dapson ve Benzoil Peroksitin Etkinlik ve Tolere Edilebilirliği: Retrospektif Bir Çalışma

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ABSTRACT

Aim: Acne vulgaris is a disease of the pilosebaceous unit and a chronic inflammatory process. This study aimed to compare topical 5% dapsone and 10% benzoyl peroxide in terms of efficacy, side effects, and patient satisfaction in mild to moderate acne.
Material and Methods: The patients who applied to the dermatology outpatient clinic with the complaint of acne between June 20, 2022, and September 20, 2022, and were diagnosed with mild and moderate acne vulgaris, were retrospectively evaluated. Forty-eight patients in the dapsone group and 53 in the benzoyl peroxide group were included in this study.
Results: At the end of the treatment, a statistically significant difference was found between the two groups in terms of ISGA values and improvement percentage in ISGA, improvement was higher in the dapsone group (both p=0.001). A statistically significant difference was found between the groups in terms of the percentage decrease in lesion counts of closed comedones, papules and pustules, inflammatory and total lesions (decreasement was higher in the dapsone group, p=0.038 for closed comedones, p=0.006 for total lesions and p<0.001 for others). There was no dissatisfied patient in the group using dapsone (p<0.001). Among the side effects, erythema was more common during the whole treatment, and dryness and burning-stinging sensation were higher at the end of the first month in the benzoyl peroxide group.
Conclusion: Topical 5% dapsone is effective in the treatment of mild to moderate acne and is safe in terms of side effects compared to topical 10% benzoyl peroxide.
Keywords: Acne vulgaris; benzoyl peroxide; dapsone.

ÖZ

Amaç: Akne vulgaris, pilosebase ünitenin bir hastalığıdır ve kronik inflamatuar bir süreçtir. Bu çalışmanın amacı hafif ve orta şiddetli akne hastalarında, topikal %5'lik dapson ile topikal %10'luk benzoil peroksiti etkinlik, yan etki ve hasta memnuniyeti açısından karşılaştırmaktır.
Gereç ve Yöntemler: Dermatoloji polikliniğine 20 Haziran 2022 ile 20 Eylül 2022 tarihleri arasında akne şikayeti ile başvuran ve hafif ve orta şiddette akne vulgaris tanısı almış olan hastalar geriye dönük olarak değerlendirildi. Bu çalışmaya, dapson grubunda 48 ve benzoil peroksit grubunda 53 hasta dahil edildi.
Bulgular: Tedavi sonunda, ISGA değerleri ve ISGA'daki iyileşme yüzdesi açısından iki grup arasında istatistiksel olarak anlamlı bir farklılık bulundu, dapson grubunda iyileşme daha fazlaydı (her ikisi için de p=0,001). Kapalı komedon, papül ve püstül, inflamatuvar lezyon ve toplam lezyonların lezyon sayılarındaki azalma yüzdeleri açısından iki grup arasında istatistiksel olarak anlamlı bir farklılık bulundu (dapson grubunda azalma daha fazlaydı, kapalı komedonlar için p=0,038, toplam lezyonlar için p=0,006 ve diğerleri için p<0,001). Dapson kullanan grupta memnun olmayan hasta yoktu (p<0,001). Yan etkiler arasında eritem tüm tedavi süresi boyunca daha sık görüldü, kuruluk ve yanma-batma hissi benzoil peroksit grubunda birinci ayın sonunda daha fazlaydı.
Sonuç: Topikal %5'lik dapson, hafif ve orta şiddetli akne tedavisinde etkilidir ve topikal %10 benzoil peroksit ile karşılaştırıldığında yan etki açısından güvenlidir.
Anahtar kelimeler: Akne vulgaris; benzoil peroksit; dapson.

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INTRODUCTION

Acne vulgaris (AV) is a chronic inflammatory process affecting the pilosebaceous unit. It is usually seen during adolescence. It has significant psychological and social impacts on patients. The treatment options for acne include topical products, systemic antibiotics, and systemic isotretinoin (1,2). Topical retinoids, benzoyl peroxide (BPO), and topical or systemic antibiotics combined with retinoids or BPO are the first-line options in treating mild to moderate acne. For nodulocystic acne, short-term systemic steroids or intralesional steroid injections may be used. BPO, one of the frequently used topicals in acne treatment, has a lipophilic nature, easily penetrates the pilosebaceous unit, and has bactericidal, anti-inflammatory, and comedolytic effects. Topical dapsone, which has both antibacterial and anti-inflammatory properties, has recently been used in the topical management of other inflammatory diseases, including acne. This retrospective study aimed to compare the efficacy, side effects, and patient satisfaction of topical 5% dapsone and 10% BPO in patients with mild and moderate AV.

MATERIAL AND METHODS

The study protocol was approved by the clinical research ethics committee of Hitit University (date: 09.11.2022, and no: 2022-96). The study was conducted in accordance with the Declaration of Helsinki and an informed consent form was obtained from all participants.

The study was conducted retrospectively by reviewing the patient files. We included the files of patients who applied to the dermatology outpatient clinic with the complaint of acne and were diagnosed with mild and moderate AV based on the investigator's static global assessment (ISGA) scores between 2 and 4, who were between 12 and 40 years of age, and who used topical 5% dapsone or topical 10% BPO once a day in the evening between June 20, 2022, and September 20, 2022. We excluded the files of patients who had previously used medicines containing same active ingredients in the study, who had severe acne with nodulocystic lesions, who were under 12 or over 40 years of age, and who had received other acne treatment within the 3 months before admission to the hospital, epilation or other application (energy-based device, peeling, dermabrasion, etc.) on the face in the last month, users of systemic corticosteroids, facial retinol, or acidic cosmetic products, those with a history of PCOS or other hormonal diseases. After all these eliminations, a total of 101 patient files with appropriate criteria, 48 patients in the dapsone group and 53 patients in the BPO group were included in the study. Demographic characteristics, ISGA scores before and after treatment, the number of non-inflammatory lesions including open and closed comedonal lesions, the number of inflammatory lesions including papules and pustules, side effects and severity scores at the end of each month (0 for absent, 1 for mild, 2 for moderate, 3 for severe), and satisfaction scores (1-not satisfied, 2-satisfied, 3-very satisfied) in files were recorded for the study.

The examination of the patients, determination of disease severity, treatments and treatment follow-ups, efficacy and side effects follow-ups, and file reviews were performed by the same doctor (SH). None of the patients in the study discontinued treatment due to side effects.

Statistical Analysis

The IBM SPSS Statistics v.25 (Armonk, NY: IBM Corp.) program was used for data recording and statistical tests. The Kolmogorov-Smirnov test was used to determine whether the continuous variables fit the normal distribution. Among the continuous variables, those that fit the normal distribution were expressed as the mean and standard deviation, and those that did not fit the normal distribution were expressed as the median and min-max. Categorical variables were expressed as numbers and percentages. The chi-square test was used to compare independent groups in terms of categorical variables. In cases where the smallest expected value was less than 5, the Fisher exact test was used instead of the chi-square. The Wilcoxon test was used to investigate the difference between dependent groups in terms of variables that did not fit the normal distribution, and the Mann-Whitney U test was used for independent groups. For the statistical significance level, $p<0.05$ was accepted.

RESULTS

A total of 101 patient files, 48 patients in the dapsone group and 53 patients in the BPO group were included in the study. The mean age of the patients using dapsone was 22.38±7.94 years, while it was 20.53±8.18 years for those using BPO. The percentage of males was 18.8% (n=9) in the dapsone group and 18.9% (n=10) in the BPO group. There was no statistically significant difference between the groups in terms of gender and age (p=0.988, and p=0.103, respectively).

When the groups were investigated in terms of ISGA scores (p=0.469), open (p=0.530) and closed (p=0.208) comedone counts, non-inflammatory lesions (p=0.110), papules (p=0.385) and pustules (p=0.529), inflammatory lesion (p=0.265), and total lesion counts (p=0.105) before treatment, there was no significant difference (Table 1).

Both groups showed a significant difference in terms of pre- and post-treatment ISGA values and the number of lesions ($p<0.001$ for all). This indicates that both drugs were effective in treatment.

Table 1. General demographic and clinical characteristics of the patients

	Dapsone (n=48)	BPO (n=53)	p
Age (years)	22.38±7.94	20.53±8.18	0.103
Gender, n (%)			
Male	9 (18.8)	10 (18.9)	0.988
Female	39 (81.2)	43 (81.1)	
Initial ISGA	2.85±0.77	2.96±0.76	0.469
Initial ISGA, n (%)			
2	18 (37.5)	16 (30.2)	
3	19 (39.6)	23 (43.4)	0.736
4	11 (22.9)	14 (26.4)	
Initial NIL	42.43±9.66	43.49±9.52	0.110
Open comedone	10.96±3.04	11.49±3.49	0.530
Closed comedone	31.19±6.71	32.00±6.42	0.208
Initial IL	37.17±5.82	38.53±6.51	0.265
Papule	13.71±1.54	14.09±1.88	0.385
Pustule	23.46±4.34	23.87±4.14	0.529
Initial TL	80.02±15.27	81.47±15.11	0.105

ISGA: investigator's static global assessment, NIL: non-inflammatory lesion count, IL: inflammatory lesion count, TL: total lesion count, BPO: benzoyl peroxide

There was a statistically significant difference between the groups in ISGA values and improvement percentage in ISGA at the end of the treatment (both p=0.001). The dapsone group had lower ISGA values and a higher improvement percentage in ISGA after 3-month treatment. There was also a significant difference between the groups both in the number of lesions and the decrease percentage in the number of lesions for closed comedones (p=0.021, and p=0.038, respectively), papules (both p<0.001) and pustules (both p<0.001), inflammatory lesions (both p<0.001), total lesions (p=0.004, and p=0.006, respectively). The dapsone group had lower counts of all lesion types and a higher improvement percentage in lesion counts after 3 months of treatment. No difference was found in terms of both lesion counts and percentage reduction for open comedones (p=0.062, and p=0.115, respectively), and non-inflammatory lesions (p=0.340, and p=0.284) between groups (Table 2).

Table 2. ISGA measurements, lesion counts, and percent decrease at the end of the third month

	3rd-month of Treatment			Percent Decrease at the End of Treatment		
	Dapsone (n=48)	**BPO (n=53)**	**p**	**Dapsone (n=48)**	**BPO (n=53)**	**p**
ISGA	0.84 [0-3]	1.38 [0-3]	**0.001**	71 [0-100]	51.83 [0-100]	**0.001**
NIL	8.6 [0-38]	17.84 [0-38]	0.062	80.67 [28.3-100]	58.45 [20.83-100]	0.115
Open comedone	2.72 [0-11]	4.98 [0-10]	0.340	76.02 [16.67-100]	56.87 [14.29-100]	0.284
Closed comedone	5.88 [0-27]	12.86 [0-29]	**0.021**	82.19 [28.95-100]	58.99 [18.18-100]	**0.038**
IL	5.84 [0-28]	11.52 [0-27]	**<0.001**	84.71 [30-100]	69.76 [32.5-100]	**<0.001**
Papule	2.6 [0-11]	4.94 [0-10]	**<0.001**	81.43 [28.57-100]	65.38 [23.08-100]	**<0.001**
Pustule	3.24 [0-18]	6.58 [0-18]	**<0.001**	86.66 [30.77-100]	72.23 [30.77-100]	**<0.001**
TL	14.44 [0-66]	29.36 [0-64]	**0.004**	82.53 [29.55-100]	63.83 [27.27-100]	**0.006**

ISGA: investigator's static global assessment, NIL: non-inflammatory lesion count, IL: inflammatory lesion count, TL: total lesion count, BPO: benzoyl peroxide, descriptive statistics were presented as median [minimum-maximum]

The patient satisfaction levels were found statistically significantly different between the groups (p<0.001). None of the patients in the dapsone group were dissatisfied, and the proportion of very satisfied patients was lower in the BPO group (Table 3).

There was a statistically significant difference between the groups in terms of erythema at the end of each month during the 3 months of treatment (p<0.001, p=0.022, and p=0.016, respectively). Erythema was more common in the BPO group in all monthly follow-ups. When the severity of erythema for each month was compared between the two groups, there was only a statistically significant difference at the end of the 1st month (p=0.029, severe erythema was not seen in the dapsone group, but was more common in the BPO group), but not at the end of the 2nd month (p>0.999). At the end of the 3rd month, no erythema was observed in the dapsone group.

There was also a statistically significant difference between the two groups in terms of dryness at the end of the 1st and 2nd month (p<0.001, and p=0.049, respectively), but not in the 3rd month (p=0.059). Dryness was also more common in patients using BPO. When the severity of dryness was compared between the groups, there was a significant difference at the end of the 1st month (p=0.001, severe dryness was not observed in the dapsone group, but was more common in the BPO group), but not at the end of 2nd, and 3rd months (p=0.101, and 0.182, respectively).

When the difference between the two groups in terms of burning-stinging was investigated, there was a significant difference at the end of the 1st month (p<0.001, more common in the BPO group), but not at the end of 2nd, and 3rd months (p=0.111, and p=0.101, respectively). When the severity of the burning-stinging sensation was compared, there was a significant difference at the end of each three months of treatment (p<0.001 for all), between the two groups (Table 4).

Table 3. Satisfaction levels of the patients

	Dapsone (n=48)	**BPO (n=53)**	**p**
Satisfaction, n (%)			
Very satisfied	22 (45.8)	4 (7.5)	
Satisfied	26 (54.2)	31 (58.5)	**<0.001**
Not satisfied	0 (0.0)	18 (34.0)	

BPO: benzoyl peroxide

DISCUSSION

Dapsone (4-amino4-diphenyl sulfone) is a drug from the sulfone group discovered in 1908 and it is mainly used to treat leprosy, but also in the treatment of dermatitis herpetiformis, vasculitis, and neutrophilic dermatoses. It competitively inhibits dihydropteroate synthetase with para-aminobenzoic acid (inhibits dihydrofolic acid production) and has both anti-inflammatory and antimicrobial activity (3-5). It has an antibacterial effect on Cutibacterium acnes (4). Some studies have shown that dapsone inhibits neutrophil migration by suppressing interleukin (IL)-8 release, which is important in neutrophil chemotaxis, prevents B2 integrin from binding to neutrophils, suppresses myeloperoxidase-induced ionization and cytotoxicity of neutrophils, and inhibits leukotriene B4-mediated chemotactic response of neutrophils by preventing its binding to neutrophils (6-9).

Systemic usage has side effects such as hemolytic anemia, methemoglobinemia, agranulocytosis, peripheral neuropathy, vertigo, headache and hearing loss, nausea-vomiting, abdominal pain, and eosinophilic pneumonia (4,10,11). Checking glucose-6-phosphate dehydrogenase levels, liver function tests and complete blood count before treatment and repeating the tests during the treatment period may reduce side effects. There are also cases of dapsone-associated photodermatitis reported during the treatment of linear Ig-A dermatosis with oral dapsone (12).

Table 4. Side effect data in groups by month

	Dapsone (n=48)			BPO (n=53)		
	1st-month	**2nd-month**	**3rd-month**	**1st-month**	**2nd-month**	**3rd-month**
Erythema, n (%)	3 (6.3)	1 (2.1)	0 (0.0)	27 (50.9)	8 (15.1)	6 (11.3)
Erythema, n (%)						
Mild	2 (4.2)	1 (2.1)	0 (0.0)	5 (9.4)	3 (5.7)	5 (9.4)
Moderate	1 (2.1)	0 (0.0)	0 (0.0)	2 (3.8)	3 (5.7)	1 (1.9)
Severe	0 (0.0)	0 (0.0)	0 (0.0)	20 (37.7)	2 (3.8)	0 (0.0)
Dryness, n (%)	6 (12.5)	4 (8.3)	3 (6.3)	30 (56.6)	12 (22.6)	10 (18.9)
Dryness, n (%)						
Mild	4 (8.3)	4 (8.3)	3 (6.3)	3 (5.7)	5 (9.4)	8 (15.1)
Moderate	2 (4.2)	0 (0.0)	0 (0.0)	5 (9.4)	3 (5.7)	2 (3.8)
Severe	0 (0.0)	0 (0.0)	0 (0.0)	22 (41.5)	4 (7.5)	0 (0.0)
Burning/Stinging, n (%)	10 (20.8)	8 (16.7)	5 (10.4)	35 (66)	16 (30.2)	12 (22.6)
Burning/Stinging, n (%)						
Mild	6 (12.5)	8 (16.7)	5 (10.4)	2 (3.8)	5 (9.4)	10 (18.9)
Moderate	4 (8.3)	0 (0.0)	0 (0.0)	2 (3.8)	6 (11.3)	2 (3.8)
Severe	0 (0.0)	0 (0.0)	0 (0.0)	31 (58.5)	5 (9.4)	0 (0.0)

BPO: benzoyl peroxide

No serious side effects have been reported in topical use, and we did not see any serious side effects in our study.

There was no significant difference between the two groups compared in this study in terms of demographic characteristics, pre-treatment ISGA values, and the number of lesions. When each group was evaluated separately, a statistically significant difference was found in the ISGA values and the lesion counts before and after the 3-month treatment, which shows that both treatment methods are effective for mild and moderate acne independently. When the groups were compared in terms of percentage improvements in the number of lesions, there was a higher improvement in the number of papules, pustules, and inflammatory lesions as well as the number of closed comedones in the dapsone group. Considering the main effect of dapsone, it is understandable that it improves inflammatory lesions, but the mechanism of the 68% improvement in closed comedones is not fully understood and this issue requires further studies. When the two groups were compared in terms of patient satisfaction, it was seen that there were no dissatisfied patients in the dapsone group, and the number of very satisfied patients in the BPO group was lower compared to the dapsone group. Side effects were more common in patients using BPO, especially in the 1st month of the treatment. The possibility of side effects is well-known in the application site of BPO.

The literature review found that most studies compared topical dapsone with placebo, and there were only a few studies in combination with systemic treatments.

Faghihi et al. (13) compared the efficacy and side-effect profile of systemic 20 mg/day isotretinoin + 5% dapsone and systemic 20 mg/day isotretinoin + placebo treatments in a 12-week treatment period in 58 moderate and severe acne patients aged 18-25 years in their placebo-controlled, randomized study. They found a significant improvement in the inflammatory lesion counts at the end of the treatment in the dapsone group compared to the other group, but no difference in the acne score between the placebo group. In our study, we observed a significant improvement in the number of inflammatory lesions in the group using dapsone. However, in our study, there was also a significant difference between the two groups in closed comedones and disease severity (improvement was greater in the dapsone group). They also found that dapsone was more effective in adult females.

Del Rosso et al. (14) conducted a 16-week study of 20 acne patients with trunk involvement and reported that the patients using 7.5% dapsone once a day had a decrease of 74% in the number of inflammatory lesions, 69% in the number of non-inflammatory lesions, and 72% in the total number of lesions. In our study, we also found similar results, we observed an improvement of 84% in inflammatory lesions, 65% in non-inflammatory lesions, and 74% in total lesion counts.

Tanghetti et al. (15) investigated the tolerability and efficacy of 5% dapsone applied twice daily in male and female acne patients and observed higher recovery rates and greater reductions in the number of lesions in female patients after 12 weeks of treatment. When we looked at the recovery percentages at the end of treatment in our study, we observed higher improvements in ISGA scores, closed comedones, papules, pustules, and inflammatory lesions in females.

Draelos et al. (16) evaluated 3010 people in 2 multicenter, 12-week, double-blind, randomized phase 3 studies and showed that 5% dapsone applied twice a day had a significant effect on acne scores compared to the control group (40.5% and 32.8% decrease, respectively). In our study, we found higher improvement rates in the severity score (71% for the dapsone group, and 51% for the BPO group). In the study, they also observed a significant decrease in both non-inflammatory (32% and 24%) and inflammatory (47.5% and 41.8%) acne lesions in the dapsone group compared to the control group (16). In our study, we found higher recovery rates in both the dapsone and BPO-using groups compared to this study. In the follow-ups, they did not see any abnormality (even in those with G-6PD deficiency) in laboratory tests. Side effects such as 21.8% dryness, 20% erythema, 1.4% burning sensation, 1% itching, and 0.1% irritation were observed in the dapsone group. In our study, the rate of erythema and dryness was lower. However, burning and stinging sensations were seen more frequently in our study.

Darjani et al. (17) compared 30 patients using 5% topical dapsone + 100 mg/day systemic doxycycline and 30 patients using 5% BPO + 100 mg/day systemic doxycycline in terms of recovery, side effects, and satisfaction in their randomized study. After 12 weeks of treatment, they found no significant difference between the groups in terms of the number of both inflammatory and non-inflammatory lesions. In the 4th, 8th, and 12th weeks of treatment, skin dryness was more common in the dapsone group than in the other group. Although erythema and irritation were seen more in the group using BPO, this difference was not found to be significant. 78% of the patients in the dapsone group and 69% in the BPO group were satisfied with the treatment result.

Jawade et al. (18) demonstrated that dapsone 5% gel was efficacious and well-tolerated in non-inflammatory and inflammatory acne lesions at the end of 12 weeks.

Kamoji et al. (19) showed that 0.1% adapalene and 1% clindamycin had good efficacy with fewer side effects than dapsone gel 5% for mild to moderate AV.

One of the limitations of the study is its retrospective nature. The treatment periods of the patients included in the study coincided with the summer period (in the cold months, the severity of side effects and patient satisfaction could be different) is among the shortcomings of the study. As there were no severe acne patients among the study patients, the results can not apply to severe acne.

CONCLUSION

Using topical 5% dapsone in the treatment of mild and moderate AV is found effective and safe in terms of side effects compared to topical 10% BPO. Case-control and/or split-face studies with large numbers of patients are needed to clarify this issue.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of Hitit University (09.11.2022, 2022-96).

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REFERENCES

1. Hsu P, Litman GI, Brodell RT. Overview of the treatment of acne vulgaris with topical retinoids. Postgrad Med. 2011;123(3):153-61.
2. Kraft J, Freiman A. Management of acne. CMAJ. 2011;183(7):E430-5.
3. Sago J, Hall RP. Dapsone. Dermatol Ther. 2002;15(4):340-51.
4. Wozel G, Blasum C. Dapsone in dermatology and beyond. Arch Dermatol Res. 2014;306(2):103-24.
5. Paniker U, Levine N. Dapsone and sulfapyridine. Dermatol Clin. 2001;19(1):79-86.
6. Schmitdt E, Reimer S, Kruse N, Bröcker EB, Zillikens D. The IL-8 release from human keratinocytes, mediated by anti-bodies to bullous pemphigoid autoantigen 180, is inhibited by dapsone. Clin Exp Immunol. 2001;124(1):157-62.
7. Booth SA, Moddy CE, Dahl MV, Herron MJ, Nelson RD. Dapsone suppresses intergrin-mediated neutrophil adherence function. J Invest Dermatol. 1992;98(2):130-40.
8. Kazmierowski JA, Ross JE, Peizner DS, Wuepper KD. Dermatitis herpetiformis: effects of sulfones and sulfonamides on neutrophil myeloperoxidase-mediated iodination and cytotoxicity. J Clin Immunol. 1984;4(1):55-64.
9. Wozel G, Lehmann B. Dapsone inhibits the generation of 5-lipoxygenase products in human polymorphonuclear. Skin Pharmacol. 1995;8(4):196-202.
10. Ross CM. The treatment of acne vulgaris with dapsone. Br J Dermatol. 1961;73(10):367-70.
11. Jaffuel D, Lebel B, Hillaire-Buys D, Pene J, Godard P, Michel FB, et al. Eosinophilic pneumonia induced by dapsone. BMJ. 1998;317(7152):181.
12. Stöckel S, Meurer M, Wozel G. Dapsone-induced photodermatitis in a patient with linear IgA dermatosis. Eur J Dermatol. 2001;11(1):50-3.
13. Faghihi G, Rakhshanpour M, Abtahi-Naeini B, Nilforoushzadeh MA. The efficacy of 5% dapsone gel plus oral isotretinoin versus oral isotretinoin alone in acne vulgaris: A randomized double-blind study. Adv Biomed Res. 2014;3:177.
14. Del Rosso JQ, Kircik L, Tanghetti E. Management of truncal acne vulgaris with topical dapsone 7.5% gel. J Clin Aesthet Dermatol. 2018;11(8):45-50.
15. Tanghetti E, Harper JC, Oefelein MG. The efficacy and tolerability of dapsone 5% gel in female vs male patients with facial acne vulgaris: gender as a clinically relevant outcome variable. J Drugs Dermatol. 2012;11(12):1417-21.
16. Draelos ZD, Carter E, Maloney JM, Elewski B, Poulin Y, Lynde C, et al. Two randomized studies demonstrate the efficacy and safety of dapsone gel, 5% for the treatment of acne vulgaris. J Am Acad Dermatol. 2007;56(3):439.e1-10.
17. Darjani A, Aboutaleb E, Alizadeh N, Rafiei R, Nejad KG, Nabatchii S, et al. Efficacy, safety, and tolerability of dapsone 5% gel and benzoyl peroxide 5% gel in combination with oral doxycycline in treating moderate acne vulgaris: a randomized clinical trial. Iran J Dermatol. 2022;25(2):132-41.
18. Jawade SA, Singh A. Efficacy of dapsone 5% gel in treatment of acne vulgaris. Int J Res Dermatol. 2016;2(4):77-81.
19. Kamoji SG, Huggi G, Pise GA, Nayak JJ, Dastikop SV, Patil MN. A double blind randomized study to compare efficacy of 5% dapsone gel vs combination of adapalene-clindamycin gel in the treatment of mild to moderate acne vulgaris. J Dermat Cosmetol. 2018;2(4):202-5.

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Detection of Mucous Retention Cysts Using Deep Learning Methods on Panoramic Radiographs

Panoramik Radyografilerde Mukos Retansiyon Kistlerinin Derin Öğrenme Yöntemleri Kullanılarak Tespiti

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ABSTRACT

Aim: This study aimed to perform clinical diagnosis and treatment planning of mucous retention cysts with high accuracy and low error using the deep learning-based EfficientNet method. For this purpose, a hybrid approach that distinguishes healthy individuals from individuals with mucous retention cysts using panoramic radiographic images was presented.
Material and Methods: Radiographs of patients who applied to the Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Fırat University between 2020 and 2022 and had panoramic radiography for various reasons were evaluated retrospectively. A total of 161 radiographs, 82 panoramic radiographs with mucous retention cysts and 79 panoramic radiographs without mucous retention cysts, were included in the study. In the classification process, deep feature representations or feature maps of the images were created using eight different deep learning models of EfficientNet from B0 to B7. The efficient features obtained from these networks were given as input to the support vector machine classifier, and healthy individuals and patients with mucous retention cysts were classified.
Results: As a result of the model training, it was determined that the EfficientNetB6 model performed the best. When all performance parameters of the model were evaluated together, the accuracy, precision, sensitivity, specificity, and F1 score values were obtained 0.878, 0.785, 0.916, 0.857, and 0.846, respectively.
Conclusion: The proposed hybrid artificial intelligence model showed a successful classification performance in the diagnosis of mucous retention cysts. The study will shed light on other future studies that will serve the same purpose.
Keywords: Deep learning; panoramic radiography; maxillary sinus; cyst.

ÖZ

Amaç: Bu çalışmada derin öğrenme tabanlı EfficientNet yöntemi kullanılarak mukos retansiyon kistlerinin yüksek doğruluk ve düşük hata ile klinik tanı ve tedavi planlamasının yapılması amaçlanmıştır. Bu amaçla panoramik radyografik görüntüler kullanılarak sağlıklı bireyleri mukos retansiyon kisti olan bireylerden ayıran hibrit bir yaklaşım sunulmuştur.
Gereç ve Yöntemler: Fırat Üniversitesi Diş Hekimliği Fakültesi Ağız, Diş ve Çene Radyolojisi Anabilim Dalı'na 2020 ve 2022 yılları arasında başvuran ve çeşitli nedenlerle panoramik radyografi çekilmiş olan hastaların radyografileri geriye dönük olarak değerlendirilmiştir. Mukos retansiyon kisti bulunan 82 panoramik radyografi ve mukus retansiyon kisti bulunmayan 79 panoramik radyografi olmak üzere toplamda 161 radyografi bu çalışmaya dahil edilmiştir. Sınıflandırma sürecinde EfficientNet'in B0'dan B7'ye kadar sekiz farklı derin öğrenme modeli kullanılarak görüntülerin derin özellik temsilleri veya özellik haritaları oluşturulmuştur. Bu ağlardan elde edilen verimli özellikler, destek vektör makinesi sınıflandırıcısına girdi olarak verilmiş ve sağlıklı bireyler ile mukos retansiyon kisti olan hastalar sınıflandırılmıştır.
Bulgular: Model eğitimleri sonucunda EfficientNetB6 modelinin en iyi performansı sergilediği belirlenmiştir. Modelin tüm performans parametreleri birlikte değerlendirildiğinde, doğruluk, kesinlik, duyarlılık, özgüllük ve F1 puanı değerleri sırasıyla 0,878, 0,785, 0,916, 0,857 ve 0,846 olarak elde edilmiştir.
Sonuç: Önerilen hibrit yapay zeka modelinin mukos retansiyon kisti teşhisinde başarılı bir sınıflandırma performansı göstermiştir. Bu çalışmanın aynı amaca hizmet edecek gelecekteki diğer çalışmalara ışık tutacağı düşünülmektedir.
Anahtar kelimeler: Derin öğrenme; panoramik radyografi; maksiller sinüs; kist.

INTRODUCTION

Panoramic radiography is the most preferred imaging method for diagnosis and treatment planning in dentistry due to its features such as low cost, low radiation dose, and comprehensive view of the anatomical structures in the maxillofacial region (1). Maxillary sinuses are structures adjacent to the oral mucosa, their borders and contents can be viewed in panoramic images and they are important anatomical structures that should be taken into consideration in dentistry practices (2). Mucous retention cysts (MRC) and pseudocysts are defined as dome-shaped, well-circumscribed radiopacities, formed because of fluid accumulation in the sinus membrane. MRC is called a "true cyst" because it has a thin epithelial lining that is formed due to obstruction of the salivary gland ducts, whereas "pseudocysts" lack an epithelial wall and result from diffuse subepithelial accumulation of inflammatory exudate. Since both are radiologically indistinguishable, many investigators have described them as sharply circumscribed, dome-shaped radiopaque formations arising from the antral wall, without differentiating between MRC and pseudocysts on radiographic images (2,3).

Inflammatory and traumatic causes such as allergy, barotrauma, and rhinitis are frequently considered in the etiology of mucosal cysts; and headache, nasal congestion, facial pain, and postnasal discharge are rarely suspected. The pain in this area can be confused with toothache, and it is known that dental infections also cause MRC (4,5). The presence of MRC increases the possibility of complications in surgical procedures planned for reasons such as missing teeth and residual ridge insufficiency in the posterior maxilla (3,6). Therefore, during the clinical examination, a careful sinus examination and the detection of MRC, which often does not cause subjective findings, are required.

Deep learning methods, which aim to solve problems that are solved by human intelligence and skills, with artificial intelligence (AI) are rapidly developing in the field of health. These methods, supported by digital data, have begun to be widely used in dentistry, where data flow is continuous. It is possible to detect and distinguish anatomical and pathological structures in panoramic radiographs with AI-based deep learning methods (7,8). Convolutional neural network (CNN) is one of the AI-based deep learning methods and has been used in many research such as the diagnosis of caries, periapical lesions, cysts, tumors, and cancers on dental radiological images (8-10). The increasing use of these AI-supported innovative technologies helps more careful radiographic interpretations by preventing asymptomatic lesions from being undiagnosed due to the pain-focused dental approach of most physicians and missed by inexperienced or unaware physicians, contributing to higher sensitivity and fewer errors. In addition, it aims to contribute to the education of physicians during the training process, facilitate the work of physicians, and improve the management of patients and treatment results (11). EfficientNet, one of the advanced deep learning models, used for the accurate diagnosis of MRC, provides reliable results due to its high sensitivity and low error rates (12,13).

In this study, it was aimed to contribute to the clinical diagnosis and treatment planning of MRC with higher precision and fewer errors by using AI-based deep learning methods, to improve clinical decision-making processes and to manage patients' health conditions more effectively. For this purpose, a hybrid approach is presented to distinguish healthy individuals from individuals with MRC using panoramic radiographs.

MATERIAL AND METHODS

The radiographs of patients applied to the Department of Oral and Maxillofacial Radiology of Fırat University Faculty of Dentistry between 2020 and 2022 and were taken panoramic radiography for various reasons were evaluated retrospectively. A total of 205 panoramic radiographs, which were taken on the Planmeca Promax (Helsinki, Finland) 2D Digital Panoramic X-ray Device, were evaluated and 44 films with poor image quality were excluded. 82 panoramic radiographs with MRC and 79 panoramic radiographs without MRC were included in the study (Figure 1). Ethical approval was obtained from the Fırat University Non-Interventional Clinical Research Ethics Committee (23.02.2023, 03-15).

Model

This study presents a hybrid approach to distinguish between healthy individuals and individuals with MRC using panoramic images. The proposed model consists of three stages; data preprocessing, feature extraction, and classification. After the dataset was created, the images were resized according to the standard input size of the EfficientNet deep network in the first stage. Images of 600*600 size were converted to 224*224 size to be fed to the CNN model. In the second stage, eight different EfficientNet models from B0 to B7 and feature maps representing deep features of the images were generated. In the last step, the features obtained from each model were used as input to the support vector machine (SVM) classifier to distinguish healthy individuals and individuals with MRC. Sensitivity, specificity, precision, accuracy, and F1-score criteria were used to compare the performance of the networks. These evaluation criteria were considered to determine the classification ability of each model and to evaluate the overall effectiveness of the study. The flow diagram including all stages of the proposed study was presented in detail (Figure 2).

Data Collection and Data Preprocessing

In this stage, the panoramic radiographs of the patients who applied to the Department of Oral and Maxillofacial Radiology of Fırat University Faculty of Dentistry were used. The radiographic images used in this study were selected from patients with an informed consent form. Patient records were protected in accordance with medical ethics rules. The data obtained at this stage was resized to be given as input to the EfficientNet network in the next stage.

Feature Extraction and Classification

The EfficientNet deep network model was used in the feature extraction stage of the study. By optimizing the size of models, EfficientNet reduces the computational cost and provides high accuracy. Although the success of the models used for the first time in the ImageNet dataset increases in parallel with the model complexity, the computational costs of these models are quite high. As a solution to this problem, EfficientNet uses eight different

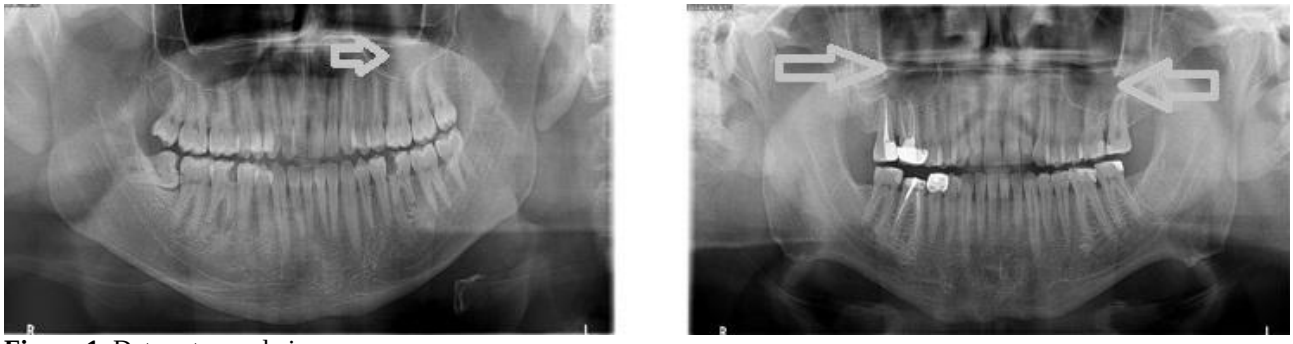

Figure 1. Data set sample images

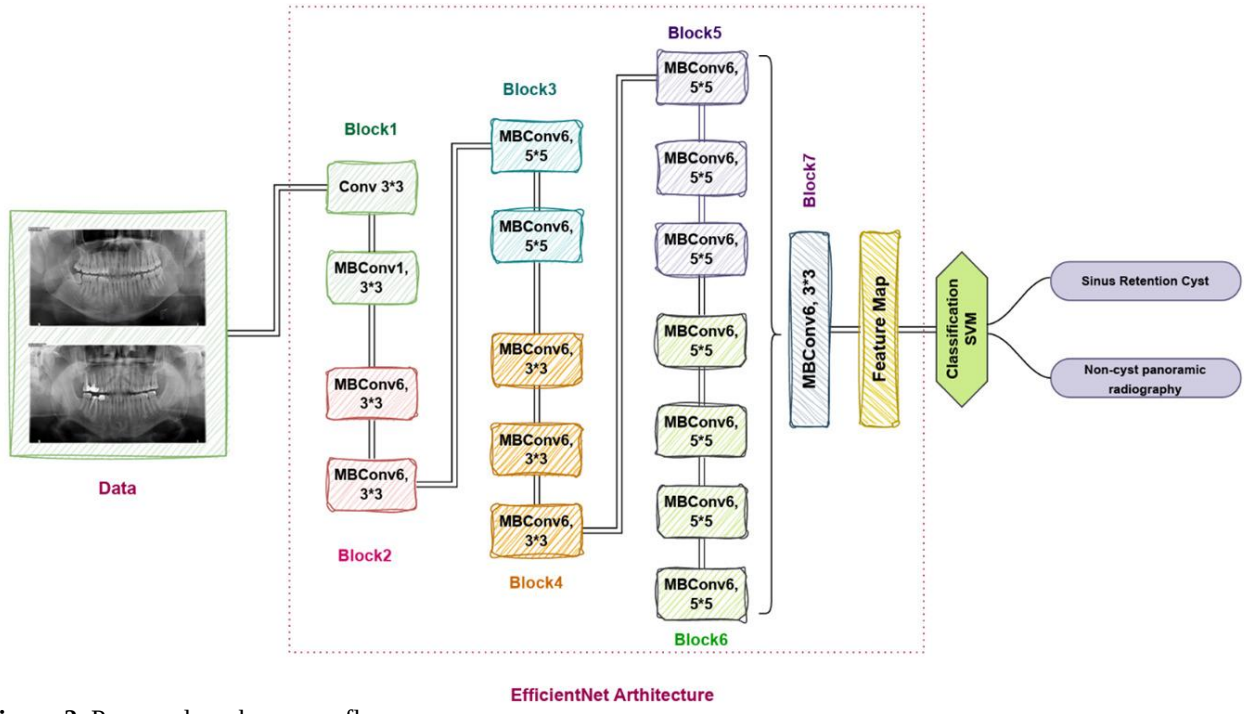

Figure 2. Proposed work process flow

CNN versions from B0 to B7, greatly increase the accuracy rate without adding more parameters (14). EfficientNet uses a new activation function known as Swish instead of the traditional ReLU activation function. This helps the model to learn and extract features. EfficientNet achieves significant success by providing more effective and efficient approaches for smaller models (15). In this study, feature maps of the data were created using 8 different versions of the EfficientNet model, from 0 to 7. Each version establishes a balanced relationship between depth, width, and resolution dimensions. This diversity provides suitable options for feature extraction in a variety of tasks such as data mining, image processing, and classification and flexibility for various application scenarios.

Classification is a data mining function that assigns features to specific groups. Its main purpose is to precisely anticipate the target class for each sample in the data (16). Classification has important applications in computer vision, medicine, engineering, and many other fields. It plays an important role in determining the conditions of patients, especially in the diagnosis of diseases in medicine. In this stage, features obtained from EfficientNet network versions were classified using SVM for each version. The results obtained were evaluated using precision, sensitivity, specificity, F1-Score, and accuracy metrics (17). Calculations of the evaluation metrics used are given with the complexity matrix (Figure 3).

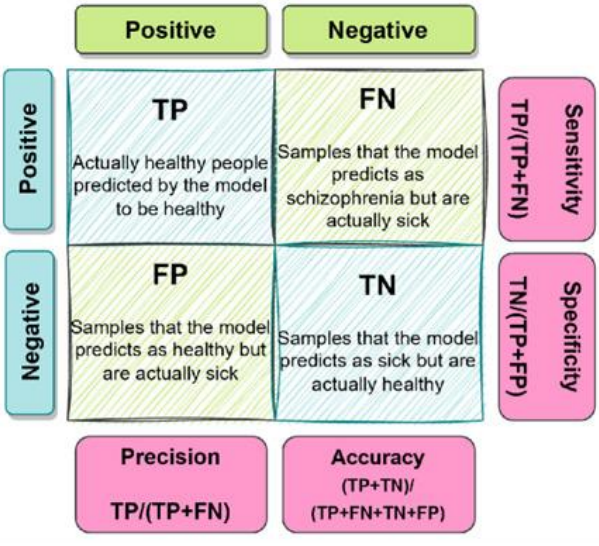

Figure 3. Performance evaluation metrics

These evaluations in the classification stage investigate whether EfficientNet networks can distinguish between healthy people and people with MRC. The metrics given in Figure 3 were used to objectively evaluate the performance of the classification algorithms and understand the results.

RESULTS

In the first stage of the proposed method, feature extraction was performed on panoramic radiographs with the feature extractor EfficientNet models (B0 to B7) to detect MRC. The extracted features were represented in 1xn size according to the output feature of the EfficientNet model and were given as input to the SVM algorithm.

The accuracy value alone may not be sufficient to reliably evaluate the performance of the proposed model. Therefore, various metrics such as F1 score, sensitivity, and specificity were also used in the experiments. Sensitivity measures the test's ability to identify true positive results, while specificity measures the rate of missing false positive results in those without the disease condition. When used together, these measurements help fully evaluate the model's performance. In this study, it is observed that the highest performance is achieved with the EfficientNetB6 model (Table 1). As the parameters of the model, the rates of 0.878, 0.785, 0.916, 0.857, and 0.846 were obtained for accuracy, precision, sensitivity, specificity, and F1 score, respectively.

The proposed system demonstrated a high classification performance by helping to diagnose MRC. The findings show that the image processing and machine learning methods of the proposed study can be used effectively in the diagnosis of MRC. Experimental results show that the model stands out as a potential clinical aid in the MRC diagnostic process.

DISCUSSION

A correct and complete examination is possible by evaluating the patient history (anamnesis) and radiological findings as a whole and integrating them with current technological developments. The use of AI in head and neck imaging during radiological detection of anomalies that cannot be noticed by the human eye or anomalies that are overlooked due to physician inexperience and fatigue has been rising in the last 20 years (18). For this purpose, digital imaging methods such as panoramic radiography and advanced imaging methods such as cone-beam computed tomography (CBCT), ultrasonography (USG), and magnetic resonance imaging (MRI) have been rapidly integrated into this new system (7,19-22). Panoramic radiographs, which are the most frequently used routinely, have become pioneers in providing sufficient data for AI-based learning methods because they allow the evaluation of a wide area including teeth and jaws, have low radiation dose, cost-effective, easily accessible and easy to apply (1,8). The development of CNN provides useful results to clinicians in the detection of normal structures, diagnosis of abnormal structures, treatment planning, and follow-up (22,23). In this study, a hybrid classification process was carried out using EfficientNet and SVM, one of the CNN models, to detect MRC in panoramic radiographs, which do not give any subjective findings, and diagnosis is often missed by clinicians due to overlapping of anatomical structures such as the nasal floor and hard palate.

Murata et al. (24) have aimed to diagnose maxillary sinusitis from radiographs using deep learning methods with 400 healthy individuals and 400 patients with inflamed maxillary sinus. They increased the amount of data for healthy and patient groups up to 6000 samples, increasing the total number of data up to 12000. They designed the learning process as 200 epochs and included 120 data, 60 patients with diseases and 60 healthy, in the training input set. They reached 0.875 accuracy, 0.867 sensitivity, 0.883 specificity, and 0.875 area under the curve (AUC) performance values. The obtained results were examined mutually with the predictions of two radiologists and two research assistants. They stated that there is not a significant difference with radiologists, but they achieved a higher performance compared to research assistants. In the proposed study, the accuracy rate was calculated as 0.878 compared to this study, a higher success rate was achieved with the proposed method even though we used a smaller data set. The reasons for the difference in model performance include many different factors such as choosing the right model and setting the parameters correctly.

Kuwana et al. (25) used the radiographic images of 416 inflamed maxillary sinuses and 171 maxillary sinus cysts to detect cysts and inflammation in the maxillary sinus in panoramic radiographs. The obtained data were divided into 3 different groups, training, test1, and test2 to be used in the training and testing process. Using the training data, they carried out a learning process of 1000 epochs with the DetectNet model. They tested the training model obtained separately with test1 and test2 data and evaluated them mutually. They stated that the model they proposed showed 1.0 accuracy for inflamed and healthy sinuses, while it showed 0.98 and 0.89 accuracy in cyst detection. In the proposed study, in addition to only incorporating deep learning methods into the process, machine learning

Table 1. Performance metrics obtained as a result of the study

Model	Accuracy	Precision	Sensitivity	Specificity	F1 Score
EfficientNetB0	0.7879	0.8125	0.7647	0.8125	0.7879
EfficientNetB1	0.8182	0.8667	0.7647	0.8750	0.8125
EfficientNetB2	0.8182	0.9231	0.7059	0.9375	0.8000
EfficientNetB3	0.8182	0.8462	0.7333	0.8889	0.7857
EfficientNetB4	0.7879	0.7857	0.7333	0.8333	0.7586
EfficientNetB5	0.8485	0.8125	0.8667	0.8333	0.8387
EfficientNetB6	**0.8788**	**0.7857**	**0.9167**	**0.8571**	**0.8462**
EfficientNetB7	0.8485	0.8235	0.8750	0.8235	0.8485

methods are also included in the study by presenting a hybrid approach. Compared to their study, accuracy was approximately 0.02 lower. The reasons for this situation include factors such as the number of data and the deep network model. It is seen that if data is expanded and the number of models is diversified, the performance will increase accordingly.

Another study aimed to create effective models for the detection of maxillary sinuses in panoramic radiographs and diagnosis of sinusitis by transferring the deep learning source model from one institution to another. 350 panoramic radiographs from source A and 25, 50, 100, 150, or 225 panoramic radiographs from source B were included in the study, and target models named T25, T50, T100, T150, and T225 were created. The study showed that the maxillary sinus detection performance of the source model was high when test data from source A was used, but its performance was low when test data from source B was used. B's test data proves that the T25 model has better detection performance. In addition, the T50 model is highly sensitive for the maxillary sinusitis diagnosis (26). In the present study, a versatile hybrid method is proposed, as opposed to using only a deep model. However, an approach is being considered in which various models can be tried to achieve higher performance and diversity.

Another study investigated the maxillary sinusitis diagnosis performance of the model with a large panoramic radiography data set in source A, using the transfer learning method with a limited number of Waters' radiography in source B. The model was created with VGG-16 using a data set consisting of 800 training and 60 validation data for 200 training rounds. Also tested with 180 Waters' and 180 panoramic images from source B. The target model was used for transfer learning over several 200 training rounds on training and validation sets of Waters' radiographs. By applying test Waters' images to the source and target models, the performance of both models was evaluated. When Waters' images are used as the test set, the target model works better than the source model, showing that transfer learning is used effectively by using a limited number of data to increase maxillary sinusitis diagnostic performance (27). In parallel with this study, significant results were obtained with a limited number of data in this proposed study. This is an indication that the problem of limited data can be minimized when correct model selection and parameter setting are performed. When the limitations and main contributions of the study are evaluated, the restricted number of studies in the literature constitutes the main contribution of the proposed study to the literature. The studies in the literature are carried out using only deep network models rather than hybrid methods. In the proposed study, a hybrid method is presented by combining deep learning and machine learning models. The small number of data used in the study is one of the limitations affecting the performance of the study. Another limitation is that we can not create our deep architecture due to a lack of hardware. It is thought that the operating performance will improve when the number of data increases, the necessary hardware is provided and the architectural design is carried out manually which is specific to the problem.

CONCLUSION

This study has shown that MRC in the maxillary sinuses can be detected and safely identified using an AI-based hybrid method. In line with the obtained results, it can be stated that if the specified limitations are improved in the following stages, there will be an increase in operating performance.

Ethics Committee Approval: The study was approved by the non-interventional clinical research ethics committee of Fırat University (23.02.2023, 03-15).

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REFERENCES

1. Roman JCM, Fretes VR, Adorno CG, Silva RG, Noguera JLV, Legal-Ayala H, et al. Panoramic dental radiography image enhancement using multiscale mathematical morphology. Sensors (Basel). 2021;21(9):3110.
2. Meer S, Altini M. Cysts and pseudocysts of the maxillary antrum revisited. SADJ. 2006;61(1):10-3.
3. Anitua E, Alkhraisat MH, Torre A, Eguia A. Are mucous retention cysts and pseudocysts in the maxillary sinus a risk factor for dental implants? A systematic review. Med Oral Patol Oral Cir Bucal. 2021;26(3): e276-83.
4. Nemati P, Jafari-Pozve N, Aryanezhad SS. Association between mucous retention cyst of paranasal sinuses and nasal septum deviation. Adv Oral Maxillofac Surg. 2023;10:100415.
5. Rastegar H, Osmani F. Evaluation of mucous retention cyst prevalence on digital panoramic radiographs in the local population of Iran. Radiol Res Pract. 2022;2022:8650027.
6. Beaumont C, Zafiropoulos GG, Rohmann K, Tatakis DN. Prevalence of maxillary sinus disease and abnormalities in patients scheduled for sinus lift procedures. J Periodontol. 2005;76(3):461-7.
7. Carrillo-Perez F, Pecho OE, Morales JC, Paravina RD, Della Bona A, Ghinea R, et al. Applications of artificial intelligence in dentistry: A comprehensive review. J Esthet Restor Dent. 2022;34(1):259-80.
8. Khanagar SB, Al-Ehaideb A, Maganur PC, Vishwanathaiah S, Patil S, Baeshen HA, et al. Developments, application, and performance of artificial intelligence in dentistry - A systematic review. J Dent Sci. 2021;16(1):508-22.

9. Agrawal P, Nikhade P. Artificial intelligence in dentistry: past, present, and future. Cureus. 2022;14(7):e27405.
10. Ilhan B, Lin K, Guneri P, Wilder-Smith P. Improving oral cancer outcomes with imaging and artificial intelligence. J Dent Res. 2020;99(3):241-8.
11. Vodanović M, Subašić M, Milošević D, Savić Pavičin I. Artificial intelligence in medicine and dentistry. Acta Stomatol Croat. 2023;57(1):70-84.
12. Kurt Z, Işık Ş, Kaya Z, Anagün Y, Koca N, Çiçek S. Evaluation of EfficientNet models for COVID-19 detection using lung parenchyma. Neural Comput Appl. 2023;35(16):12121-32.
13. Gunwant H, Joshi A, Sharma M, Gupta D. Automated medical diagnosis and classification of skin diseases using Efficinetnet-B0 convolutional neural network. In: Castillo O, Melin P, editors. New perspectives on hybrid intelligent system design based on fuzzy logic, neural networks and metaheuristics. Springer, Cham; 2022. p.3-19.
14. Duong LT, Nguyen PT, Di Sipio C, Di Ruscio D. Automated fruit recognition using EfficientNet and MixNet. Comput Electron Agric. 2020;171:105326.
15. Atila Ü, Uçar M, Akyol K, Uçar E. Plant leaf disease classification using EfficientNet deep learning model. Ecol Inform. 2021;61:101182.
16. Açıkoğlu M, Arslan Tuncer S. Classification of 1D and 2D EEG signals for seizure detection in the newborn using convolutional neural networks. BEU J Sci. 2022;11(1):194-202.
17. Çelik F, Aydemir E. Prediction of difficult tracheal intubation by artificial intelligence: a prospective observational study. Duzce Med J. 2021;23(1):47-54.
18. Rajaram Mohan K, Mathew Fenn S. Artificial intelligence and its theranostic applications in dentistry. Cureus. 2023;15(5):e38711.
19. Mureșanu S, Almășan O, Hedeșiu M, Dioșan L, Dinu C, Jacobs R. Artificial intelligence models for clinical usage in dentistry with a focus on dentomaxillofacial CBCT: a systematic review. Oral Radiol. 2023;39(1):18-40.
20. Baydar O, Ulusoy AC, Alpöz E. Artificial intelligence in maxillofacial ultrasonography applications. EÜ Dişhek Fak Derg. 2022;43(Ozel Sayi):11-7. Turkish.
21. Fujima N, Kamagata K, Ueda D, Fujita S, Fushimi Y, Yanagawa M, et al. Current state of artificial intelligence in clinical applications for head and neck MR imaging. Magn Reson Med Sci. 2023;22(4):401-14.
22. Li M, Punithakumar K, Major PW, Le LH, Nguyen KT, Pacheco-Pereira C, et al. Temporomandibular joint segmentation in MRI images using deep learning. J Dent. 2022;127:104345.
23. Sivari E, Senirkentli GB, Bostanci E, Guzel MS, Acici K, Asuroglu T. Deep learning in diagnosis of dental anomalies and diseases: a systematic review. Diagnostics (Basel). 2023;13(15):2512.
24. Murata M, Ariji Y, Ohashi Y, Kawai T, Fukuda M, Funakoshi T, et al. Deep-learning classification using convolutional neural network for evaluation of maxillary sinusitis on panoramic radiography. Oral Radiol. 2019;35(3):301-7.
25. Kuwana R, Ariji Y, Fukuda M, Kise Y, Nozawa M, Kuwada C, et al. Performance of deep learning object detection technology in the detection and diagnosis of maxillary sinus lesions on panoramic radiographs. Dentomaxillofac Radiol. 2021;50(1):20200171.
26. Mori M, Ariji Y, Katsumata A, Kawai T, Araki K, Kobayashi K, et al. A deep transfer learning approach for the detection and diagnosis of maxillary sinusitis on panoramic radiographs. Odontology. 2021;109(4):941-8.
27. Kotaki S, Nishiguchi T, Araragi M, Akiyama H, Fukuda M, Ariji E, et al. Transfer learning in diagnosis of maxillary sinusitis using panoramic radiography and conventional radiography. Oral Radiol. 2023;39(3):467-74.

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The Impact of Adhering to a Mediterranean Diet on the Severity of Overuse Tendinopathy Symptoms

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ABSTRACT

Aim: The Mediterranean diet's high carbohydrate content and antioxidant and anti-inflammatory properties make it appealing to athletes. Tendinopathy etiology is influenced by nutrition. Athletes with high adherence to the Mediterranean diet may have a lower severity of tendinopathy. This study aimed to investigate the effect of adherence to the Mediterranean diet on tendinopathy severity.
Material and Methods: Exercise habits, physical activity level, body region, stage of the injury, age, body mass index, gender, smoking status, and presence of chronic diseases of individuals older than 18 years of age and who were diagnosed with tendinopathy in the sports medicine clinic were recorded in this cross-sectional study. The Revised Oslo Sports Trauma Research Centre Overuse Injury and Health Problems Questionnaires and the Mediterranean diet adherence screener were applied to participants.
Results: A total of 152 participants, 52% (n=79) of whom were female, with lateral epicondylopathy (n=71), Achilles tendinopathy (n=25), plantar fasciitis (n=23), patellar tendinopathy (n=13), and other tendinopathies were included in the study. Upon examination of the relationship between the level of adherence to the Mediterranean diet and the severity of complaints due to overuse, no significant differences were found between groups (p=0.935, and p=0.927, respectively). A weak negative correlation was found between weekly exercise duration and adherence to the Mediterranean diet (r_s=-0.237; p=0.016).
Conclusion: No evidence was found that adherence to the Mediterranean diet was associated with differences in the severity of tendinopathy complaints. Athletes with high weekly exercise durations do not adopt the Mediterranean diet as an optimal dietary approach.
Keywords: Mediterranean diet; athletes; tendinopathy; diet patterns.

ÖZ

Amaç: Akdeniz diyetinin yüksek karbonhidrat içeriği ve antioksidan ve anti-inflamatuvar özellikleri bu diyeti sporcular için cazip kılmaktadır. Tendinopati etiyolojisi beslenmeden etkilenmektedir. Akdeniz diyetine bağlılığı yüksek olan sporcularda tendinopati şiddeti daha düşük olabilir. Bu çalışmanın amacı Akdeniz diyetine bağlılığın tendinopati şiddeti üzerindeki etkisini araştırmaktır.
Gereç ve Yöntemler: Bu kesitsel çalışmada spor hekimliği kliniğinde tendinopati tanısı almış olan 18 yaş üstü bireylerin egzersiz alışkanlıkları, fiziksel aktivite düzeyi, vücut bölgesi, yaralanma evresi, yaş, vücut kitle indeksi, cinsiyet, sigara içme durumu ve kronik hastalık varlık durumları kayıt edildi. Katılımcılara Revize Edilmiş Oslo Spor Travması Araştırma Merkezi Aşırı Kullanım Yaralanması ve Sağlık Sorunları Anketleri yanı sıra Akdeniz diyetine uyum tarama aracı uygulandı.
Bulgular: Lateral epikondilopati (n=71), Aşil tendinopatisi (n=25), plantar fasiit (n=23), patellar tendinopati (n=13) ve diğer tendinopatileri olan, %52'si (n=79) kadın olmak üzere toplam 152 katılımcı bu çalışmaya dahil edildi. Akdeniz diyetine uyum düzeyi ile şikâyetlerin şiddeti arasında ilişki olup olmadığı incelendiğinde, gruplar arasında anlamlı bir fark olmadığı bulundu (sırasıyla p=0,935 ve p=0,927). Haftalık egzersiz süresi ile Akdeniz diyetine bağlılık düzeyi arasında zayıf düzeyde negatif bir korelasyon olduğu bulundu (r_s=-0,237; p=0,016).
Sonuç: Akdeniz diyetine uyumun tendinopati şikâyetlerinin şiddetindeki farklılıklarla ilişkili olduğuna dair bir kanıt bulunamadı. Haftalık egzersiz süreleri yüksek olan sporcular, Akdeniz diyetini en uygun bir beslenme yaklaşımı olarak benimsememektedir.
Anahtar kelimeler: Akdeniz diyeti; sporcular; tendinopati; diyet kalıpları.

INTRODUCTION

The Mediterranean diet is a dietary pattern that promotes good health. It is characterized by a high intake of olive oil and low intake of saturated fats and includes plenty of fruits, vegetables, cereals, legumes, nuts, a moderate amount of fish and chicken (2-4 times per week), and low levels of red meat (1-2 times per month). Additionally, it is common to consume small amounts of red wine (1 glass per day for females, two glasses per day for males) with meals (1). The Mediterranean diet's high carbohydrate content and antioxidant and anti-inflammatory properties make it appealing to athletes. Numerous studies have demonstrated that the Mediterranean diet possesses anti-inflammatory properties, resulting in a reduction of tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and c-reactive protein (CRP) levels (2,3).

Tendinopathy refers to pain and loss of function in tendons caused by mechanical loading (4). It is a prevalent injury among athletes and the general population. Treatment can be challenging, whether the condition is acute or chronic, and it may become a persistent injury that is unresponsive to treatment (5,6). Tendon overload is linked to markers of inflammation and matrix degradation, which can lead to tendinopathy. This condition is characterized by matrix remodeling due to inflammation and damage caused by cytokines and inflammatory cells. The presence of increased macrophage numbers in tendinopathy also indicates inflammation (6,7). Studies on mixed inflammatory processes suggest that inflammation may persist in tendinopathy (5).

The easy adoption of the Mediterranean diet by athletes may be attributed to its richness in carbohydrates and possession of antioxidant and anti-inflammatory properties (8). A review of the effects of nutrition on tendon health indicates that a variety of nutrients, including amino acids, vitamins, and trace minerals, may be beneficial in improving tendon growth and healing. It has been proposed that nutritional interventions comprising multiple nutrients may be more efficacious in tendon and collagen metabolism (9).

Tendinopathy etiology is known to be influenced by various habits, including nutrition (10,11). The hypothesis is that individuals with high adherence to the Mediterranean diet will have a lower severity of tendinopathy. This study aimed to investigate the effect of adherence to the Mediterranean diet on tendinopathy severity.

MATERIAL AND METHODS

The study received ethical approval from the Süleyman Demirel University Health Sciences Ethics Committee on 24.10.2022 (number: 61/4). Participants over 18 years of age diagnosed with tendinopathy due to overuse injury, as determined by sports medicine examination between November 2022 and April 2024, were included in this cross-sectional study. Healthy individuals were not included in the study. Participants were included in the study after signing the informed consent.

Descriptive Information Form

The descriptive characteristics, such as age, body mass index, gender, smoking status, presence of chronic diseases, exercise habits, physical activity level (12), body region, and stage of injury by self-answering (12), of the participants included in the study were recorded. Additionally, the diagnosis made by the sports medicine after the examination was also documented.

The Revised Oslo Sports Trauma Research Centre Overuse Injury Questionnaire (OSTRC-O) and Questionnaire on Health Problems (OSTRC-H)

The questionnaires aim to evaluate athletes' health concerning their sports involvement, training adjustments, performance, and any pain or symptoms they may experience (13). The questionnaire was converted into two separate measurement tools evaluating overuse injuries and general health, and adjustments had to be made in the answer options to the questions over time. The score of the questionnaire, whose Turkish validity and reliability was conducted by Özal, Kafa, and Güzel following a thesis study in 2023, ranges from 0 to 100, with a higher score indicating more severe health problems (14).

The Mediterranean Diet Adherence Screener (MEDAS)

It is a 14-item scale developed to examine the adherence levels of individuals to the Mediterranean diet (15). Depending on the answer given to the questions related to nutrition, a score of 1 or 0 is obtained, and the total score is obtained by summing the answers given to all questions. The score that can be obtained from the scale is in the range of 0-14. A total score of 7 and above indicates that the individual has an acceptable degree of compliance with the Mediterranean diet (Group acceptable), and a score of 9 and above indicates that the individual has strict compliance with the Mediterranean diet (Group compatible). Turkish validity and reliability of the scale were performed by Pehlivanoğlu et al. (16).

Statistical Analysis

IBM SPSS v.23 package was used for the analyses. Since the data were not normally distributed, the Kruskal-Wallis test with Dunn's test and the chi-square test with Monte Carlo simulation were used to analyze the difference between the groups formed according to the level of adherence to the Mediterranean diet. Spearman correlation analysis was used to analyze the relationship between adherence to the Mediterranean diet and other variables. Data were presented as n (%), and median (interquartile range, IQR) [minimum-maximum]. A p-value of <0.05 was accepted as the statistical significance level.

RESULTS

A total of 152 participants, 52% (n=79) of whom were female, were included in the study. The injured body regions were elbow (50%, n=76), ankle (16.4%, n=25), foot (15.1%, n=23), knee (8.6%, n=13), thigh (2.6%, n=4), and other regions, respectively.

The diagnoses made by the sports medicine included lateral epicondylopathy (46.7%, n=71), Achilles tendinopathy (16.4%, n=25), plantar fasciitis (15.1%, n=23), patellar tendinopathy (8.6%, n=13), medial epicondylopathy (2.6%, n=4), and other tendinopathies.

When the participants were grouped according to their MEDAS score, it was determined that females had a higher level of adherence to the Mediterranean diet (p=0.049). However, age, body mass index, smoking, presence of chronic disease, physical activity level, and stage of injury did not make a difference in terms of adherence to the Mediterranean diet (Table 1).

When it was examined whether the level of adherence to the Mediterranean diet caused a difference in the severity level of complaints, no significant difference was found between the groups (Table 2).

It was found that weekly exercise duration was negatively correlated with adherence to the Mediterranean diet at a low level (r_s=-0.237; p=0.016), while there was no other significant correlation (Table 3).

DISCUSSION

This study of 152 patients with tendinopathy found no difference in Mediterranean diet adherence on tendinopathy severity. Females demonstrated a higher level of adherence to the Mediterranean diet. Age, body mass index, smoking, presence of chronic disease, physical activity level, and stage of injury did not influence adherence to the Mediterranean diet. Furthermore, the adherence of athletes with more weekly exercise time did not correlate with higher adherence to the Mediterranean diet.

Numerous studies have investigated the relationship between chronic diseases and dietary patterns (17-22). It has been suggested that various dietary patterns may exhibit anti-inflammatory or pro-inflammatory properties (23). The Western diet is generally considered pro-inflammatory, whereas the Mediterranean diet is generally considered anti-inflammatory (24).

The Mediterranean diet is a traditional eating style that is specific to the Mediterranean region. It emphasizes a high proportion of fruits, vegetables, whole grains, olive oil, legumes, nuts, and seeds while limiting the consumption

Table 1. Descriptive characteristics of participants

	Adherence Level to the Mediterranean Diet			p
	Incompatible (n=63)	**Acceptable (n=66)**	**Compatible (n=23)**	
MEDAS (score)	5 (4-6) [2-6]	7 (7-8) [7-8]	9 (9-10) [9-12]	**<0.001**
Age (year)	42 (31-49) [18-64]	46 (31-53) [18-61]	48 (35-53) [18-69]	0.267
Body mass index (kg/m^2)	26.8 (24.2-30.1) [17.8-42.4]	25.6 (22.9-28.3) [18.4-44.4]	26.9 (23.1-28.9) [20-34.2]	0.516
Gender, n (%)				
Female	28 (44.4) [a]	34 (51.5) [a,b]	17 (73.9) [b]	**0.049**
Male	35 (55.6) [a]	32 (48.5) [a,b]	6 (26.1) [b]	
Smoking status, n (%)				
Smoking	15 (23.8)	13 (19.7)	5 (21.7)	
Quit	15 (23.8)	15 (22.7)	7 (30.4)	0.900
Never smoked	33 (52.4)	38 (57.6)	11 (47.8)	
Chronic disease, n (%)	16 (25.4)	22 (33.3)	10 (43.5)	0.281
Duration of regular sport (year)	10 (5-15) [1-40]	10 (2-15) [0.5-45]	6 (3-12.5) [0.5-46]	0.457
Weekly exercise duration (min)	285 (180-480) [60-3000]	180 (131.25-360) [60-720]	300 (75-600) [30-720]	0.229
Physical activity level, n (%)				
Professional	14 (22.2)	12 (18.2)	4 (17.4)	
Semi-professional	15 (23.8)	16 (24.2)	4 (17.4)	0.719
Recreational	21 (33.3)	30 (45.5)	10 (43.5)	
Sedentary	13 (20.6)	8 (12.1)	5 (21.7)	
Stage of injury, n (%)				
Acute	1 (1.6)	1 (1.6)	1 (4.3)	
Subacute	6 (9.5)	4 (6.3)	2 (8.7)	0.925
Persistent	31 (49.2)	32 (47.6)	10 (43.5)	
Chronic	25 (39.7)	29 (44.4)	10 (43.5)	

MEDAS: Mediterranean diet adherence screener, a,b: denotes a significant difference between groups with different exponential letters, descriptive statistics were presented as n (%) for categorical variables and median (interquartile range, IQR) [minimum-maximum] for numerical variables

Table 2. The level of complaints developing due to overuse according to the level of adherence to the Mediterranean diet

	Adherence Level to the Mediterranean Diet			p
	Incompatible (n=63)	**Acceptable (n=66)**	**Compatible (n=23)**	
OSTRC-O (score)	68 (42-84) [0-100]	68 (50.75-92) [16-100]	76 (50-84) [24-100]	0.935
OSTRC-O, n (%)				
0-25 score	5.1 (3)	4.5 (3)	4.8 (1)	
26-50 score	25.4 (16)	19.7 (13)	23.8 (6)	0.744
51-75 score	28.8 (18)	37.9 (25)	19.0 (4)	
76-100 score	40.7 (26)	37.9 (25)	52.4 (12)	
OSTRC-H (score)	68 (45.5-80) [0-100]	68 (41.25-90) [0-100]	75.5 (47-84) [24-100]	0.927
OSTRC-H, n (%)				
0-25 score	10.5 (7)	6.7 (4)	9.1 (2)	
26-50 score	21.1 (13)	23.3 (16)	22.7 (5)	0.902
51-75 score	28.1 (18)	31.7 (21)	18.2 (4)	
76-100 score	40.3 (25)	38.3 (25)	50.0 (12)	

OSTRC-O: The Oslo Sports Trauma Research Center Overuse Injury Questionnaire, OSTRC-H: The Oslo Sports Trauma Research Center Questionnaire on Health Problems, descriptive statistics were presented as n (%) for categorical variables and median (interquartile range, IQR) [minimum-maximum] for numerical variables

Table 3. Correlation of demographic characteristics with MEDAS, OSTRC-O, and OSTRC-H scores

		MEDAS	OSTRC-O	OSTRC-H
Age	r_s	0.158	-0.058	-0.068
	p	0.052	0.484	0.424
Body mass index	r_s	-0.061	-0.095	-0.057
	p	0.456	0.253	0.503
Duration of regular sport	r_s	-0.164	0.017	0.044
	p	0.102	0.868	0.669
Weekly exercise duration	r_s	-0.237	-0.119	-0.120
	p	**0.016**	0.232	0.235
MEDAS	r_s		0.054	0.056
	p		0.521	0.515
OSTRC-O	r_s	0.054		0.965
	p	0.521		**<0.001**
OSTRC-H	r_s	0.056	0.965	
	p	0.515	**<0.001**	

MEDAS: Mediterranean diet adherence screener, OSTRC-O: The Oslo Sports Trauma Research Center Overuse Injury Questionnaire, OSTRC-H: The Oslo Sports Trauma Research Center Questionnaire on Health Problems, r_s: Spearman's rho

of unhealthy foods such as red meat, processed foods, sugary sweets, and saturated fats. Additionally, fish consumption is encouraged several times a week, while red meat consumption is reduced. The Mediterranean diet is a dietary pattern that is high in fiber, antioxidants, omega-3 fatty acids, and other nutrients. It is associated with numerous health benefits, including heart health, weight control, and the prevention of cancer and chronic diseases (18). The Mediterranean diet is a leading healthy dietary recommendation worldwide. However, it may not provide sufficient calories for individuals who engage in sports. Therefore, it is recommended that a modified Mediterranean diet should be prepared specifically for athletes (2).

Additionally, this study found that compliance with the Mediterranean diet decreased as sports participation levels increased. A study conducted on female futsal athletes found low compliance with the Mediterranean diet. This lack of compliance was found to be unrelated to body composition and performance (25). It is important to note that this study was conducted on female futsal athletes only. Additionally, no relationship was found between the Mediterranean diet and body mass index and performance in this study.

A study conducted in Spain analyzed the compliance of cycling and triathlon athletes with the Mediterranean diet. The results showed that male athletes were less compliant than their female counterparts (26). This could be a result of a calorie deficit. This is consistent with the findings of Griffiths et al. (2), who reported that females were more compliant with the Mediterranean diet. Therefore, it can be concluded that gender may play a role in compliance with the Mediterranean diet.

Despite the ability of tendons to adapt to loading, repetitive use can lead to injuries such as tendinopathy. Tendinopathy is characterized by pain during activity, local tenderness to palpation, swelling, and decreased performance. This may prevent sports participation or lead to decreased performance (27). Tendinopathy is an inflammatory process (6). Increased inflammation may prolong the healing process, but since it is an indispensable part of the healing process, dramatic reduction of inflammation may also have a negative effect on healing. This negative effect may prolong the return to sport, particularly in athletes, and may result in time, training/match, and financial losses. Therefore, it would be incorrect to support the use of an unmodified Mediterranean diet in athletes even though the Mediterranean diet did not seem to affect the severity of tendinopathy in this study.

A systematic review was conducted to evaluate 19 trials about the effects of habitual diet, alcohol exposure, and the use of dietary supplements. The review included four studies investigating the effects of alcohol exposure and 14 studies investigating the use of dietary supplements. The results demonstrated that the number of high-quality studies was limited, and there was significant heterogeneity in methodology across studies (diet, tendon region, outcome measure, population, etc.). Despite these limitations, the findings suggest that providing precise dietary recommendations for the prevention and treatment of tendinopathy remains challenging (28).

Limitations and Strengths

It would have been beneficial to recruit a bigger number of participants and analyze them using the sports discipline. However, adherence to the Mediterranean diet decreased as the sport intensity increased. It would have been advantageous to classify the severity of tendinopathy using a radiological measurement method. A questionnaire was preferred because tendinopathies from different body regions were included. It would be possible to examine the effect of the Mediterranean diet by selecting tendinopathy belonging to a single body region. This may be the subject of further investigation. While there are studies investigating the relationship between chronic diseases and a Mediterranean diet in the literature, no study on this specific subject was identified. Consequently, the results presented in this study are considered valuable contributions to knowledge in this area.

CONCLUSION

The findings of this study indicate that the Mediterranean diet has not much effect on the severity of tendinopathy. Furthermore, the results suggest that athletes with high weekly exercise durations do not adopt the Mediterranean diet as an optimal dietary approach. Further research is needed to gain a more comprehensive understanding of the effects of the Mediterranean diet on sports and athletes.

Ethics Committee Approval: The study was approved by the Health Sciences Ethics Committee of Süleyman Demirel University (24.10.2022, 61/4).

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REFERENCES

1. Urquiaga I, Echeverría G, Dussaillant C, Rigotti A. Origin, components and mechanisms of action of the Mediterranean diet. Rev Med Chile. 2017;145(1):85-95. Spanish.
2. Griffiths A, Matu J, Whyte E, Akin-Nibosun P, Clifford T, Stevenson E, et al. The Mediterranean dietary pattern for optimising health and performance in competitive athletes: a narrative review. Br J Nutr. 2022;128(7):1285-98.
3. Nani A, Murtaza B, Sayed Khan A, Khan NA, Hichami A. Antioxidant and anti-inflammatory potential of polyphenols contained in Mediterranean diet in obesity: molecular mechanisms. Molecules. 2021;26(4):985.
4. Scott A, Squier K, Alfredson H, Bahr R, Cook JL, Coombes B, et al. ICON 2019: International Scientific Tendinopathy Symposium Consensus: Clinical Terminology. Br J Sports Med. 2020;54(5):260-2.
5. Lipman K, Wang C, Ting K, Soo C, Zheng Z. Tendinopathy: injury, repair, and current exploration. Drug Des Devel Ther. 2018;12:591-603.
6. Kaya İ, Örsçelik A, Solmaz İ, Karaaslan B, Şafak Yılmaz E. Neutrophil to lymphocyte ratio may be used as a predictor in tendinopathy. Eur Res J. 2023;9(4):645-51.
7. Andarawis-Puri N, Flatow EL, Soslowsky LJ. Tendon basic science: Development, repair, regeneration, and healing. J Orthop Res. 2015;33(6):780-4.
8. Bifulco M, Cerullo G, Abate M. Is the Mediterranean diet pattern a good choice for athletes? Nutr Today. 2019;54(3):121-3.
9. Curtis L. Nutritional research may be useful in treating tendon injuries. Nutrition. 2016;32(6):617-9.
10. Loiacono C, Palermi S, Massa B, Belviso I, Romano V, Di Gregorio A, et al. Tendinopathy: pathophysiology, therapeutic options, and role of nutraceutics. A narrative literature review. Medicina (Kaunas). 2019;55(8):447.
11. Wonders J. Lifestyle factors as risk factors and treatment options in tendinopathy. Arch Med. 2021;13(5):22.
12. Baker RT, Burton D, Pickering MA, Start A. Confirmatory factor analysis of the disablement in the physically active scale and preliminary testing of short-form versions: A calibration and validation study. J Athl Train. 2019;54(3):302-18.
13. Clarsen B, Myklebust G, Bahr R. Development and validation of a new method for the registration of overuse injuries in sports injury epidemiology: the Oslo Sports Trauma Research Centre (OSTRC) Overuse Injury Questionnaire. Br J Sports Med. 2013;47(8):495-502.
14. Clarsen B, Bahr R, Myklebust G, Andersson SH, Docking SI, Drew M, et al. Improved reporting of overuse injuries and health problems in sport: an update of the Oslo Sports Trauma Research Center questionnaires. Br J Sports Med. 2020;54(7):390-6.
15. Schröder H, Fitó M, Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, et al. A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. J Nutr. 2011;141(6):1140-5.
16. Özkan Pehlivanoğlu EF, Balcıoğlu H, Ünlüoğlu İ. Turkish validation and reliability of Mediterranean diet adherence screener. Osmangazi J Med. 2020;42(2):160-4. Turkish.
17. Dominguez LJ, Di Bella G, Veronese N, Barbagallo M. Impact of Mediterranean diet on chronic non-communicable diseases and longevity. Nutrients. 2021;13(6):2028.
18. Dinu M, Pagliai G, Casini A, Sofi F. Mediterranean diet and multiple health outcomes: an umbrella review of meta-analyses of observational studies and randomised trials. Eur J Clin Nutr. 2018;72(1):30-43.
19. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. N Engl J Med. 2018;378(25):e34.
20. Bonaccio M, Di Castelnuovo A, Costanzo S, Gialluisi A, Persichillo M, Cerletti C, et al. Mediterranean diet and mortality in the elderly: a prospective cohort study and a meta-analysis. Br J Nutr. 2018;120(8):841-54.
21. Martínez-González MA, Gea A, Ruiz-Canela M. The Mediterranean diet and cardiovascular health. Circ Res. 2019;124(5):779-98.
22. Dominguez LJ, Barbagallo M. Dietary approaches and supplements in the prevention of cognitive decline and Alzheimer's disease. Curr Pharm Des. 2016;22(6):688-700.
23. Norde MM, Collese TS, Giovannucci E, Rogero MM. A posteriori dietary patterns and their association with systemic low-grade inflammation in adults: a systematic review and meta-analysis. Nutr Rev. 2021;79(3):331-50.
24. Hébert JR, Shivappa N, Wirth MD, Hussey JR, Hurley TG. Perspective: the dietary inflammatory index (DII)-lessons learned, improvements made, and future directions. Adv Nutr. 2019;10(2):185-95.
25. Rubio-Arias JÁ, Ramos Campo DJ, Ruiloba Nuñez JM, Carrasco Poyatos M, Alcaraz Ramón PE, Jiménez Díaz FJ. Adherence to a Mediterranean diet and sport performance in a elite female athletes futsal population. Nutr Hosp. 2015;31(5):2276-82. Spanish.
26. Muros JJ, Zabala M. Differences in Mediterranean diet adherence between cyclists and triathletes in a sample of Spanish athletes. Nutrients. 2018;10(10):1480.
27. Magnusson SP, Langberg H, Kjaer M. The pathogenesis of tendinopathy: balancing the response to loading. Nat Rev Rheumatol. 2010;6(5):262-8.
28. Hijlkema A, Roozenboom C, Mensink M, Zwerver J. The impact of nutrition on tendon health and tendinopathy: a systematic review. J Int Soc Sports Nutr. 2022;19(1):474-504.

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Evaluation of the Efficacy of Pitavastatin on Pain Response in Rats with Thermal Plantar and Dynamic Plantar Tests

Pitavastatinin Ratlarda Termal Plantar ve Dinamik Plantar Testler ile Ağrı Yanıtına Etkinliğinin Değerlendirilmesi

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ABSTRACT

Aim: Pitavastatin (Pita) is a member of the statin family, a well-known hypolipidemic agent, and some statin members are effective in treating pain. This study aimed to evaluate the antinociceptive effects of Pita by measuring nociception in rats with different doses and durations.

Material and Methods: This study consisted of six groups including saline oral as a non-drug control, 1 mg/kg Pita single dose, 3 mg/kg Pita single dose, 1 mg/kg Pita for 14 days, 3 mg/kg Pita for 14 days, and 20 mg/kg diclofenac for 14 days. Thermal plantar and mechanical plantar tests were used to observe pain threshold changes. Correlations between Pita doses, durations, and behavioral pain responses were evaluated. The sciatic nerves were evaluated histopathologically.

Results: Pita showed a significant antinociceptive effect in the dynamic plantar test at doses of 1 mg/kg for 14 days by increasing the mechanical threshold from 25.43±4.79 g to 32.28±2.27 g (p=0.041) and 3 mg/kg for 14 days by increasing the mechanical threshold from 27.41±2.36 g to 34.35±2.58 g (p=0.039). Also, Pita increased the thermal latency from 8.95±1.28 s to 11.71±1.49 s in the thermal plantar test at a single dose of 3 mg/kg (p=0.004). Although the antinociceptive effects of Pita were proven in dynamic plantar and thermal plantar tests, these findings did not reach a significant level at histopathological evaluation.

Conclusion: These results suggest that Pita has an antinociceptive effect and when used in conjunction with the right dosage and timing, might be favored for the treatment of pain.

Keywords: Pain; pitavastatin; antiallodynic effect; antihyperalgesic effect.

ÖZ

Amaç: Pitavastatin (Pita), iyi bilinen bir hipolipidemik ajan olan statin ailesinin bir üyesidir ve bazı statin üyelerinin ağrı tedavisinde etkili olduğu gösterilmiştir. Bu çalışmada sıçanlarda farklı doz ve sürelerde nosisepsiyon ölçülerek Pita'nın antinosiseptif etkilerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Bu çalışma ilaçsız kontrol olarak salin oral, tek doz 1 mg/kg Pita, tek doz 3 mg/kg Pita, 14 gün boyunca 1 mg/kg Pita, 14 gün boyunca 3 mg/kg Pita ve 14 gün boyunca 20 mg/kg diklofenak olmak üzere altı gruptan oluşmaktadır. Ağrı eşiği değişikliklerini gözlemlemek için termal plantar ve mekanik plantar testler kullanıldı. Pita dozları, süreleri ve davranışsal ağrı tepkileri arasındaki korelasyonlar değerlendirildi. Siyatik sinirler histopatolojik olarak değerlendirildi.

Bulgular: Pita, 14 gün boyunca uygulanan 1 mg/kg dozunda mekanik eşiği 25,43±4,79 g'dan 32,28±2,27 g'a çıkararak (p=0,041) ve 14 gün boyunca uygulanan 3 mg/kg dozunda ise mekanik eşiği 27,41±2,36 g'dan 34,35±2,58 g'a çıkararak (p=0,039) dinamik plantar testte anlamlı bir antinosiseptif etki gösterdi. Ayrıca Pita, termal plantar testte 3 mg/kg tek dozda termal latansı 8,95±1,28 sn'den 11,71±1,49 sn'ye çıkardı (p=0,004). Dinamik plantar ve termal plantar testlerde Pita'nın antinosiseptif etkileri kanıtlanmış olmakla birlikte, bu bulgular histopatolojik değerlendirmede anlamlı bir düzeye ulaşmadı.

Sonuç: Bu sonuçlar Pita'nın antinosiseptif etkiye sahip olduğunu ve uygun doz ve zamanlama ile kullanıldığında ağrı tedavisinde tercih edilebileceğini düşündürmektedir.

Anahtar kelimeler: Ağrı; pitavastatin; antiallodinik etki; antihiperaljezik etki.

INTRODUCTION

Pain serves as a protective mechanism for living organisms through the activation of nociceptors (1). Despite ongoing scientific progress in comprehending the genetics, pathology, molecular biology, and neurophysiology associated with pain, the preventive measures for adverse conditions—including the precise identification of its source, alleviation, and detrimental impacts on the life process—remain inadequately understood (2).

It may indicate the potential for innovative and more efficacious methods for pain management, considering various factors, including the limitations of existing treatment modalities that fail to eradicate the pain symptom, exhibit adverse side effects, and carry a risk of dependency (3,4).

Statins are involved in the regulation of cholesterol synthesis by enhancing the levels of apolipoprotein A1 (Apo-A1) and inhibiting the activity of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a mechanism that was initially identified in 1976 (5,6). The use of statins has been associated with a decrease in both mortality and morbidity in patients with various cardiovascular diseases (7,8).

Statins, beyond their established role in lowering lipid levels, have demonstrated various biological, anti-inflammatory properties, modulation of cell proliferation, enhancement of endothelial function, and influences on coagulation and platelet activity, as indicated by several studies (9-12). Among these agents, pitavastatin (Pita) is particularly notable for its strong ability to inhibit the enzyme HMG-CoA reductase (13,14).

Systemic administration of statins has demonstrated efficacy in preclinical investigations, indicating a potential to inhibit the onset of mechanical allodynia and thermal hyperalgesia in various experimental animal models (15-19).

Despite the encouraging results regarding the use of statins for alleviating nerve pain in animal subjects, the underlying mechanisms that contribute to these beneficial effects remain inadequately understood. Furthermore, the specific impact of statins on pain thresholds and their effectiveness in the context of chronic versus acute pain conditions has yet to be thoroughly elucidated.

Therefore, this investigation aimed to demonstrate the effectiveness of Pita, a member of the statin family, on pain threshold. It is hoped that the findings obtained from this study will contribute to the first-line literature in revealing the antiallodynic, antihyperalgesic activity of Pita in neurological animal models. After demonstrating its effectiveness on pain threshold in the initial stage, this study will help determine whether Pita will be effective in complex neuropathic pain conditions.

MATERIAL AND METHODS

Animals and Experimental Design

A total of thirty-six adult male Wistar rats (250-260 g) from the local medical sciences experimental research center were used in the experiments. All experiments were conducted strictly with the National Institute of Health Guidelines for the Care, and Use of Laboratory Animals. Experimental protocols were approved by Kahramanmaraş Sütçü İmam University Animal Experiments Ethics Committee (2020/02-04, approval date: 27.02.2020). Rats were kept in their cages until the day of the experiment in animal housing rooms where sound insulation was provided, and temperature (22-24 °C) was controlled under a 12:12-h light/dark cycle (06:00 am to 06:00 pm) with 40-60% relative humidity. The rats were housed in hygienic stainless steel cages where they could comfortably continue feeding and water consumption.

Drugs and Chemicals

Pita, supplied by Abdi Ibrahim Drug Company (Istanbul, Turkey), was homogenously dissolved in tap water, and delivered to rats per oral (p.o.) with orogastric gavage. In the study protocol, 1 mg/kg, and 3 mg/kg administration doses of Pita were determined based on the basic literature (20,21). Diclofenac Na was obtained from Deva Holding (Istanbul, Turkey), and anti-inflammatory doses of diclofenac 20 mg/kg were based on doses that we observed in our previous studies (22).

Experimental Design and Groups

The rats were brought to the laboratory 14 days before and on the day of the experiment and kept for 20 minutes to adapt to the environment. To reduce extra stress factors on the rats, the same researcher carried out the drug applications each time, and care was taken to guarantee that there was no excessive distance between the place where the experiments were carried out and the colony. Since the experiment involved researcher-animal contact, and some animal practices, before the experiments rats were acclimated to the person, and system at least three times for 20-30 s for 3 days. Animals were allocated to six groups designed as follows. Control: saline oral (non-drug control), Pita 1mg1d: 1 mg/kg Pita single dose (low dose and short-term administration), Pita 3mg1d: 3 mg/kg Pita single dose (high dose and short-term administration), Pita 1mg14d: 1 mg/kg Pita for 14 days, (low dose and long-term administration), Pita 3mg14d: 3 mg/kg Pita for 14 days (high dose and long-term administration), and Diclofenac 20mg14d: 20 mg/kg diclofenac administration for 14 days (positive control). Before drug administrations, thermal plantar, and dynamic plantar pain assessments were protocoled to standardize pain responses across all groups. The treatment regimen started on the first day. Thermal and dynamic plantar pain tests were repeated on the second day of the treatment regimen for those who took the drug as a single dose, and on the fifteenth day for those who took the drug for 14 days (Figure 1).

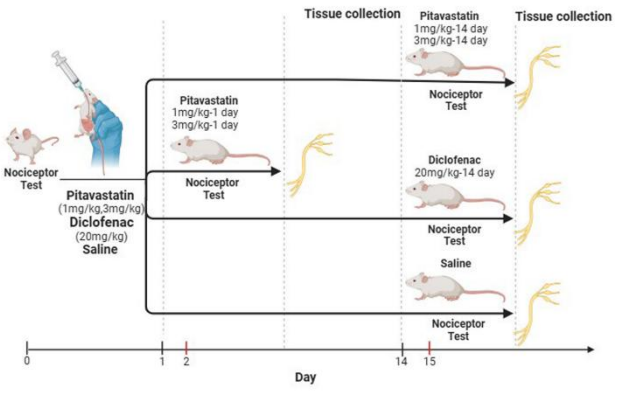

Figure 1. Experimental design (created by biorender.com)

Nociception Test Procedures

Thermal Plantar Test

A thermal plantar test (Commat, Ankara, Turkey) was used to measure the latency of rats, which reflects the delay in paw withdrawal time in the presence of thermal hyperalgesia. Each animal was kept in plexiglass chambers for 10 minutes to adapt to the test environment in which they were previously placed. Under the glass plate on which the rats were placed, a manually moving radial heat source was appropriately held to give a thermal stimulus to the left/right posterior mid-plantar region. Thermal stimulus administration, 1% of the maximum intensity was used to focus the area of the hind paw. Stimulus intensity; the hind paw was adjusted to give a maximum of 25% infrared ray intensity, which would cause a total thermal latency of 8-10 s. The system is designed in such a way that when the rat pulls back its paw, which feels the pain that develops as a result of the stimulus, the infrared generator is automatically turned off via a photocell, and the timer displays the value that will determine the latency on the digital screen. The control mechanism of the system is designed to close in 25 seconds (cut-off latency) so that the animal cannot be exposed to too much stimulus when it does not retract its paw due to excessive desensitization (23).

Dynamic Plantar Test (Von Frey Filament Test)

Dynamic plantar aesthesiometer modified from a version of the von Frey filament test (Ugo Basile, Comerio, Italy), was used for the measurement of animal sensitivity to the non-noxious light mechanic touch of the paw. The mechanical allodynia measurement was determined by measuring the withdrawal threshold to a truly non-destructive mechanical stimulus applied to the rat's paw. Sudden withdrawals against mechanical stimuli were defined as mechanical allodynia (24).

Histopathological Evaluation

After being fixed in 10% buffered formalin, the sciatic nerve samples were prepared for paraffin block processing. Hematoxylin, and Eosin (H&E) staining was applied after the slices were cut at a thickness of 5 µm using a rotatory microtome. The images were examined under a Carl Zeiss Axio Imager A2 microscope at different magnifications. The sciatic nerve longitudinal sections were scored using the method described previously (25,26). In the histopathological evaluation, edema, vacuolization, mononuclear cellular infiltration, and axonal and myelin degenerations were considered and scored using a scale ranging from 0 to 3.

Statistical Analysis

For numerical data, Levene and Kolmogorov-Smirnov tests were employed to examine the variance homogeneity and normal distribution assumptions. The numerical data were defined with mean and standard deviation. Categorical variables were expressed as percentages and numbers. Repeated measures analysis of variance test was utilized to compare pre- and post-treatment values across multiple groups and post hoc Tukey and Bonferroni methods were used to examine in detail for the comparisons of pre- and post-treatment in each group. Fisher-Freeman-Halton test was used for categorical variables. The SPSS v.17.0 (SPSS Statistics for Windows, version 17.0. Chicago: SPSS Inc.) program was utilized for statistical analysis. The p-value of <0.05 accepted the criteria for statistical significance.

RESULTS

Nociception Test Result

The Effect of Diclofenac 20 mg/kg on Mechanical Threshold and Thermal Latency

Following the administration of diclofenac 20 mg/kg, the mechanical threshold after the mechanical stimulus, and thermal latency after the thermal stimulus were statistically significantly improved. Diclofenac 20 mg/kg significantly increased the mechanic threshold from 28.62±4.19 grams (g) to 37.62±4.31 g (p=0.009, Table 1). Diclofenac 20 mg/kg also markedly improved the thermal latency, which increased from 8.52±1.64 seconds (s) to 10.68±0.72 s (p=0.022, Table 2). Figures 2 and 3 also showed the alterations in thresholds and latencies caused by diclofenac 20 mg/kg.

The Effect of Pita 1 mg/kg on Mechanical Threshold and Thermal Latency

Pita 1 mg/kg for 14 days exerted a significant antinociceptive effect in the mechanical test. Pita 1 mg/kg for 14 days significantly increased the mechanical threshold from 25.43±4.79 g to 32.28±2.27 g (p=0.041). Mechanical and thermal plantar test results showed that a single dose of 1 mg/kg Pita did not reveal a significant improvement in pain responses (p=0.597, and p=0.287, respectively). The effects of Pita 1 mg/kg on paw withdrawal responses to mechanical stimuli were shown in Table 1 and Figure 2.

The Effect of Pita 3 mg/kg on Mechanical Threshold and Thermal Latency

Pita 3 mg/kg for 14 days exerted a significant antinociceptive effect in the mechanical test. Pita 3 mg/kg for 14 days increased the mechanical threshold from 27.41±2.36 g to 34.35±2.58 g (p=0.039). The effects of Pita 3 mg/kg on the paw withdrawal responses to mechanical

Table 1. Pre- and post-treatment dynamic plantar test responses, mechanic threshold (g) of groups

Groups	Pre-treatment	Post-treatment	p
Control	26.51±3.16	28.45±2.21	0.550
Pita 1mg1d	31.58±7.50	29.87±5.38	0.597
Pita 3mg1d	27.93±10.37	33.29±3.69	0.105
Pita 1mg14d	25.43±4.79	32.28±2.27	**0.041**
Pita 3mg14d	27.41±2.36	34.35±2.58	**0.039**
Diclofenac 20mg14d	28.62±4.19	37.62±4.31	**0.009**

Pita: pitavastatin, g: grams, data were reported as mean±standard deviation

Table 2. Pre- and post-treatment thermal plantar test responses, thermal latencies (s) of groups

Groups	Pre-treatment	Post-treatment	p
Control	9.22±0.41	9.14±0.63	0.931
Pita 1mg1d	9.31±2.24	10.28±1.82	0.287
Pita 3mg1d	8.95±1.28	11.71±1.49	**0.004**
Pita 1mg14d	8.33±0.53	8.48±1.44	0.868
Pita 3mg14d	9.69±1.39	9.42±1.80	0.766
Diclofenac 20mg14d	8.52±1.64	10.68±0.72	**0.022**

Pita: pitavastatin, s: seconds, data were reported as mean±standard deviation

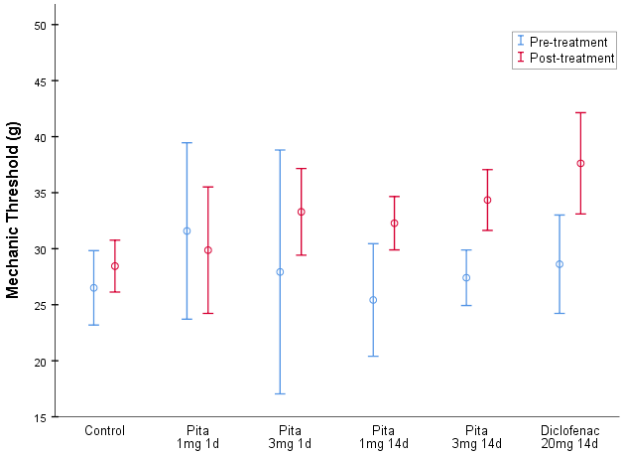

Figure 2. Pita at different doses and durations showed its effectiveness on mechanical allodynia with a significant increase in post-treatment mechanical threshold (g) in groups of Pita 1mg14d, Pita 3mg14d, and diclofenac 20mg14d compared to pretreatment values

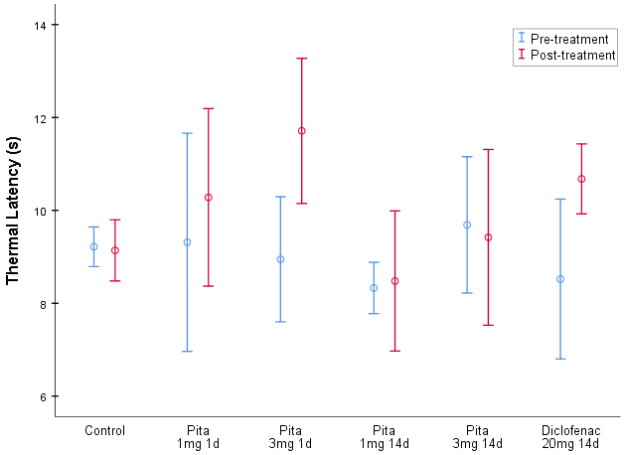

Figure 3. Pita at different doses and durations showed its effectiveness on thermal hyperalgesia with a significant increase in post-treatment thermal latencies (s) in groups of Pita 3mg1d and diclofenac 20mg14d compared to pretreatment values

stimuli were shown in Table 1, and Figure 2. In addition, thermal plantar test results showed that a single dose of Pita 3 mg/kg revealed a significant improvement in pain responses. Pita 3 mg/kg single dose enhanced the thermal latency from 8.95±1.28 s to 11.71±1.49 s (p=0.004). The effects of Pita 3 mg/kg on the paw withdrawal responses to the thermal stimuli were shown in Table 2 and Figure 3.

Histopathological Results

Histopathological examination showed that the sciatic nerves of the control group had a normal histoarchitecture with well-organized myelin sheath, and axons (Figure 4a). Conversely, Pita 1mg1d, Pita 3mg1d, Pita 1mg14d, Pita 3mg14d, and diclofenac 20mg14d groups exhibited degenerated myelinated axons, with separated distributed nerve fibers, mononuclear cellular infiltration, vacuolization as well as edema (Figure 4b, 4c, 4d, 4e, 4f). Diclofenac 20mg14d group sections showed the worst histological picture (Figure 4f). However, when the score results of these parameters were evaluated, no significant difference was seen between the experimental study, and control groups (Table 3).

DISCUSSION

Statins are classified as inhibitors of HMG-CoA reductase and are employed in the management of conditions characterized by elevated cholesterol levels (27). In addition to their ability to reduce cholesterol levels, statins are recognized for a variety of other beneficial properties, including anti-inflammatory effects and pain-relieving capabilities (28,29). The results of this investigation indicate that Pita may exhibit an antinociceptive effect, as evidenced by its impact on both thermal and mechanical thresholds, which were assessed through thermal and mechanical plantar test responses administered at the appropriate dosage and timing.

The selection of diclofenac as the positive control group was based on its recognized analgesic effectiveness and the alignment of test outcomes with findings from prior studies (22). Allodynia and hyperalgesia are recognized as manifestations of sensitization occurring in both peripheral and central processes (30,31). Peripheral and central sensitization processes may potentially be connected to neurochemical alterations and neuroinflammatory consequences in the pain perception process. Studies suggest that statins may possess anti-neuroinflammatory properties (32). Neuroinflammation is an immune system response with complex subcomponents that neural tissues

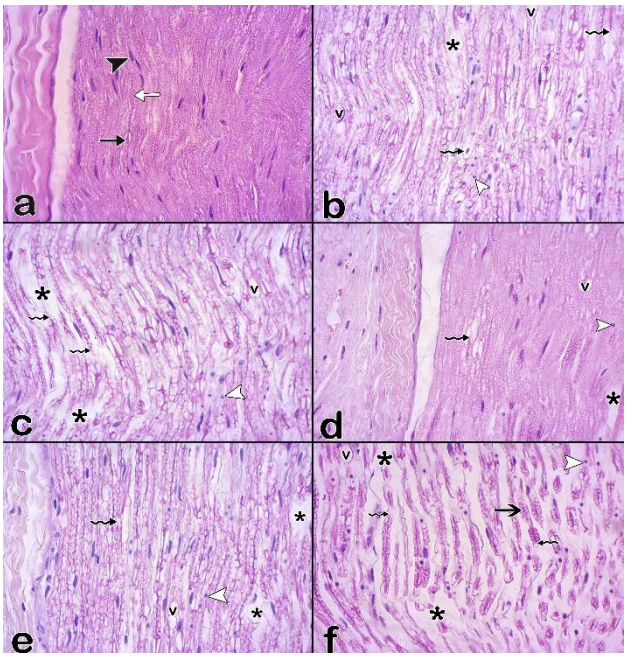

Figure 4. Effect of Pita on sciatic nerve histopathological changes, **a)** the control group showed normal axons (black arrow) with intact myelin sheaths (white arrow) and Schwann cell (arrowhead), **b)** Pita 1mg1d, **c)** Pita 3mg1d, **d)** Pita 1mg14d, **e)** Pita 3mg14d, **f)** Diclofenac 20mg14d groups showed axonal degeneration (wavy arrows), vacuolization (v), edema (asterisks) and mononuclear cell infiltration (white arrowheads), the diclofenac 20mg14d group also showed the fragmentation (black arrow) of myelin and axons (Hematoxylin, and Eosin (H&E), 400x)

Table 3. Comparison of histopathological evaluation results between groups

	Control	Pita 1mg1d	Pita 3mg1d	Pita 1mg14d	Pita 3mg14d	Diclofenac 20mg14d	p
Axonal Degeneration, n (%)							
Absent	3 (50.0%)	1 (16.7%)	2 (33.3%)	1 (16.7%)	1 (16.7%)	0 (0.0%)	0.437
Mild	3 (50.0%)	3 (50.0%)	1 (16.7%)	5 (83.3%)	4 (66.7%)	4 (66.7%)	
Moderate	0 (0.0%)	2 (33.3%)	2 (33.3%)	0 (0.0%)	1 (16.7 %)	1 (16.7%)	
Severe	0 (0.0%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	1 (16.7%)	
Infiltration, n (%)							
Absent	4 (66.7%)	2 (33.3%)	2 (33.3%)	3 (50.0%)	2 (33.3%)	1 (16.7%)	0.877
Mild	2 (33.3%)	4 (66.7%)	3 (50.0%)	3 (50.0%)	3 (50.0%)	4 (66.7%)	
Moderate	0 (0.0%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	1 (16.7%)	1 (16.7%)	
Severe	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Vacuolization, n (%)							
Absent	3 (50.0%)	2 (33.3%)	1 (16.7%)	2 (33.3%)	2 (33.3%)	0 (0.0%)	0.478
Mild	3 (50.0%)	2 (33.3%)	3 (50.0%)	4 (66.7%)	3 (50.0%)	5 (83.3%)	
Moderate	0 (0.0%)	2 (33.3%)	2 (33.3%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	
Severe	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (16.7%)	
Edema, n (%)							
Absent	4 (66.7%)	1 (16.7%)	1 (16.7%)	3 (50.0%)	3 (50.0%)	2 (33.3%)	0.540
Mild	2 (33.3%)	4 (66.7%)	3 (50.0%)	2 (33.3%)	1 (16.7%)	1 (16.75)	
Moderate	0 (0.0%)	1 (16.7%)	2 (33.3%)	1 (16.7%)	2 (33.3%)	3 (50.0%)	
Severe	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Myelin Degeneration, n (%)							
Absent	4 (66.7%)	2 (33.3%)	2 (33.3%)	3 (50.0%)	2 (33.3%)	2 (33.3%)	0.984
Mild	2 (33.3%)	3 (50.0%)	2 (33.3%)	2 (33.3%)	3 (50.0%)	2 (33.3%)	
Moderate	0 (0.0%)	1 (16.7%)	2 (33.3%)	1 (16.7%)	1 (16.7%)	1 (16.7%)	
Severe	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (16.7%)	

can exhibit during various processes (33). In previous studies, simvastatin and atorvastatin have been reported to exhibit antihyperalgesic, and antiallodynic effects, as well as antinociceptive consequences in animal models of neuropathic pain (18,34).

In this research, the antiallodynic effect demonstrated statistical significance in the groups receiving Pita at dosages of 1 mg/kg for 14 days and 3 mg/kg for 14 days, particularly in relation to mechanical allodynia, according to data from a modernized von Frey application employed in plantar mechanical responses in the assessment of nociception. However, Pita in short-term dosages (1 mg/kg single dose and 3 mg/kg single dose) is not as effective as long-term administration.

The C fiber group, comprising polymodal nociceptors, is responsible for responding to temperature changes in nociception. The C fiber group, which includes polymodal nociceptors, plays a crucial role in detecting variations in temperature as part of the nociceptive process. The current study's thermal plantar test responses with C fiber supplementation revealed that Pita significantly prolonged thermal latency in the 3 mg/kg single dose treatment group compared to the pretreatment condition.

While there was a relative improvement in thermal delay reductions in the other dose-time application groups, this did not reach statistical significance. This finding is consistent with the effect of Pita on thermal hyperalgesia in the partial sciatic nerve-induced neuropathy model (35). Previous studies have mentioned that the antinociceptive effect may occur through several regulatory effects such as inflammatory pathways, and oxidative stress (28,36).

Atorvastatin has been shown to have neuroinflammatory activity in the ischemic stroke model (1 or 10 mg/kg) and in the chronic constriction-induced nerve injury model (3, 10, and 30 mg/kg by oral gavages for 14 days) (28,37).

If neuropathic pain is to be evaluated from a different perspective, another important relationship between neuropathic pain and RhoA/Rho kinase stands out. Both RhoA and Rho-related kinase (Rho kinase) play important roles in the maintenance of chronic pain states (38). In another study, simvastatin reduced nociceptive behaviors by blocking p38 mitogen-activated protein kinase, and microglial RhoA (39). Statins have been also shown to have an inhibitory effect on the Rho/Rho kinase signaling pathway (40). This phenomenon can also be considered as an example of a pleiotropic effect, which refers to the lipid-independent actions of statins, including their capacity to inhibit Rho-Rho kinase activity. In this context, it seems plausible that the antinociceptive properties of Pita may affect pain thresholds even in the absence of a neuropathic disorder.

In the study investigating the potential effects of a group of analgesic drugs on the sciatic nerve, more severe effects were observed with diclofenac compared to the other drugs such as lornoxicam, morphine, and pethidine groups (25). Similarly, in the diclofenac group in the present study, separated nerve fibers, mononuclear cellular infiltration, vacuolization, edema, and degenerated myelinated axons were reported based on the histopathological evaluation of their effects on the sciatic nerve. Additionally, reflex behaviors including withdrawal thresholds against heat, and pressure noxious stimuli for pain sensitivity measurement, were examined in this study, and histopathological evaluation consistency with the diclofenac compared to the control group.

This research has some limitations. Firstly, the effectiveness of Pita on pain has not been discussed with a neuropathy model. Secondly, the study methodology did not include molecular techniques such as assessing inflammatory markers. It is necessary to add a detail here

that, under initial conditions, the study was planned in two stages; the initial instance is to reveal the effectiveness of Pita on pain threshold within the framework of dose-time data, and in the second stage; it is planned to support this effect with experimental neuropathy models.
In subsequent research, it would be beneficial to incorporate and analyze Pita treatment protocols across various animal models, alongside the molecular findings observed. The findings from this investigation demonstrate the antiallodynic and antihyperalgesic properties of Pita, as evidenced by thermal and dynamic plantar tests conducted at varying dosages and time intervals, complemented by histological analysis. Additional research is required to clarify the mechanisms through which Pita exerts its antinociceptive effects, particularly in relation to different molecular markers within neuropathy models.

CONCLUSION

In this study, Pita was shown to have antiallodynic and antihyperalgesic effects at different doses, and times with thermal, and dynamic plantar tests, and histological data. These results suggest that Pita has an antinociceptive effect and may be preferred for pain management. Further studies are needed to clarify how Pita's antinociceptive activity mediates its role in pain pathology through various molecular markers on neuropathy models.

Ethics Committee Approval: The study was approved by the local ethics committee on animal experiments at Kahramanmaraş Sütçü İmam University (27.02.2020, 04).

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REFERENCES

1. Muley MM, Krustev E, McDougall JJ. Preclinical assessment of inflammatory pain. CNS Neurosci Ther. 2016;22(2):88-101.
2. Max MB, Stewart WF. The molecular epidemiology of pain: a new discipline for drug discovery. Nat Rev Drug Discov. 2008;7(8):647-58.
3. Jain KK. Gene therapy for pain. Expert Opin Biol Ther. 2008;8(12):1855-66.
4. Meert TF, Vermeirsch HA. A preclinical comparison between different opioids: antinociceptive versus adverse effects. Pharmacol Biochem Behav. 2005;80(2):309-26.
5. Lennernäs H, Fager G. Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. Similarities and differences. Clin Pharmacokinet. 1997;32(5):403-25.
6. Wasim R, Ansari TM, Ahsan F, Siddiqui MH, Singh A, Shariq M, et al. Pleiotropic benefits of statins in cardiovascular diseases. Drug Res (Stuttg). 2022;72(9):477-86.
7. Sirtori CR. The pharmacology of statins. Pharmacol Res. 2014;88:3-11.
8. Mantell G. Lipid lowering drugs in atherosclerosis--the HMG-CoA reductase inhibitors. Clin Exp Hypertens A. 1989;11(5-6):927-41.
9. Chen XY, Li K, Light AR, Fu KY. Simvastatin attenuates formalin-induced nociceptive behaviors by inhibiting microglial RhoA and p38 MAPK activation. J Pain. 2013;14(11):1310-9.
10. Grundy SM. Statin trials and goals of cholesterol-lowering therapy. Circulation. 1998;21;97(15):1436-9.
11. Takemoto M, Liao JK. Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme reductase inhibitors. Arterioscler Thromb Vasc Biol. 2001;21(11):1712-9.
12. Bhalla S, Singh N, Jaggi AS. Statins: do they aggravate or ameliorate neuropathic pain? J Pain. 2014;15(11):1069-80.
13. Hoy SM. Pitavastatin: A review in hypercholesterolemia. Am J Cardiovasc Drugs. 2017;17(2):157-68.
14. Duggan ST. Pitavastatin: a review of its use in the management of hypercholesterolaemia or mixed dyslipidaemia. Drugs. 2012;72(4):565-84.
15. Shi XQ, Lim TKY, Lee S, Zhao YQ, Zhang J. Statins alleviate experimental nerve injury-induced neuropathic pain. Pain. 2011;152(5):1033-43.
16. Bhalla S, Singh N, Jaggi AS. Dose-related neuropathic and anti-neuropathic effects of simvastatin in vincristine-induced neuropathic pain in rats. Food Chem Toxicol. 2015;80:32-40.
17. Cameron N, Cotter M, Inkster M, Nangle M. Looking to the future: diabetic neuropathy and effects of rosuvastatin on neurovascular function in diabetes models. Diabetes Res Clin Pract. 2003;61(Suppl 1):S35-9.
18. Pathak NN, Balaganur V, Lingaraju MC, More AS, Kant V, Kumar D, et al. Antihyperalgesic and anti-inflammatory effects of atorvastatin in chronic constriction injury-induced neuropathic pain in rats. Inflammation. 2013;36(6):1468-78.
19. Pan HC, Yang DY, Ou YC, Ho SP, Cheng FC, Chen CJ. Neuroprotective effect of atorvastatin in an experimental model of nerve crush injury. Neurosurgery. 2010;67(2):376-89.
20. Iqbal R, Akhtar MS, Hassan MQ, Jairajpuri Z, Akhtar M, Najmi AK. Pitavastatin ameliorates myocardial damage by preventing inflammation and collagen deposition via reduced free radical generation in isoproterenol-induced cardiomyopathy. Clin Exp Hypertens. 2019;41(5):434-43.
21. Hu G, Ito O, Rong R, Sakuyama A, Miura T, Ito D, et al. Pitavastatin upregulates nitric oxide synthases in the kidney of spontaneously hypertensive rats and Wistar-Kyoto rats. Am J Hypertens. 2018;31(10):1139-46.

22. Altintas Aykan D, Yaman S. Evaluation of the effects of tadalafil on pain response in thermal plantar and dynamic plantar tests in rats. Ann Med Res. 2020;27(3):749-54.
23. Mert T, Metin TO, Sahin E, Yaman S, Sahin M. Neuroprotective and anti-neuropathic actions of pulsed magnetic fields with low frequencies in rats with chronic peripheral neuropathic pain. Brain Res Bull. 2021;177:273-81.
24. Mert T, Metin TO, Sahin M, Yaman S. Antiinflammatory properties of antiLy6G antibody disappear during magnetic field exposure in rats with carrageenan induced acute paw inflammation. Naunyn Schmiedebergs Arch Pharmacol. 2020;393(11):2107-15.
25. Balaha M, Kandeel S, Kabel A. Phloretin either alone or in combination with duloxetine alleviates the STZ-induced diabetic neuropathy in rats. Biomed Pharmacother. 2018;101:821-32.
26. Bostan H, Cabalar M, Altinay S, Kalkan Y, Tumkaya L, Kanat A, et al. Sciatic nerve injury following analgesic drug injection in rats: A histopathological examination. North Clin Istanb. 2018;5(3):176-85.
27. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. Fundam Clin Pharmacol. 2005;19(1):117-25.
28. Jung KH, Chu K, Jeong SW, Han SY, Lee ST, Kim JY, et al. HMG-CoA reductase inhibitor, atorvastatin, promotes sensorimotor recovery, suppressing acute inflammatory reaction after experimental intracerebral hemorrhage. Stroke. 2004;35(7):1744-9.
29. Chu LW, Chen JY, Yu KL, Cheng KI, Wu PC, Wu BN. Neuroprotective and anti-inflammatory activities of atorvastatin in a rat chronic constriction injury model. Int J Immunopathol Pharmacol. 2012;25(1):219-30.
30. Viana F. Nociceptors: thermal allodynia and thermal pain. Handb Clin Neurol. 2018;156:103-19.
31. Weber MS, Prod'homme T, Steinman L, Zamvil SS. Drug insight: using statins to treat neuroinflammatory disease. Nat Clin Pract Neurol. 2005;1(2):106-12.
32. Skaper SD, Facci L, Zusso M, Giusti P. An inflammation-centric view of neurological disease: beyond the neuron. Front Cell Neurosci 2018;12:72.
33. Corso CR, Martins DF, Borges SC, Beltrame OC, Telles JEQ, Buttow NC, et al. Effect of simvastatin on sensorial, motor, and morphological parameters in sciatic nerve crush induced-neuropathic pain in rats. Inflammopharmacology. 2018;26(3):793-804.
34. Goel R, Kumar N, Kumar Saxena P, Pratap Singh A, Bana S. Pitavastatin attenuates neuropathic pain induced by partial sciatic nerve in Wistar rats. J Pharm Pharmacol. 2023;75(1):66-75.
35. Pathak NN, Balaganur V, Lingaraju MC, Kant V, Latief N, More AS, et al. Atorvastatin attenuates neuropathic pain in rat neuropathy model by down-regulating oxidative damage at peripheral, spinal and supraspinal levels. Neurochem Int. 2014;68:1-9.
36. Jung KH, Chu K, Jeong SW, Han SY, Lee ST, Kim JY, et al. HMG-CoA reductase inhibitor, atorvastatin, promotes sensorimotor recovery, suppressing acute inflammatory reaction after experimental intracerebral hemorrhage. Stroke. 2004;35(7):1744-9.
37. Chen XY, Li K, Light AR, Fu KY. Simvastatin attenuates formalin-induced nociceptive behaviors by inhibiting microglial RhoA and p38 MAPK activation. J Pain. 2013;14(11):1310-9.
38. Tatsumi S, Mabuchi T, Katano T, Matsumura S, Abe T, Hidaka H, et al. Involvement of Rho-kinase in inflammatory and neuropathic pain through phosphorylation of myristoylated alanine-rich C-kinase substrate (MARCKS). Neuroscience. 2005;131(2):491-8.
39. Gillon JT, Smith SE, Lowden MR. Atorvastatin as novel treatment for neuropathic pain: a case report. Clin J Pain. 2013;29(12):e46-8.
40. Nohria A, Prsic A, Liu PY, Okamoto R, Creager MA, Selwyn A, et al. Statins inhibit Rho kinase activity in patients with atherosclerosis. Atherosclerosis. 2009;205(2):517-21.

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The Self-Concept, Family Functioning, Psychological and Emotional Symptoms, in Children and Adolescents with Neurofibromatosis Type 1

Nörofibromatozis Tip 1 Tanılı Çocuk ve Ergenlerde Benlik Kavramı, Aile İşlevleri, Ruhsal ve Emosyonel Semptomlar

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ABSTRACT

Aim: This study aimed to examine the self-concept and related psychological factors in children and adolescents with neurofibromatosis type 1 (NF1).
Material and Methods: A total of 71 participants (35 patients and 36 controls) were included in this study. As measurement tools the Piers-Harris children's self-concept scale (PHSCS), family assessment device (FAD), Turgay DSM-IV-based screening and rating scale for disruptive behavior disorders (T-DSM-IV-S), children's depression inventory (CDI), Beck depression inventory (BDI), state-trait anxiety inventory (STAI), children's sleep habits questionnaire (CSHQ), and autism spectrum quotient-adolescent (AQ-adolescent) were used.
Results: NF1 patients had lower intellectual functioning/academic achievement (p=0.002) and popularity/social appreciation (p<0.001) subscale scores in addition to total self-esteem (p=0.002) scores compared to the control group. Inattentiveness (p=0.024), state anxiety (p=0.028), and trait anxiety (p=0.012) symptoms were more common in these patients, and autistic features were more conspicuous in adolescents (p<0.001). Mother's depression (p=0.045), and state and trait anxiety (p=0.016, and p=0.006, respectively) levels were higher in the NF1 group. Depression, state anxiety, and hyperactivity were found predictors of self-concept in children and adolescents with NF1 (p<0.001, p=0.036, and p=0.016, respectively).
Conclusion: Turkish children and adolescents with NF1 face substantial challenges related to their self-concept and psychological health, particularly in academic and social domains, compared to their peers. It seems important to be aware of poor self-concept and related psychological factors in NF patients early in life and to develop intervention programs to address this issue.
Keywords: Self-concept; anxiety; neurofibromatosis 1.

ÖZ

Amaç: Bu çalışma, nörofibromatozis tip 1 (NF1) tanısı olan çocuk ve ergenlerde benlik kavramını ve ilişkili psikolojik faktörleri incelemeyi amaçlamaktadır.
Gereç ve Yöntemler: Bu çalışmaya toplam 71 katılımcı (35 hasta ve 36 kontrol) dahil edildi. Ölçme aracı olarak Piers-Harris çocuklar için benlik kavramı ölçeği (PHBKÖ), aile değerlendirme ölçeği (ADÖ), Turgay yıkıcı davranım bozuklukları için DSM-IV'e dayalı tarama ve değerlendirme ölçeği (YDB- TDÖ), çocuklar için depresyon ölçeği (ÇDÖ), Beck depresyon envanteri (BDE), durumluk-sürekli kaygı envanteri (DSKE), çocuk uyku alışkanlıkları anketi (ÇUAA) ve otizm spektrum anketi-ergen formu (OSA-ergen) uygulandı.
Bulgular: NF1 hastaları, kontrol grubuna kıyasla daha düşük toplam benlik kavramı puanlarının (p=0,002) yanı sıra daha düşük zihinsel işlevsellik/akademik başarı (p=0,002) ve popülerlik/sosyal beğeni (p<0,001) alt ölçek puanlarına sahipti. Dikkat eksikliği (p=0,024), durumluk anksiyete (p=0,028) ve sürekli anksiyete (p=0,012) semptomları bu hastalarda daha sıktı ve ergenlerde otistik özellikler (p<0,001) daha belirgindi. Annelerin depresyon (p=0,045) ve durumluk ve sürekli kaygı (sırasıyla p=0,016 ve p=0,006) düzeyleri NF1 grubunda daha yüksekti. NF1 tanılı çocuk ve ergenlerde depresyon, durumluk anksiyete ve hiperaktivite, benlik kavramının yordayıcıları olduğunu gösterdi (sırasıyla, p<0,001, p=0,036 ve p=0,016).
Sonuç: NF1 tanısı olan Türk çocuk ve ergenler, akranlarına kıyasla, özellikle akademik ve sosyal alanlarda olmak üzere benlik kavramları ve psikolojik sağlıkları ile ilgili önemli zorluklarla karşılaşmaktadır. NF hastalarındaki zayıf benlik kavramının ve ilişkili psikolojik faktörlerin yaşamın erken dönemlerinde farkında olmak ve buna yönelik müdahale programları geliştirmek önemli görünmektedir.
Anahtar kelimeler: Benlik kavramı; anksiyete; nörofibromatozis 1.

INTRODUCTION

Neurofibromatosis (NF) is a progressive, multisystemic, genetic disorder that primarily affects the nervous system and skin, increasing the risk of tumors and affecting the skeletal, endocrine, reproductive, and cardiovascular systems. Additionally, elevates the risk of developing mental disorders (1,2). These include NF type 1 (NF1, known as Von Recklinghausen disease), NF type 2 (NF2), and schwannomatosis (SWN) (3). NF1 and NF2 are autosomal dominant disorders caused by mutations in genes located on chromosomes 17 and 22, respectively. They can be inherited or caused by de novo mutations in germ cells, and each mechanism is responsible for approximately half of the cases. The pooled prevalence of NF1 and NF2 was 1:3164 (95% CI, 1:2132-1:4712) and 1:50.000 (95% CI, 1:32.829-1:65.019), respectively (4). The exact prevalence of mosaic cases is not known but is estimated at 1:36.000 to 1:40.000 (5). NF1 is a RASopathy caused by mutations in the neurofibromin gene and characterized by café-au-lait patches, Lisch nodules, and neurofibromas (5). Neuropsychologically, NF1 is characterized by problems in visual-spatial, visual-perceptive, and executive functions along with maintaining and shifting attention. These problems may increase with age and may be present in mosaic cases (6). Up to 80.0% of children with NF1 may have psychiatric disorders while 25.0-36.8% may be diagnosed with autism spectrum disorder (ASD). Otherwise, 40.0% of NF1 individuals have attention deficit hyperactivity disorder (ADHD) (6-10). In the preschool period, up to 68.0% of these children may display delays in speech/language development and motor coordination problems. Preclinical studies suggest that learning and memory in NF1 may be disrupted by p21RAS activity and long-term potentiation, whereas lesions in the basal ganglia, thalamus, brain stem, and cerebellum may contribute to the development of ADHD (5-10). A genetic disorder with far-reaching consequences, such as NF1, may also affect self-concept in addition to causing psychopathology (11).

The set of beliefs (also known as self-schemas) about oneself is known as one's self-concept. It can be also termed as, self-identity, self-perspective, self-construction, and self-structure (12,13). It differs from self-awareness, self-knowledge, self-esteem, and the social self, as it interacts with them to form the self as a gestalt. Self-schemas may pertain to the past, the current, and the future, as well as various domains of daily functioning (e.g., academic, social, etc.). They affect behavior and are composed of cognitive/descriptive components rather than evaluations or opinions (12,13). Although it develops and is elaborated upon throughout one's lifespan, it is most malleable in childhood and adolescence (12-14). Age, gender, developmental, social, and educational processes, as well as chronic disorders affect its development (11-15). Studies conducted on self-concept and related constructs among patients with NF1 at various ages suggest that children may focus on the effects of the disorder on physical abilities and pain (11,16), while adolescents report broader effects on self-concept (17,18), and that the clinical severity of the disorder may not correlate with changes in self-concept (11).

Studies conducted on patients with NF1 at various ages suggest that they may have reduced self-esteem compared to their peers (19). Having peers with NF1, attending dedicated support groups, receiving care at specialized clinics, and genetic counseling were found to predict higher self-esteem (19). Almost five to seven million people in Turkey are thought to be affected by rare genetic or metabolic disorders, including NF (20). Limited knowledge of these disorders, limited numbers of specialist physicians and centers, elevated costs of treatment, and high rates of consanguineous marriages further contribute to this problem (20). Studies on Turkish children with NF are limited to single centers (21,22) and focus on the neurocognitive abilities of affected children and their correlates (23-25). To the best of our knowledge, no research has evaluated self-concept, family functioning, or reported emotional/psychological symptoms among Turkish children with NF. Therefore, this study aimed to assess self-concept and related characteristics in Turkish children and adolescents with NF1 and compare them with healthy controls.

MATERIAL AND METHODS

Study Center, Sampling, and, Ethics

This is a cross-sectional, case-control study of children and adolescents diagnosed with NF1 and is in the process of treatment and follow-up in the Pediatric Oncology Department of Mersin University conducted between May 15, 2021, and October 15, 2021. The following were the inclusion criteria for the patient group: 1) diagnosis of NF1 according to the National Institutes of Health (NIH) criteria (26); 2) age between 8 and 18 years (including 8 and 18 years old); 3) absence of hearing loss and visual impairment; 4) absence of tumors, hepatic, and renal disorders; 5) normal ambulation skills; 6) fluency in Turkish; 7) ability to complete scales at a level that can be analyzed; and 8) acceptance of participation in this study and approval of the informed consent form for both the parents and children. The following were the inclusion criteria for the control group: 1) age between 8 and 18 years (including 8 and 18 years); 2) absence of hearing loss and visual impairment; 3) absence of any chronic illness, medication use, major trauma, surgical history, and physical disability; 4) fluency in Turkish and ability to complete scales at a level that can be analyzed; 5) absence of psychopathology in the diagnostic and statistical manual of mental disorders, 5th edition (DSM-5)-based psychiatric interviews (27); and 6) acceptance of participation in this study and approval of the informed consent form by both the parents and themselves. Sixty patients with NF were followed up in the Department of Pediatric Oncology, and 35 met the inclusion and exclusion criteria. Therefore, 71 participants (35 patients and 36 controls) were included in this study. The study was conducted in accordance with the principles of the Declaration of Helsinki, following the approval from the ethics committee of Mersin University Faculty of Medicine (08.05.2019, 186).

Data Collection Tools

Piers-Harris Children's Self-Concept Scale (PHSCS)

This scale was developed by Piers (28) to evaluate the self-concept and adapted to Turkish by Öner (29). It consists of 80 items with six subscales and is standardized for those aged 9-20. The internal consistency of the PHSCS was determined as 0.87 for the children sample,

and 0.86 for the adolescent sample. The scale score is between 0 and 80 points, with high scores indicating positive self-concept. PHSCS ≤39 is classified as reflecting "poor self-esteem", and PHSCS ≥40 is "average, and normal self-concept." In this study, the cut-off score was set at 40 to distinguish between poor and average/normal self-concept (29). In this study, the children completed the PHSCS.

Family Assessment Device (FAD)

This scale measures family functioning on seven distinct subscales by means of this 4-point Likert-type self-report scale: problem-solving, communication, roles, emotional responsiveness, attention span, behavior control, and general functions. It comprised 60 items. Mean scores greater than 2 are recognized as indicators of a trend toward poorer family functioning (30). The reliability and validity of the Turkish version have already been determined by Bulut (31). In the study, Cronbach's alpha value was found as 0.91 for general functioning, and ranging from 0.38 to 0.86 for the subscales.

Turgay DSM-IV-Based Screening and Rating Scale for Disruptive Behavior Disorders (T-DSM-IV-S)

This 4-point, Likert-type scale evaluates symptoms of attention deficit, hyperactivity/impulsivity, oppositional defiant, and conduct disorder according to parents'/teachers' reports. It consisted of 41 items. Its validity and reliability were established by Ercan et al. (32), and Cronbach's alpha coefficients of the subscales were found to be 0.88 for attention deficit, 0.95 for hyperactivity/impulsivity, 0.89 for oppositional defiant, and 0.85 for conduct disorder. High scores indicate increased symptom levels. The children's mothers completed the scale.

Children's Depression Inventory (CDI)

This self-report scale consists of 27 items evaluating symptoms of depression and is standardized between the ages of 6 and 17 years (33). The cut-off score was 19, and the maximum score was 54. Öy (34) conducted a validity and reliability study of the CDI's Turkish version, and Cronbach's alpha value was determined as 0.77. Children completed the CDI in this study.

Beck Depression Inventory (BDI)

This self-report scale consists of 21 items evaluating symptoms of depression, each of which is scored between 0 and 3 (35). Hisli (36) conducted a validity and reliability study of the BDI's Turkish version, and the internal consistency coefficient of the scale was found as 0.74. The mothers completed the BDI for themselves.

State-Trait Anxiety Inventory (STAI)

This is a 40-item, 4-point Likert-type scale, 20 items tapping state, and 20 items evaluating trait anxiety. A total score can range from 20 to 80 with each item receiving a value between 1 and 4 (37). Öner and Le Compte (38) conducted a reliability and validity study on the Turkish version. Cronbach's alpha value was found as 0.93 for STAI-State and 0.85 for STAI-Trait in the study. The mothers and children completed the STAI.

Children's Sleep Habits Questionnaire (CSHQ)

This scale evaluates the sleep habits and associated problems in children by 3-point Likert-type 33 items (39). Fiş et al. (40) performed a validity and reliability study of the CSHQ's Turkish version, and Cronbach's alpha coefficient was determined as 0.78. The parents completed the CSHQ for their children.

Autism Spectrum Quotient-Adolescent (AQ-Adolescent)

This is a parental report-based screening tool for measuring the degree of subthreshold autistic characteristics of adolescents with normal intelligence (41). Çetinoğlu and Aras (42) conducted a validity and reliability study in Turkey, and Cronbach's alpha value was found as 0.83. The cut-off score for the AQ-adolescent scale was 24 points. Parents completed AQ-adolescent for their children in this study.

Statistical Analysis

The Shapiro-Wilk test was used to test the assumptions of normality. The normally distributed data were compared between groups using an independent samples t-test, while the non-normally distributed data were compared using a Mann-Whitney U test. The categorical data was analyzed using either Fisher's exact test or Pearson's chi-square, depending on the expected value. Pearson's correlation analysis was used in order to examine the correlations between the continuous variables and the PHSCS score. The effects of variables that were significant in correlation analyses as predictors of PHSCS were analyzed using multiple regression analysis (backward method). Statistical analyses were performed using the IBM SPSS v.22.0 software (IBM Inc., Armonk, NY, USA), and the level of significance was considered 0.05.

RESULTS

The NF1 and control groups had mean ages of 12.9±3.4 and 13.1±3.0 years, respectively. The majority of both groups (65.7%, n=23 in NF1 and 58.8%, n=21 in control) were male. There were no significant differences in age and gender between the groups (p=0.766, and p=0.522, respectively). The frequency of having one or more psychiatric disorders in the NF1 group (82.8%, n=29) compared to the control group (2.8%, n=2) was significantly higher (p<0.001). The NF1 group had significantly lower PHSCS total (p=0.002), intellectual and school status (p=0.002), anxiety (p=0.044), and popularity/social appreciation (p<0.001) scores compared to the control group (Table 1).

According to the results of the parent T-DSM-IV-S scale, only the attention deficit subscale score was higher in the NF1 group (p=0.024). CDI scores showed no statistically significant difference (p=0.074), however, both the STAI-state (p=0.028) and STAI-trait (p=0.012) scores were significantly higher in the NF1 group. This group had

Table 1. Evaluation of subscale and total scores of the PHSCS between NF1 and the control groups

	NF1 (n=35)	Control (n=36)	p
Intellectual and school status	4.00±1.88	5.19±1.19	**0.002**
Happiness	7.03±2.24	6.64±2.31	0.473
Anxiety	5.00±2.44	6.03±1.73	**0.044**
Physical appearance and attributes	4.57±1.56	5.11±1.09	0.095
Behavioral adjustment	9.91±2.48	10.75±1.46	0.090
Popularity/Social appreciation	5.14±1.68	7.31±0.86	**<0.001**
PHSCS-Total	35.66±8.31	41.03±5.57	**0.002**

NF1: neurofibromatosis type 1, PHSCS: Piers-Harris children's self-concept scale

higher AQ-adolescent scores (p<0.001), and no significant difference in total sleep scores (p=0.461, Table 2).

In the FAD scale, only the FAD involvement score was significantly different between the groups (p=0.034). Mothers' BDI (p=0.045), STAI-trait (p=0.016), and STAI-state anxiety scores (p=0.016) were higher in the NF1 group (Table 3).

FAD affective involvement (r=-0.379, p=0.032), FAD behavior control (r=-0.443, p=0.011), T-DSM-IV-S attention deficit (r=-0.474, p=0.004), T-DSM-IV-S hyperactivity (r=-0.422, p=0.012), CDI (r=-0.599, p<0.001), STAI-trait (r=-0.605, p=0.001), and STAI-state (r=-0.547, p=0.001) scores were significantly correlated with the PHSCS total score. No correlation was found between the T-DSM-IV-S oppositional defiant (r=-0.223, p=0.199), T-DSM-IV-S conduct disorder (r=-0.115, p=0.511), mother's BDI depression (r=-0.177, p=0.331), mother's STAI-trait anxiety (r=-0.012 p=0.947), mother's STAI-state anxiety (r=-0.012 p=0,420), CSHQ (r=-0.088 p=0,622), AQ-adolescent (r=-0.264 p=0.184), and PHSCS total score. In the NF1 group, there was no correlation between T-DSM-IV-S attention deficit score (r=0.102, p=0.613), T-DSM-IV-S hyperactivity (r=0.034, p=0.865) score, and AQ-adolescent score. In the multiple regression analysis, the PHSCS total score among children with NF1 was negatively predicted by T-DSM-IV-S hyperactivity, CDI, and STAI-state scores (F=16.936; p<0.001; Table 4).

Lastly, the PHSCS total scores were divided into two groups, PHSCS ≤39 (n=22) and PHSCS ≥40 (n=13), and the study variables were compared between the groups. There were no statistically significant sex and age differences between the groups (p=0.736, and p=0.411, respectively). The CDI (p=0.017), STAI-state (p=0.006), STAI-trait (p=0.002) anxiety scores, and FAD affective involvement (p=0.033) scores were significantly higher in the group with PHSCS ≤39 (Tables 5, and Table 6).

DISCUSSION

This single-center study used a case-control cross-sectional design to evaluate self-concept and associated factors in Turkish children and adolescents with NF1 and compared them with healthy controls. The rate of psychopathology was found to be significantly elevated among the children with NF1, they had lower self-esteem (especially in terms of intellectual/academic status and popularity/social appreciation), and their state and trait anxiety were significantly elevated compared to controls. Their mothers rated them as having significantly elevated symptoms of inattention and ASD compared to the controls. Self-reported depressive and state/trait anxiety scores were also significantly elevated among the mothers of children with NF1. Families of children with NF1 have been found to show significantly different levels of parental involvement. The self-esteem of children with NF1 correlated negatively with parental involvement, behavior control, self-reported depression, state and trait anxiety, and parent-reported inattention/hyperactivity. In regression analyses, self-reported depression, state anxiety, and parent-reported hyperactivity emerged as significant predictors of self-esteem among children with NF1. Bivariate analyses showed that lower self-esteem among children with NF1 was also associated with lower affective involvement.

Up to five-fourths of children with NF1 may have psychopathology, while less than half may be diagnosed with ADHD (6-10). Supporting these results, the frequency of psychopathology among our sample of children with NF1 was reported to be 82.8%, which was significantly higher than that in controls. Mothers also rated

Table 2. Evaluation of T-DSM-IV-S, CDI, STAI, CSHQ, and AQ-Adolescent scores between NF1 and the control groups

	NF1 (n=35)	Control (n=36)	p
T-DSM-IV-S, attention deficit	13.60±8.08	9.49±6.82	**0.024**
T-DSM-IV-S, hyperactivity	10.71±8.65	8.80±5.76	0.280
T-DSM-IV-S, oppositional defiant	8.71±7.39	7.89±6.66	0.624
T-DSM-IV-S, conduct disorder	2.43±5.51	1.86±2.93	0.590
CDI	11.74±6.81	8.94±6.18	0.074
STAI-state	36.34±10.44	31.33±8.26	**0.028**
STAI-trait	41.09±9.23	35.56±8.84	**0.012**
CSHQ	55.88±11.76	53.92±10.41	0.461
AQ-adolescent	21.74±5.03	16.11±5.69	**<0.001**

T-DSM-IV-S: Turgay DSM-IV-based screening and rating scale for disruptive behavior disorders, CDI: children's depression inventory, STAI: state-trait anxiety inventory, CSHQ: children's sleep habits questionnaire, AQ-adolescent: autism spectrum quotient-adolescent, NF1: neurofibromatosis type 1

Table 3. Evaluation of FAD, mother's BDI, and mother's STAI scales between NF1 and control groups

	NF1 (n=35)	Control (n=36)	p
FAD, general functioning	1.90±0.70	1.74±0.48	0.278
FAD, problem solving	1.82±0.61	1.80±0.48	0.909
FAD, communication	1.92±0.59	1.78±0.62	0.371
FAD, roles	2.13±0.58	2.20±0.44	0.547
FAD, affective involvement	2.52±0.50	2.29±0.36	**0.034**
FAD, behavior control	2.23±0.54	2.15±0.47	0.527
FAD, affective responsiveness	2.19±0.86	1.96±0.79	0.254
Mother's BDI	16.81±10.26	12.25±8.09	**0.045**
Mother's STAI-state	42.86±10.74	36.28±11.60	**0.016**
Mother's STAI-trait	48.76±10.93	41.75±9.80	**0.006**

FAD: family assessment device, BDI: Beck depression inventory, STAI: state-trait anxiety inventory, NF1: neurofibromatosis type 1

Table 4. Predictors of PHSCS total score

	Beta	95% CI	p
(Constant)		47.587 - 61.684	**<0.001**
T-DSM-IV-S, hyperactivity	-0.291	-0.553 - -0.061	**0.016**
CDI	-0.530	-1.010 - -0.344	**<0.001**
STAI-state	-0.281	-0.437 - -0.016	**0.036**

PHSCS: Piers-Harris children's self-concept scale, T-DSM-IV-S: Turgay DSM-IV-based screening and rating scale for disruptive behavior disorders, CDI: children's depression inventory, STAI: state-trait anxiety inventory, CI: confidence interval

their children as having significantly elevated symptoms of inattention and ASD compared to the controls. Anxiety, depression, and learning disorders may also be elevated in children with NF1 (43). Partially supporting these results, children with NF1 in our study reported significantly elevated state and trait anxiety, while their depressive symptom levels were similar to those of controls. This discrepancy may be due to our dependence on self-report instruments, which may be subject to reporting and recall bias, as well as shared method variance. We did not evaluate ADHD, ASD, or learning disorders using semi-structured clinical interviews (44). Future studies on psychopathology among children with NF1 should use structured clinical interviews and parent and clinician ratings of depression, anxiety, and various psychopathologies. They may also evaluate the correlations between these psychopathologies and the severity of the NF1.

Studies conducted on patients with NF1 at various ages suggest that they may have reduced self-esteem compared to their peers (19). Barton et al. (11) reported that children with NF1 may have lower self-esteem, especially in terms of physical and sports-related skills, while their academic self-esteem may be elevated. In our study, children with NF1 reported lower self-esteem, especially in terms of intellectual and academic status and popularity. Lower self-esteem in the academic/intellectual domain may be due to the presence of learning disorders (45). Children with NF1 have also been reported to be less popular and more prone to peer bullying (46). Supporting these views, children with NF1 in our study rated themselves as less popular among their peers. Lower self-esteem in our sample was predicted by elevated self-reported depression and state anxiety, and higher parent-reported hyperactivity. There are also signals that lower family functioning may contribute to reduced self-esteem. Although the study's cross-sectional nature prevents hypotheses on causality, it may be posited that lower family support may have led to a negative self-concept and internalizing symptoms, while hyperactivity/impulsivity may lead to problems with peers, further compounding the problem (46). Future studies on self-esteem and related factors among children with NF1 should use longitudinal designs with multiple evaluation waves to test this hypothesis.

We need to consider evaluating the results in light of these limitations. First, the results are valid for the study center and may not be generalizable to children with NF1 followed up at other centers or those without access to specialized care. Second, we did not evaluate the level of social support available to children and their victimization rates in peer bullying. Third, we did not evaluate ADHD, ASD, or learning disorders in children with NF1 using psychometric instruments. Fourth, we did not evaluate the effects of NF1 symptom severity or visibility on self-esteem or psychopathology. Fifth, dependence on multiple self- and parent-report instruments may have biased our results. Sixth, we did not evaluate the effects of genetic counseling on the symptoms of children and mothers. Lastly, we did not evaluate mothers with structured clinical interviews for psychopathology. Regardless of these limitations, our results suggest that Turkish children with NF1 have lower self-esteem and higher symptoms of psychopathology compared to peers and that interventions to address these problems may be beneficial.

Table 5. Evaluation of emotional symptoms and family functioning according to the PHSCS total score in the mothers of patients with NF1

	PHSCS ≤39 (n=22)	PHSCS ≥40 (n=13)	p
FAD, general functioning	1.93±0.80	1.86±0.52	0.798
FAD, problem solving	1.70±0.64	2.01±0.54	0.166
FAD, communication	1.89±0.66	1.95±0.47	0.786
FAD, roles	2.04±0.62	2.27±0.50	0.298
FAD, affective involvement	2.64±0.58	2.31±0.25	**0.033**
FAD, behavior control	2.32±0.51	2.09±0.57	0.257
FAD, affective responsiveness	2.21±1.00	2.15±0.57	0.827
Mother's BDI	17.57±11.89	15.36±6.31	0.572
Mother's STAI-state	41.73±11.53	44.77±9.37	0.426
Mother's STAI-trait	47.82±12.38	50.50±7.80	0.503

PHSCS: Piers-Harris children's self-concept scale, NF1: neurofibromatosis type 1, FAD: family assessment device, BDI: Beck depression inventory, STAI: state-trait anxiety inventory

Table 6. Evaluation of the emotional and behavioral symptoms, sleep habits, and autistic features according to the PHSCS total score in the patients with NF1

	PHSCS ≤39 (n=22)	PHSCS ≥40 (n=13)	p
T-DSM-IV-S, attention deficit	15.05±7.91	11.15±8.08	0.172
T-DSM-IV-S, hyperactivity	11.73±8.95	9.00±8.16	0.375
CDI	13.82±6.34	8.23±6.31	**0.017**
STAI-state	39.95±10.05	30.23±8.22	**0.006**
STAI-trait	44.64±8.64	35.08±6.98	**0.002**
CSHQ	54.45±11.96	58.50±11.41	0.345
AQ-adolescent	22.24±3.72	20.90±6.87	0.581

PHSCS: Piers-Harris children's self-concept scale, NF1: neurofibromatosis type 1, T-DSM-IV-S: Turgay DSM-IV-based screening and rating scale for disruptive behavior disorders, CDI: children's depression inventory, STAI: state-trait anxiety inventory, CSHQ: children's sleep habits questionnaire, AQ-adolescent: autism spectrum quotient-adolescent

CONCLUSION

The study highlights that Turkish children and adolescents with NF1 face substantial challenges related to their self-concept and psychological health compared to their peers. Elevated rates of anxiety, ADHD symptoms, and reduced self-esteem, particularly in academic and social domains, underscore the complex impact of NF1 on daily life and emotional well-being. These findings emphasize the importance of comprehensive care strategies that address not only medical needs but also psychosocial support for affected individuals and their families. Future research should explore longitudinal effects and interventions to mitigate these challenges effectively.

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REFERENCES

1. Elbeltagy M, Abbassy M. Neurofibromatosis type1, type 2, tuberous sclerosis and Von Hippel-Lindau disease. Childs Nerv Syst. 2023;39(10):2791-806.
2. Brar KS, Trivedi C, Kaur N, Adnan M, Patel H, Beg U, et al. Prevalence of psychiatric comorbidities in patients with neurofibromatosis. Prim Care Companion CNS Disord. 2023;25(5):23m03514.
3. National Institute of Neurological Disorders and Stroke. Neurofibromatosis. Bethesda, MD: NINDS Publication; 2023.
4. Lee TJ, Chopra M, Kim RH, Parkin PC, Barnett-Tapia C. Incidence and prevalence of neurofibromatosis type 1 and 2: a systematic review and meta-analysis. Orphanet J Rare Dis. 2023;18(1):292.
5. Friedman JM. Neurofibromatosis 1. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, et al., editors. GeneReviews®. Seattle (WA): University of Washington, Seattle; 2018.
6. Beaussart ML, Barbarot S, Mauger C, Roy A. Systematic review and meta-analysis of executive functions in preschool and school-age children with neurofibromatosis type 1. J Int Neuropsychol Soc. 2018;24(9):977-94.
7. Molosh AI, Shekhar A. Neurofibromatosis type 1 as a model system to study molecular mechanisms of autism spectrum disorder symptoms. Prog Brain Res. 2018;241:37-62.
8. Sánchez Marco SB, López Pisón J, Calvo Escribano C, González Viejo I, Miramar Gallart MD, Samper Villagrasa P. Neurological manifestations of neurofibromatosis type 1: our experience. Neurologia (Engl Ed). 2022;37(5):325-33.
9. Torres Nupan MM, Velez Van Meerbeke A, López Cabra CA, Herrera Gomez PM. Cognitive and behavioral disorders in children with neurofibromatosis type 1. Front Pediatr. 2017;5:227.
10. Smith TF, Kaczorowski JA, Acosta MT. An executive functioning perspective in neurofibromatosis type 1: from ADHD and autism spectrum disorder to research domains. Childs Nerv Syst. 2020;36(10):2321-32.
11. Barton B, North K. The self-concept of children and adolescents with neurofibromatosis type 1. Child Care Health Dev. 2007;33(4):401-8.
12. Gore JS, Cross SE. Defining and measuring self-concept change. Psychol Stud. 2011;56(1):135-41.
13. Elliott GC. Dimensions of the self-concept: a source of further distinctions in the nature of self-consciousness. J Youth Adolesc. 1984;13(4):285-307.
14. Cole DA, Maxwell SE, Martin JM, Peeke LG, Seroczynski AD, Tram JM, et al. The development of multiple domains of child and adolescent self-concept: a cohort sequential longitudinal design. Child Dev. 2001;72(6):1723-46.
15. Ahn JA, Lee S. Peer attachment, perceived parenting style, self-concept, and school adjustments in adolescents with chronic illness. Asian Nurs Res (Korean Soc Nurs Sci). 2016;10(4):300-4.
16. Krab LC, Oostenbrink R, de Goede-Bolder A, Aarsen FK, Elgersma Y, Moll HA. Health-related quality of life in children with neurofibromatosis type 1: contribution of demographic factors, disease-related factors, and behavior. J Pediatr. 2009;154(3):420-5.
17. Ejerskov C, Lasgaard M, Østergaard JR. Teenagers and young adults with neurofibromatosis type 1 are more likely to experience loneliness than siblings without the illness. Acta Paediatr. 2015;104(6):604-9.
18. Hummelvoll G, Antonsen KM. Young adults' experience of living with neurofibromatosis type 1. J Genet Couns. 2013;22(2):188-99.
19. Rosnau K, Hashmi SS, Northrup H, Slopis J, Noblin S, Ashfaq M. Knowledge and self-esteem of individuals with neurofibromatosis type 1 (NF1). J Genet Couns. 2017;26(3):620-7.
20. Aksu B. Rare disease and orphan drug situations in Turkey and around the world. A J Health Sci. 2019;1(2):95-109.
21. Demir Gündoğan B, Tuğ Bozdoğan S, Ayhan YS, Müjde C, Bişgin A, Çitak EÇ. Clinical findings and mutational spectrum of neurofibromatosis type 1 patients in a single center of south part of Turkey. Turk J Med Sci. 2021;[Epub ahead of print]. doi: 10.3906/sag-2101-401.
22. Gonca Kaçar A, Kılınc Oktay B, Çınar Özel S, Ocak S, Güneş N, Uludağ Alkaya D, et al. Neurofibromatosis type 1 in children: a single-center experience. Turk Arch Pediatr. 2021;56(4):339-43.
23. Aydin S, Kurtcan S, Alkan A, Guler S, Filiz M, Yilmaz TF, et al. Relationship between the corpus callosum and neurocognitive disabilities in children with NF-1: diffusion tensor imaging features. Clin Imaging. 2016;40(6):1092-5.
24. Terzi YK, Oğuzkan-Balci S, Anlar B, Erdoğan-Bakar E, Ayter S. Learning disability and oligodendrocyte myelin glycoprotein (OMGP) gene in neurofibromatosis type 1. Turk J Pediatr. 2011;53(1):75-8.
25. Erdoğan-Bakar E, Cinbiş M, Ozyürek H, Kiriş N, Altunbaşak S, Anlar B. Cognitive functions in neurofibromatosis type 1 patients and unaffected siblings. Turk J Pediatr. 2009;51(6):565-71.
26. Legius E, Messiaen L, Wolkenstein P, Pancza P, Avery RA, Berman Y, et al. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. Genet Med. 2021;23(8):1506-13.
27. American Psychiatric Association (APA). Diagnostic and statistical manual of mental disorders, 5th ed. Washington, DC: APA; 2013.

28. Piers EV. Revised manual for the Piers-Harris Children's Self Concept Scale, 2nd ed. Los Angeles, CA: Western Psychological Services; 1984.
29. Öner N. Manual of the Piers-Harris children's self-concept scale, 1st ed. Ankara: Turkish Psychologists Association Publications; 1996. Turkish.
30. Epstein NB, Baldwin LM, Bishop DS. The McMaster family assessment device. J Marital Fam Ther. 1983;9(2):171-80.
31. Bulut I. Handbook of family assessment device, 1st ed. Ankara: Özgüzeliş Matbaası; 1990. Turkish.
32. Ercan ES, Amado S, Somer O, Cıkoglu S. Development of a test battery for the assessment of attention deficit hyperactivity disorder. Turk J Child Adolesc Ment Health. 2001;8(3):132-44. Turkish.
33. Kovacs M. The children's depression inventory (CDI). Psychopharmacol Bull. 1985;21(4):995-8.
34. Oy B. Reliability and validity study of the children's depression inventory. Turk J Psychiatry. 1991;2(2):137-40. Turkish.
35. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry. 1961;4:561-71.
36. Hisli N. Validity and reliability of Beck Depression Inventory in university students. Turk J Psychol. 1989;7(23):3-13. Turkish.
37. Gaudry E, Vagg P, Spielberger CD. Validation of the state-trait distinction in anxiety research. Multivariate Behav Res. 1975;10(3):331-41.
38. Öner N, Le Compte A. Handbook of state-trait anxiety inventory. İstanbul: Boğaziçi University Press; 1998.
39. Owens JA, Spirito A, McGuinn M. The children's sleep habits questionnaire (CSHQ): psychometric properties of a survey instrument for school-aged children. Sleep. 2000;23(8):1043-51.
40. Perdahlı Fiş N, Arman A, Ay P, Topuzoglu A, Guler AS, Gökçe Imren S, et al. The validity and the reliability of Turkish Version of Children's Sleep Habits Questionnaire. Anatolian J Psychiatry. 2010;11(2):151-60.
41. Baron-Cohen S, Hoekstra RA, Knickmeyer R, Wheelwright S. The autism-spectrum quotient (AQ)-adolescent version. J Autism Dev Disord. 2006;36(3):343-50.
42. Cetinoglu E, Aras S. The autism-spectrum quotient (AQ) adolescent's version in Turkey: factor structure, reliability and validity. J Autism Dev Disord. 2022;52(7):3260-70. Turkish.
43. Wang DL, Smith KB, Esparza S, Leigh FA, Muzikansky A, Park ER, et al. Emotional functioning of patients with neurofibromatosis tumor suppressor syndrome. Genet Med. 2012;14(12):977-82.
44. Ünal F, Öktem F, Çetin Çuhadaroğlu F, Çengel Kültür SE, Akdemir D, Foto Özdemir D, et al. Reliability and validity of the schedule for affective disorders and schizophrenia for school-age children-present and lifetime version, DSM-5 November 2016-Turkish adaptation (K-SADS-PL-DSM-5-T). Turk J Psychiatry. 2019;30(1):42-50. Turkish.
45. Descheemaeker MJ, Ghesquière P, Symons H, Fryns JP, Legius E. Behavioural, academic and neuropsychological profile of normally gifted Neurofibromatosis type 1 children. J Intellect Disabil Res. 2005;49(Pt 1):33-46.
46. Cavallo ND, Maggi G, Ferraiuolo F, Sorrentino A, Perrotta S, Carotenuto M, et al. Neuropsychiatric manifestations, reduced self-esteem and poor quality of life in children and adolescents with neurofibromatosis type 1 (NF1): the impact of symptom visibility and bullying behavior. Children (Basel). 2023;10(2):330.

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Drug-Drug Interactions in the Red Zone of the Emergency Department: A Retrospective Study

Acil Servis Kırmızı Alanda İlaç-İlaç Etkileşimleri: Retrospektif Bir Çalışma

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ABSTRACT

Aim: This study aimed to assess the potential for drug-drug interactions in adult patients admitted to the emergency departments.
Material and Methods: This cross-sectional study included 410 patients who were admitted to the red zone of the emergency departments, examined, treated, and received multiple medications. Drug-drug interaction analysis was conducted using LexiInteract software.
Results: The median age of patients was 63 (range, 19-96) years, with 55.4% (n=227) being female and 44.6% (n=183) were male. A total of 1,230 medications were identified among the patients. In 181 (44.1%) patients, 330 possible drug-drug interactions were detected. While there was no significant difference in the rate of drug-drug interactions between male and female patients (p=0.658), this rate was higher in patients aged 65 years and over (p=0.048) and patients with polypharmacy (p<0.001). Also, the interaction rates were higher in patients admitted with cerebrovascular disease (p=0.038) and trauma (p=0.002). According to the Lexicomp© drug information system, potential drug-drug interactions were classified into risk category C (n=299, 72.9%), risk category D (n=22, 5.4%), and risk category X (n=9, 2.2%). The most frequently interacting drug pairs were Furosemide-Salbutamol in category C, Enoxaparin-Acetylsalicylic acid in category D, and Dexketoprofen-Acetylsalicylic acid in category X.
Conclusion: Nearly half of the patients treated in the red zone of the emergency department were at risk of drug interactions. Assessing the risk of drug-drug interactions is essential before initiating medical instructions in critical areas of emergency department patient care, and follow-up should be organized about potential adverse effects.
Keywords: Drug interaction; emergency service; red zone; clinical pharmacist.

ÖZ

Amaç: Bu çalışmanın amacı, acil servislere başvuran yetişkin hastalarda ilaç-ilaç etkileşimi potansiyelini değerlendirmektir.
Gereç ve Yöntemler: Bu kesitsel çalışmaya, acil servisin kırmızı bölgesine kabul edilen, muayene edilen, tedavi edilen ve birden fazla ilaç alan 410 hasta dahil edilmiştir. İlaç-ilaç etkileşimi analizi LexiInteract yazılımı kullanılarak gerçekleştirilmiştir.
Bulgular: Hastaların ortanca yaşı 63 (aralık, 19-96) yıl olup %55,4'ü (n=227) kadın ve %44,5'i (n=183) erkektir. Hastalar arasında toplam 1.230 ilaç tespit edilmiştir. 181 (%44,1) hastada 330 adet olası ilaç-ilaç etkileşimi tespit edilmiştir. Erkek ve kadın hastalar arasında ilaç-ilaç etkileşimi oranları bakımından anlamlı bir fark bulunmazken (p=0,658), 65 yaş ve üzeri hastalarda (p=0,048) ve çoklu ilaç kullanımı olan hastalarda (p<0,001) bu oran daha yüksekti. Ayrıca, serebrovasküler hastalık (p=0,038) ve travma (p=0,002) ile başvuran hastalarda da etkileşim oranları daha yüksek idi. Lexicomp© ilaç bilgi sistemine göre, olası ilaç-ilaç etkileşimleri risk kategorisi C (n=299, %72,9), risk kategorisi D (n=22, %5,4) ve risk kategorisi X (n=9, %2,2) olarak sınıflandırıldı. En sık etkileşime giren ilaç çiftleri, C kategorisinde Furosemid-Salbutamol, D kategorisinde Enoksaparin-Asetilsalisilik asit ve X kategorisinde Deksketoprofen-Asetilsalisilik asit idi.
Sonuç: Acil servisin kırmızı bölgesinde tedavi edilen hastaların neredeyse yarısı ilaç etkileşimi riski altındaydı. Acil servis hasta bakımının kritik alanlarında tıbbi talimatlara başlamadan önce ilaç-ilaç etkileşimi riskinin değerlendirilmesi esastır ve potansiyel yan etkilerle ilgili olarak takip düzenlenmelidir.
Anahtar kelimeler: İlaç etkileşimi; acil servis; kırmızı bölge; klinik eczacı.

INTRODUCTION

A healthcare system without adequate medical care can lead to illness, mortality, and economic hardship for communities (1). While the therapeutic effects of multiple medications can often be beneficial, some combinations pose serious risks, increasing the likelihood of drug-drug interactions (DDIs) (2). Challenges such as overcrowding, high-stress levels, understaffing, rapid patient turnover, and insufficient communication among multidisciplinary teams exacerbate these risks in emergency departments (EDs) (3).

In the Turkish healthcare system, EDs are classified into three triage levels, indicated by red, yellow, and green colors in descending order of priority. The red triage code denotes life-threatening situations that require urgent and simultaneous examination and treatment, necessitating immediate patient transfer to the red zone (4).

DDIs, adverse drug events (ADEs), allergic reactions, and medication errors are among the most critical issues associated with drug use in EDs, with ADEs being among the most frequently reported errors (5). DDIs, defined as altered toxicity or efficacy when medications are administered concurrently (6), significantly contribute to increases in ADEs, hospital admissions, ED visits, and rehospitalizations (7,8). They also drive up healthcare costs and hospitalization rates (2,9). Reports suggest that between 5% and 20% of severe drug reactions from DDIs lead to hospitalization or death (10). Therefore, managing DDIs is crucial to enhancing drug safety. Early identification and reporting of potential drug-drug interactions (pDDIs) can prevent numerous complications, ultimately improving patient safety and quality of life.

This study aimed to determine the frequency and clinical severity of pDDIs in patients admitted to EDs.

MATERIAL AND METHODS

Study Group

This cross-sectional study was conducted with red zone patients at the Emergency Medicine Clinic of the Mersin City Training and Research Hospital. Patients under 18 years old, those referred to departments outside the red zone of the ED, and those with inaccessible records were excluded from the study. Based on average monthly admissions to the ED, a minimum sample size of 355 patients was calculated to achieve a 95% confidence level and a 5% margin of error, with a final sample size of 410 patients chosen to account for a 15% attrition rate. A total of 410 adult patients admitted to the red zone of the ED were included. The inclusion criteria were patients over 18 years of age referred to the red zone of the ED between April 1, 2021, and April 1, 2022, with at least two drug administrations in their medical history and accessible medical records. Exclusion criteria included patients under 18 years old, patients referred to areas other than the red zone of the ED during the specified period, and patients with no recorded drug administration history. The study protocol received approval from the Mersin University Clinical Research Ethics Committee (06.04.2022, 232).

Data Collection

Drug evaluations included only medications administered within the first 24 hours of a patient's registration in the red zone, even if the patient was monitored for a longer period. Patient demographic and medication data were retrospectively obtained from the medical records. The concurrent use of five or more drugs was classified as polypharmacy. The LexiInteract software (Lexicomp Online®, Lexi-Comp, Inc., Hudson, Ohio) was utilized to assess co-prescriptions with known interactions. LexiInteract, typically not used in routine hospital practice, was employed here specifically for research purposes. In this system, pDDIs are graded: X for combinations with an unfavorable risk-benefit ratio and a recommendation to avoid, D for combinations where a treatment change should be considered, and C for cases requiring close monitoring. Clinically relevant exposures included grade X, D, or more than two grade C pDDIs in a patient's treatment regimen. Additionally, data reliability was categorized as excellent, acceptable, or moderate based on the quality of supporting information, and interaction significance was classified as major, moderate, or minor. Drugs were further categorized according to the Anatomic Therapeutic Chemical (ATC) Drug Classification system developed by the World Health Organization Collaborating Centre.

Statistical Analysis

The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess the normality of the data, revealing that the data did not follow a normal distribution. Descriptive statistics were presented as counts with percentages and as medians with interquartile ranges. Chi-square or Fisher's exact test was used for group comparisons. The correlation between DDI risk categories and patient characteristics was assessed using Spearman's correlation coefficient. All statistical analyses were conducted using IBM SPSS v.25 for Windows, with statistical significance set at $p<0.05$.

RESULTS

Basic Characteristics of Patients

Of the 410 patients included, 227 (55.4%) were female, and 183 (44.6%) were male. The median age of all patients was 63 (range, 19-96) years, and 195 (47.6%) of the patients were over the age of 65. The most common diagnoses among the 109 identified upon admission were respiratory disease (n=117, 28.5%), cardiovascular disease (n=63, 15.4%), and chest pain (n=54, 13.2%). The total number of drugs prescribed to patients in this study was 1,230. The median number of medications per patient was 3 (range, 0-8), and polypharmacy was observed in 12.9% (n=53) of the total patients. A total of 54 different types of medications were used across the study group. Detailed patient diagnoses were presented in Table 1 and the most used medications were detailed in Table 2.

Frequency and Qualities of pDDIs

A total of 330 pDDIs were identified in 181 (44.1%) patients. One pDDIs were observed in 105 (25.6%) patients, while eleven patients (2.7%) had five or more pDDIs (Table 3).

While the rate of patients with at least one interaction was 45.4% (n=83) in males, this rate was 43.2% (n=98) in females (p=0.658). While the number of patients aged 65 years and over who had at least one drug interaction was 96 (49.2%), this number was 85 (39.5%) in those under 65 years of age (p=0.048). While 45 (84.9%) of 53 patients with polypharmacy had at least one interaction, this rate was 38.1% (n=136) in those without polypharmacy (p<0.001).

When the presence of interaction was analyzed according to the other clinical characteristics of the patients, it was seen that diagnoses at the admission did not show a significant difference in having at least one interaction, except for the patients admitted due to cerebrovascular disease (p=0.038) and trauma (p=0.002), details were shown in Table 4.

A total of 330 pDDIs were identified and categorized using the Lexicomp© drug information system as risk category C (n=299, 72.9%), risk category D (n=22, 5.4%), and risk category X (n=9, 2.2%). In examining the relationships between the number of drugs used, age, and risk categories, a significant correlation was found between age and risk category C (r_s=0.163, p=0.001). Significant correlations were observed in both C (r_s=0.450, p<0.001) and D (r_s=0.180, p<0.001) risk categories with the number of drugs used (Table 5).

Regarding reliability, while 127 (31.0%) of the pDDIs were classified as good, 192 (46.8%) were classified as fair, and 11 (2.7%) of them were classified as excellent. The

Table 1. Characteristics of the study group

Characteristics	**(n=410)**
Age (year), median (IQR) [min-max]	63 (48-77) [19-96]
Gender, n (%)	
Male	183 (44.6)
Female	227 (55.4)
Diagnosis, n (%)	
Respiratory disturbances	117 (28.5)
Cardiovascular disorders	63 (15.4)
Chest pain	54 (13.2)
Pain	23 (5.6)
Trauma (Fracture, injury, etc.)	23 (5.6)
Gastrointestinal problems	22 (5.4)
Cerebrovascular diseases	12 (2.9)
Epilepsy - Neurological	12 (2.9)
Fever	7 (1.7)
Polypharmacy, n (%)	53 (12.9)
Number of drugs, median (IQR) [min-max]	3 (2-4) [0-8]

IQR: interquartile range (25^{th}-75^{th} percentile)

Table 2. Most used drugs in patients (active ingredients)

ATC Groups, and Active Ingredients	**n**
A - Digestive System and Metabolism	
Pantoprazole	130
Metoclopramide	48
C - Cardiovascular System	
Furosemide	111
H - Endocrine System (Except Gender Hormones and Insulin)	
Methylprednisolone	91
R - Respiratory System	
Salbutamol	85
Ipratropium bromide/Salbutamol	82
Budesonide	55
N - Nervous System	
Paracetamol	75
Acetylsalicylic acid	69

ATC: anatomic therapeutic chemical

Table 3. Frequency of pDDIs among patients

Number of pDDI per Patient	**n (%)**
Patients with 1 pDDI	105 (25.6)
Patients with 2 pDDIs	38 (9.3)
Patients with 3 pDDIs	23 (5.6)
Patients with 4 pDDIs	4 (1.0)
Patients with 5 or more pDDIs	11 (2.7)
Total Patients with pDDIs	181 (44.1)

pDDI: potential drug-drug interaction

Table 4. Comparison of the presence of interaction* according to the demographic and clinical characteristics

	Gender		**p**
	Female (n=227)	**Male (n=183)**	
Interaction, n (%)	98 (43.2%)	83 (45.4%)	0.658
	Age Group		**p**
	≥65 (n=195)	**<65 (215)**	
Interaction, n (%)	96 (49.2%)	85 (39.5%)	**0.048**
	Polypharmacy		**p**
	+ (n=53)	**- (n=357)**	
Interaction, n (%)	45 (84.9%)	136 (38.1%)	**<0.001**
	Respiratory Disturbances		**p**
	+ (n=117)	**- (n=293)**	
Interaction, n (%)	54 (46.2%)	127 (43.3%)	0.605
	Cardiovascular Disorders		**p**
	+ (n=63)	**- (n=347)**	
Interaction, n (%)	34 (54.0%)	147 (42.4%)	0.088
	Chest Pain		**p**
	+ (n=54)	**- (n=356)**	
Interaction, n (%)	23 (42.6%)	158 (44.4%)	0.805
	Pain		**p**
	+ (n=23)	**- (n=387)**	
Interaction, n (%)	7 (30.4%)	174 (45.0%)	0.173
	Trauma		**p**
	+ (n=23)	**- (n=387)**	
Interaction, n (%)	3 (13.0%)	178 (46.0%)	**0.002**
	Gastrointestinal Problems		**p**
	+ (n=22)	**- (n=388)**	
Interaction, n (%)	11 (50.0%)	170 (43.8%)	0.570
	Cerebrovascular Diseases		**p**
	+ (n=12)	**- (n=398)**	
Interaction, n (%)	9 (75.0%)	172 (43.2%)	**0.038**
	Epilepsy - Neurological		**p**
	+ (n=12)	**- (n=398)**	
Interaction, n (%)	7 (58.3%)	174 (43.7%)	0.382
	Fever		**p**
	+ (n=7)	**- (n=403)**	
Interaction, n (%)	1 (14.3%)	180 (44.7%)	0.140

*: number of patients with at least one interaction

Table 5. Correlation between the risk categories and number of drugs used and age of the patients

		Risk Category		
		C	**D**	**X**
Age	r_s	0.163	-0.027	0.003
	p	**0.001**	0.581	0.949
Number of Drugs	r_s	0.450	0.180	0.080
	p	**<0.001**	**<0.001**	0.106

LexiInteract categorization further classified the severity of interactions as 16 (3.9%) were major, 72 (17.6%) were classified as minor, and 242 (59.0%) were classified as moderate. Seventy-nine distinct interaction pairs were detected among patients admitted to the ED. The most frequently interacting drug pairs in category C were Furosemide-Salbutamol with 54 interactions, Glyceryl trinitrate-Furosemide with 27 interactions, and Furosemide-Methylprednisolone with 21 interactions. In category D, while Enoxaparin-Acetylsalicylic acid interaction was more common with 9 interactions, Phenytoin-Dexamethasone with 4 interactions, and Enoxaparin-Clopidogrel with 2 interactions were observed. For category X, the interactions included Dexketoprofen-Acetylsalicylic acid with 7 interactions, Atropine-Ipratropium and Salbutamol with one, and Pheniramine-Ipratropium and Salbutamol with one interaction (Table 6). Additionally, Furosemide, Diltiazem, and Metoprolol were each involved in multiple interaction pairs, forming 16, 11, and 7 distinct interactions, respectively (Table 7).

Overall, nine ATC drug groups were involved in the pDDIs, with the most common categories being drugs related to digestion and metabolism (21.6%, 266/1230), followed by the cardiovascular system (21.5%, 265/1230) and the respiratory system (20.5%, 252/1230).

DISCUSSION

The use of multiple medications poses a substantial risk of DDIs, with potential adverse outcomes ranging from toxicity to treatment failure and even death (11,12). Most studies on this topic have focused on patients presenting to EDs with high medication counts and/or older adults. However, the severity of cases in the ED varies, and more medications may be required as patient acuity increases.

Our study findings revealed that nearly half (44.1%) of critically ill patients in the ED were at risk of a DDI. Of the 181 pDDIs identified among the 410 patients, 72.9% were moderate interactions, while 2.2% were classified as contraindicated. Another study on neuroleptic malignant syndrome (NMS) in ED patients highlighted that DDIs could significantly alter plasma drug concentrations. In that study, interactions between ciprofloxacin and quetiapine were implicated in causing NMS in a patient. Therefore, it is essential for clinicians to evaluate DDIs carefully when managing patients on psychiatric medications (13).

Critically ill patients treated in the ED are at risk for DDIs, regardless of age. As the number of medications administered increases, so does the likelihood of DDIs. The present study found that both the number of DDIs and the risk of interactions increased with patient age. Similarly,

Table 6. Most frequently interacting drug pairs

Interaction Pair	Risk Category	n	Mechanisms	Severity	Reliability Rating
Furosemide - Salbutamol	C	54	Beta2-agonists may enhance the hypokalemic effect of loop diuretics.	Minor	Good
Glyceryl trinitrate - Furosemide	C	27	Blood pressure-lowering agents may enhance the hypotensive effect of hypotension-associated agents.	Moderate	Fair
Furosemide - Methylprednisolone	C	21	Corticosteroids (systemic) may enhance the hypokalemic effect of loop diuretics.	Moderate	Fair
Enoxaparin - Acetylsalicylic acid	D	9	Agents with antiplatelet properties may enhance the anticoagulant effect of enoxaparin.	Moderate	Fair: Reported in the prescribing information
Phenytoin - Dexamethasone	D	4	Phenytoin may decrease the serum concentration of dexamethasone (systemic). Dexamethasone (systemic) may decrease/increase the serum concentration of phenytoin.	Major	Fair
Dexamethasone - Rocuronium	D	2	Neuromuscular-blocking agents (nondepolarizing) may enhance the adverse neuromuscular effect of corticosteroids (systemic). Increased muscle weakness, possibly progressing to polyneuropathies and myopathies, may occur.	Major	Excellent
Enoxaparin - Clopidogrel	D	2	Agents with antiplatelet properties may enhance the anticoagulant effect of enoxaparin.	Moderate	Fair: Reported in the prescribing information
Tramadol - Iohexol	D	2	Agents with seizure threshold-lowering potential may enhance the adverse/toxic effect of iohexol. Specifically, the risk for seizures may be increased.	Major	Fair
Dexketoprofen - Acetylsalicylic acid	X	7	Salicylates may enhance the adverse/toxic effect of dexketoprofen. Dexketoprofen may diminish the therapeutic effect of salicylates. Salicylates may decrease the serum concentration of dexketoprofen.	Major	Fair
Atropine - Ipratropium and Salbutamol	X	1	Ipratropium (oral inhalation) may enhance the anticholinergic effect of anticholinergic agents.	Moderate	Fair
Pheniramine - Ipratropium and Salbutamol	X	1	Ipratropium (oral inhalation) may enhance the anticholinergic effect of anticholinergic agents.	Moderate	Fair

Table 7. Top 10 drugs with the most different drug interaction pairs

Drugs	Number of Interaction Pairs	The ATC Group That Forms the Most Interaction Pairs
Furosemide	16	C - Cardiovascular System
Diltiazem	11	C - Cardiovascular System
Metoprolol	7	C - Cardiovascular System
Metoclopramide	7	A - Digestive System and Metabolism
Acetylsalicylic acid	7	N - Nervous System
Glyceryl trinitrate	7	C - Cardiovascular System
Captopril	6	C - Cardiovascular System
Salbutamol	5	R - Respiratory System
Hyoscine-N-butylbromide	5	A - Digestive System and Metabolism
Dexketoprofen	5	M - Musculoskeletal System
Methylprednisolone	5	H - Endocrine System (Except Gender Hormones and Insulin)

ATC: anatomic therapeutic chemical

Hovstadius et al. (14) reported a significant positive correlation between polypharmacy and older age, adding to previous findings that highlighted a high prevalence of polypharmacy among older ED patients in the UK. Additionally, prior research has associated factors such as female sex, polypharmacy, and chronic conditions with advancing age, all of which contribute to an elevated DDI risk (15).

Among patients aged 65 and older, 96 (49.2%) experienced at least one DDI, while 99 (50.8%) had none. The absence of a significant difference in DDIs in this study may be attributed to the inclusion of only patients in the red zone, despite the median age being comparable to other studies. Another significant finding was the evaluation of diagnoses; aside from trauma, and cerebrovascular disease. Further research is warranted to investigate preventable DDIs in patients with these specific medical conditions.

With advancements in health information and computerized decision-support systems, various online databases —some available free of charge— now provide critical medical information on medications and assess potential DDIs. Commonly used databases include Clinical Pharmacology, Micromedex, Medscape, Lexi-Comp Online, Facts & Comparisons 4.0, Epocrates Online Premium, RxList.com, and Drugs.com. Differences in evaluation criteria and DDI results can occur across these platforms. A study evaluating the clinical decision-support capabilities of these databases found that paid databases generally answered a greater number of DDI-related queries compared to free databases (16).

Another comparison of five DDI databases identified Lexi-Interact and Micromedex as the most authoritative, comprehensive, and user-friendly options. Despite the utility of these databases, using multiple databases alongside expert intervention is recommended to enhance accuracy (17). Lexi-Interact, in particular, is widely used across various professions and conditions due to its ease of use (18-20). It offers quick, accessible information on the risk, severity, and safety of pDDIs, and this study provides additional recommendations for the prevention and management of pDDIs (21).

In this study, the Lexicomp© drug information system was used as the primary tool to identify pDDIs, with 330 identified interactions, 72.9% of which were classified under risk category C. The primary factors contributing to DDI risk include the high patient volume in the ED, the presence of multiple comorbidities requiring numerous medications, and the need to administer drugs without comprehensive knowledge of patient's medical histories to expedite emergency procedures. Given their high-pressure environments, EDs are inherently high-risk areas for DDIs. The implementation of clinical decision-support systems, managed by clinical pharmacists, could help mitigate drug-related problems in these settings.

To ensure patient safety in clinical environments, alerting systems are essential, particularly as the use of databases grows. DDI decision support is crucial for healthcare professionals, including prescribers, pharmacologists, pharmacists, and nurses. Therefore, hospital administrators and technology providers should develop electronic alert systems and preventive measures accessible to all healthcare professionals to improve prescribing practices and enhance patient safety. Additionally, longitudinal studies are needed to observe patients over extended periods, as this would provide a deeper understanding of DDIs' real-world consequences in clinical practice. The results of such research will be invaluable in shaping future DDI management strategies.

This study had several limitations. Although the hospital confirmed that the patients in the ED generally represented the population’s ethnic and demographic composition, the single-center design introduced a risk of selection bias. Another limitation was that only data from patients in the ED's red zone were analyzed, excluding those receiving care in other sections. Consequently, the findings may not reflect the entire ED population, as data from all emergency areas would likely result in lower DDI rates. Additionally, we examined only DDIs occurring within the first 24 hours of admission, without accounting for the timing of drug administration, which may have affected DDI identification. Lastly, critical patient care is a dynamic process, with the potential for DDIs to arise from medications administered to address complications or new conditions during patient follow-up.

CONCLUSION

This study highlights the prevalence of pDDIs in the red zone of the ED. These findings may help raise clinician awareness of the interactions associated with commonly used drug combinations in critically ill patients. Future directions include evaluating the prevalence and impact of these pDDIs across multiple centers and investigating their relevance in outpatient settings.

Ethics Committee Approval: The study was approved by the clinical research ethics committee of Mersin University (06.04.2022, 232).

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REFERENCES

1. Aronson JK. Medication errors: definitions and classification. Br J Clin Pharmacol. 2009;67(6):599-604.
2. Moura CS, Acurcio FA, Belo NO. Drug-drug interactions associated with length of stay and cost of hospitalization. J Pharm Pharm Sci. 2009;12(3):266-72.
3. Gentile S, Vignally P, Durand AC, Gainotti S, Sambuc R, Gerbeaux P. Nonurgent patients in the emergency department? A French formula to prevent misuse. BMC Health Serv Res. 2010;10:66.
4. Okuno MFP, Cintra RS, Vancini-Campanharo CR, Batista REA. Drug interaction in the emergency service. Einstein (São Paulo). 2013;11(4):462-6.
5. Zhao M, Liu CF, Feng YF, Chen H. Potential drug-drug interactions in drug therapy for older adults with chronic coronary syndrome at hospital discharge: A real-world study. Front Pharmacol. 2022;13:946415.
6. Becker ML, Kallewaard M, Caspers PW, Visser LE, Leufkens HG, Stricker BH Hospitalisations and emergency department visits due to drug-drug interactions: a literature review. Pharmacoepidemiol Drug Saf. 2007;16(6):641-51.
7. Limandri BJ. Adverse events, drug interactions, and treatment adherence. J Psychosoc Nurs Ment Health Serv. 2020;58(2):9-13.
8. Thomsen LA, Winterstein AG, Søndergaard B, Haugbølle LS, Melander A. Systematic review of the incidence and characteristics of preventable adverse drug events in ambulatory care. Ann Pharmacother. 2007;41(9):1411-26.
9. Gülçebi İdriz Oğlu M, Küçükibrahimoğlu E, Karaalp A, Sarikaya Ö, Demirkapu M, Onat F, et al. Potential drug-drug interactions in a medical intensive care unit of a university hospital. Turk J Med Sci. 2016;46(3):812-9.
10. Cakmak F, Ikizceli I, Ozturk D, Altinbilek E, Karakus Yilmaz B, Biberoglu S, et al. Emergency department length of stay for critically ill patients followed up in red zone. Signa Vitae. 2021;17(1):63-8.
11. Malki MA, Pearson ER. Drug-drug-gene interactions and adverse drug reactions. Pharmacogenomics J. 2020;20(3):355-66.
12. Davies EA, O'Mahony MS. Adverse drug reactions in special populations - the elderly. Br J Clin Pharmacol. 2015;80(4):796-807.
13. Rohail MU, Khan A, Pflaum RM, Patel M, Moody MA. An atypical case of neuroleptic malignant syndrome associated with ciprofloxacin and quetiapine. Cureus. 2023;15(3):e36178.
14. Hovstadius B, Petersson G. Factors leading to excessive polypharmacy. Clin Geriatr Med. 2012;28(2):159-72.
15. Dookeeram D, Bidaisee S, Paul JF, Nunes P, Robertson P, Maharaj VR, et al. Polypharmacy and potential drug-drug interactions in emergency department patients in the Caribbean. Int J Clin Pharm. 2017;39(5):1119-27.
16. Clauson KA, Marsh WA, Polen HH, Seamon MJ, Ortiz BI. Clinical decision support tools: analysis of online drug information databases. BMC Med Inform Decis Mak. 2007;7:7.
17. Kheshti R, Aalipour M, Namazi S. A comparison of five common drug-drug interaction software programs regarding accuracy and comprehensiveness. J Res Pharm Pract. 2016;5(4):257-63.
18. Babushkina EA, Belokopytova LV, Grachev AM, Meko DM, Vaganov EA. Variation of the hydrological regime of Bele-Shira closed basin in Southern Siberia and its reflection in the radial growth of Larix sibirica. Reg Environ Change. 2017;17(6):1725-37.
19. Dagdelen MS, Gulen D, Ceylan I, Girgin NK. Evaluation of potential drug-drug interactions in intensive care unit. Eur Rev Med Pharmacol Sci. 2021;25(18):5801-6.
20. Ramsdale E, Mohamed M, Yu V, Otto E, Juba K, Awad H, et al. Polypharmacy, potentially inappropriate medications, and drug-drug interactions in vulnerable older adults with advanced cancer initiating cancer treatment. Oncologist. 2022;27(7):e580-8.
21. Zhao M, Liu CF, Feng YF, Chen H. Potential drug-drug interactions in drug therapy for older adults with chronic coronary syndrome at hospital discharge: A real-world study. Front Pharmacol. 2022;13:946415.

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Evaluation of the Reliability and Quality of YouTube Videos about Insulin Injection in Children

Çocuklarda İnsülin Enjeksiyonu ile İlgili YouTube Videolarının Güvenilirliği ve Kalitesinin Değerlendirilmesi

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ABSTRACT

Aim: Social media platforms, especially YouTube, have become indispensable channels where patients apply for information on medical issues. This study aimed to evaluate the reliability and quality of videos on YouTube about insulin injection in children.
Material and Methods: The first 100 videos with the search "insulin injection in children" were analyzed. The distribution of video types, viewing rates, like ratios, number of comments, and video power indexes (VPI) were recorded. The content qualities were assessed by the global quality scale (GQS), modified DISCERN score, the Journal of the American Medical Association (JAMA) benchmark score, and the patient education materials assessment tool for audio/visual (PEMAT-A/V) materials.
Results: Forty-seven (47%) videos were analyzed finally. The median score of the GQS was 4, modified DISCERN and JAMA was 3. The median PEMAT A/V understandability and actionability scores were 84 and 75. Videos by professionals were found to have better results than the non-professional ($p<0.001$). Correlation analyses revealed positive correlations between video analytics, such as number of views, likes and comments, view rate, VPI, and content quality scales, including GQS, modified DISCERN, JAMA, and PEMAT-A/V scores ($p<0.001$).
Conclusion: Although many of the videos examined were of professional origin and their quality scores were higher, the rate of videos from non-professional origin was close to half. The quality scores of these non-professional videos were quite low in all used scales. It is important that patients and their relatives prefer videos uploaded by professional sources to access information from platforms such as YouTube.
Keywords: Diabetes mellitus; insulin injection; child; YouTube.

ÖZ

Amaç: Başta YouTube olmak üzere sosyal medya platformları, hastaların tıbbi konularda bilgi almak için başvurdukları vazgeçilmez mecralar haline gelmiştir. Bu çalışma, çocuklarda insülin enjeksiyonu ile ilgili YouTube videolarının güvenilirliğini ve kalitesini değerlendirmeyi amaçlamaktadır.
Gereç ve Yöntemler: "Çocuklarda insülin enjeksiyonu" aramasını içeren ilk 100 video analiz edilmiştir. Video türlerinin dağılımı, izlenme oranları, beğenilme oranları, yorum sayıları ve video güç endeksleri (video power index, VPI) kaydedilmiştir. İçerik kaliteleri küresel kalite ölçeği (global quality scale, GQS), modifiye DISCERN skoru, Journal of the American Medical Association (JAMA) kıyaslama skoru ve görsel/işitsel materyaller için hasta eğitim materyalleri değerlendirme aracı (patient education materials assessment tool for audio/visual, PEMAT-A/V) ile değerlendirilmiştir.
Bulgular: Çalışmada 47 (%47) video analiz edilmiştir. GQS puanı ortancası 4, modifiye edilmiş DISCERN ve JAMA 3 idi. Ortanca PEMAT A/V anlaşılabilirlik ve eyleme geçirilebilirlik puanları ise 84 ve 75 idi. Profesyoneller tarafından yüklenen videoların profesyonel olmayanlara göre daha iyi sonuçlara sahip olduğu görülmüştür ($p<0,001$). Korelasyon analizi, görüntülenme, beğeni ve yorum sayısı, görüntüleme oranı, VPI gibi video analitikleri ile GQS, modifiye DISCERN, JAMA ve PEMAT-A/V puanlarını içeren içerik kalitesi arasında pozitif korelasyonlar olduğu göstermiştir ($p<0,001$).
Sonuç: İncelenen videoların birçoğu profesyonel kaynaklı olmasına ve kalite puanları daha yüksek olmasına rağmen, profesyonel olmayan kaynaklı videoların oranının yarıya yakın olduğu gözlenmiştir. Profesyonel olmayan bu videoların kalite puanlarının da kullanılan tüm ölçeklerde oldukça düşük olduğu tespit edilmiştir. Hasta ve hasta yakınlarının YouTube gibi platformlardan bilgiye ulaşmak için profesyonel kaynaklar tarafından yüklenen videoları tercih etmeleri önemlidir.
Anahtar kelimeler: Diabetes mellitus; insülin enjeksiyonu; çocuk; YouTube.

INTRODUCTION

Globally, there are 422 million diabetes mellitus (DM) patients according to the 2017 report of the World Health Organization (1). The International Diabetes Federation also reported that there are nearly 1,11 million children and adolescents having type 1 DM with 132,600 new cases diagnosed yearly in this age group (1). The most common metabolic disease in childhood is still type 1 DM (2). Therefore, the management of type 1 DM among children and adolescents is crucial.

The gold standard way of treatment for children with type 1 DM is insulin therapy. Insulin therapy should be started immediately after the diagnosis because the child's metabolism can deteriorate rapidly (3). To prevent micro and macrovascular complications in patients, intensive insulin therapy is recommended (4). But the problem with this intensive therapy is hypoglycemia which can be a mortal complication. So, delivery of the insulin mimicking the endogenous insulin secretion by the pancreas is very important (5). Because the insulin is a peptide hormone, the gastric acid destroys it if taken orally. Also, the intradermal, intramuscular, and intravenous daily self-administrations are not suitable to control blood glucose levels. The most preferred method of insulin administration is the subcutaneous route (5). There are some limitations of this route like pain and lipodystrophy at the injection site; the compliance of the patients, especially the lower aged children, to this method can be difficult (6). Although there are some new insulin administration methods like continuous subcutaneous insulin injection with pump, nasal, or inhaled insulin therapy, the most used method of insulin delivery remains the use of insulin pens (6). Insulin pens are more effective and accurate when compared to traditional vial and syringe methods (7). While insulin pens have numerous advantages, the usage technique and adjustment of the dosage need some expertise, and children and/or their caregivers should learn these well (8). Hospitals give the necessary education about these methods with their professional teams (nurses, etc.) to the children and their families but the patients need sometimes educational videos.

Access to the internet is now easy nearly all over the world. The social media platforms are the popular sources of information about health issues. Especially the video sharing channels are preferred by most users to gain information (9). YouTube is the leading video platform on the internet according to 2023 data (10) and it is one of the most preferred sources of health information (11). While it has a lot of valuable information about health problems, there are some important concerns regarding the negative impacts. The most important one is false information and its uncontrolled spread. The misinformation about DM care given by uncontrolled data can cause serious health outcomes such as incorrect insulin injections, difficulties in DM control, and even irreversible morbidities. Therefore, the verification of the reliability and quality of the information given in these videos is essential. There are plenty of studies in the literature developing some scales and methods to evaluate the quality of medical content online (12). There is limited data in the literature about YouTube videos concerning insulin and DM (13,14). However, there is no research evaluating the videos about "insulin injection in children" on the YouTube platform. The purpose of this study was to evaluate the videos about insulin injection in children on YouTube in terms of reliability, quality, understandability, applicability, and accuracy.

MATERIAL AND METHODS

Data Collection

The video search was performed on https://youtube.com/ on August 10, 2024, using the keyword "insulin injection in children". The browser's search history and all cookies were cleared, and personal Google or YouTube accounts had not been logged in to prevent personalized results.

The default selection was set to relevance-based ranking to mimic an ordinary user. Literature has shown that; most of the users click on results within the first pages of internet search (15). Currently, results from the YouTube search engine are displayed in the form of an infinite scrolling list, not as pages. For this reason, the first 100 videos for the keyword were analyzed to have a reliable result.

Videos that were related to the subject, in English and between 1-10 minutes were included. Videos under 10 minutes and more than 1 minute have been shown to be more effective in giving information to the user (16). Videos that are shorter than 1 minute and longer than 10 minutes, not related to the subject, not in English, and duplicated and advertisement videos were excluded. The uniform resource locators (URLs) of the videos meeting the inclusion criteria were saved for further analysis.

Analysis of Videos

Information about the videos such as country of origin, source, image type (animation/real), number of views, days since publication, quality of image, number of likes and dislikes, number of comments, and video duration (in seconds) were recorded. The view rate (number of views/days since publication) and like rate (number of likes x 100 / number of likes + dislikes) were calculated and recorded. The video power index (VPI) was computed using the formula: like rate x view rate / 100.

The purposes of the videos were grouped regarding the content as, technical information about insulin injection in children, general information about DM in children, social life of the diabetic children, and dosing and calculations of insulin usage in children with DM. The uploaders of the videos were categorized into five groups: 1) healthcare professionals (HP), 2) academic health organizations (AH), 3) patient and/or their caregivers (PT), 4) TV/educational websites (TV), and 5) non-academic healthcare systems (non-AH). Videos from groups 1 and 2 were regarded as professional and the other sources as non-professional sources.

The videos were evaluated for quality, accuracy, reliability, understandability, and actionability by utilizing the modified DISCERN score, the Journal of the American Medical Association (JAMA) benchmark score, the global quality scale (GQS), and the patient education materials evaluation tool audio/visual (PEMAT-A/V).

The modified DISCERN scale has five questions for the assessment of the reliability of the information given in the video. Every question can take either 1 or 0 points; higher scores indicate increased reliability and less bias in the presented content. The scale is a reliable and valid

instrument for assessing the quality of written health information. This modified tool has been used in many studies especially to evaluate the reliability of YouTube content (17).

JAMA benchmark score is used to evaluate the quality of the given information according to authorship, citation of sources, currency, and conflict of interest. All the criteria used in this score can take 1 point; a maximum score of 4 points shows the highest level of accuracy and reliability. To ensure the veracity and integrity of online content, it is essential that website authorship is formally defined to include authors, contributors, links, and credentials. Furthermore, citations must include references and sources used for content and copyright information. In addition, disclosures should include details about sponsorship, advertising, commercial funding, and potential conflicts of interest. Finally, currency should include the date and timeliness of the information published (18).

GQS is widely used and measures the quality of the video based on usefulness. The scale enables investigators to assess the flow, ease of use, and quality of videos. A score of 4 or 5 indicates high quality, 3 indicates intermediate quality, and 1 or 2 points indicate low quality (19,20).

PEMAT-A/V was used to assess the clarity and quality of the patient education materials. The video and/or audio material was evaluated to ensure that is the information given effectively communicated to the patients in a clear, understandable, and actionable manner. The tool has 13 parts for the understandability domain (PEMAT-A/V U) and 5 parts for the actionability domain (PEMAT-A/V A). Percentages of each domain were reported in this validated instrument (21).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics v.22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The normality assumption for continuous variables was investigated by the Shapiro-Wilk test. Descriptive statistics were presented as median, interquartile range, minimum, and maximum for continuous data, and numbers and percentages were reported for categorical data. The Mann-Whitney U test for two groups and the Kruskal-Wallis test followed by the Bonferroni corrected post hoc test for three or more groups were used to compare groups. Spearman's rho was calculated for analyzing correlations between video characteristics and quality scores. A p-value of <0.05 was considered statistically significant.

RESULTS

The first 100 videos were watched. 12 of the videos were less than 60 seconds and 6 were more than 600 seconds; 11 videos were not in English, 8 videos were the same, 10 videos were advertising, and 6 videos were not related to the subject. Finally, 53 (53%) of the videos were excluded and the remaining 47 (47%) videos were analyzed.

Thirty-eight (80.9%) of the videos were real and the remaining 9 (19.1%) were animation. 29 (61.7%) of the videos were professional. Most of the videos were about the technical information. The general characteristics of the videos was summarized in Table 1.

The median duration of the 47 videos was 315 seconds. The median time since upload was 1765 days, number of views was 44,196, the number of likes was 418, the number of dislikes was 13, the number of comments was 22, the like rate was 95, the view rate was 21.8 and VPI was 20.6. The median score of the GQS was 4, modified DISCERN was 31 and JAMA was 3. The median scores of PEMAT-A/V understandability and actionability were 84 and 75, respectively (Table 2).

The content quality of the videos was analyzed according to the source, and videos uploaded by professional (HP and AH groups) sources were found to have better content quality results than non-professional (PT, TV, and non-AH groups) ones (Tables 3 and 4). When the type of the videos was grouped as real or animation, there wasn't any statistically significant difference between them according to the content quality assessments (Table 5).

The content of the videos was grouped as technical information (T), general information (G), the social life of the child (S), and dosing calculation (D). There wasn't any statistically significant difference between them in VPI, GQS, modified DISCERN, and JAMA. The PEMAT A/V understandability scores of the T videos (p=0.005) and D videos (p=0.009) were significantly higher than the S videos. Also, the PEMAT A/V actionability scores of the T videos (p=0.007) and D videos (p=0.005) were significantly higher than the S videos (Table 6).

The image qualities of the videos were also assessed, and the videos were grouped as having standard or high definition. The content qualities of the videos according to VPI, GQS, modified DISCERN, JAMA, PEMAT-A/V U, and PEMAT-A/V A having high-definition image quality were better than the standard definition videos (p<0.001, p=0.002, p<0.001, p<0.001, p<0.001, and p<0.001, respectively, Table 7).

Table 1. Distribution of video types

	n (%)
Country	
USA	27 (57.4)
UK	9 (19.1)
Canada	4 (8.5)
Australia	2 (4.3)
India	3 (6.4)
Singapore	2 (4.3)
Source	
Healthcare professionals	17 (36.2)
Academic health organizations	12 (25.5)
Patient	8 (17.0)
TV/educational website	5 (10.6)
Non-academic healthcare systems	5 (10.6)
Professionality	
Professional	29 (61.7)
Non-professional	18 (38.3)
Type	
Real	38 (80.9)
Animation	9 (19.1)
Video Content	
Technical info	28 (59.6)
General info	9 (19.1)
Social life of a child	6 (12.8)
Dosing calculation	4 (8.5)
Image Quality	
Standard (480p)	14 (29.8)
High (≥720p)	33 (70.2)

USA: United States of America, UK: United Kingdom

Table 2. Descriptive statistics of video characteristics

	Mean±SD	Median	IQR	Min-Max
Time since upload (days)	2062.74±1294.65	1765	1150-2920	280-5020
Duration (seconds)	303.38±139.26	315	203-388	80-592
Number of views	313306.32±598189.05	44196	13191-193187	467-2474391
Number of likes	2180.68±5426.28	418	101-1018	2-33218
Number of dislikes	86.43±220.55	13	3-38	0-1255
Number of comments	254.12±763.49	22	6-161	0-4300
Like rate	92.79±9.08	95	93-98	61-100
View rate	168.77±366.18	21.8	8.1-128.2	0.5-1682.6
VPI	162.09±354.61	20.6	7.6-126.9	0.3-1665.8
GQS	3.77±1.13	4	3-5	2-5
DISCERN	3.11±1.17	3	2-4	1-5
JAMA	2.57±1.25	3	1-4	0-4
PEMAT A/V U	69.85±26.22	84	44-92	20-100
PEMAT A/V A	58.62±36.40	75	33-100	0-100

VPI: video power index, GQS: global quality scale, JAMA: Journal of the American Medical Association, PEMAT-A/V: patient education materials assessment tool audio/visual, U: understandability, A: actionability, SD: standard deviation, IQR: interquartile range (25^{th}-75^{th} percentile)

Table 3. Analyses of content quality of videos by source

	Health Professionals (HP)	Academic Health Organizations (AH)	Patient and/or their caregivers (PT)	TV/Educational Website (TV)	Non-Academic Healthcare Systems (non-AH)	p
	Median (IQR) [min-max]	Median (IQR) [min-max]	Median (IQR) [min-max]	Median (IQR) [min-max]	Median (IQR) [min-max]	
VPI	110.9 (20.9-354.8) [0.4-1665.8]	19.4 (10.6-122.4) [1.2-1433.3]	6.9 (1.4-20.2) [0.3-52.8]	1.4 (0.9-43.5) [0.7-75.1]	36.3 (19.4-93.0) [19.2-126.9]	**0.011**
GQS	5 (4-5) [2-5]	4 (4-5) [3-5]	2.5 (2-3.75) [2-4]	3 (2-3.5) [2-4]	4 (2.5-4.5) [2-5]	**0.003**
DISCERN	4 (3.5-4) [1-5]	4 (3-4.75) [2-5]	2 (1.25-2.75) [1-3]	2 (1.5-2.5) [1-3]	3 (2-3) [2-3]	**<0.001**
JAMA	4 (2.5-4) [0-4]	3 (3-4) [1-4]	1 (1-1.75) [1-3]	2 (1-2) [1-2]	2 (1.5-3) [1-3]	**0.002**
PEMAT A/V U	92 (83.5-96) [27-100]	89.5 (84-91.75 [72-100]	38 (23.25-52.25) [22-55]	40 (26.5-53.5) [20-63]	63 (49.5-79.5) [45-90]	**<0.001**
PEMAT A/V A	100 (75-100) [0-100]	75 (75-100) [50-100]	25 (6.25-33) [0-33]	0 (0-29) [0-33]	33 (33-75) [33-100]	**<0.001**

VPI: video power index, GQS: global quality scale, JAMA: Journal of the American Medical Association, PEMAT-A/V: patient education materials assessment tool audio/visual, U: understandability, A: actionability, IQR: interquartile range (25^{th}-75^{th} percentile), post hoc test results of groups; VPI: HP vs PT: p=0.002, HP vs TV: p=0.009; GQS: HP vs PT: p=0.002, HP vs TV: p=0.012, AH vs PT: p=0.003, AH vs TV: p=0.012; DISCERN: HP vs PT: p=0.001, HP vs TV: p=0.004, HP vs Non-AH: p=0.050, AH vs PT: p=0.001, AH vs TV: p=0.005; JAMA: HP vs PT: p=0.001, HP vs TV: p=0.013, AH vs PT: p=0.002, AH vs TV: p=0.019; PEMAT A/V U: HP vs PT: p<0.001, HP vs TV: p=0.001, AH vs PT: p<0.001, AH vs TV: p=0.003; PEMAT A/V A: HP vs PT: p<0.001, HP vs TV: p=0.001, AH vs PT: p=0.001, AH vs TV: p=0.001

Table 4. Content quality of videos by professionality

	Professionals	Non-professionals	p
	Median (IQR) [min-max]	Median (IQR) [min-max]	
VPI	57.2 (14.2-322.6) [0.4-1665.8]	15.5 (1.3-40.4) [0.3-126.9]	**0.008**
GQS	4 (4-5) [2-5]	3 (2-4) [2-5]	**<0.001**
DISCERN	4 (3-4) [1-5]	2 (2-3) [1-3]	**<0.001**
JAMA	4 (3-4) [0-4]	1.5 (1-2) [1-3]	**<0.001**
PEMAT A/V U	91 (84-92) [27-100]	44 (31.5-57) [20-90]	**<0.001**
PEMAT A/V A	75 (75-100) [0-100]	29 (0-33) [0-100]	**<0.001**

VPI: video power index, GQS: global quality scale, JAMA: Journal of the American Medical Association, PEMAT-A/V: patient education materials assessment tool audio/visual, U: understandability, A: actionability, IQR: interquartile range (25^{th}-75^{th} percentile)

Table 5. Content quality of videos by type

	Real	Animation	p
	Median (IQR) [min-max]	Median (IQR) [min-max]	
VPI	23.6 (9.8-154.6) [0.4-1433.3]	19.2 (2.5-42.9) [0.3-1665.8]	0.262
GQS	4 (3-5) [2-5]	5 (2.5-5) [2-5]	0.351
DISCERN	3 (2-4) [1-5]	3 (2-5) [1-5]	0.548
JAMA	3 (1-4) [0-4]	3 (1.5-4) [1-4]	0.393
PEMAT A/V U	84 (44-92) [20-100]	83 (47-90) [27-100]	0.871
PEMAT A/V A	75 (31-100) [0-100]	75 (16.5-100) [0-100]	0.879

VPI: video power index, GQS: global quality scale, JAMA: Journal of the American Medical Association, PEMAT-A/V: patient education materials assessment tool audio/visual, U: understandability, A: actionability, IQR: interquartile range (25^{th}-75^{th} percentile)

Table 6. Analyses of content quality of videos by content

	Technical Info (T)	General Info (G)	Social Life of a Child (S)	Dosing Calculation (D)	p
	Median (IQR) [min-max]	Median (IQR) [min-max]	Median (IQR) [min-max]	Median (IQR) [min-max]	
VPI	58.2 (6.2-317.9) [0.5-1665.8]	19.1 (11.7-50.4) [0.4-1163.4]	10.4 (0.6-24.5) [0.3-36.3]	22.5 (19.6-114.4) [19.5-144.1]	0.197
GQS	4 (3-5) [2-5]	4 (2.5-4.5) [2-5]	2 (2-3.5) [2-5]	4 (4-4.75) [4-5]	0.114
DISCERN	3 (2-4) [1-5]	3 (2-4) [2-4]	2 (1-3) [1-3]	4 (3.25-4.75) [3-5]	0.050
JAMA	3 (1.25-4) [1-4]	3 (1.5-3.5) [0-4]	1 (1-2.25) [1-3]	3.5 (3-4) [3-4]	0.076
PEMAT A/V U	87 (54-92) [22-100]	83 (49.5-90) [36-92]	38.5 (25.25-49.5) [20-63]	90.5 (88.5-91.75) [88-92]	**0.024**
PEMAT A/V A	75 (33-100) [0-100]	75 (29-75) [0-100]	16.5 (0-33) [0-33]	87.5 (75-100) [75-100]	**0.019**

GQS: global quality scale, JAMA: Journal of the American Medical Association, PEMAT-A/V: patient education materials assessment tool audio/visual, U: understandability, A: actionability, VPI: video power index, IQR: interquartile range (25^{th}-75^{th} percentile), post hoc test results of groups; PEMAT A/V U: T vs S: p=0.005, S vs D: p=0.009; PEMAT A/V A: T vs S: p=0.007, S vs D: p=0.005

Correlation analyses revealed positive correlations between video analytics, such as number of views, likes, dislikes, comments, and view rate, VPI, and content quality scales, including GQS, modified DISCERN, JAMA, and PEMAT-A/V U and A scores (Table 8).

Table 7. Content quality of videos by image quality

	Standard (480p)	High (≥720p)	p
	Median (IQR) [min-max]	Median (IQR) [min-max]	
VPI	6.9 (0.9-19.1) [0.3-59.1]	57.2 (19.4-316.7) [0.7-1665.8]	**<0.001**
GQS	2.5 (2-4) [2-5]	4 (4-5) [2-5]	**0.002**
DISCERN	2 (1.75-2.25) [1-4]	4 (3-4) [1-5]	**<0.001**
JAMA	1 (1-2) [0-4]	3 (2-4) [1-4]	**<0.001**
PEMAT A/V U	38 (27-64.5) [22-91]	90 (63.5-92) [20-100]	**<0.001**
PEMAT A/V A	25 (0-37.25) [0-75]	75 (49.5-100) [0-100]	**<0.001**

VPI: video power index, GQS: global quality scale, JAMA: Journal of the American Medical Association, PEMAT-A/V: patient education materials assessment tool audio/visual, U: understandability, A: actionability, IQR: interquartile range (25^{th}-75^{th} percentile)

DISCUSSION

This study evaluated the quality and usefulness of the YouTube videos about "insulin injection in children" focusing on the first 100 videos related to the subject. In general, the evaluated 47 videos exhibit medium quality using the GQS, modified DISCERN, JAMA, and PEMAT A/V understandability and actionability scales. The quality of the content of the videos uploaded by the professional sources was better than the non-professional ones according to all used scales. While there are some studies in the literature evaluating the online contents related to DM, insulin injection, and resistance (13,14,22-24), this study is the first to specifically analyze the utility of YouTube videos on the subject of insulin injection in children.

Diabetes mellitus is a complex problem and the patient, whether a child or adolescent, should be informed about the course and treatment of the disease. The most common metabolic disorder in all over the world is still type 1 DM and insulin therapy is the mainstay of the management of this condition (25). Education of the children with type 1 DM is very important in the management. One of the musts that a child with type 1 DM should learn is the insulin injection technique (26). The technique should be explained well to the child and his/her family, and the initial applications should be carried out under the supervision of a professional team. However, it is also obvious that information materials will be needed so that the child and his/her family will not have difficulties when applying this treatment in their own lives. YouTube

Table 8. Correlation coefficients between content quality scores and video analytics

	GQS		DISCERN		JAMA		PEMAT-A/V U		PEMAT-A/V A	
	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p
Time since upload	-0.008	0.960	-0.143	0.339	-0.001	0.996	-0.053	0.722	0.037	0.804
Video duration	-0.065	0.663	0.054	0.720	-0.014	0.927	-0.008	0.957	-0.008	0.957
Number of views	0.616	**<0.001**	0.635	**<0.001**	0.637	**<0.001**	0.727	**<0.001**	0.695	**<0.001**
Number of likes	0.562	**<0.001**	0.648	**<0.001**	0.643	**<0.001**	0.727	**<0.001**	0.720	**<0.001**
Number of dislikes	0.320	**0.028**	0.415	**0.004**	0.427	**0.003**	0.455	**0.001**	0.432	**0.002**
Number of comments	0.458	**0.003**	0.585	**<0.001**	0.542	**<0.001**	0.689	**<0.001**	0.641	**<0.001**
Like rate	0.275	0.062	0.270	0.066	0.244	0.099	0.324	**0.026**	0.353	**0.015**
View rate	0.643	**<0.001**	0.716	**<0.001**	0.675	**<0.001**	0.761	**<0.001**	0.730	**<0.001**
VPI	0.653	**<0.001**	0.727	**<0.001**	0.689	**<0.001**	0.771	**<0.001**	0.744	**<0.001**

GQS: global quality scale, JAMA: Journal of the American Medical Association, PEMAT-A/V: patient education materials assessment tool audio/visual, U: understandability, A: actionability, VPI: video power index, r_s: Spearman's rho

has significant potential as a health education tool, as video-assisted learning has been shown to be valuable for patients' knowledge acquisition (27). It should be kept in mind that uncontrolled and easily accessible online information may cause serious problems in the diagnosis and treatment of some diseases. At this point, it is important that the information provided on YouTube is also examined by professionals in the field and that patients are guided in line with the results obtained (9,11,20).

Evaluation and standardization of the quality and reliability of health-related content can be achieved by using appropriate quality assessment scales. The most frequently used scales for this purpose include GQS, modified DISCERN, and JAMA (9,11,20). In addition to these commonly used scales, the PEMAT A/V was used in this study. While the use of educational materials such as videos is increasing, scales specifically developed to evaluate them are limited. The PEMAT A/V has been shown to be superior to other quality assessment tools in its ability to reliably assess video or audio materials. In addition, PEMAT A/V is the first tool to measure actionability, an increasingly desirable goal of patient education materials. Although the use of PEMAT A/V in such studies complicates the study process, it increases the reliability of the results (21). It should be noted that the actionability scores of the videos analyzed in this study were lower than the understandability scores. It is possible to say that the quality of educational videos related to insulin injection in children will increase significantly with the development of video content having high actionability scores.

Videos about health information on YouTube generally show medium or low quality. In a study evaluating the reliability of YouTube videos about health-related information, the quality of the contents was found to be average-low according to modified DISCERN and GQS scales (11). In a recent study evaluating the insulin pen injection videos on YouTube as a patient education resource, they found reliable results, but nearly half of the videos were misleading (14). Barlas et al. (13) evaluated the YouTube videos about insulin resistance recently and they also found that 54% of the videos had very poor quality. Sixty-two YouTube videos about DM were evaluated by Mylavarapu et al. (24) and they found average results of GQS and reliability scores. In this study, the mean scores were found to be consistent with the literature in all the data obtained from the GQS, modified DISCERN, JAMA, and two forms of the PEMAT A/V scales.

When we look at the results of this study, one can see that videos with high educational quality have more views. However, the view rate or VPI may not always indicate that the video is quality and reliable. Scales such as the GQS, modified DISCERN, JAMA, and PEMAT A/V scores assess specific content quality elements, but the VPI indicates the perceived value of video content. Some studies on medical videos have shown that videos that are popular among viewers may lack content quality (9,28). To increase the view rate of informative content about insulin injection in children by the target audience, it is valuable to create a video format that considers the standards set by quality scales.

Educational videos uploaded by professionals had higher quality and reliability than those of non-professionals in most of the studies (29-31). However, some studies have reported contrary results (13). In this study, it was found that videos uploaded from professional sources have higher quality rates than non-professional sources according to all quality scales used.

In further evaluation, video analytics such as number of views, likes, dislikes, comments, view rate, VPI, and used content quality scales showed a positive correlation. This result is consistent with the studies in the literature (32,33). In light of these results, we can say that patients and/or their relatives can understand quality and reliable information and determine their preferences accordingly. Standardization of the videos uploaded to the YouTube platform in the field of health can be effective in improving quality. For example, the inclusion of certain criteria to improve quality, such as indicating the source of information and indicating areas of uncertainty before videos are presented to internet users, may contribute positively to health accreditation.

This study is not free from some limitations. First, only videos published in English were examined, which prevents the generalization of the study results. Secondly, although the aim was to identify the most relevant videos, being stuck with a single keyword for video selection could be seen as a limiting factor. Furthermore, focusing only on videos published on the YouTube platform and excluding content from other websites and social media platforms may not cover all the information available on insulin injection in children on online platforms.

CONCLUSION

This study is the first to analyze YouTube videos about insulin injection in children specifically. Although many of the videos examined were of professional origin and their quality scores were higher, the rate of the videos of non-professional origin was close to half. Unfortunately, the quality scores of these videos were quite low in all used scales. It is important that patients and their relatives are not prevented from accessing information from platforms such as YouTube, but rather are guided correctly by health professionals.

Ethics Committee Approval: Since our study was not an experimental study including human or animal subject, ethics committee approval was not required.

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REFERENCES

1. Zuberi Z, Sauli E, Cun L, Deng J, Li WJ, He XL, et al. Insulin-delivery methods for children and adolescents with type 1 diabetes. Ther Adv Endocrinol Metab. 2020;11:2042018820906016.
2. Neu A, Bürger-Büsing J, Danne T, Dost A, Holder M, Holl RW, et al. Diagnosis, therapy and follow-up of diabetes mellitus in children and adolescents. Exp Clin Endocrinol Diabetes. 2019;127(S 01):S39-72.
3. Bangstad HJ, Danne T, Deeb LC, Jarosz-Chobot P, Urakami T, Hanas R; International Society for Pediatric and Adolescent Diabetes. ISPAD clinical practice consensus guidelines 2006-2007. Insulin treatment. Pediatr Diabetes. 2007;8(2):88-102.
4. Diabetes Control and Complications Trial Research Group; Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329(14):977-86.
5. Shah VN, Moser EG, Blau A, Dhingra M, Garg SK. The future of basal insulin. Diabetes Technol Ther. 2013;15(9):727-32.
6. Shah RB, Patel M, Maahs DM, Shah VN. Insulin delivery methods: Past, present and future. Int J Pharm Investig. 2016;6(1):1-9.
7. Olsen BS, Lilleøre SK, Korsholm CN, Kracht T. Novopen Echo® for the delivery of insulin: a comparison of usability, functionality and preference among pediatric subjects, their parents, and health care professionals. J Diabetes Sci Technol. 2010;4(6):1468-75.
8. Hanas R, de Beaufort C, Hoey H, Anderson B. Insulin delivery by injection in children and adolescents with diabetes. Pediatr Diabetes. 2011;12(5):518-26.
9. Madathil KC, Rivera-Rodriguez AJ, Greenstein JS, Gramopadhye AK. Healthcare information on YouTube: A systematic review. Health Informatics J. 2015;21(3):173-94.
10. Statista.com [Internet]. Most popular websites worldwide as of November 2023, by unique visitors. [Cited: 2024 October 24]. Available from: https://www.statista.com/statistics/1201889/most-visited-websites-worldwide-unique-visits/.
11. Osman W, Mohamed F, Elhassan M, Shoufan A. Is YouTube a reliable source of health-related information? A systematic review. BMC Med Educ. 2022;22(1):382.
12. Sui W, Sui A, Rhodes RE. What to watch: Practical considerations and strategies for using YouTube for research. Digit Health. 2022;8:20552076221123707.
13. Barlas T, Ecem Avci D, Cinici B, Ozkilicaslan H, Muhittin Yalcin M, Eroglu Altinova A. The quality and reliability analysis of YouTube videos about insulin resistance. Int J Med Inform. 2023;170:104960.
14. Gülhan Güner S, Tezel M, Özsaban A. The content, reliability, and quality of insulin pen injection videos on YouTube as patient education resource. Comput Inform Nurs. 2024;42(11):809-16.
15. Urman A, Makhortykh M. You are how (and where) you search? Comparative analysis of web search behavior using web tracking data. J Comput Soc Sci. 2023;6(2):1-16.
16. Srinivasacharlu A. Using YouTube in colleges of education. Shanlax Int J Educ. 2020;8(2):21-4.
17. Charnock D, Shepperd S, Needham G, Gann R. DISCERN: an instrument for judging the quality of written consumer health information on treatment choices. J Epidemiol Community Health. 1999;53(2):105-11.
18. Silberg WM, Lundberg GD, Musacchio RA. Assessing, controlling, and assuring the quality of medical information on the Internet: Caveant lector et viewor--Let the reader and viewer beware. JAMA. 1997;277(15):1244-5.
19. Drozd B, Couvillon E, Suarez A. Medical YouTube videos and methods of evaluation: literature review. JMIR Med Educ. 2018;4(1):e3.
20. Gurler D, Buyukceran I. Assessment of the medical reliability of videos on social media: detailed analysis of the quality and usability of four social media platforms (Facebook, Instagram, Twitter, and YouTube). Healthcare (Basel). 2022;10(10):1836.
21. Shoemaker SJ, Wolf MS, Brach C. Development of the Patient Education Materials Assessment Tool (PEMAT): a new measure of understandability and actionability for print and audiovisual patient information. Patient Educ Couns. 2014;96(3):395-403.
22. Kong W, Song S, Zhao YC, Zhu Q, Sha L. TikTok as a health information source: assessment of the quality of information in diabetes-related videos. J Med Internet Res. 2021;23(9):e30409.
23. Gimenez-Perez G, Robert-Vila N, Tomé-Guerreiro M, Castells I, Mauricio D. Are YouTube videos useful for patient self-education in type 2 diabetes? Health Informatics J. 2020;26(1):45-55.
24. Mylavarapu M, Maheta D, Clarke S, Parmar K, Mohammed M, Vuyyuru CS. Diabetes mellitus on YouTube: A cross-sectional observational study to assess the quality and reliability of videos. Cureus. 2023;15(8):e43704.
25. Danne T, Phillip M, Buckingham BA, Jarosz-Chobot P, Saboo B, Urakami T, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Insulin treatment in children and adolescents with diabetes. Pediatr Diabetes. 2018;19(Suppl 27):115-35.
26. White NH, Sun W, Cleary PA, Danis RP, Davis MD, Hainsworth DP, et al. Prolonged effect of intensive therapy on the risk of retinopathy complications in patients with type 1 diabetes mellitus: 10 years after the Diabetes Control and Complications Trial. Arch Ophthalmol. 2008;126(12):1707-15.
27. El-Jawahri A, Paasche-Orlow MK, Matlock D, Stevenson LW, Lewis EF, Stewart G, et al. Randomized, controlled trial of an advance care planning video decision support tool for patients with advanced heart failure. Circulation. 2016;134(1):52-60.
28. Desai T, Shariff A, Dhingra V, Minhas D, Eure M, Kats M. Is content really king? An objective analysis of the public's response to medical videos on YouTube. PLoS One. 2013;8(12):e82469.
29. Şahin A, Şahin M, Türkcü FM. YouTube as a source of information in retinopathy of prematurity. Ir J Med Sci. 2019;188(2):613-7.

30. Ozsoy-Unubol T, Alanbay-Yagci E. YouTube as a source of information on fibromyalgia. Int J Rheum Dis. 2021;24(2):197-202.
31. De La Torre AB, Joe S, Lee VS. An evaluation of YouTube videos as a surgical instructional tool for endoscopic endonasal approaches in otolaryngology. Ear Nose Throat J. 2024;103(7):NP440-9.
32. Altun A, Askin A, Sengul I, Aghazada N, Aydin Y. Evaluation of YouTube videos as sources of information about complex regional pain syndrome. Korean J Pain. 2022;35(3):319-26.
33. Özcan F, Gürçay E. Is the information about lateral epicondylitis on the YouTube platform reliable and of good quality? Phys Sportsmed. 2023;51(5):458-62.

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Long-term Results of Proximal Pole Scaphoid Nonunion Treated with Retrograde Fixation and Non-vascularized Cancellous Bone Autograft

Retrograd Fiksasyon ve Vaskülerize Olmayan Kansellöz Kemik Otogrefti ile Tedavi Edilen Proksimal Kutup Skafoid Kaynamamasının Uzun Dönem Sonuçları

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ABSTRACT

Aim: To evaluate the long-term clinical and functional results of patients diagnosed with scaphoid bone proximal pole nonunion and who underwent retrograde fixation with non-vascularized cancellous autograft from the distal radius and a cannulated, headless screw via a volar approach.

Material and Methods: Twenty-nine patients, 2 female, and 27 male, who underwent surgery with the diagnosis of scaphoid proximal pole nonunion were evaluated in this study. All patients underwent retrograde fixation with a non-vascularized cancellous autograft from the distal radius and a headless cannulated screw using a volar approach and the same rehabilitation program was applied. The clinical and functional scores of the patients were evaluated before surgery and at the last follow-up.

Results: The mean age of the patients was 30.66±7.34 (range, 18-40) years. The median symptom duration was 11 (range, 6-18), and the median follow-up period after surgery was 55 (range, 36-67) months. When the maximum isometric strength of the forearm muscles of the healthy and surgical sides was evaluated at the last follow-up, the mean hand-grip strength test values of the healthy side were significantly higher than the pathological side ($p<0.001$). In addition, the mean flexion and radial deviation values of the healthy side were found to be statistically significantly higher than the surgical side in terms of the wrist joint range of motion at the last follow-up ($p=0.006$, and $p=0.022$, respectively).

Conclusion: Non-vascularized cancellous autograft and retrograde fixation with a volar approach is an effective surgical treatment option for proximal pole nonunion of the scaphoid bone.

Keywords: Scaphoid nonunion; proximal pole; retrograde fixation; non-vascularized cancellous bone autograft.

ÖZ

Amaç: Skafoid kemik proksimal kutup kaynamama tanısı almış olan ve volar yaklaşımla distal radiusdan nonvaskülarize kanselloz otogreft ve bir adet kanüllü, başsız vida ile retrograd fiksasyon uygulanan hastaların uzun dönem klinik ve fonksiyonel sonuçlarını değerlendirmektir.

Gereç ve Yöntemler: Bu çalışmada skafoid proksimal kutup kaynamama tanısı ile cerrahi yapılan, 2 kadın ve 27 erkek olmak üzere yirmi dokuz hasta değerlendirildi. Tüm hastalara volar yaklaşımla distal radiustan nonvaskülarize kanselloz otogreft ve bir adet başsız kanüllü vida ile retrograd fiksasyon yapıldı ve aynı rehabilitasyon programı uygulandı. Hastaların klinik ve fonksiyonel skorları cerrahi öncesi ve son kontrolde değerlendirildi.

Bulgular: Hastaların ortalama yaşı 30,66±7,34 (aralık, 18-40) yıl idi. Ortanca semptom süresi 11 (aralık, 6-18) ay ve cerrahi sonrası ortanca takip süresi 55 (aralık, 36-67) ay idi. Son kontrolde sağlam ve cerrahi uygulanan tarafların ön kol kaslarının maksimum izometrik kuvveti değerlendirildiğinde, sağlam tarafın ortalama el kavrama gücü test değerleri patolojik tarafa göre anlamlı derecede daha yüksekti ($p<0,001$). Buna ek olarak, son kontrolde el bilek eklem hareket açıklığı açısından sağlam tarafın ortalama fleksiyon ve radial deviasyon değerlerinin cerrahi uygulanan tarafa göre istatistiksel olarak anlamlı derecede daha yüksek olduğu bulundu (sırasıyla $p=0,006$ ve $p=0,022$).

Sonuç: Skafoid kemik proksimal kutup kaynamama durumunda, volar yaklaşımla nonvaskülarize kanselloz otogreft ve retrograd fiksasyon etkili bir cerrahi tedavi seçeneğidir.

Anahtar kelimeler: Skafoid kaynamaması; proksimal kutup; retrograd fiksasyon; nonvaskülerize kansellöz kemik otogrefti.

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INTRODUCTION

Scaphoid fractures are the most common fractures among the carpal bone fractures (1-3). Nonunion is observed in 5-25% of scaphoid fractures after treatment, and nonunion is more common, especially in elderly patients, smokers, and when there is a delay in the initial treatment of the fracture (4,5). In addition, scaphoid nonunion is frequently encountered in untreated cases, in patients with undiagnosed cases, and in patients who do not heal despite conservative treatment (3,6). In addition to standard radiographs, computed tomography is frequently used in diagnosis, especially in surgical patients, but magnetic resonance imaging is also frequently used to evaluate avascular necrosis (4). In standard radiographs used in diagnosis, diagnosis is often made late or overlooked because the scaphoid fracture line cannot be clearly determined (2).

In the literature, the risk of developing nonunion is reported in scaphoid fractures diagnosed six weeks after the fracture and without radiological evidence of union despite eight weeks of conservative treatment (1,4,6). The incidence of nonunion is not fully known because some patients continue to be asymptomatic (2). It has been reported that chronic wrist pain and progressive arthrosis may develop in symptomatic cases with nonunion (5). Scaphoid fractures are classified according to their location in the scaphoid as tuberosity (17%), proximal pole (6%), middle (66%) and distal pole (11%). This classification helps in determining the treatment methods to be applied, the vascularity of the fractured part, the potential for healing, or whether surgical treatment is required (3). The vascularity of the scaphoid bone is provided by the palmar and dorsal branches of the radial artery. The dorsal branch of the radial artery is solely responsible for the vascularity of the proximal pole (7). The necessity of surgery in the treatment of proximal pole nonunion is a common opinion of all hand surgeons, but there is no method accepted as a gold standard in the literature in terms of fixation method, volar/dorsal surgical approach, or grafting technique (8-10). In the cadaver model study by Daly et al. (11), in which they compared the biomechanical strength of retrograde and antegrade fixation in proximal pole scaphoid fractures, it was emphasized that antegrade and retrograde screw fixation were equivalent in terms of biomechanical strength. In the systematic evaluation and meta-analysis study conducted by Duncumb et al. (12) regarding the selection of bone graft in scaphoid nonunion surgery, it was emphasized that vascularized bone graft application did not provide significantly superior results compared to non-vascularized graft in the management of scaphoid nonunion. In addition, the authors stated that the fixation type or source of the graft used did not affect the union rates. Although successful results have been reported in the literature with vascular or non-vascular cancellous bone grafts and dorsal antegrade/volar retrograde fixation methods for proximal pole nonunion surgery, there is no consensus (13,14).

The aim of this study was to compare the clinical and functional long-term results of patients who underwent surgery with the diagnosis of scaphoid proximal pole nonunion and were treated with a cancellous graft from the distal radius and one headless screw with retrograde fixation using a volar approach.

MATERIAL AND METHODS

Of the 32 patients who underwent surgery due to the diagnosis of scaphoid proximal pole nonunion in our clinic between December 2009 and June 2013, 29 patients who met the inclusion criteria were included in the study. All patients who received nonvascularized cancellous autograft from the distal radius with a volar approach and underwent retrograde fixation surgery with a headless cannulated screw were evaluated clinically and functionally, retrospectively. This study was approved by the Duzce University Faculty of Medicine Non-invasive Health Research Ethics Committee (21.10.2019, 186) and all patients were informed about the study, an informed consent form and approval were obtained.

The inclusion criteria for the study were; between the ages of 18-45, proximal pole nonunion, volar approach, retrograde fixation, non-vascularized cancellous bone graft from the distal volar radius, a headless cannula screw, and magnetic resonance imaging before surgery. Exclusion criteria for the study were; being under 18 or over 45 years of age, dorsal approach, having previously undergone hand surgery for any reason, avascular proximal pole on magnetic resonance imaging (MRI), scaphoid middle or distal 1/3 nonunion, and Kirshner wire. Three patients were excluded from the study because it was observed that regular wrist joint range of motion measurements were not recorded during routine outpatient clinic control.

Demographic data of the patients were evaluated as follows; age, gender, surgical side, dominant extremity, etiology, smoking, duration of symptoms, follow-up period, grip strength test measurement (Jamar® Hydraulic Hand Dynamometer) before surgery and at the last follow-up, pain level before surgery and at the last follow-up, functional tests for the wrist, surgical satisfaction level, and healthy/pathological wrist range of motion.

The clinical and functional scores of the patients were evaluated with the scales of patient-rated tennis elbow evaluation (PRTEE), quick-disabilities of the arm, shoulder, and hand (Q-DASH), and visual analog scale (VAS) scores measured before surgery and at the last follow-up. The PRTEE score (maximum 100 points) evaluates the pain (50 points) in the affected arm and the function (100/2 points) in the affected arm (specific activities, activities of daily living). Q-DASH is a regional outcome measure developed for upper extremity musculoskeletal disorders and contains 11 questions. In order to calculate the score of the scale, which is reported to be used instead of DASH, at least 10 of the 11 questions must be answered. Each question is scored on a 5-point scale and a final score ranging from 0 (no disability) to 100 (severe disability) is calculated. VAS is used to convert some values that cannot be measured numerically into numerical values. The two extreme definitions of the parameter to be evaluated are written at both ends of a 100 mm line and the patient is asked to indicate where their own condition fits on this line by drawing a line, putting a dot, or marking. For example, for pain, I have no pain at one end and very severe pain is written at the other end and the patient marks their current condition on this line. The length of the distance from the point where there is no pain to the point marked by the patient indicates the patient's pain.

Surgical Technique and Rehabilitation

All patients underwent surgery under general anesthesia and with a tourniquet. A skin incision was made from the distal part of the flexor carpi radialis tendon to the level of the palmar rim of the distal radius and then to the scaphoid tubercle and scaphotrapezial joint. After retracting the superficial palmar branch of the radial artery, the flexor carpi radialis tendon was retracted to the ulnar side. After the capsule was opened obliquely from the scaphoid tubercle to the palmar rim of the radius, the radioscaphocapitate ligament was retracted. The long radiolunate ligament was incised to the palmar edge of the radius and the nonunion line was revealed. All fibrous tissues and sclerotic bone tissues in both the proximal and distal nonunion lines were removed with the help of a curette, burr, or osteotome. After adequate debridement, the distal radius was reached through the same volar proximal skin incision and sufficient pure cancellous bone autograft was taken by opening a window in the radius. After the grafting process was completed, retrograde fixation was achieved with a suitable size, 1.6 mm cannulated, headless screw (ACUMED), and the reduction was checked with scopy. The incision lines were closed according to the anatomy (Figure 1).

In all patients, the wrist was immobilized with a short arm splint for 6 weeks to allow for the healing of the soft tissues and radioscaphocapitate ligament after surgery, and then all patients underwent wrist rehabilitation with the same physical therapy protocol.

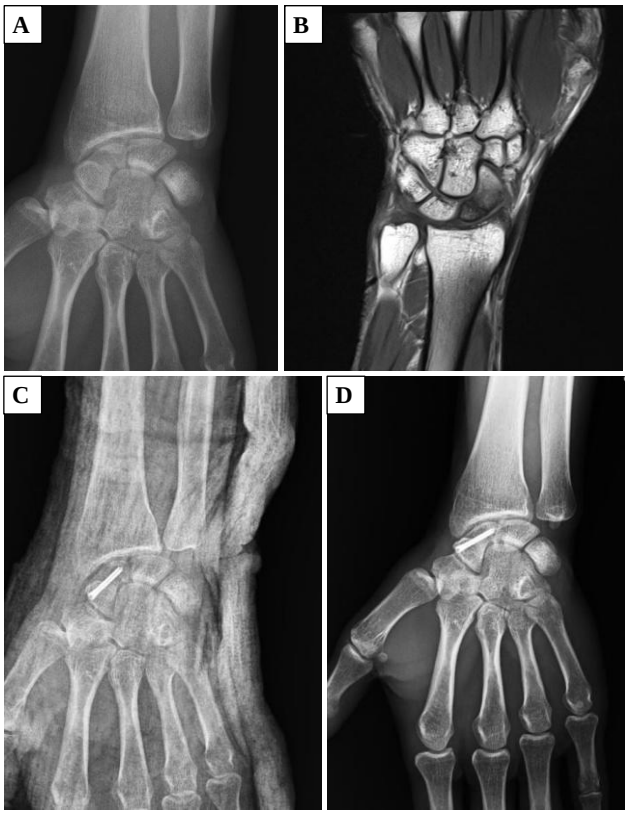

Figure 1. Long-term radiographic follow-up of a 29-year-old male patient who underwent scaphoid proximal pole nonunion surgery. **A)** Anteroposterior wrist radiograph, **B)** Coronal magnetic resonance imaging at 11 months after trauma (preoperative), Anteroposterior wrist radiograph **C)** 1 day, and **D)** 50 months after surgery with the complete union of the scaphoid

Statistical Analysis

Statistical analyses were performed with the Number Cruncher Statistical Systems (NCSS) 2007 statistical software (Utah, USA). In addition to descriptive statistical methods (mean, standard deviation, median, interquartile range, minimum, maximum) in the evaluation of the data, the distribution of the variables was examined with the Shapiro-Wilk normality test. In the comparison of the first and last measurements of the variables showing normal distribution the paired samples t-test was used, while the Wilcoxon test was used for variables not showing normal distribution. The independent samples t-test was used to compare two groups and categorical variables were analyzed with Fisher's exact test. The results were evaluated at the significance level of $p<0.05$.

RESULTS

Of the 29 patients included in the study, 2 were female and 27 were male, and their mean age was 30.66±7.34 (range, 18-40) years. The median symptom duration until surgery was 11 (range, 6-18) months. The median follow-up period after surgery was 55 (range, 36-67) months. When the functional scores of the patients were compared, the median VAS, Q-DASH, and PRTEE values measured at the last follow-up period were found to be statistically significantly lower than the values measured before surgery ($p<0.001$ for all, Table 1). When we evaluated the maximum isometric strength of the forearm muscles of the healthy and surgical sides at the last follow-up period after surgery, the mean hand-grip strength test values of the healthy side were found to be statistically significantly higher than the pathological side ($p<0.001$). In addition, when the wrist joint range of motion of the healthy and surgical sides was evaluated at the last follow-up period, no statistically significant difference was observed between the mean extension ($p=0.075$) and ulnar deviation ($p=0.234$) values, but the mean flexion ($p=0.006$) and radial deviation ($p=0.022$) values of the healthy side were found to be statistically significantly higher than the surgical side (Table 2). Nonunion was detected in a total of 4 patients, 3 male and 1 female, at their last follow-up. The mean age ($p=0.011$), and advanced osteoarthritis findings ($p=0.001$) of the nonunion group were found to be significantly higher than the union group (Table 3).

DISCUSSION

In this study, where we evaluated the long-term results of patients who underwent surgical treatment with a volar approach and non-vascularized bone graft from the distal radius for proximal pole nonunion in scaphoid bone fractures, 86% complete union was achieved. We concluded that there was a significant clinical improvement compared to pre-surgical values in the clinical evaluation made with PRTEE and Q-DASH measurements. It was found that especially extension and ulnar deviation movements were almost completely gained when we evaluated the range of motion of the joint in comparison with the healthy wrist.

Despite the diversity and lack of consensus in management of nonunion after scaphoid bone fractures, studies have reported very successful long-term results (1,15,16). In a study by Reigstad et al. (15) evaluating scaphoid fracture nonunion with a long-term follow-up, they concluded that

Table 1. Comparison of clinical and functional test values of patients before and after (final evaluation) surgery

	Preoperative		Final evaluation		p
	Mean±SD	Median (IQR) [min-max]	Mean±SD	Median (IQR) [min-max]	
VAS	6.89±1.49	7 (6-8) [4-10]	1.03±1.48	1 (0-1) [0-5]	**<0.001**
Q-DASH	61.91±18.57	65.9 (43.2-70.5) [20.5-88.6]	6.97±9.45	0 (0-12.5) [0-36.4]	**<0.001**
PRTEE	66.21±11.92	66 (57-75) [40-90]	8.60±7.62	7 (0-15) [0-23.5]	**<0.001**

VAS: visual analog scale, Q-DASH: quick-disabilities of the arm, shoulder, and hand, PRTEE: patient-rated tennis elbow evaluation, SD: standard deviation, IQR: interquartile range

Table 2. Hand grip strength and joint range of motion values of the wrist with nonunion surgery and healthy wrist

	Healthy Wrist	Lesion Wrist	p
Grip Strength	82.52±14.05	65.69±21.82	**<0.001**
Flexion	78.38±11.72	72.93±13.30	**0.006**
Extension	69.00±10.10	66.21±10.17	0.075
Radial Deviation	29.00±5.35	25.93±6.82	**0.022**
Ulnar Deviation	41.76±5.93	39.62±8.96	0.234

Table 3. Comparison of patients with and without union after nonunion surgery due to scaphoid fracture

	Nonunion	Union	p
Age (years), mean±SD	39.00±1.55	29.32±7.02	**0.011**
Gender, n (%)			
Male	3 (75.0)	24 (96.0)	0.261
Female	1 (25.0)	1 (4.0)	
Fracture, n (%)			
Right	2 (50.0)	10 (40.0)	>0.999
Left	2 (50.0)	15 (60.0)	
Smoking, n (%)	2 (50.0)	12 (48.0)	>0.999
Osteoarthritis, n (%)			
None	0 (0.0)	19 (76.0)	**0.001**
Mild	1 (25.0)	3 (12.0)	
Moderate	0 (0.0)	3 (12.0)	
Extremely	3 (75.0)	0 (0.0)	

SD: standard deviation

the applied surgery reduced pain, improved wrist functions, and slowed down degenerative changes. In another scaphoid nonunion study by Jaminet et al. (16) evaluating the radiological results of 286 patients at the end of ten years, they emphasized that the prognosis of scaphoid nonunion could be very good with the application of a correct treatment algorithm. Similar to the literature, in this study, we obtained very successful results both clinically and functionally in proximal pole nonunion, which we followed up for a long time.

It is still a matter of debate whether the bone graft to be used in proximal pole nonunions of the scaphoid bone is vascularized or non-vascularized (17-20). Rancy et al. (19) emphasized that non-vascularized autograft and rigid fixation are quite effective in achieving union despite vascular ischemia and histopathological osteonecrosis in their prospective study on proximal pole nonunions of the scaphoid. In addition, the authors stated that vascularized bone grafting is rarely necessary for internal fixation of scaphoid nonunions. Similarly, Luchetti et al. (20) achieved 90% union after cancellous non-vascularized autograft taken from the distal radius and fixation in proximal pole nonunions and also stated that non-vascularized cancellous autograft and antegrade fixation a useful options in the treatment of proximal pole scaphoid nonunions.

The management of scaphoid nonunion surgery is quite difficult due to reasons such as the geometry of the scaphoid bone, the fracture site, and the vascular supply pattern (21). In particular, the decision to choose the volar or dorsal approach is very important in scaphoid proximal pole nonunions. The dorsal approach is the most preferred method in proximal pole nonunions, when the literature is examined, (22-28). For example, in the meta-analysis study conducted by Merrel et al. (26) in terms of scaphoid nonunion surgery, they emphasized that there was a 67% recovery rate for proximal pole nonunions in surgeries performed with the dorsal approach. In another study conducted by Weber et al. (27) in proximal pole scaphoid nonunions with the volar approach, they emphasized that union, joint range of motion, and grip strength were at a good level. Finsen et al. (28) evaluated bone chip graft and Kirschner wire fixation in scaphoid nonunion surgery with a 10-year follow-up period and reported that bone union was quite successful in another study. The authors also emphasized that this method is excellent for proximal pole nonunions. In contrast to these studies, some studies adopt retrograde fixation with a volar approach in proximal scaphoid fracture surgery (11,29). In a cadaver study by Daly et al. (11), they compared the biomechanical strength of retrograde fixation in proximal pole scaphoid fractures and divided 22 scaphoids into two groups to create proximal pole fractures. They compared antegrade fixation with a dorsal approach and retrograde fixation with a volar approach biomechanically in this current study. The authors emphasized that antegrade and retrograde screw configurations are equivalent in terms of biomechanical strength. In another clinical study by Jeon et al. (29), comparing percutaneous screw application with volar and dorsal approaches in scaphoid fractures, they stated that the screws were placed more parallel to the long axis of the scaphoid and more perpendicular to the fracture line with the dorsal approach, but they emphasized that there was no significant difference in terms of functional outcome and bone union.

In nonunion cases developing after scaphoid bone fractures, carpal instability, collapse or advanced osteoarthritic changes in the radio scaphoid joint may be observed after the conservative or surgical approaches (25,30,31). In a long-term follow-up study by Daecke et al. (31) evaluating the formation of carpal osteoarthritis after scaphoid nonunion treatment with bone graft and cannulated screws, it was stated that high patient satisfaction and good

function were observed after the healing of scaphoid nonunion. In addition, the authors emphasized that correct anatomical reconstruction of the non-arthritic joint with cannulated screws in long-term follow-ups suggests the onset of osteoarthritic changes in most patients. In this study, nonunion was detected in 4 of 29 patients, and advanced osteoarthritic changes developed in the radio scaphoid joint of the patients.

There are some limitations of this study in which we evaluated patients who underwent retrograde fixation with a volar approach for proximal pole scaphoid fracture nonunions. First, it is a retrospective study with a small number of patients. Second, the patient group consists of young patients, and therefore the clinical and functional results may have been quite good. Finally, the proximal fragment was not separated in terms of size, and all proximal pole nonunions were included in the study.

CONCLUSION

Although the volar approach is not preferred in terms of the shape of the scaphoid bone, fracture pattern, and screw configuration in proximal pole nonunions of scaphoid fractures, non-vascularized cancellous autograft and retrograde fixation with the volar approach is a highly effective surgical treatment option in proximal pole nonunions of the scaphoid bone.

Ethics Committee Approval: The study was approved by the Non-invasive Health Research Ethics Committee of Düzce University (21.10.2019, 186).

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REFERENCES

1. Rhee PC, Jones DB Jr, Shin AY, Bishop AT. Evaluation and treatment of scaphoid nonunions: a critical analysis review. JBJS Rev. 2014;2(7):e4.
2. Huang YC, Liu Y, Chen TH. Long-term results of scaphoid nonunion treated by intercalated bone grafting and Herbert's screw fixation--a study of 49 patients for at least five years. Int Orthop. 2009;33(5):1295-300.
3. Steinmann SP, Adams JE. Scaphoid fractures and nonunions: diagnosis and treatment. J Orthop Sci. 2006;11(4):424-31.
4. Yeo JH, Kim JY. Surgical strategy for scaphoid nonunion treatment. J Hand Surg Asian Pac Vol. 2018;23(4):450-62.
5. Ammori MB, Elvey M, Mahmoud SS, Nicholls AJ, Robinson S, Rowan C, et al. The outcome of bone graft surgery for nonunion of fractures of the scaphoid. J Hand Surg Eur Vol. 2019;44(7):676-84.
6. Rogers MJ, Ohlsen SM, Huang JI. Fixation techniques for scaphoid nonunion. J Am Acad Orthop Surg. 2023;31(15):783-92.
7. Taleisnik J, Kelly PJ. The extraosseous and intraosseous blood supply of the scaphoid bone. J Bone Joint Surg Am. 1966;48(6):1125-37.
8. Aibinder WR, Wagner ER, Bishop AT, Shin AY. Bone grafting for scaphoid nonunions: is free vascularized bone grafting superior for scaphoid nonunion? Hand (N Y). 2019;14(2):217-22.
9. Papatheodorou LK, Papadopoulos DV, Graber MM, Sotereanos DG. Dorsal capsular-based vascularized distal radius graft for proximal pole scaphoid nonunion with avascular necrosis. Injury. 2021;52(12):3635-9.
10. Miller EA, Huang JI. Traditional bone grafting in scaphoid nonunion. Hand Clin. 2024;40(1):105-16.
11. Daly CA, Boden AL, Hutton WC, Gottschalk MB. Biomechanical strength of retrograde fixation in proximal third scaphoid fractures. Hand (N Y). 2019;14(6):760-4.
12. Duncumb JW, Robinson PG, Williamson TR, Murray IR, Campbell D, Molyneux SG, et al. Bone grafting for scaphoid nonunion surgery: a systematic review and meta-analysis. Bone Joint J. 2022;104-B(5):549-58.
13. Rancy SK, Wolfe SW, Jerome JTJ. Predictors of failure for vascularized and nonvascularized bone grafting of scaphoid nonunions: a systematic review. J Hand Microsurg. 2021;14(4):322-35.
14. Fujihara Y, Yamamoto M, Hidaka S, Sakai A, Hirata H. Vascularised versus non-vascularised bone graft for scaphoid nonunion: Meta-analysis of randomised controlled trials and comparative studies. JPRAS Open. 2022;35:76-88.
15. Reigstad O, Grimsgaard C, Thorkildsen R, Reigstad A, Rokkum M. Long-term results of scaphoid nonunion surgery: 50 patients reviewed after 8 to 18 years. J Orthop Trauma. 2012;26(4):241-5.
16. Jaminet P, Götz M, Gonser P, Schaller HE, Lotter O. Treatment of scaphoid nonunion: radiologic outcome of 286 patients in 10 years. Eplasty. 2019;19:e5.
17. Ribak S, Medina CE, Mattar R Jr, Ulson HJ, Ulson HJ, Etchebehere M. Treatment of scaphoid nonunion with vascularised and nonvascularised dorsal bone grafting from the distal radius. Int Orthop. 2010;34(5):683-8.
18. Tambe AD, Cutler L, Murali SR, Trail IA, Stanley JK. In scaphoid non-union, does the source of graft affect outcome? Iliac crest versus distal end of radius bone graft. J Hand Surg Br. 2006;31(1):47-51.
19. Rancy SK, Swanstrom MM, DiCarlo EF, Sneag DB, Lee SK, Wolfe SW. Success of scaphoid nonunion surgery is independent of proximal pole vascularity. J Hand Surg Eur Vol. 2018;43(1):32-40.
20. Luchetti TJ, Rao AJ, Fernandez JJ, Cohen MS, Wysocki RW. Fixation of proximal pole scaphoid nonunion with non-vascularized cancellous autograft. J Hand Surg Eur Vol. 2018;43(1):66-72.
21. McInnes CW, Giuffre JL. Fixation and grafting after limited debridement of scaphoid nonunions. J Hand Surg Am. 2015;40(9):1791-6.

22. Wagner ER, Spencer CC, Dawes AM, Gottschalk MB, Daly CA. Management of proximal pole scaphoid nonunions: a critical analysis review. JBJS Rev. 2021;9(4):e19.00181.
23. Waters PM, Stewart SL. Surgical treatment of nonunion and avascular necrosis of the proximal part of the scaphoid in adolescents. J Bone Joint Surg Am. 2002;84(6):915-20.
24. Severo AL, Lemos MB, Lech OLC, Barreto Filho D, Strack DP, Candido LK. Bone graft in the treatment of nonunion of the scaphoid with necrosis of the proximal pole: a literature review. Rev Bras Ortop. 2017;52(6):638-43.
25. Matsuki H, Ishikawa J, Iwasaki N, Uchiyama S, Minami A, Kato H. Non-vascularized bone graft with Herbert-type screw fixation for proximal pole scaphoid nonunion. J Orthop Sci. 2011;16(6):749-55.
26. Merrell GA, Wolfe SW, Slade JF 3rd. Treatment of scaphoid nonunions: quantitative meta-analysis of the literature. J Hand Surg Am. 2002;27(4):685-91.
27. Weber MB, Bishop AT, Shin AY. Osteochondral autograft transplantation for proximal pole scaphoid nonunions. J Hand Surg Am. 2023;48(7):655-64.
28. Finsen V, Hofstad M, Haugan H. Most scaphoid non-unions heal with bone chip grafting and Kirschner-wire fixation. Thirty-nine patients reviewed 10 years after operation. Injury. 2006;37(9):854-9.
29. Jeon IH, Micic ID, Oh CW, Park BC, Kim PT. Percutaneous screw fixation for scaphoid fracture: a comparison between the dorsal and the volar approaches. J Hand Surg Am. 2009;34(2):228-36.e1.
30. Lim TK, Kim HK, Koh KH, Lee HI, Woo SJ, Park MJ. Treatment of avascular proximal pole scaphoid nonunions with vascularized distal radius bone grafting. J Hand Surg Am. 2013;38(10):1906-12.e1.
31. Daecke W, Wieloch P, Vergetis P, Jung M, Martini AK. Occurrence of carpal osteoarthritis after treatment of scaphoid nonunion with bone graft and Herbert screw: a long-term follow-up study. J Hand Surg Am. 2005;30(5):923-31.

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Clinical and Histopathological Effects of Isotretinoin on Neuroregeneration in Experimental Spinal Cord Injury

Deneysel Omurilik Yaralanmasında İsotretinoinin Nörorejenerasyon Üzerine Klinik ve Histopatolojik Etkileri

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ABSTRACT

Aim: Spinal cord injury is an important problem, and a fully effective treatment for it has not yet been developed. Isotretinoin is a retinoid known for its anti-inflammatory effect. The present study aimed to evaluate whether isotretinoin has a positive impact on neural tissue in post-injury damage.
Material and Methods: A total of 36 rats were randomly divided into 6 groups as control, sham, and injury with 14-day 7.5 mg/kg/day, 28-day 7.5 mg/kg/day, 14-day 15 mg/kg/day, and 28-day 15 mg/kg/day isotretinoin groups. Laminectomy was performed and spinal cord injury was produced by using the clip compression technique. Neurological examination was performed on days 1, 7, 14, and 28. After the treatment period, all rats were sacrificed, and their spinal cord samples were collected for histopathological assessment.
Results: Groups receiving 7.5 mg/kg/day ($p=0.048$) and 15 mg/kg/day ($p<0.001$) isotretinoin showed a significantly increased inclined plane angle on day 14 compared with the sham group. In hematoxylin and eosin, and Luxol fast blue staining, the 28-day isotretinoin of 15 mg/kg/day group and the control group had similar histopathological findings in motor neurons and glial cells. The cleaved caspase 3 expression was significantly diminished in the groups of 28-day isotretinoin 7.5 mg/kg/day, 14-day isotretinoin 15 mg/kg/day, and 28-day isotretinoin 15 mg/kg/day, compared to the sham group, along with the reduction in apoptosis.
Conclusion: Isotretinoin reduces neuronal apoptosis, diminishes pathological tissue damage, and improves functional recovery after injury. The observed prominent neuroprotective effects introduce isotretinoin as a promising therapy for spinal cord injury.
Keywords: Isotretinoin; neuroregeneration; spinal cord injury.

ÖZ

Amaç: Omurilik yaralanması önemli bir sorundur ve henüz tam olarak etkili bir tedavi geliştirilememiştir. İsotretinoin, anti-inflamatuar etkisiyle bilinen bir retinoiddir. Bu çalışmanın amacı, yaralanma sonrası sinir dokusu üzerinde isotretinoinin olumlu bir etkisinin olup olmadığını değerlendirmektir.
Gereç ve Yöntemler: Toplam 36 sıçan kontrol, sham ve travma ile 14 günlük 7,5 mg/kg/gün, 28 günlük 7,5 mg/kg/gün, 14 günlük 15 mg/kg/gün ve 28 günlük 15 mg/kg/gün isotretinoin grupları olmak üzere 6 gruba ayrıldı. Sıçanlara laminektomi yapıldı ve omurilik yaralanması klip kompresyon tekniği kullanılarak oluşturuldu. Nörolojik muayene 1, 7, 14 ve 28. günlerde yapıldı. Tedavi süresinden sonra tüm sıçanlar öldürüldü ve histopatolojik değerlendirme için omurilik örnekleri toplandı.
Bulgular: 7,5 mg/kg/gün ($p=0,048$) ve 15 mg/kg/gün ($p<0,001$) isotretinoin alan gruplar, sham grubuna kıyasla 14. günde önemli ölçüde artmış eğik düzlem açısı gösterdi. Hematoksilen ve eozin ile Luxol fast blue ile boyamada 15 mg/kg/gün isotretinoin 28 günlük grubu ve kontrol grubunun motor nöronlarında ve glial hücrelerinde benzer histopatolojik bulgular görüldü. Cleaved kaspaz 3 ekspresyonu, sham grubuna kıyasla 28 günlük isotretinoin 7,5 mg/kg/gün, 14 günlük isotretinoin 15 mg/kg/gün ve 28 günlük isotretinoin 15 mg/kg/gün gruplarında anlamlı bir şekilde azaldı ve apoptozisde azalma olduğu görüldü.
Sonuç: İsotretinoin, nöronal apoptozu azaltır, patolojik doku hasarını azaltır ve yaralanma sonrası fonksiyonel iyileşmeye etki eder. Gözlemlenen belirgin nöroprotektif etkiler, isotretinoini omurilik yaralanması için umut verici bir tedavi olarak tanıtmaktadır.
Anahtar kelimeler: İsotretinoin; nörorejenerasyon; omurilik yaralanması.

INTRODUCTION

Acute spinal cord injury (ASCI) remains a critical and uncontrollable health issue, leading to significant morbidity and mortality. The consequences extend beyond individual health, affecting psychiatric well-being and socioeconomic stability. Individuals with ASCI often require ongoing care, and in severe cases, they may experience tetraplegia and need respiratory support, posing substantial burdens on families and national economies due to high treatment and care costs and loss of productivity. Despite advancements, effective treatment for ASCI remains elusive, with current approaches limited to supportive measures (1,2).

ASCI typically results from high-energy incidents such as traffic accidents, falls, workplace injuries, and sports activities, predominantly affecting the cervical spinal cord and dorsolumbar junction (3,4). The injury process in ASCI is twofold: primary damage, an immediate and unavoidable consequence of trauma, and secondary damage, which results from endogenous cell death pathways activated by the initial injury. Clinical interventions focus on mitigating secondary damage, primarily responsible for neurological dysfunction (5). Factors such as free radicals, lipid peroxidation, neurotransmission system disruptions, inflammation, ion channel dysregulation, and apoptosis contribute significantly to secondary damage (6). Current clinical trials aim to prevent these secondary injury mechanisms.

Retinoids, known for their roles in cell growth, differentiation, and tumor suppression, also possess immunomodulating effects. Isotretinoin (13-cis-retinoic acid, $C_{20}H_{28}O_2$), a first-generation retinoid, has been widely used to treat severe acne since the 1980s due to its anti-inflammatory properties, which it exerts through its effects on neutrophils and T lymphocytes (7-13).

Isotretinoin, a vitamin A derivative, plays a crucial role in the development of vertebrate tissues by regulating gene expression involved in cell proliferation, differentiation, and apoptosis (14). This study aimed to investigate the potential benefits of isotretinoin on neural tissues that have been damaged by ASCI.

MATERIAL AND METHODS

The study was conducted at the Experimental Animal Laboratory at the Düzce University and received funding from the Düzce University Scientific Research Projects. Ethics approval was granted by the Düzce University Experimental Animal Ethics Committee, decision number 2020/11/03. The study adhered to the EU Directive 2010/63/EU regarding animal experiments, ensuring the minimum number of animals were utilized.

Animals

This study used 36 adult female Sprague-Dawley rats, aged 2-4 weeks and weighing 200-250 grams. The rats were housed in standard cages under controlled conditions, including a 12-hour light/dark cycle and a consistent room temperature of 22±2°C. They were acclimated for seven days before beginning the experimental procedures.

Experimental Groups

The 36 rats were randomly assigned to six experimental groups, each consisting of six rats: group I (Control), only underwent laminectomy at the T7-T9 level without further treatment; group II (Sham), underwent laminectomy at the T7-T9 level, followed by one minute of spinal cord compression using an aneurysm clip, with the dura kept intact; group III (Isotretinoin 14/7.5), received isotretinoin at 7.5 mg/kg/day by gavage for 14 days post-compression; group IV (Isotretinoin 28/7.5), received isotretinoin at 7.5 mg/kg/day by gavage for 28 days post-compression; group V (Isotretinoin 14/15) also received isotretinoin at 15 mg/kg/day by gavage for 14 days post-compression; and group VI (Isotretinoin 28/15), received isotretinoin at 15 mg/kg/day by gavage for 28 days post-compression. Each group was carefully monitored to assess the impact of isotretinoin on neural tissue recovery after spinal cord injury (SCI).

Spinal Cord Injury

Before anesthesia, all rats were evaluated to ensure normal motor function. Anesthesia was administered intramuscularly using 50 mg/kg ketamine hydrochloride (Ketas, Pfizer; Istanbul, Turkey) and 10 mg/kg xylazine (Rompun, Bayer; Istanbul, Turkey). The rats were positioned prone, and their backs were shaved and disinfected with polyvidone iodine (Batticon, Adeka; Samsun, Turkey). A midline vertical incision was made between T5 and T12, guided by the interscapular distance and ribs. Bilateral paravertebral muscles were dissected, and a mastoid retractor was used (Figure 1A). Laminectomy was performed at T7-T9. In the control group, the surgical wound was closed post-laminectomy (Figure 1B). For the standard SCI procedure, the thoracic vertebrae were exposed, and laminectomy was performed for 1 minute using a 70 g closing-force aneurysm clip (Yasargil FE 721 Aesculap; Istanbul, Turkey) (Figure 1C), as previously described (14,15). Hemostasis was ensured, and the paravertebral muscles and skin were sutured with 3/0 Vicryl, following anatomical layers. The rats were allowed to recover at room temperature.

Evaluation of Recovery

The recovery was assessed using the inclined plane test and motor examination based on the Drummond and Moore (D&M) criteria. The inclined plane method, developed by Rivlin and Tator (16) in 1977, records the highest angle at which the experimental animal can remain stationary without slipping for 5 seconds. The rat was placed parallel to the ground on the inclined plane, and the table was tilted at various angles. The angle at which the rat slipped was noted on days 1, 7, 14, and 28. To evaluate functional recovery in all rats, post-traumatic motor examinations were conducted on days 1, 7, 14, and 28 using the D&M scale (14,17).

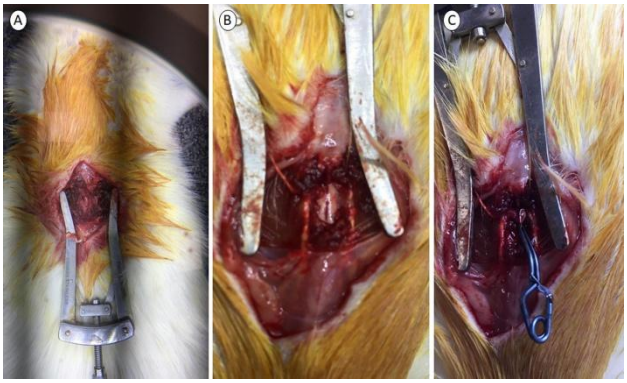

Figure 1. A) Retractor placed, **B)** Dura after laminectomy, **C)** Aneurysm clip application

Specimen Collection

At the conclusion of day 14, rats in groups I, II, III, and V were sacrificed. Similarly, rats in groups IV and VI were euthanized at the end of their 28-day follow-up period. The previously incised skin was reopened to access the site where the ASCI was induced using the clip-on dura after the laminectomy. The damaged section of the spinal cord was then carefully excised.

Histopathological Examination

Spinal cord specimens were preserved in 4% formaldehyde and embedded in paraffin. Thin sections, each 5 μm thick, were prepared from these paraffin blocks on silanized slides. After deparaffinization, some sections were stained with hematoxylin and eosin to assess tissue integrity and structure. Additional sections underwent Luxol fast blue staining to highlight and examine neuronal extensions. Immunohistochemical staining for cleaved caspase 3 was used to identify cells undergoing apoptosis. All stained histopathological samples were examined using light microscopy (Olympus Cx41-AxioCam Zeiss).

Statistical Analysis

The statistical analysis was done with the IBM SPSS v.22 package program. The normality assumption was assessed with the Shapiro-Wilk test. The Kruskal-Wallis test was used to compare the groups, followed by post hoc Bonferroni correction. The data were summarized with median, interquartile range, and minimum-maximum. Fisher-Freeman-Halton test was used to analyze categorical variables and given in number and percentage. A p-value of <0.05 indicated statistical significance.

RESULTS

Results from Motor Examination

A statistically significant difference was found between the experimental groups in terms of the inclined plane angles recorded on day 14 (p<0.001, Table 1). Compared to the control group, the inclined plane angles recorded on day 14 were statistically significantly lower in the sham, isotretinoin 7.5 mg/kg/day, and isotretinoin 15 mg/kg/day groups (p<0.001, p<0.001, and p=0.045, respectively). The groups of isotretinoin 7.5 mg/kg/day and isotretinoin 15 mg/kg/day had a significantly increased value of inclined plane angle, compared to the sham group (p=0.048, and p<0.001, respectively). There was no significant difference between the isotretinoin 7.5 mg/kg/day and isotretinoin 15 mg/kg/day groups in terms of the inclined plane angles measured on day 14 (p=0.180).

There was a statistically significant difference between the groups on day 14 in terms of the values measured in reference to the D&M criteria (p<0.001, Table 1). Again, compared to the control group, the D&M criteria scores of day 14 were statistically significantly lower in the sham, isotretinoin 7.5 mg/kg/day, and isotretinoin 15 mg/kg/day groups (p<0.001, p<0.001, and p=0.001, respectively). The D&M scores measured in the isotretinoin 7.5 mg/kg/day and isotretinoin 15 mg/kg/day groups were found to be similar to that of the sham group (p=0.291, and p=0.111, respectively). There was no significant difference between the isotretinoin 7.5 mg/kg/day and isotretinoin 15 mg/kg/day groups on day 14 either (p=0.510).

The inclined plane angles measured on day 28 were found to be different between the groups at a statistically significant level (p<0.001, Table 2). While the inclined plane angles of day 28 were statistically significantly lower in the sham and isotretinoin 15 mg/kg/day groups than in the control group (p<0.001, p=0.003, respectively), there was a significant difference between the sham group and the isotretinoin 7.5 mg/kg/day group (p=0.139). The inclined plane angle was found to be significantly higher in the isotretinoin 15 mg/kg/day group than in the sham group (p=0.003). There was no significant difference between the isotretinoin 7.5 mg/kg/day and isotretinoin 15 mg/kg/day groups in terms of inclined plane angle (p=0.834).

There was a statistically significant difference between the experimental groups also in D&M criteria scores measured on day 28 (p<0.002, Table 2). The D&M criteria scores of day 28 were statistically significantly lower in the sham, isotretinoin 7.5 mg/kg/day, and isotretinoin 15 mg/kg/day

Table 1. Inclined plane angles and Drummond and Moore criteria scores on day 14

	Control (n=6)	Sham (n=6)	Isotretinoin 14/7.5 (n=12)	Isotretinoin 14/15 (n=12)	p
Inclined plane angle	64.5 (1) [64-66]	44 (3) [42-46]	47 (1) [46-49]	48.5 (1) [48-50]	**<0.001**
D&M score	4 (0) [4-4]	0 (2) [0-4]	1 (0) [0-1]	1 (0) [1-1]	**<0.001**
D&M score, n (%)					
0	0 (0.0%)	4 (66.7%)	2 (16.7%)	0 (0.0%)	
1	0 (0.0%)	1 (16.7%)	10 (83.3%)	12 (100%)	**<0.001**
4	6 (100%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	

D&M: Drummond and Moore, Isotretinoin 14/7.5: received isotretinoin at 7.5 mg/kg/day for 14 days, Isotretinoin 14/15: received isotretinoin at 15 mg/kg/day for 14 days, descriptive statistics were as median (interquartile range) [minimum-maximum] for numerical data, and as frequency (percentage) for categorical data

Table 2. Inclined plane angles and Drummond and Moore criteria scores on day 28

	Control (n=6)	Sham (n=6)	Isotretinoin 28/7.5 (n=6)	Isotretinoin 28/15 (n=6)	p
Inclined plane angle	68.5 (2) [66-69]	46 (2) [45-48]	50 (1) [50-51]	52 (1) [52-54]	**<0.001**
D&M score	4 (0) [4-4]	0.5 (2) [0-4]	1 (0) [0-1]	1.5 (1) [1-2]	**0.002**
D&M score, n (%)					
0	0 (0.0%)	3 (50.0%)	1 (16.7%)	0 (0.0%)	
1	0 (0.0%)	2 (33.3%)	5 (83.3%)	3 (50.0%)	**<0.001**
2	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (50.0%)	
4	6 (100%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	

D&M: Drummond and Moore, Isotretinoin 28/7.5: received isotretinoin at 7.5 mg/kg/day for 28 days, Isotretinoin 28/15: received isotretinoin at 15 mg/kg/day for 28 days, descriptive statistics were as median (interquartile range) [minimum-maximum] for numerical data, and as frequency (percentage) for categorical data

groups than in the control group (p=0.001, p=0.001, and p=0.033, respectively). The D&M criteria scores in the isotretinoin 7.5 mg/kg/day and isotretinoin 15 mg/kg/day groups were similar to that of the sham group (p=0.914 and p=0.211, respectively), while there was no significant difference between the isotretinoin 7.5 mg/kg/day and isotretinoin 15 mg/kg/day groups (p=0.254).

Histopathological Findings

Hematoxylin and Eosin Staining

The hematoxylin and eosin staining did not reveal any histopathological changes in motor neurons and glial cells in the control group (Figure 2A). In the rats in the sham group, it was observed that the cytoplasm of the motor neuron turned pink and its nucleus turned purple, which indicated that their cell integrity was disrupted (Figure 2B). In the 14-day isotretinoin of 7.5 mg/kg/day group, cytoplasmic volumes of the motor neurons were decreased, the nuclei that were supposed to be visualized in purple were not definable and there were edematous areas between cell extensions. In glial cells, no histopathological changes were observed compared to the control group and the sham group (Figure 2C). In the 28-day isotretinoin of 7.5 mg/kg/day group, the cytoplasmic volumes of the motor neurons decreased and the cytoplasmic limits of some neurons could not be identified. In glial cells, no histopathological changes were observed compared to the control and sham groups (Figure 2D). In the 14-day isotretinoin of 15 mg/kg/day group, the cytoplasmic margins of the motor neurons, and the nuclei of purple color were all identifiable and there was no edema between the cell extensions. In glial cells, no histopathological changes were observed compared to the control group and the sham group (Figure 2E). On day 28, it was observed that the cytoplasmic margins and nucleus of motor neurons could be selected similarly to the control group. In glial cells, histopathological findings were found to be similar to those in the control group (Figure 2F).

Luxol Fast Blue Staining

There were no histopathological changes in motor neurons and glial cells observed in the Luxol fast blue staining of the control group (Figure 3A). In the Luxol fast staining of the sham group, dendrite extensions originating from motor neurons were observed (Figure 3B). In the 14-day isotretinoin of 7.5 mg/kg/day group, the motor neurons were seen to have diminished volume of cell bodies, and their extensions were not well identified (Figure 3C). In the 28-day isotretinoin of 7.5 mg/kg/day group, the volumes of the motor neurons were close to the control group and the cell extensions were identifiable (Figure 3D). In the 14-day isotretinoin of 15 mg/kg/day group, the cytoplasmic volumes and cell extensions of the motor neurons were found to be similar to the control group (Figure 3E). In the 14-day isotretinoin of 15 mg/kg/day group, the cytoplasmic volumes and cell extensions of the motor neurons were found to be the same as the control group (Figure 3F).

Immunohistochemical Staining of Cleaved Caspase 3

The immunohistochemical staining of cleaved caspase 3 did not reveal any immunohistochemical reaction in the motor neurons of the control group. Only the glial cells exhibited a reaction, interpreted as a physiological process (Figure 4A). It was observed that the expression of cleaved caspase 3 in motor neurons significantly increased in the sham group compared to the control group. The pronounced brown color, indicative of a dense expression pattern, was observed in some motor neurons. Additionally, the reactions in glial cells were higher than those in the control group (Figure 4B). In the 14-day isotretinoin treatment at 7.5 mg/kg/day group, the expression of cleaved caspase 3 in motor neurons and glial cells was higher than in the control group but lower than in the sham group (Figure 4C). Similarly, in the group treated with isotretinoin at 7.5 mg/kg/day for 28 days, the expression was similar to the control group and decreased compared to

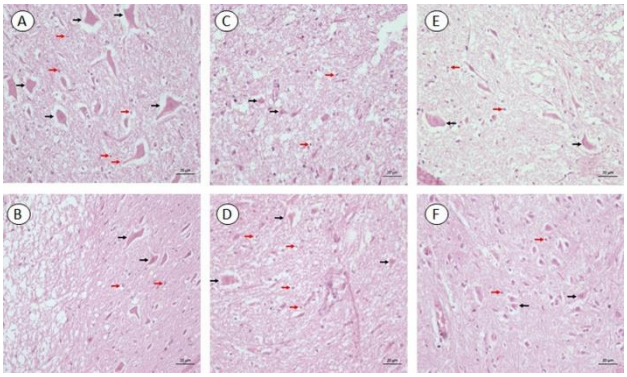

Figure 2. A) Control, **B)** sham, and **C)** 14-day 7.5 mg/kg/day, **D)** 28-day 7.5 mg/kg/day, **E)** 14-day 15 mg/kg/day, **F)** 28-day 15 mg/kg/day isotretinoin groups (black arrow points at motor neuron, red arrow at the glial cell, H&Ex400)

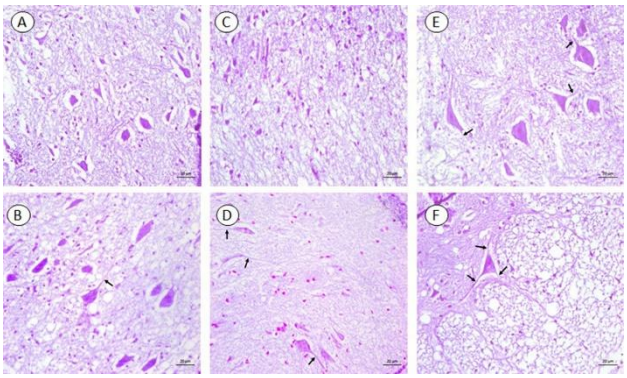

Figure 3. A) Control, **B)** sham, and **C)** 14-day 7.5 mg/kg/day, **D)** 28-day 7.5 mg/kg/day, **E)** 14-day 15 mg/kg/day, **F)** 28-day 15 mg/kg/day isotretinoin groups (black arrow points at dendrite extensions of the motor neuron) (Luxol fast bluex400)

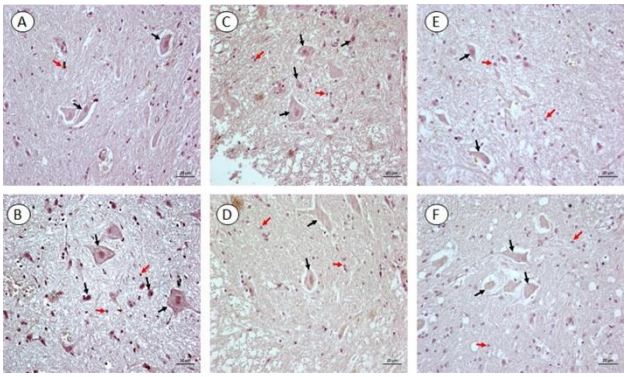

Figure 4. A) Control, **B)** sham, and **C)** 14-day 7.5 mg/kg/day, **D)** 28-day 7.5 mg/kg/day, **E)** 14-day 15 mg/kg/day, **F)** 28-day 15 mg/kg/day isotretinoin groups (black arrow points at motor neuron, red arrow at glial cell) (cleaved caspase 3x400)

the sham group (Figure 4D). The 14-day isotretinoin treatment at 15 mg/kg/day group showed cleaved caspase 3 expression levels similar to the control group and decreased compared to the sham group (Figure 4E). The 28-day isotretinoin treatment at 15 mg/kg/day group exhibited expression levels identical to those of the control group (Figure 4F).

Cleaved Caspase 3 Antibody

The cleaved caspase 3 expression was significantly increased in the sham group and the 14-day isotretinoin of 7.5 mg/kg/day group compared to the control group. The cleaved caspase 3 expression was statistically significantly decreased in the 28-day isotretinoin of 7.5 mg/kg/day, and 14-day and 28-day isotretinoin of 15 mg/kg/day groups, compared to the sham group. The cleaved caspase 3 expressions in the 28-day isotretinoin of 7.5 mg/kg/day, and 14-day and 28-day isotretinoin of 15 mg/kg/day groups were the same as in the control group. There was no statistically significant difference between the 14-day and the 28-day isotretinoin of 7.5 mg/kg/day and 14-day isotretinoin of 15 mg/kg/day groups.

DISCUSSION

Neurological dysfunction is usually because of secondary damage rather than the primary damage in SCI (5). The continuation of tissue damage due to the secondary damage mechanisms occurring after trauma has led to numerous experimental studies conducted to discover the treatment agents that are involved in this process (18-20). In the study, we investigate the therapeutic effect of isotretinoin on SCI.

Trauma itself causes the primary injury of sensitive spinal cord tissue that triggers secondary injury by enhanced inflammation, apoptosis, and free radical formation (21,22). Secondary damage mechanisms such as neurogenic shock, hemorrhages, ischemic reperfusion injury, secondary adverse effects of calcium, fluid-electrolyte imbalance, mitochondrial dysfunction, and apoptosis cause real damage to the spinal cord and healing mechanism (23). Caspase 3 is activated in the cells upon the resulting perfusion disorder, and apoptosis becomes dominant (21,22). As a result, SCI initiates a process that disrupts the blood-spinal cord barrier, leading to the infiltration of immune cells and the activation of inflammatory signaling pathways (24). Inflammation is responsible for secondary damage to the core and surrounding regions of the injury site (25). Vascular damage causes apoptosis, edema, and destruction of the white matter of the spinal cord (18,26).

Neutrophils, monocytes, microglia, and T-lymphocytes gather at the damaged area upon the inflammation response in ASCI. Neutrophils are immune cells that first arrive in the damaged area, activating other inflammatory cells and glial cells and secreting the cytokine, protease, and free radicals of the cascade that result in neuronal damage and death (27). Therefore, considered to be potentially effective in suppressing the inflammation; steroids, opioid antagonists, calcium channel blockers, volume expanders, tirilazad mesylate, and especially methylprednisolone due to its anti-inflammatory effect were widely used to suppress the inflammation (28). However, although intensive experimental and clinical studies have been carried out on this subject to date, such a molecule that has proven effective in this regard has not yet been described.

Isotretinoin is a molecule that acts as an anti-inflammatory agent by reducing the migration and movement of neutrophils to the tissue. It is also reported to stimulate the natural immune system and have an anti-inflammatory effect as it reduces T cell response (7,9). It has been shown to significantly diminish the levels of TNF-α, IL-4, IL-17, and IFN-γ released from T lymphocytes and have immunoregulatory effects (10-13). Isotretinoin also acts on matrix metalloproteinases (MMP). It is reported to reduce alleviate scar formation by reducing MMP-9 and MMP-13 (11,29).

In our study, we used isotretinoin, which has not been studied on a rat model before and whose anti-inflammatory effect is well known. Different groups of rats were formed considering that its effects may potentially vary according to the dose and term of its administration. To determine the dose of isotretinoin to be administered to rats, the rat dose of 7.5 mg/kg/day was taken as the base dose, after being calculated as the equivalent to the standard dose of isotretinoin (1 mg/kg/day) used for treating nodulocystic acne (30). The high dose was taken as twice this base dose.

Motor deficits were not found in the rats of the control group where only laminectomy was performed. It was planned to exclude any such rats that develop motor deficits. The inclined plane angles in the 7.5 mg/kg/day and 15 mg/kg/day isotretinoin groups were statistically lower than in the control group, while these inclined plane angles were increased significantly compared to the sham group. This finding indicated that isotretinoin rehabilitated walking on the inclined plane and resulted in improved motor examination. We found that this effect continued in the 28-day terms as well, but there was no further improvement as the dose increased. According to the evaluation based on the D&M criteria scores, we found that the low-high doses or short-long terms of isotretinoin administration did not make a difference when compared to the sham group. We believe that the reason why we were unable to detect the differences in the inclined plane angles in the D&M criteria scores is that we have not been able to correctly evaluate the traumatized subjects.

When cell integrity was evaluated by hematoxylin and eosin staining, it was observed that 7.5 mg/kg/day isotretinoin did not generate the expected changes in glial cells. In the 28-day isotretinoin of 7.5 mg/kg/day group, showed that the cytoplasmic volumes of motor neurons were found to diminish, and the cytoplasmic margins of some neurons were not identifiable. In glial cells, no histopathological changes were observed compared to the control and sham groups. In 14-day of increased treatment dose, it was seen that edema decreased in the motor neurons, the nuclei were identifiable, as well as that the cytoplasmic margins and nuclei of the motor neurons were identifiable similarly to the control group, while glial cells did not change reveal any changes as the term extended. Histopathological findings of the glial cells were found to be similar to that of the control group. These findings show that, as the dose and number of days of isotretinoin administration increase, isotretinoin significantly reduces necrosis and provides a level of improvement that makes a difference in histopathological terms.

In the examination by Luxol fast staining, it was observed that, in the 14-day isotretinoin of 7.5 mg/kg/day, the volume of the cell bodies of motor neurons diminished and

their extensions were not well identified, while the volumes of motor neurons were close to the control group and their cell extensions were identifiable in the 28-day term of the administration of the same dose. In the groups where the dose was 15 mg/kg/day, it was observed that the recovery was detected in the staining examinations on day 14. When the duration of treatment was increased to 28 days, the findings were found to be the same.

In the cleaved caspase 3 immunohistochemical staining where we intended to expose the cells' progress toward apoptosis, the 14-day isotretinoin of 7.5 mg/kg/day group had decreased staining of motor neurons and glial cells compared to the control group. It was determined that the staining decreased even more when the time was extended. It was also found that the cleaved caspase 3 expression of motor neurons and glial cells in the groups with increased dose was similar to the control group, while it was lower than the sham group. When the treatment period was 28 days, the cleaved caspase 3 expression continued to be the same as in the control group and its effect was maintained. These results show that isotretinoin corrects and restores the edema, ischemia, and membrane damage occurring as a result of the primary damage caused by trauma in ASCI. In addition, cleaved caspase 3 staining also demonstrated that it alleviates apoptosis, which is a very important step in secondary damage. Given the fact that these effects get more potent as the dose of isotretinoin and its duration of use increases, it makes sense to associate these effects with isotretinoin use. We believe that the motor recovery revealed in the inclined plane angle evaluation is thanks to the anti-inflammatory, antiapoptotic, and immunoregulatory effects of isotretinoin.

CONCLUSION

While the primary objective of this study is not to develop a standalone treatment agent for spinal cord trauma, it aims to identify a therapeutic agent that can complement both medical and surgical interventions. Based on the clinical and histopathological results obtained in the present study, it was understood that isotretinoin can be useful in ASCI.

Ethics Committee Approval: The study was approved by the Local Ethics Committee on Animal Experiments of Düzce University (16.11.2020, 11/03).

Conflict of Interest: None declared by the authors.

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REFERENCES

1. Yang R, Cai X, Li J, Liu F, Sun T. Protective effects of mir-129-5p on acute spinal cord injury rats. Med Sci Monit. 2019;25:8281-8.
2. Aytar MH, Civi S, Kaymaz M, Ergun E, Kaymaz FF, Pasaoglu A. The effect of quetiapine on treatment of experimental acute spinal cord injury. Turk Neurosurg. 2018;28(1):105-10.
3. Schwab ME, Bartholdi D. Degeneration and regeneration of axons in the lesioned spinal cord. Physiol Rev. 1996;76(2):319-70.
4. Demediuk P, Saunders RD, Clendenon NR, Means ED, Anderson DK, Horrocks LA. Changes in lipid metabolism in traumatized spinal cord. Prog Brain Res. 1985;63:211-26.
5. Tator CH. Review of experimental spinal cord injury with emphasis on the local and systemic circulatory effects. Neurochirurgie. 1991;37(5):291-302.
6. Kwon BK, Tetzlaff W, Grauer JN, Beiner J, Vaccaro AR. Pathophysiology and pharmacologic treatment of acute spinal cord injury. Spine J. 2004;4(4):451-64.
7. Karadağ AS. İzotretinoin. In: Sarıcaoğlu H, Ünal İ, Karaman G, Ferahbaş Kesikoğlu A, Karadağ, Şikar Aktürk A, et al., editors. Akne ve Rozase Tanı ve Tedavi, İstanbul: Galenos Yayınevi, 2018. p:242-58.
8. Bagatin E, Costa CS. The use of isotretinoin for acne - an update on optimal dosing, surveillance, and adverse effects. Expert Rev Clin Pharmacol. 2020;13(8):885-97.
9. Melnik BC. The role of transcription factor FoxO1 in the pathogenesis of acne vulgaris and the mode of isotretinoin action. G Ital Dermatol Venereol. 2010;145(5):559-71.
10. Karadag AS, Ertugrul DT, Bilgili SG, Takci Z, Akin KO, Calka O. Immunoregulatory effects of isotretinoin in patients with acne. Br J Dermatol. 2012;167(2):433-5.
11. Papakonstantinou E, Aletras AJ, Glass E, Tsogas P, Dionyssopoulos A, Adjaye J, et al. Matrix metalloproteinases of epithelial origin in facial sebum of patients with acne and their regulation by isotretinoin. J Invest Dermatol. 2005;125(4):673-84.
12. Thielitz A, Krautheim A, Gollnick H. Update in retinoid therapy of acne. Dermatol Ther. 2006;19(5):272-9.
13. Zouboulis CC. Isotretinoin revisited: pluripotent effects on human sebaceous gland cells. J Invest Dermatol. 2006;126(10):2154-6.
14. Kılıç G, Polat Ö, Şensoy G, Soylu H. Effects of isotretinoin and acitretin on neuroregeneration in experimental spinal cord injury. Acta Orthop Traumatol Turc. 2023;57(4):127-33.
15. Tascioglu T, Karatay M, Erdem Y, Tekiner A, Celik H, Sahin O, et al. Simvastatin in an experimental spinal cord injury model: a histopathological and biochemical evidence based study. Bratisl Lek Listy. 2020;121(10):722-6.
16. Rivlin AS, Tator CH. Objective clinical assessment of motor function after experimental spinal cord injury in the rat. J Neurosurg 1977;47(4):577-81.
17. Gürkan G, Sayin M, Kizmazoglu C, Erdogan MA, Yigitturk G, Erbak Yilmaz H, et al. Evaluation of the neuroprotective effects of ozone in an experimental spine injury model. J Neurosurg Spine. 2020;33(3):406-14.

18. Amar AP, Levy ML. Pathogenesis and pharmacological strategies for mitigating secondary damage in acute spinal cord injury. Neurosurgery. 1999;44(5):1027-40.
19. Lou J, Lenke LG, Ludwig FJ, O'Brien MF. Apoptosis as a mechanism of neuronal cell death following acute experimental spinal cord injury. Spinal Cord. 1998;36(10):683-90.
20. Lu J, Ashwell KW, Waite P. Advances in secondary spinal cord injury: Role of Apoptosis. Spine (Phila Pa 1976). 2000;25(14):1859-66.
21. Adams JM, Cory S. Life-or-death decisions by the Bcl-2 protein family. Trends Biochem Sci. 2001;26(1):61-6.
22. Curtin JF, Cotter TG. Live and let die: regulatory mechanisms in Fas-mediated apoptosis. Cell Signal. 2003;15(11):983-92.
23. Dumont RJ, Okonkwo DO, Verma S, Hurlbert RJ, Boulos PT, Ellegala DB, et al. Acute spinal cord injury, part I: pathophysiologic mechanisms. Clin Neuropharmacol. 2001;24(5):254-64.
24. Yakovlev AG, Faden AI. Sequential expression of c-fos protooncogene, TNF-alpha, and dynorphin genes in spinal cord following experimental traumatic injury. Mol Chem Neuropathol. 1994;23(2-3):179-90.
25. Hausmann O. Post-traumatic inflammation following spinal cord injury. Spinal Cord. 2003;41(7):369-78.
26. Arac D, Erdi MF, Keskin F, Kenan M, Cuce G, Aydemir FHY, et al. Neuroprotective effects of milrinone on experimental acute spinal cord injury: rat model. World Neurosurg. 2021;147:e225-33.
27. Rossignol S, Schwab M, Schwartz M, Fehlings MG. Spinal cord injury: time to move? J Neurosci. 2007;27(44):11782-92.
28. Topsakal C, Erol FS, Ozveren MF, Yilmaz N, Ilhan N. Effects of methylprednisolone and dextromethorphan on lipid peroxidation in an experimental model of spinal cord injury. Neurosurg Rev. 2002;25(4):258-66.
29. Mollan SP, Woodcock M, Siddiqi R, Huntbach J, Good P, Scott RA. Does use of isotretinoin rule out a career in flying? Br J Ophthalmol. 2006;90(8):957-9.
30. Goldsmith LA, Bolognia JL, Callen JP, Chen SC, Feldman SR, Lim HW, et al. American Academy of Dermatology Consensus Conference on the safe and optimal use of isotretinoin: summary and recommendations. J Am Acad Dermatol. 2004;50(6):900-6.

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Investigation of Anti-inflammatory, Antioxidative, and Cardioprotective Effects of Combined Metformin and Exercise in Rats

Sıçanlarda Kombine Metformin ve Egzersizin Anti-inflamatuar, Antioksidatif ve Kardiyoprotektif Etkilerinin Araştırılması

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ABSTRACT

Aim: This study aimed to investigate the anti-inflammatory, antioxidative and cardioprotective effects of exercise and metformin treatment applied alone or in combination.
Material and Methods: In this study, 42 male Wistar rats were used. The rats were separated into six groups as control (CONT), exercise (EXE), 100 mg/kg metformin (M100), 200 mg/kg metformin (M200), 100 mg/kg metformin+exercise (M100+EXE), and 200 mg/kg metformin+exercise (M200+EXE). Exercise was applied for 10 weeks including exercise training. Metformin was administered 30 minutes before exercise. At the end of the study, levels of C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-10 (IL-10), cardiac troponin-I (cTn-I), creatine kinase-muscle/brain (CK-MB), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) in serum samples from rats were quantified using the ELISA method.
Results: The combined application of metformin and exercise significantly decreased cTn-I, CK-MB, MDA, TNF-α, CRP and IL-6 levels ($p<0.001$). In contrast, it increased SOD, CAT, GPx, and IL-10 levels significantly ($p<0.001$). Glucose levels of groups treated alone or in combination were found statistically significantly less than CONT group ($p<0.001$).
Conclusion: The findings of this study reveal that both metformin and exercise administration, alone or in combination, exert significant anti-inflammatory, antioxidant, and cardioprotective effects in Wistar rats. These results suggest that combining metformin therapy with regular exercise may offer a synergistic approach to reducing cardiovascular risk factors and enhancing antioxidant defenses.
Keywords: Longevity; cardiovascular diseases; hyperglycemia; oxidative stress; metformin; exercise.

ÖZ

Amaç: Bu çalışmanın amacı egzersiz ve metformin tedavisinin tek başına veya birlikte uygulanmasının anti-enflamatuar, antioksidatif ve kardiyoprotektif etkilerinin araştırılmasıdır.
Gereç ve Yöntemler: Bu çalışmada 42 adet erkek Wistar sıçan kullanılmıştır. Sıçanlar kontrol (CONT), egzersiz (EXE), 100 mg/kg metformin (M100), 200 mg/kg metformin (M200), 100 mg/kg metformin+egzersiz (M100+EXE) ve 200 mg/kg metformin+egzersiz (M200+EXE) olmak üzere altı gruba ayrılmıştır. Egzersiz, egzersiz eğitimi de dahil olmak üzere 10 hafta boyunca uygulanmıştır. Metformin egzersizden 30 dakika önce uygulanmıştır. Çalışma sonunda sıçanlardan alınan serum örneklerinde, C-reaktif protein (CRP), tümör nekroz faktörü-alfa (TNF-α), interlökin-6 (IL-6), interlökin-10 (IL-10), kardiyak troponin-I (cTn-I), kreatin kinaz-kas/beyin (CK-MB), katalaz (CAT), süperoksit dismutaz (SOD), glutatyon peroksidaz (GPx) ve malondialdehit (MDA) ELISA yöntemi kullanılarak ölçülmüştür.
Bulgular: Metformin ve egzersizin birlikte uygulanması cTn-I, CK-MB, MDA, TNF-α, CRP ve IL-6 düzeylerini anlamlı şekilde azaltmıştır ($p<0,001$). Buna karşılık, SOD, CAT, GPx ve IL-10 düzeylerini de önemli şekilde artırmıştır ($p<0,001$). Tek başına veya kombinasyon halinde tedavi edilen grupların glukoz seviyeleri CONT grubundan istatistiksel olarak anlamlı şekilde daha düşük bulunmuştur ($p<0,001$).
Sonuç: Bu çalışmanın bulguları, hem metformin hem de egzersiz uygulamasının, tek başına veya kombinasyon halinde, Wistar sıçanlarında önemli anti-inflamatuar, antioksidan ve kardiyoprotektif etkiler gösterdiğini ortaya koymaktadır. Bu sonuçlar, metformin tedavisinin düzenli egzersizle birleştirilmesinin, kardiyovasküler risk faktörlerini azaltmak ve antioksidan savunmaları güçlendirmek için sinerjik bir yaklaşım sunabileceğini düşündürmektedir.
Anahtar kelimeler: Uzun ömür; kardiyovasküler hastalıklar; hiperglisemi; oksidatif stress; metformin; egzersiz.

INTRODUCTION

Cardiovascular diseases (CVDs) are among the top causes of mortality worldwide. In 2019, an estimated 17.9 million people died from CVDs, accounting for 32% of total worldwide mortality. 85% of these fatalities were caused by heart attacks or strokes (1). Most CVDs can be avoided by addressing lifestyle risks such as tobacco use, poor nutrition, being overweight, lack of exercise, and problematic alcohol consumption (2). Myocardial infarction (MI), sometimes known as a heart attack, is the leading cause of cardiovascular mortality in most nations. MI occurs when one or more of the coronary arteries that feed blood to the heart get clogged, depriving a portion of the heart of oxygen and nutrients and eventually leading to myocardial necrosis (3). Many risk factors for MI are modifiable and can therefore be preventable in many cases. Changes in blood creatine kinase-muscle/brain (CK-MB), lactate dehydrogenase (LDH), and troponin-T/troponin-I levels can be used to determine cardiac injury after MI (4). These alterations are followed by large increases in superoxide anion and hydroxyl radical levels (5). As a result, antioxidants have emerged as an effective technique for preventing oxidative damage in cardiac injury (6).

Metformin, a biguanide often used for diabetic therapy, has been shown to improve ventricular function (6). Metformin therapy has been linked to increased phosphorylation of protein kinase triggered by adenosine monophosphate (AMP), affecting intracellular pathways and mitochondrial function. Experimental findings indicate that administering metformin before and during ischemia-reperfusion may impact these protective mechanisms and sustain left ventricular function regardless of glucose status (7,8). Furthermore, studies conducted in patients with acute myocardial infarction (AMI) have shown that metformin, compared to other antihyperglycemic strategies, is associated with lower peak levels of creatine kinase (CK), myocardial CK-MB band, and troponins in patients with type 2 diabetes (T2DM), as well as improved survival following STEMI (9,10).

Exercise is well-established to enhance oxygen delivery and utilization, thereby improving cardiorespiratory fitness. Furthermore, during high-intensity, short-duration exercise, muscle glycogen serves as the primary energy source (11). Exercise capacity is defined as the maximum level of physical effort that an individual is capable of sustaining. Despite the pronounced cytokine response to exercise, there is limited indirect or direct evidence linking oxidative stress with proinflammatory cytokine production. One study reported that acute exercise-induced oxidative stress did not influence cytokine release 30 minutes post-exercise (12). However, following a repeated cycling exercise for three days (90 minutes/day), it was found that plasma interleukin-6 (IL-6) concentration significantly increased at the 30th minute of exercise on the first day, while reactive oxygen species (ROS) production occurred 60 minutes later (13).

In light of the above information, it is suggested that the increased oxidative stress induced by exercise can be modulated by the antioxidant activity of metformin. This study aimed to investigate the anti-inflammatory, antioxidative, and cardioprotective effects of exercise and metformin treatment when applied alone or in combination.

MATERIAL AND METHODS

Animals

The rats used in this study were obtained from the Experimental Animals Application and Research Center of Düzce University. Experiments were conducted in a laboratory environment with a room temperature of 23°C, 60±5% humidity, and a 12:12 hour light-dark cycle. A total of, forty-two male Wistar rats, weighing 230±30 g and aged 2-3 months, with ad libitum access to food and water, were used in this study. Ethical approval for this study was obtained from the Düzce University's Local Ethics Committee for Animal Research with the code 2021/11/01. The Animal Research: Reporting of in Vivo Experiments (ARRIVE) standards require the reporting of all study methodologies.

Groups, Substances, and Doses

The rats were divided into six groups, each consisting of seven rats: control (CONT), exercise (EXE), 100 mg/kg metformin (M100), 200 mg/kg metformin (M200), 100 mg/kg metformin+exercise (M100+EXE), and 200 mg/kg metformin+exercise (M200+EXE). Metformin (Biovision Inc., Milpitas, CA, USA) dissolved in saline was given intraperitoneally (i.p.) at dosages of 100 and 200 mg/kg. Metformin and saline were given 30 minutes before the exercise (Figure 1). Anesthesia was administered intramuscularly (i.m.) with 90 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride. All drugs were prepared fresh daily.

Treadmill Exercise Protocols

Exercise training was given to the rats to train them to run regularly ensuring they would not have difficulty in subsequent running-based exercises. Exercise training protocols for the treadmill were adapted from the literature (14-16). Rats in the exercise groups (EXE, M100+EXE, and M200+EXE) were given a 2-week acclimatization period to adjust to their new surroundings, followed by 8 weeks of exercise training. The rats were trained for 15 minutes each day to run on a horizontal treadmill (May Time 0804, Animal Treadmill). For the training of the animals, the treadmill speed was set to 2 m/min for the first 2 days (Days 1 and 2). Then, the speed was increased to 5 m/min between Days 3 and 5, and for the final 5 days (Days 6 to 10), it was raised to 8 m/min. The exercise protocol described in the literature was followed for

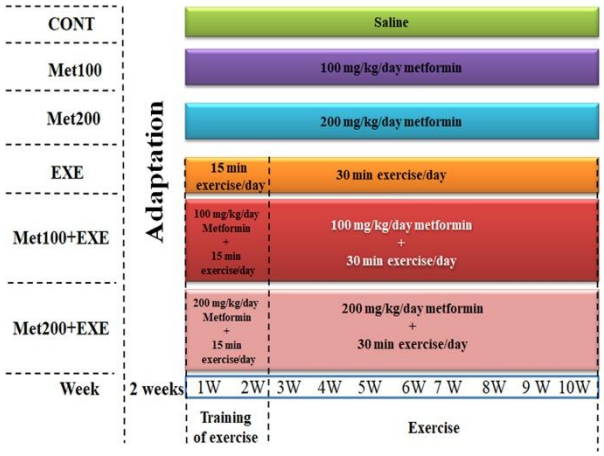

Figure 1. Representative illustration of the experimental procedure

the main study. The rats were exercised for 30 minutes a day, 6 days a week, between 08.00 am and 10.00 am for 10 weeks. Every day, the rats were put through three phases of exercise: 2 m/min for 5 minutes, 5 m/min for 5 minutes, and 8 m/min for 20 minutes. Rats in the CONT, M100, and M200 groups spent an equal amount of time on a static treadmill with no activity.

Termination of the Study

Blood was drawn from the rats' hearts via cardiac puncture under ketamine/xylazine anesthesia 24 hours following the last injection. The animals were subsequently sacrificed via cervical dislocation under anesthesia. Blood samples were centrifuged at 4000 rpm for 15 minutes, and serum was kept at -80°C until analysis.

Determination of Biochemical Biomarkers

Cardiac troponin-I (cTn-I), CK-MB, tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), IL-6, interleukin-10 (IL-10), malondialdehyde (MDA), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) in serum samples from rats were quantified using the sandwich enzyme-linked immunosorbent assay (ELISA) method. ELISA kits from SunRed (SunRed, China) and ELK (ELK Biotechnology CO., Ltd., Wuhan, China) were used in the study. Following the manufacturer's protocols, IL-10 (Cat: 201-11-0109), IL-6 (Cat: 201-11-0136), TNF-α (Cat: 201-11-0765), CRP (Cat: 201-11-0054), CK-MB (Cat: 201-11-0312), cTn-I (Cat: 201-11-0640), GPx (ELK2222), CAT (ELK5986), SOD (ELK5616), and MDA (ELK8616) levels were determined by ELISA reader was measured in serum.

Statistical Analysis

Compliance with normal distribution was evaluated by the Shapiro-Wilk test. All data were analyzed using means and standard deviations. The groups were compared using a one-way analysis of variance (ANOVA), and the different groups were identified using Tukey's multiple comparisons test. A p-value of ≤0.05 was accepted as statistically significant. All statistical analyses were performed using the IBM SPSS software v.22.0.

RESULTS

Cardioprotective Effect of Combined Administration of Metformin and Exercise

When the serum CK-MB levels of the groups were compared, a statistically significant difference was observed between groups (p<0.001, Table 1, Figure 2A). The EXE group had significantly greater CK-MB levels compared to the CONT, M100, M200, M100+EXE, and M200+EXE groups (p=0.001, p=0.010, p=0.003, p<0.001, and p=0.001, respectively).

When cTn-I levels were examined between groups, there was a substantial difference (p<0.001, Table 1, Figure 2B). In-depth analysis revealed that the EXE group had significantly greater cTn-I levels than the CONT, M100, M200, M100+EXE, and M200+EXE groups (p=0.008, p=0.010, p<0.001, p<0.001, and p<0.001, respectively).

Pro- and Anti-inflammatory Effects of Combined Application of Metformin and Exercise

When serum TNF-α levels of the groups were compared, a statistically significant difference was identified between the groups (p<0.001, Table 1, Figure 3A). The EXE group had significantly greater TNF-α levels compared to the CONT, M100, M200, M100+EXE, and M200+EXE groups (p<0.001, p<0.001, p=0.020, p<0.001, and p=0.002, respectively).

When the groups were compared in terms of CRP levels, a statistically significant difference was found between the groups (p<0.001, Table 1, Figure 3B). The EXE group showed significantly greater CRP levels compared to the CONT, M100, M200, M100+EXE, and M200+EXE groups (p<0.001 for all comparisons). Similarly, the M200+EXE group had significantly greater CRP levels than the M100 group (p=0.050).

When IL-6 levels were examined between groups, there was a substantial difference (p<0.001, Table 1, Figure 3C). The EXE group had significantly greater IL-6 levels compared to the CONT, M100, and M200 groups (p<0.001 for all comparisons). Similarly, the M100+EXE (p=0.010) and M200+EXE (p=0.020) groups had significantly greater IL-6 levels compared to the CONT group.

When IL-10 levels were examined between groups, there was a substantial difference (p<0.001, Table 1, Figure 3D). The CONT group had significantly reduced IL-10 levels compared to the EXE, M100, M200, M100+EXE, and M200+EXE groups (p<0.001 for all comparisons).

Oxidative and Antioxidative Effect of Combined Application of Metformin and Exercise

When the groups were compared in terms of serum MDA levels, a statistically significant difference was determined between the groups (p<0.001, Table 1, Figure 4A). The EXE group showed significantly greater MDA levels compared to the CONT, M100, M200, M100+EXE, and M200+EXE groups (p<0.001 for all comparisons). In contrast, the CONT group had significantly lower MDA levels than M100+EXE and M200+EXE groups (p=0.007, and p=0.001, respectively). Similarly, the M100 group had significantly lower MDA levels than M100+EXE (p=0.007) and M200+EXE (p=0.001) groups.

Serum GPx levels were examined between groups, there was a substantial difference (p<0.001, Table 1 Figure 4B). The CONT group had significantly lower GPx levels than the EXE, M100, M200, M100+EXE, and M200+EXE groups (p<0.001 for all comparisons). Additionally, the EXE group showed substantially reduced GPx levels compared to the M100, M200, M100+EXE, and M200+EXE groups (p<0.001 for all comparisons).

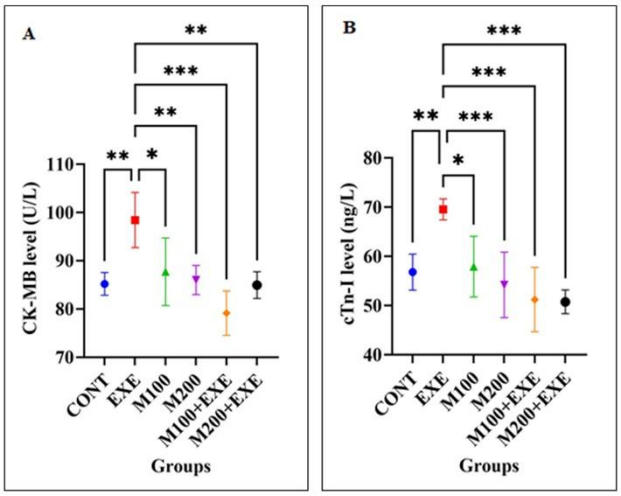

Figure 2. The effect of combined metformin and exercise on cardiac biomarkers (*p<0.05, **p<0.01 and ***p<0.001)

A statistically significant difference was found in CAT levels, between groups (p<0.001, Table 1, Figure 4C). The CONT group had significantly lower CAT levels compared to the M100+EXE and M200+EXE groups (p=0.001, and p=0.005, respectively). Similarly, the EXE group had significantly lower CAT levels than the M100+EXE and M200+EXE groups (p=0.008, and p=0.030, respectively). Serum SOD levels were examined between groups, there was a substantial difference (p<0.001, Table 1, Figure 4D). The CONT group showed significantly reduced SOD levels compared to the EXE, M100, M200, M100+EXE, and M200+EXE groups (p<0.001 for all comparisons).

Table 1. Effect of combined exercise and metformin on biochemical biomarkers

	CONT	EXE	M100	M200	M100+EXE	M200+EXE	p
CK-MB (U/L)	85.19±2.35$^{\Delta}$	98.43±5.72	87.73±6.99$^{\Delta}$	86.00±3.01$^{\Delta}$	79.14±4.60$^{\Delta}$	84.94±2.76$^{\Delta}$	**<0.001**
cTn-I (ng/L)	56.80±3.64$^{\Delta}$	69.56±2.14	57.92±6.17$^{\Delta}$	54.20±6.64$^{\Delta}$	51.22±6.53$^{\Delta}$	50.76±2.41$^{\Delta}$	**<0.001**
TNF-α (ng/L)	64.01±3.88$^{\Delta}$	74.79±2.67	62.46±4.43$^{\Delta}$	67.08±3.96$^{\Delta}$	64.43±4.37$^{\Delta}$	65.40±3.22$^{\Delta}$	**<0.001**
CRP (ng/mL)	34.02±3.14$^{\Delta}$	46.40±3.23	31.90±2.94$^{\Delta\#}$	33.10±2.07$^{\Delta}$	35.20±1.90$^{\Delta}$	36.40±1.61$^{\Delta}$	**<0.001**
IL-6 (pg/mL)	34.45±2.73$^{\Delta}$	47.80±5.25	37.63±5.04$^{\Delta}$	36.20±2.14$^{\Delta}$	42.52±2.96$^{\Delta}$	42.25±3.76$^{\Delta}$	**<0.001**
IL-10 (ng/mL)	19.18±1.37	30.13±4.06*	32.27±3.05*	33.35±7.30*	29.89±2.13*	31.26±3.27*	**<0.001**
MDA (pg/mL)	641.5±64.67$^{\Delta}$	1415±157.90	663.5±65.33$^{\Delta}$	742.30±82.99$^{\Delta}$	826.8±44.52$^{*\Delta\#}$	863.20±54.02$^{*\Delta}$	**<0.001**
GPx (pg/mL)	85.68±3.27	101.30±2.65*	125.60±9.52$^{*\Delta}$	124.10±10.53$^{*\Delta}$	133.80±2.94$^{*\Delta}$	127.00±8.34$^{*\Delta}$	**<0.001**
CAT (ng/mL)	11.15±0.27	12.39±0.39	15.26±5.51	14.54±3.77	18.71±0.69$^{*\Delta}$	17.74±2.00$^{*\Delta}$	**<0.001**
SOD (U/mL)	0.755±0.12	1.611±0.37*	1.679±0.04*	1.809±0.19*	1.991±0.24*	1.761±0.28*	**<0.001**
Glucose (mg/dL)	206.30±10.42	180.90±5.90*	175.00±21.59*	179.00±14.87*	167.10±7.19*	175.30±4.64*	**<0.001**

CONT: control, EXE: exercise, M100: 100 mg/kg metformin, M200: 200 mg/kg metformin, M100+EXE: 100 mg/kg metformin+exercise, M200+EXE: 200 mg/kg metformin+exercise, CK-MB: creatine kinase-muscle/brain, cTn-I: cardiac troponin-I, TNF-α: tumor necrosis factor-alpha, CRP: C-reactive protein, IL-6: interleukin-6, IL-10: interleukin-10, MDA: malondialdehyde, GPx: glutathione peroxidase, CAT: catalase, SOD: superoxide dismutase, *: significant difference compared to CONT group; Δ: significant difference compared to EXE group; #: significant difference compared to M100 group

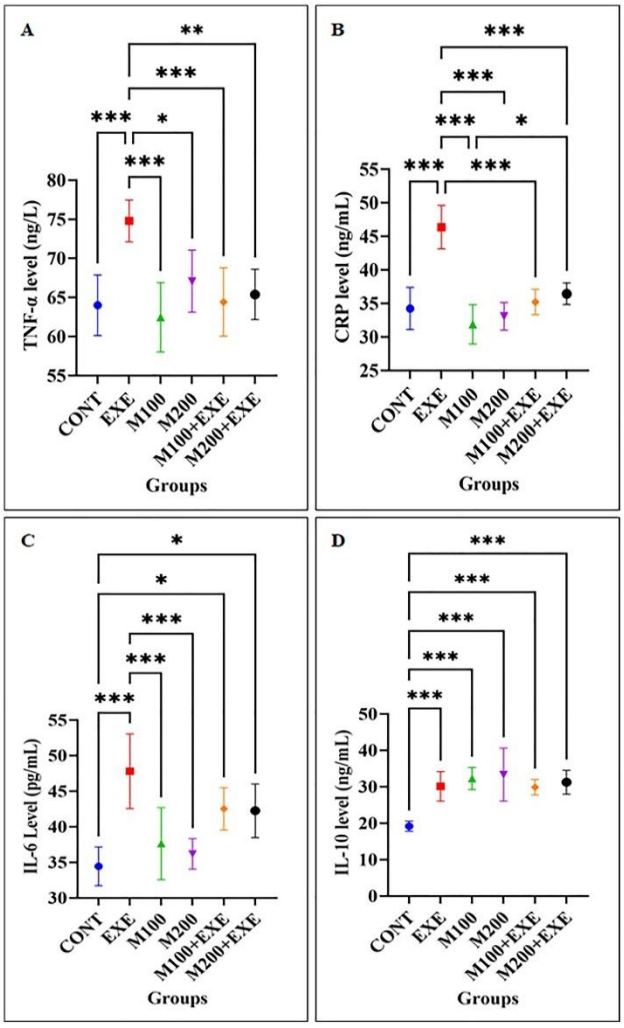

Figure 3. The effect of combined administration of metformin and exercise on oxidative and antioxidative parameters (*p<0.05, **p<0.01, and ***p<0.001)

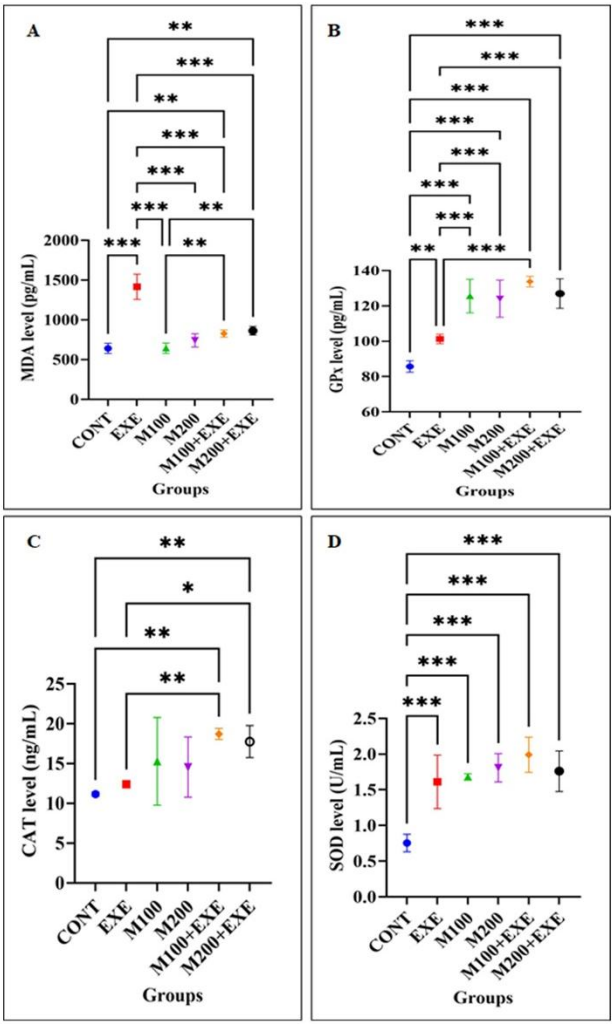

Figure 4. The effect of combined administration of metformin and exercise on pro- and anti-inflammatory parameters (*p<0.05, **p<0.01, and ***p<0.001)

The Effect of the Combination of Metformin and Exercise on Blood Glucose Levels

In terms of blood glucose levels, a statistically significant difference was determined between the groups ($p<0.001$, Table 1, Figure 5). The CONT group had significantly higher blood glucose levels than the EXE, M100, M200, M100+EXE, and M200+EXE groups ($p=0.005$, $p<0.001$, $p=0.002$, $p<0.001$, and $p<0.001$, respectively).

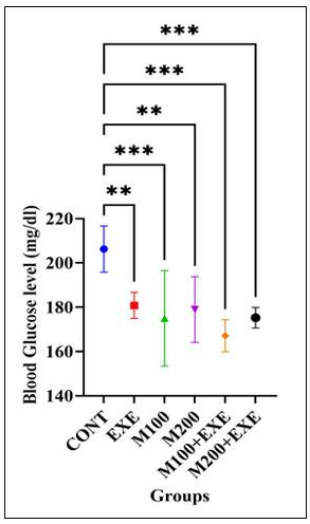

Figure 5. The effect of combined administration of metformin and exercise on blood glucose level (**p<0.01, and ***p<0.001)

DISCUSSION

In this study, the effects of alone or combined exercise and metformin administration on serum cardiac biomarkers, glucose, oxidant, and antioxidant parameters were evaluated. The CK-MB and cTn-I levels were found to be higher in the group subjected to exercise alone, whereas the CK-MB and cTn-I levels were lower in the groups where metformin was administered alone or in combination with exercise. Similarly, the TNF-α and CRP levels were lower in the groups where metformin was administered alone or with exercise compared to the EXE group. The IL-6 levels were lower in the groups where only metformin was administered compared to the EXE group, while the IL-6 levels were higher in the groups where exercise and metformin were administered together compared to the CONT group. The IL-10 levels were found to be higher in all groups where either exercise, metformin, or their combination was applied compared to the CONT group. However, the blood glucose levels were found to be lower than those in the CONT group. The MDA level, an oxidative marker, was higher only in the exercise group, while it was lower in the groups where metformin was administered alone or in combination with exercise. Conversely, the levels of antioxidant parameters SOD, CAT, and GPx were particularly higher in the groups where metformin and exercise were administered together.

Studies aimed at increasing the healthspan—the period of life free from chronic diseases—are on the rise (17,18). Recently, it has been shown that chronic diseases grow increasingly resistant to treatment as people age, and while medication may lower the chance of death from one disease, it may raise the risk of death from others (18). The present medical strategy is limited to therapy after illness onset, sustaining competing risks. Preventing disease onset is an alternate technique for shortening the duration of illness. According to a widely recognized definition of healthspan, a therapy targeted at prolonging healthspan begins before the onset of chronic disease and stops when chronic disease begins (17). While there are acknowledged flaws with this definition, it is useful in distinguishing a time of general health from one defined by the buildup of chronic disorders. Therefore, strategies to preserve healthspan are inherently preventive.

The therapeutic benefits of exercise in preventing and treating various chronic diseases are indisputable (19). Regular exercise not only prevents the onset of disease but also slows the progression of disease in the absence of physical activity. Aerobic capacity refers to a physiological system's robust ability to provide and utilize oxygen during high-intensity activity. Low aerobic capacity increases the risk of several illnesses, including CVD, cancer, and diabetes (20). Individuals with high or moderate aerobic capacity, on the other hand, are at a much-decreased risk of chronic illnesses and early mortality (18). Intense endurance exercise alters immune system homeostasis, resulting in leukocytosis and immunological suppression from neutrophils in the systemic circulation (21,22). Numerous studies have found increases in IL-6, IL-10, IL-1ra, and IL-8 during prolonged endurance exercise (22-24). However, during and after short-term intense exercise and eccentric contraction exercise, changes in the levels of these cytokines appear to be negligible (23,25). These data imply that cytokine responses are more closely connected to exercise intensity and duration (physiological load/stress) than to exercise-induced muscle injury (23,26).

Metformin is a biguanide widely used in the treatment of T2DM (27). Its antihyperglycemic effect is not due to stimulation of insulin secretion, rather its influence on peripheral tissues renders them more susceptible to insulin action. Metformin's influence on glucose levels has been demonstrated in vitro and in vivo by its capacity to diminish hepatic glucose production while increasing peripheral glucose absorption (28). Metformin primarily targets the liver, but it also influences metabolic processes in skeletal muscle, adipose tissue, the gut, the brain, and cardiovascular tissues (29-32). Metformin reduces hepatic glucose synthesis by decreasing gluconeogenesis and boosting glucose absorption in skeletal muscle (29,32). In addition to its glucose-lowering efficacy, studies in preclinical models over the past two decades have recognized other beneficial effects of metformin (33). Different durations and doses of metformin administration have been shown to reduce cTn-I and CK-MB levels (34-36). The antioxidant activity of metformin in cardiomyocytes occurs through AMP-activated protein kinase (AMPK) activation. This reduces ROS production in animal models of heart failure. Metformin can protect cardiomyocytes from oxidative stress induced by H_2O_2 or TNF-α (33). A multicenter prospective study reported that a 2-year metformin treatment in prediabetic patients with stable angina and nonobstructive coronary stenosis reduced inflammation and oxidative stress markers. The same study also reported improvements in epicardial endothelial dysfunction (37). A decrease in CRP, IL-6, and TNF-α levels after a 3-month metformin treatment was reported in patients with carotid artery atherosclerosis (38). In patients with metabolic syndrome, a 7-day metformin pretreatment significantly reduced CRP, CK-MB, and cTn-I levels (39).

The combination of exercise and metformin administration appears logical for two reasons. Firstly, although their targets (liver and skeletal muscle) are different, they are complementary. Secondly, both metformin and exercise partly exhibit their beneficial effects through AMPK. Metformin and exercise capacity are intricately linked. Muscle contraction activates AMPK. There is mounting evidence that metformin boosts AMPK activation in the liver, muscle, and other tissues (40). Due to energy depletion, glycolysis, and phosphocreatine energy systems are activated to maintain normal cellular metabolism (41). Metformin has anti-inflammatory properties, albeit the mechanisms are not fully understood (42). However, metformin has been shown to exert anti-inflammatory effects in various disease models and humans. Studies have reported that metformin reduces TNF-α, IL-1β, and IL-6, and increases IL-10 (43,44). Another study reported that metformin reduces CRP levels (45).

Metformin and exercise were given to prediabetic male Wistar rats that had been fed a high-fat diet and sugary beverages for four weeks (46). Rats that received both exercise and metformin showed lower levels of inflammatory factors, improved lipid metabolism, and reduced mitochondrial oxidative stress levels. Another study reported that the combination of exercise and metformin alleviated diabetes-induced cardiac complications (47). In a study investigating the effects of the combination of metformin and exercise on inflammation and apoptosis mechanisms in rats, it was found that the combined application of metformin and exercise reduced TNF-α and IL-6 levels (48). In a study examining the effects of exercise and metformin in adult patients with T2DM, 699 patients were subjected to either exercise or metformin treatment for twenty-four weeks. By the end of the therapy, both groups had normalized their fasting blood glucose, HbA1c, BMI, and lipid profile values (49).

Hyperglycemia triggers the excessive production of ROS, which can lead to harmful effects through various pathways, including lipid peroxidation and the production of highly reactive aldehydes like MDA. It also leads to the degradation of antioxidant defense systems such as GPx, SOD, and CAT (50). In streptozotocin (STZ)-induced diabetic rats, the combined application of metformin and exercise significantly reduced the MDA levels and improved antioxidant status (CAT and SOD), thereby significantly reducing oxidative stress compared to the diabetic group (51).

CONCLUSION

This study highlights the beneficial effects of combining exercise with metformin in reducing oxidative stress and improving antioxidant defenses. The significant reduction in MDA levels and enhancement in antioxidant enzyme activities, such as SOD, CAT, and GPx, suggests that this combination can effectively reduce oxidative damage and improve overall cellular health. This, in turn, may contribute to lower levels of cardiac biomarkers like CK-MB and cTn-I, indicating a potential positive impact on cardiovascular health. While these findings support the therapeutic potential of combining exercise with metformin, further research is essential to fully elucidate the cardioprotective mechanisms underlying this combination. Ultimately, this may pave the way for broader adoption and implementation of these interventions in promoting cardiovascular health, both in individuals with existing conditions and in those seeking preventive measures.

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REFERENCES

1. Luengo-Fernandez R, Little M, Gray A, Torbica A, Maggioni AP, Huculeci R, et al. Cardiovascular disease burden due to productivity losses in European Society of Cardiology countries. Eur Heart J Qual Care Clinical Outcomes. 2024;10(1):36-44.
2. Gök A, Beyazçiçek E. Experimental animal models in heart disease. Duzce Med J. 2024;26(S1):79-86.
3. Nabofa WEE, Alashe OO, Oyeyemi OT, Attah AF, Oyagbemi AA, Omobowale TO, et al. Cardioprotective effects of curcumin-nisin based poly lactic acid nanoparticle on myocardial infarction in guinea pigs. Sci Rep. 2018;8(1):16649.
4. Hemalatha KL, Stanely Mainzen Prince P. Anti-inflammatory and anti-thrombotic effects of zingerone in a rat model of myocardial infarction. Eur J Pharmacol. 2016;791:595-602.
5. Zhu P, Liu J, Shi J, Zhou Q, Liu J, Zhang X, et al. Melatonin protects ADSCs from ROS and enhances their therapeutic potency in a rat model of myocardial infarction. J Cell Mol Med. 2015;19(9):2232-43.
6. Ansari MA, Iqubal A, Ekbbal R, Haque SE. Effects of nimodipine, vinpocetine and their combination on isoproterenol-induced myocardial infarction in rats. Biomed Pharmacother. 2019;109:1372-80.
7. Yin M, van der Horst IC, van Melle JP, Qian C, van Gilst WH, Silljé HH, et al. Metformin improves cardiac function in a nondiabetic rat model of post-MI heart failure. Am J Physiol Heart Circ Physiol. 2011;301(2):H459-68.
8. Gundewar S, Calvert JW, Jha S, Toedt-Pingel I, Ji SY, Nunez D, et al. Activation of AMP-activated protein kinase by metformin improves left ventricular function and survival in heart failure. Circ Res. 2009;104(3):403-11.

9. Lexis CP, Wieringa WG, Hiemstra B, van Deursen VM, Lipsic E, van der Harst P, et al. Chronic metformin treatment is associated with reduced myocardial infarct size in diabetic patients with ST-segment elevation myocardial infarction. Cardiovasc Drug Ther. 2014;28(2):163-71.
10. Mellbin LG, Malmberg K, Norhammar A, Wedel H, Rydén L; DIGAMI 2 Investigators. Prognostic implications of glucose-lowering treatment in patients with acute myocardial infarction and diabetes: experiences from an extended follow-up of the Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction (DIGAMI) 2 Study. Diabetologia. 2011;54(6):1308-17.
11. Das S, Behera SK, Srinivasan A, Xavier AS, Selvarajan S, Kamalanathan S, et al. Effect of metformin on exercise capacity: a meta-analysis. Diabetes Res Clin Pract. 2018;144:270-8.
12. Steinberg JG, Ba A, Brégeon F, Delliaux S, Jammes Y. Cytokine and oxidative responses to maximal cycling exercise in sedentary subjects. Med Sci Sports Exerc. 2007;39(6):964-8.
13. Suzuki K, Totsuka M, Nakaji S, Yamada M, Kudoh S, Liu Q, et al. Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. J Appl Physiol (1985). 1999;87(4):1360-7.
14. Zhao N, Yan QW, Xia J, Zhang XL, Li BX, Yin LY, et al. Treadmill exercise attenuates Aβ-induced mitochondrial dysfunction and enhances mitophagy activity in APP/PS1 transgenic mice. Neurochem Res. 2020;45(5):1202-14.
15. Cetinkaya A, Demir S, Orallar H, Kayacan Y, Beyazcicek E. The effects of treadmill exercise on oxidative stress in Mongolian gerbils with penicillin-induced epilepsy. Exp Biomed Res. 2018;1(1):10-16.
16. Kayacan Y, Bahadir A, Cetinkaya A, Orallar H, Cakir S, Beyazcicek E, et al. Penicillin-induced epileptiform ECoG activity in gerbils: effects of physical exercise and a Diospyros kaki extract. Neurophysiology. 2016;48(5):367-74.
17. Kaeberlein M. How healthy is the healthspan concept? Geroscience. 2018;40(4):361-4.
18. Miller BF, Thyfault JP. Exercise-pharmacology interactions: metformin, statins, and healthspan. Physiology (Bethesda). 2020;35(5):338-47.
19. Ruegsegger GN, Booth FW. Health benefits of exercise. Cold Spring Harb Perspect Med. 2018;8(7):a029694.
20. Willis BL, Gao A, Leonard D, Defina LF, Berry JD. Midlife fitness and the development of chronic conditions in later life. Arch Intern Med. 2012;172(17):1333-40.
21. Ma S, Tominaga T, Kanda K, Sugama K, Omae C, Hashimoto S, et al. Effects of an 8-week protein supplementation regimen with hyperimmunized cow milk on exercise-induced organ damage and inflammation in male runners: A randomized, placebo controlled, cross-over study. Biomedicines. 2020;8(3):51.
22. Goh J, Lim CL, Suzuki K. Effects of endurance-, strength-, and concurrent training on cytokines and inflammation. In: Schumann M, Rønnestad BR, editors. Concurrent aerobic and strength training: Scientific basics and practical applications. Springer, Cham; 2019. p.125-38.
23. Suzuki K. Characterization of exercise-induced cytokine release, the impacts on the body, the mechanisms and modulations. Int J Sports Exerc Med. 2019;5(3):122.
24. Suzuki K, Tominaga T, Ruhee RT, Ma S. Characterization and modulation of systemic inflammatory response to exhaustive exercise in relation to oxidative stress. Antioxidants (Basel). 2020;9(5):401.
25. Peake JM, Roberts LA, Figueiredo VC, Egner I, Krog S, Aas SN, et al. The effects of cold water immersion and active recovery on inflammation and cell stress responses in human skeletal muscle after resistance exercise. J Physiol. 2017;595(3):695-711.
26. Suzuki K. Cytokine response to exercise and its modulation. Antioxidants (Basel). 2018;7(1):17.
27. Iranshahy M, Rezaee R, Karimi G. Hepatoprotective activity of metformin: A new mission for an old drug? Eur J Pharmacol. 2019;850:1-7.
28. Ajzashokouhi AH, Bostan HB, Jomezadeh V, Hayes AW, Karimi G. A review on the cardioprotective mechanisms of metformin against doxorubicin. Hum Exp Toxicol. 2020;39(3):237-48.
29. Konopka AR, Esponda RR, Robinson MM, Johnson ML, Carter RE, Schiavon M, et al. Hyperglucagonemia mitigates the effect of metformin on glucose production in prediabetes. Cell Rep. 2016;15(7):1394-400.
30. Konopka AR, Laurin JL, Schoenberg HM, Reid JJ, Castor WM, Wolff CA, et al. Metformin inhibits mitochondrial adaptations to aerobic exercise training in older adults. Aging Cell. 2019;18(1):e12880.
31. Kulkarni AS, Brutsaert EF, Anghel V, Zhang K, Bloomgarden N, Pollak M, et al. Metformin regulates metabolic and nonmetabolic pathways in skeletal muscle and subcutaneous adipose tissues of older adults. Aging Cell. 2018;17(2):e12723.
32. Madiraju AK, Qiu Y, Perry RJ, Rahimi Y, Zhang XM, Zhang D, et al. Metformin inhibits gluconeogenesis via a redox-dependent mechanism in vivo. Nat Med. 2018;24(9):1384-94.
33. Triggle CR, Mohammed I, Bshesh K, Marei I, Ye K, Ding H, et al. Metformin: Is it a drug for all reasons and diseases? Metabolism. 2022;133:155223.
34. Zilinyi R, Czompa A, Czegledi A, Gajtko A, Pituk D, Lekli I, et al. The cardioprotective effect of metformin in doxorubicin-induced cardiotoxicity: the role of autophagy. Molecules. 2018;23(5):1184.
35. Kelleni MT, Amin EF, Abdelrahman AM. Effect of metformin and sitagliptin on doxorubicin-induced cardiotoxicity in rats: impact of oxidative stress, inflammation, and apoptosis. J Toxicol. 2015;2015:424813.
36. Sheta A, Elsakkar M, Hamza M, Solaiman A. Effect of metformin and sitagliptin on doxorubicin-induced cardiotoxicity in adult male albino rats. Hum Exp Toxicol. 2016;35(11):1227-39.
37. Sardu C, Paolisso P, Sacra C, Mauro C, Minicucci F, Portoghese M, et al. Effects of metformin therapy on coronary endothelial dysfunction in patients with prediabetes with stable angina and nonobstructive coronary artery stenosis: The CODYCE Multicenter Prospective Study. Diabetes Care. 2019;42(10):1946-55.
38. Xu W, Deng YY, Yang L, Zhao S, Liu J, Zhao Z, et al. Metformin ameliorates the proinflammatory state in patients with carotid artery atherosclerosis through sirtuin 1 induction. Transl Res. 2015;166(5):451-8.

39. Li J, Xu JP, Zhao XZ, Sun XJ, Xu ZW, Song SJ. Protective effect of metformin on myocardial injury in metabolic syndrome patients following percutaneous coronary intervention. Cardiology. 2014;127(2):133-9.
40. Stephenne X, Foretz M, Taleux N, van der Zon GC, Sokal E, Hue L, et al. Metformin activates AMP-activated protein kinase in primary human hepatocytes by decreasing cellular energy status. Diabetologia. 2011;54(12):3101-10.
41. Ouyang JY, Parakhia RA, Ochs RS. Metformin activates AMP kinase through inhibition of AMP deaminase. J Biol Chem. 2011;286(1):1-11.
42. Hammad AM, Ibrahim YA, Khdair SI, Hall FS, Alfaraj M, Jarrar Y, et al. Metformin reduces oxandrolone-induced depression-like behavior in rats via modulating the expression of IL-1β, IL-6, IL-10 and TNF-α. Behav Brain Res. 2021;414:113475.
43. Beyazcicek O, Beyazcicek E, Kubur UB, Gok A. Effect of the combination of exercise and metformin on osteocalcin, insulin, interleukin-6, glucose levels, and body weights in rats. Niger J Clin Pract. 2024;27(6):766-73.
44. Liu J, Lu J, Zhang L, Liu Y, Zhang Y, Gao Y, et al. The combination of exercise and metformin inhibits TGF-β1/Smad pathway to attenuate myocardial fibrosis in db/db mice by reducing NF-κB-mediated inflammatory response. Biomed Pharmacother. 2023;157:114080.
45. Selvi P, Elizabeth AA. Effect of metformin on C-reactive protein in type 2 diabetes mellitus patients. J Pharm Res Int. 2021;33(23B):53-61.
46. Yu F, Xing C, Fan Y, Liu Y, Su P, Yang Q, et al. Aerobic exercise and metformin on intermuscular adipose tissue (IMAT): insights from multimodal MRI and histological changes in prediabetic rats. Diabetol Metab Syndr. 2023;15(1):221.
47. Shabab S, Mahmoudabady M, Gholamnezhad Z, Niazmand S, Fouladi M, Mousavi Emadi Z. Endurance exercise prevented diabetic cardiomyopathy through the inhibition of fibrosis and hypertrophy in rats. Rev Cardiovasc Med. 2024;25(5):173.
48. Özüdoğru E, Atay E, Savran M, Aşci H, Özmen Ö, Topsakal Ş. Protective effects of swimming exercises and metformin on cardiac and aortic damage caused by a high-fat diet in obese rats with type 2 diabetes, by regulating the Bcl2/Bax signaling pathway. Turk J Med Sci. 2023;53(6):1582-92.
49. Sanghani NB, Chokshi SA. Comparative study of structured physical exercise and metformin on glycemic control and body mass on obese type 2 diabetic. J Cardiovasc Dis Res. 2023;14(3):336-42.
50. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI. Markers of oxidative stress during diabetes mellitus. J Biomark. 2013;2013:378790.
51. Fouladi M, Mahmoudabady M, Gholamnezhad Z, Shabab S, Niazmand S, Salmani H. Impact of endurance exercise training on biomarkers of aortic endothelial damage in diabetic rats. Cardiovasc Ther. 2024;2024(1):6025911.

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Therapeutic Effects of Trolox and Neocuproine on Experimental Mild Traumatic Brain Injury in Rats

Troloks ve Neokuproin'in Sıçanlarda Deneysel Hafif Travma Sonucu Beyin Hasarı Üzerindeki Terapötik Etkisi

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ABSTRACT

Aim: This study aimed to evaluate the therapeutic efficacy of Trolox and neocuproine treatment in an experimental rat model of mild traumatic brain injury (mTBI).
Material and Methods: Forty rats were grouped as Control, TBI, Trolox, and neocuproine. After the Marmarou Weight Drop Model was used to create TBI, 80 mg/kg/day Trolox (i.p.), and 100 μM/day Neocuproine (i.p.) treatments were applied in the 2nd hour. Open field, attention, and short-term memory novel object recognition tests were performed to measure locomotor activity. IL-1β, IL-10, TNF-α, and TGF-β levels in the hippocampus tissues of the rats were analyzed by the ELISA method.
Results: Open field and novel object recognition test results showed that the total path traveled was significantly decreased in the TBI group compared to the control group ($p<0.001$). A significant increase in locomotor functions was observed in the Trolox ($p=0.018$) and neocuproine ($p=0.002$) groups compared to TBI. Short-term memory test results decreased in TBI compared to control ($p<0.001$), while they increased in the Trolox and neocuproine groups compared to TBI (both $p<0.001$). Hippocampus IL-1β ($p=0.012$) and TNF-α ($p=0.011$) levels increased, while IL-10 ($p=0.031$) and TGF-β ($p=0.007$) levels decreased compared to control in the TBI group. While all proinflammatory cytokine levels decreased, antiinflammatory cytokine levels increased in the Trolox and neocuproine groups compared to TBI.
Conclusion: The present findings showed that Trolox and neocuproine treatments in TBI provided significant improvements in short-term memory, and locomotor activity levels by reducing proinflammatory cytokine production, and increasing antiinflammatory cytokine production.
Keywords: Neocuproine; Trolox; traumatic brain injury; locomotor activity; neuroinflammation.

ÖZ

Amaç: Bu çalışmanın amacı hafif travmatik beyin hasarı (hTBH) olan deneysel rat modelinde Troloks ve neokuprin tedavilerinin terapötik etkinliğinin değerlendirilmesidir.
Gereç ve Yöntemler: Kırk rat, kontrol, TBH, Troloks ve neokuprin olarak gruplandırıldı. TBH oluşturmak amacı ile Marmarou Weight Drop Model uygulandıktan sonraki 2. saat diliminde 80 mg/kg/gün Troloks (i.p.) ve 100 μM/gün neokuproin (i.p.) tedavileri yapıldı. Lokomotor aktiviteyi ölçmek için açık alan ve dikkat ve kısa süreli hafıza yeni nesne tanıma testleri gerçekleştirildi. Sıçanların hipokampüs dokularındaki IL-1β, IL-10, TNF-α ve TGF-β düzeyleri ELISA yöntemi ile analiz edildi.
Bulgular: Açık alan ve yeni nesne tanıma test sonuçları, alınan toplam yolun TBH grubunda kontrol grubuna kıyasla önemli ölçüde azaldığını gösterdi ($p<0,001$). Troloks ($p=0,018$) ve neokuproin ($p=0,002$) gruplarında ise TBH'a kıyasla lokomotor fonksiyonlarında anlamlı bir artış gözlendi. Kısa süreli bellek test sonuçları, TBH'da kontrole kıyasla azalırken ($p<0,001$), Troloks ve neokuproin gruplarında ise TBH'a kıyasla arttı (her iki $p<0,001$). Kontrole kıyasla TBH grubunun hipokampüs IL-1β ($p=0,012$) ve TNF-α ($p=0,011$) seviyeleri artarken, IL-10 ($p=0,031$) ve TGF-β ($p=0,007$) seviyeleri azaldı. Troloks ve neokuproin gruplarında TBH'ye kıyasla tüm proinflamatuar sitokin seviyeleri azalırken, antiinflamatuar sitokin seviyeleri arttı.
Sonuç: Elde edilen bulgular, TBH'da Troloks ve neokuproin tedavilerinin proinflamatuar sitokin üretimini azaltarak ve antiinflamatuar sitokin üretimini artırarak kısa süreli bellek ve lokomotor aktivite düzeyinde anlamlı iyileşmeler sağladıklarını göstermiştir.
Anahtar kelimeler: Neokuproin; Troloks; travmatik beyin hasarı; lokomotor aktivite; nöroinflamasyon.

INTRODUCTION

Traumatic brain injury (TBI) is a substantial clinical disorder that affects survival, sequelae, mortality, and morbidity and occurs as a result of many traumatic incidents, or multiple underlying neuropathological causes (1).

Traumatic brain damage can lead to encephalopathy, cognitive deficits, behavioral abnormalities, epileptic seizures, and perhaps a neurodegenerative condition resembling Alzheimer's disease (2).

There are two forms of TBI. The first type results in central nervous system damage to the brain caused by trauma such as fractures or other traumas. Secondary TBI develops sometime after the initial brain injury and may result from pathological causes such as mitochondrial dysfunction, inflammation, neurotransmitter activity, calcium, and gene activity on a cellular basis (3,4).

α-tocopherol is a powerful antioxidant that protects the membrane from oxidative agents, and Trolox is a water-soluble analog of α-tocopherol vitamin E (5). Neocuproine is the chemical form that acts as a copper chelator (6,7). Trolox has been evaluated as a control group in experimental studies due to its antioxidant analog properties of vitamin E. In one study, it was reported that incubation of glial cells with Trolox prevented neuronal cell death through interleukin-1beta (IL-1β) expression (8). In a study on isoprostane found in the rat cerebral cortex, it was shown that treatment with antioxidant Trolox prevented isoprostane release under basal conditions (9).

A few of the studies on neocuproine in the literature are on smooth muscle activity, which is on the effect on relaxation in the rat corpus cavernosum (10). Another rat study using neocuproine showed its protective role against cardiac damage in isolated perfused rat hearts (11). In a cancer study, it was stated that neocuproine could be considered an antitumor candidate (12). Another study using the copper chelate neocuproine discussed its effects on DNA synthesis (13).

TBI continues to be a major public health concern worldwide, with the need for effective research models to better understand its pathophysiology and develop therapeutic interventions. The use of Trolox and neocuproine in TBI studies has not yet been included in the literature. It is questionable whether Trolox and neocuproine can be considered as an alternative treatment option in terms of anti-neuroinflammatory effects in eliminating abnormal locomotor activity and cognitive behaviors. The working hypothesis is that separately administering Trolox and neocuproine to TBI rats will reduce neurodegenerative processes through anti-inflammatory effects. To demonstrate the therapeutic efficacy of Trolox and neocuproine in TBI, cognitive function and locomotor activity were assessed using the open field (OF), and novel object recognition (NOR) tests. Proinflammatory, and antiinflammatory markers such as IL-1β, interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), and tumor growth factor beta (TGF-β) levels were investigated in the cerebral hippocampus preparations.

MATERIAL AND METHODS

Experimental Groups and Protocol

This study used 3-month-old Wistar Albino male rats weighing 200-250 g. The Bolu University Animal Care and Ethics Committee authorized, and oversaw the implementation of all procedures involving the use of animals in experiments (Approval no: 2024/22, Date: 07.08.2024). Forty rats were divided into four groups each with n=10, as Control, TBI, Trolox, and neocuproine. The TBI model was created using the Marmarou method. The control group was administered Trolox, and neocuproine solvent DMSO intraperitoneally (i.p.). The Trolox and neocuproine groups were administered 80 mg/kg/day Trolox (i.p.), and 100 μM/day neocuproine (i.p.) immediately after TBI. The method used to determine the therapeutic effective dose was taken into account in the relevant studies using Trolox and neocuproine, and also a single dose was preferred in this preliminary study to prove whether Trolox and neocuproine, which is the hypothesis of the study, could be a treatment option in TBI (14-17).

Secondary injury plays a role in the pathophysiology of TBI and secondary injuries begin to appear in the first hours of TBI. After the 4th hour, the blood-brain barrier is damaged and neuroinflammation and apoptotic processes begin, leading to loss of motor and cognitive function. Therefore, treatments should usually be initiated at an early stage to prevent secondary damage. Neuroinflammation and apoptotic processes play a role in the pathophysiological damage mechanism at 24, 48, and 72 hours of TBI. In our study, in order to investigate the efficacy of Trolox and neocuproine treatments on neuroinflammation in TBI-induced motor and cognitive dysfunctions, drug treatment was started at the 2nd hour of TBI and terminated at the 48th hour (4,18,19).

The NOR test evaluated learning, and memory activities, whereas the OF test evaluated locomotor activity. The subjects were sacrificed after the behavioral experiments, the brains were removed, and IL-1β, IL-10, TNF-α, and TGF-β levels in the hippocampus tissues were assessed using the ELISA method (Figure 1).

Marmarou Weight Drop Model

In this study, the most preferred among weight drop models, Marmarou's (20) weight drop method was used (Figure 2). Animals were anesthetized with 5 % isoflurane. The skull was placed between lambda, and bregma without surgical operation using a polyacrylamide adhesive, with a stainless steel disk (10 mm diameter, 3 mm depth). A moderate chronic traumatic encephalopathy (CTE) model was created by dropping a 450 g brass weight directly onto the steel disk from a height of 1 meter on the animals placed on the sponge.

Behavioral Experiments

Open Field (OF) Test

The locomotor activity is measured with the OF test. OF experiments will be conducted in a square-shaped, 80x80 cm, black mat-based setup with a wall height of 40 cm. The area was divided into 16 equal small circles of 20 cm^2. At the beginning of the experiment, rats were placed one by one in the center of this area, and their movements were examined for 3 minutes. The number of squares entered by each rat, the total distance covered, and the average speed were measured (21,22).

Novel Object Recognition (NOR) Test

The NOR test is particularly effective in measuring attention, and short-term memory studies, and has three stages: habituation, training, and retention. In the habituation stage, rats were placed in the middle of an

80×80 cm setup, 40 cm high, and allowed to explore the area for five minutes without any items. In the training stage, rats were placed in the area from the center and were given 5 minutes to examine two objects placed in the environment. During the experiments, the apparatus was cleaned with 70% ethanol to prevent the rats from moving according to their sense of smell. In the retention stage, one of the objects was replaced with a new object, and the rats' behaviors were recorded for 5 minutes. During this process, it is expected that the rats will spend more time examining the new object (23). In the NOR test, the discrimination index (%), and the time spent on the new object (sec) values were analyzed.

Discrimination Index = ((time spent on new object - time spent on old object) / total time) * 100.

Biochemical Assessment

Tissue Homogenization Process

Tissues were roughly homogenized with liquid nitrogen in a ceramic mortar. Then, brain tissue was transferred into an Eppendorf tube, its weight was weighed, and 10 times the volume of phosphate buffer was added. After homogenization for 60 seconds with a tissue homogenizer, it was centrifuged at 12000 rpm at + 4 °C for 10 minutes.

Tumor Necrosis Factor-Alpha (TNF-α) and Tumor Growth Factor Beta (TGF-β)

The measurement was carried out in compliance with the guidelines provided by the solid phase sandwich enzyme immunoassay (ELISA) kit, which is sold commercially. Specific monoclonal antibodies against rat TNF-α were placed onto 96-well plates together with standard solutions, and samples were made in serial dilutions at decreasing concentrations. TNF-α, and TGF-β molecules in the samples bound to antibodies in this antigen-antibody binding technique. It was incubated for ninety minutes at 37 °C in an incubator. Following the incubation period, 100 µl of concentrated biotinylated detection Ab was applied to each well after being diluted 1/100 with a biotinylated detection dilution solution. Following an hour of incubation at 37 °C, 750 milliliters of distilled water were added to the concentrated wash buffer, and three washes using the wash buffer solution were carried out to eliminate any unattached molecules. After that, 100 µl of streptavidin-bound peroxidase (horseradish peroxidase, HRP) conjugate was added and incubated at 37 °C for 30 minutes. After incubation, 5 washes were performed and substrate solution was added. The TNF-α and TGF-β concentrations in the samples were directly correlated with a color shift that was seen, and the reaction was stopped by adding 50 µl of stop solution to each well. Each well's absorbance value was calculated by reading at 450 nm using a microplate reader.

Interleukin (IL-1β and IL-10) Measurement

The measurement was carried out in compliance with the guidelines provided by the ELISA kit, which is sold commercially. Specific monoclonal antibodies against rat IL-1β and IL-10 were placed onto 96-well plates together with standard solutions and samples generated in serial dilutions at decreasing concentrations. The IL-1β and IL-10 molecules in the samples were attached to the antibody in this antigen-antibody binding technique. For ninety minutes, it was incubated at 37 °C in an incubator. Following the incubation period, 100 µl of concentrated biotinylated detection Ab was applied to each well after being diluted 1/100 with a biotinylated detection dilution solution. Following an hour of incubation at 37 °C, unattached molecules were eliminated by washing the concentrated wash buffer three times with the wash buffer solution made by adding 750 ml of distilled water to it. Subsequently, 100 µl of the HRP conjugate was added, and 30 minutes were spent incubating at 37 °C. This time, after incubation, a substrate solution was added and five washes were carried out. 50 µl of stop solution was given to each well to halt the reaction, and a color shift was seen that was directly correlated with the amounts of IL-1β and IL-10 in the samples. Using a microplate reader, the absorbance value of each well was measured by reading at 450 nm.

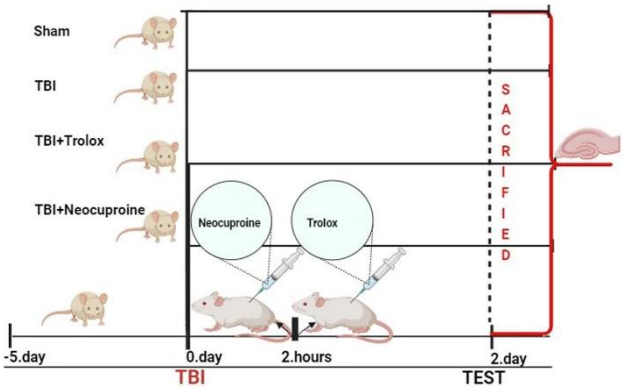

Figure 1. Experimental design (created by biorender.com)

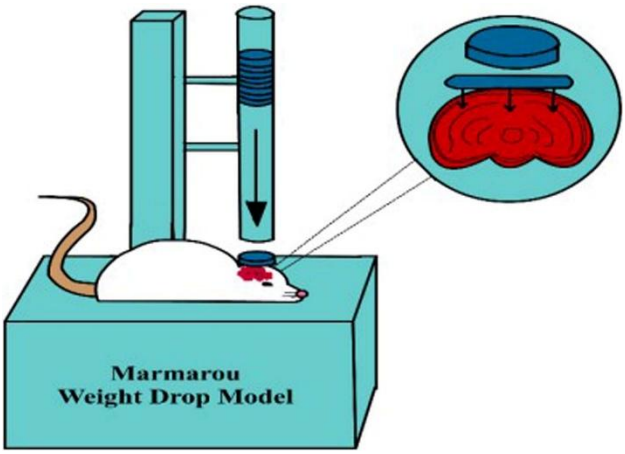

Figure 2. Marmarou weigth drop model (2)

Statistical Analysis

The IBM SPSS v.20.0 software was used to analyze the data. The Shapiro-Wilk test was used for normality assumption, and homogeneity of variances was evaluated with the Levene test. The One-way ANOVA or Welch test followed by post hoc Tukey test for pairwise comparisons between groups was used to analyze data that fit the normal distribution. The descriptive statistics of the data were presented as mean±standard deviation. A $p<0.05$ was deemed to be statistically significant.

RESULTS

The NOR test assessed learning, while the OF assessed locomotor activity (Table 1). The total distance traveled in the OF was significantly reduced in the TBI group compared to the control group ($p<0.001$). When comparing the TBI group to the control group, the total distance traveled in the

Table 1. Comparison of behavioral results and biochemical findings between experimental groups

	Control (n=10)	TBI (n=10)	Trolox (n=10)	Neocuproine (n=10)	p
TD (cm)	2112.00±72.99	1756.00±90.95	1907.00±146.59	1948.00±108.40	**<0.001**
DI (%)	13.11±1.17	8.01±1.14	11.03±1.20	11.43±1.33	**<0.001**
IL-1β (pg/100µg protein)	98.40±5.95	115.10±11.77	107.10±12.27	105.60±14.07	**0.023**
IL-10 (pg/100µg protein)	122.52±19.11	103.40±11.95	109.70±13.27	111.30±13.58	**0.047**
TNF-α (pg/100µg protein)	120.80±11.84	137.60±10.20	126.20±12.55	128.50±10.75	**0.018**
TGF-β (pg/100µg protein)	128.40±15.83	103.70±18.30	111.20±13.16	109.80±15.58	**0.009**

TBI: traumatic brain injury, TD: total distance, DI: discrimination index, IL-1β: interleukin 1 beta, IL-10: interleukin 10, TNF-α: tumor necrosis factor-alpha, TGF-β: tumor growth factor-beta, post hoc test results; TD: control vs TBI: p<0.001, control vs trolox: p=0.001, control vs neocuproine: p=0.009, TBI vs trolox: p=0.018, TBI vs neocuproine: p=0.002, trolox vs neocuproine: p=0.832; DI: control vs TBI: p<0.001, control vs trolox: p=0.003, control vs neocuproine: p=0.019, TBI vs trolox: p<0.001, TBI vs neocuproine: p<0.001, trolox vs neocuproine: p=0.875; IL-1β: control vs TBI: p=0.012, control vs trolox: p=0.337, control vs neocuproine: p=0.502, TBI vs trolox: p=0.411, TBI vs neocuproine: p=0.263, trolox vs neocuproine: p=0.991; IL-10: control vs TBI: p=0.031, control vs trolox: p=0.228, control vs neocuproine: p=0.337, TBI vs trolox: p=0.775, TBI vs neocuproine: p=0.631, trolox vs neocuproine: p=0.995; TNF-α: control vs TBI: p=0.011, control vs trolox: p=0.715, control vs neocuproine: p=0.440, TBI vs trolox: p=0.131, TBI vs neocuproine: p=0.295, trolox vs neocuproine: p=0.969; TGF-β: control vs TBI: p=0.007, control vs trolox: p=0.089, control vs neocuproine: p=0.058, TBI vs trolox: p=0.716, TBI vs neocuproine: p=0.824, trolox vs neocuproine: p=0.997

OF was considerably less (Figure 3A). Following therapy, the Trolox (p=0.018) and neocuproine (p=0.002) groups showed a substantial increase in locomotor activity in comparison to the TBI group. The NOR test was used to assess the groups' short-term memory tests (Figure 3B). The Trolox and neocuproine groups showed a substantial gain in learning compared to TBI group (both p<0.001), whereas the TBI group showed a significant decline in learning compared to the control group (p<0.001).

Effects of Neocuproine and Trolox on Locomotor Activity in TBI Groups

Biochemical Findings in Hippocampus Tissue

IL-1β, IL-10, TNF-α, and TGF-β levels in the hippocampus were shown in Table 1. Compared to the control group, IL-1β (p=0.012) and TNF-α (p=0.011) levels of the TBI group increased significantly, while the IL-10 (p=0.031) and TGF-β (p=0.007) levels decreased significantly. Both of the treatments reduced all proinflammatory cytokine levels in the Trolox and neocuproine groups while increasing anti-inflammatory cytokine levels (Figure 4).

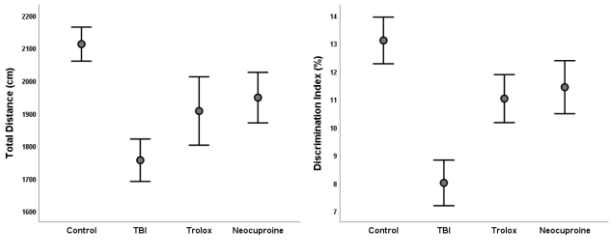

Figure 3. Behavioral results, **A)** total distance traveled (cm), and **B)** discrimination index of experimental groups

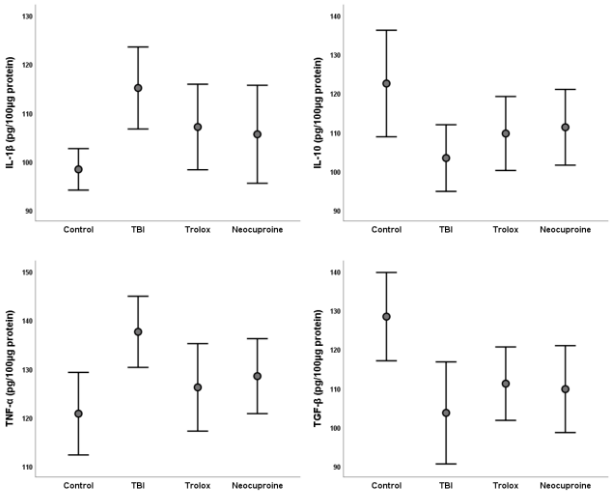

Figure 4. Results of neuroinflammation, **A)** IL-1β, **B)** IL-10, **C)** TNF-α, and **D)** TGF-β levels in the hippocampus

DISCUSSION

The problems observed in TBI cases in the clinical picture have not yet been completely resolved and treatment searches are ongoing (24). In primary brain damage and secondary brain damage caused by trauma, it is thought that secondary damage plays the leading role (18). Other mechanisms observed in the development of secondary damage and neuroinflammation cause motor function losses (25).

In the current study, it was determined that there was motor dysfunction in the experimental groups that developed TBI. It has not yet been shown that Trolox or neocuproine can be a therapeutic factor for motor function loss. Again, neither Trolox nor neocuproine has been included in a study on TBI in vivo study with its anti-inflammatory aspects. As a result of the findings presented in this study, it was observed that Trolox and neocuproine applications can have a therapeutic effect on both motor function loss of TBI and by affecting neuroinflammation levels. Motor function defects that developed with TBI in the experimental groups were recovered with Trolox and neocuproine treatment.

The Marmarou weight-drop model is a popular animal model used in TBI. The Marmarou model induces closed-head TBI without allowing for other complications caused by open-head injuries (26). In the experimental groups where the TBI model was performed, decreased locomotor activity was observed, and these results are consistent with the literature (27).

In addition, it is possible to say that while Trolox and neocuproine applied for treatment purposes for TBI regressed the loss of locomotor activity, similar gains were made for motor functions. A decrease in locomotor activity is a concurrent process with motor dysfunction (18). The therapeutic effectiveness of Trolox and neocuproine also draws attention to the similarity of the results to the control group. Another therapeutic effect that draws attention to the findings of this study is that according to the NOR test results of the short-term memory experiments of Trolox and neocuproine in rats, a significant decrease in learning

was observed in the TBI group compared to the control group, while a significant increase was observed in the Trolox and neocuproine groups compared to the TBI group.

In the clinic, it is mentioned that there may be both cognitive and physical function patterns in TBI patients (28). There is an underlined statement between the primary injury, and secondary injury processes that in patients with TBI, the secondary injury process, that is, the process in which TBI is observed at a more micro index, is associated with a poor prognosis (29). It is predicted that neuroinflammatory steps are involved in the progression of the process in the post-primary brain injury phase of clinical findings of TBI patients. In light of this information, the study aimed to determine the proinflammatory and anti-inflammatory cytokine levels in the hippocampus. The findings of the TBI in vivo rat study also suggest that diphenhydramine HCI application has a significant anti-inflammatory effect on TNF-α serum levels in the treatment groups, and reversals in brain damage findings (30).

Genes that are active in TBI may affect the severity of the injury, including pro, and anti-inflammatory cytokines, and neurotrophic genes are thought to affect repair and plasticity (31).

Considering the healing effects of Trolox and neocuproine, they can be used as preventive agents in cases where TBI may develop or occur again, with their antioxidant character as well as their therapeutic activity on TBI.

The limitations of the study are as follows: First, the dose intervals have not been tested in single applications to find the most effective dose for Trolox and neocuproine application in TBI. However, the current study is a preliminary study to evaluate whether Trolox and neocuproine have a place in TBI studies.

CONCLUSION

Trolox and neocuproine treatment resulted in significant improvements in short-term memory and locomotor activity levels in TBI. It is thought that this therapeutic effect is achieved by decreasing the production of proinflammatory cytokines and increasing the production of anti-inflammatory cytokines.

Ethics Committee Approval: The study was approved by the local ethics committee on animal experiments at Bolu Abant İzzet Baysal University (07.08.2024, 22)

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REFERENCES

1. Pop V, Badaut J. A neurovascular perspective for long-term changes after brain trauma. Transl Stroke Res. 2011;2(4):533-45.
2. Akçay G. Weight drop models in traumatic brain injury. Mid Blac Sea J Health Sci. 2023;9(2):375-84.
3. Yi JH, Hazell AS. Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury. Neurochem Int. 2006;48(5):394-403.
4. Akçay G, Baydemir R. Therapeutic effects of transcranial direct current stimulation on loss of motor function caused by experimental mild traumatic brain injury. Cukurova Med J. 2023;48(3):972-8.
5. Ricciarelli R, Azzi A, Zingg JM. Reduction of senescence-associated beta-galactosidase activity by vitamin E in human fibroblasts depends on subjects' age and cell passage number. Biofactors. 2020;46(4):665-74.
6. Göçmen C, Giesselman B, de Groat WC. Effect of neocuproine, a copper(i) chelator, on rat bladder function. J Pharmacol Exp Ther. 2005;312(3):1138-43.
7. De Man JG, Moreels TG, De Winter BY, Herman AG, Pelckmans PA. Neocuproine potentiates the activity of the nitrergic neurotransmitter but inhibits that of S-nitrosothiols. Eur J Pharmacol. 1999;24;381(2-3):151-9.
8. Viviani B, Corsini E, Binaglia M, Galli CL, Marinovich M. Reactive oxygen species generated by glia are responsible for neuron death induced by human immunodeficiency virus-glycoprotein 120 in vitro. Neuroscience. 2001;107(1):51-8.
9. Brunetti L, Orlando G, Michelotto B, Recinella L, Di Nisio C, Ciabattoni G, et al. Identification of 8-iso-prostaglandin F(2alpha) in rat brain neuronal endings: a possible marker of membrane phospholipid peroxidation. Life Sci. 2002;71(20):2447-55.
10. Göçmen C, Göktürk HS, Ertuğ PU, Onder S, Dikmen A, Baysal F. Effect of neocuproine, a selective Cu(I) chelator, on nitrergic relaxations in the mouse corpus cavernosum. Eur J Pharmacol. 2000;406(2):293-300.
11. Applebaum YJ, Kuvin J, Borman JB, Uretzky G, Chevion M. The protective role of neocuproine against cardiac damage in isolated perfused rat hearts. Free Radic Biol Med. 1990;8(2):133-43.
12. Takahashi K, Takita T, Umezawa H. Effects of o-phenanthroline, 2,2'-dipyridyl and neocuproine on the activities of bleomycin to inhibit DNA synthesis and growth of cultured cells. J Antibiot (Tokyo). 1986;39(10):1473-8.
13. Jahani S, Noroozifar M, Khorasani-Motlagh M, Torkzadeh-Mahani M, Adeli-Sardou M. In vitro cytotoxicity studies of parent and nanoencapsulated Holmium-2,9-dimethyl-1,10-phenanthroline complex toward fish-salmon DNA-binding properties and antibacterial activity. J Biomol Struct Dyn. 2019;37(17):4437-49.
14. Nunes EA, Manieri TM, Matias AC, Bertuchi FR, da Silva DA, Lago L, et al. Protective effects of neocuproine copper chelator against oxidative damage in NSC34 cells. Mutat Res Genet Toxicol Environ Mutagen. 2018;836(Pt B):62-71.

15. Sharma SS, Kaundal RK. Neuroprotective effects of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), an antioxidant in middle cerebral artery occlusion induced focal cerebral ischemia in rats. Neurol Res. 2007;29(3):304-9.
16. Park H, Seol GH, Ryu S, Choi IY. Neuroprotective effects of (-)-linalool against oxygen-glucose deprivation-induced neuronal injury. Arch Pharm Res. 2016;39(4):555-64.
17. Massaeli H, Sobrattee S, Pierce GN. The importance of lipid solubility in antioxidants and free radical generating systems for determining lipoprotein proxidation. Free Radic Biol Med. 1999;26(11-12):1524-30.
18. Akcay G, Demirdogen F, Gul T, Yilmaz A, Kotan D, Karakoc E, et al. Effects of transcranial direct current stimulation on motor and cognitive dysfunction in an experimental traumatic brain injury model. Turk Neurosurg. 2024;34(2):343-50.
19. Akcay G, Ozdemir F, Ozkinali S, Demirdogen F, Yilmaz A, Celik C. Investigation of the protective and therapeutic effects of ginger (Zingiber officinale) extracts on neuroinflammatory, motor and cognitive impairments caused by mild traumatic brain injury model. Turk Neurosurg. 2024;[Epub ahead of print]. doi: 10.5137/1019-5149.JTN.46057-23.7
20. Marmarou A, Foda MA, van den Brink W, Campbell J, Kita H. Demetriadou K. A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. J Neurosurg. 1994;80(2):291-300.
21. Berry A, Tomidokoro Y, Ghiso J, Thornton J. Human chorionic gonadotropin (a luteinizing hormone homologue) decreases spatial memory and increases brain amyloid-beta levels in female rats. Horm Behav. 2008;54(1):143-52.
22. Denenberg VH. Open-field bheavior in the rat: what does it mean? Ann N Y Acad Sci. 1969;159(3):852-9.
23. Johnston IN, Tan M, Cao J, Matsos A, Forrest DRL, Si E, et al. MR. Ibudilast reduces oxaliplatin-induced tactile allodynia and cognitive impairments in rats. Behav Brain Res. 2017;334:109-18.
24. Galgano M, Toshkezi G, Qiu X, Russell T, Chin L, Zhao LR. Traumatic brain injury: current treatment strategies and future endeavors. Cell Transplant. 2017;26(7):1118-30.
25. Yang B, Zhang R, Sa Q, Du Y. Rhamnazin ameliorates traumatic brain injury in mice via reduction in apoptosis, oxidative stress, and inflammation. Neuroimmunomodulation. 2022;29(1):28-35.
26. Thompson FJ, Hou J, Bose PK. Closed-head TBI model of multiple morbidity. Methods Mol Biol. 2016;1462:521-36.
27. Xing P, Ma K, Li L, Wang D, Hu G, Long W. The protection effect and mechanism of hyperbaric oxygen therapy in rat brain with traumatic injury. Acta Cir Bras. 2018;33(4):341-53.
28. Capizzi A, Woo J, Verduzco-Gutierrez M. Traumatic brain injury: An overview of epidemiology, pathophysiology, and medical management. Med Clin North Am. 2020;104(2):213-38.
29. Ma X, Aravind A, Pfister BJ, Chandra N, Haorah J. Animal models of traumatic brain injury and assessment of injury severity. Mol Neurobiol. 2019;56(8):5332-45.
30. Kurniawan VR, Islam AA, Adhimarta W, Zainuddin AA, Widodo D, Nasrullah, et al. The role of diphenhydramine HCl on tumor necrosis factor-α levels in Wistar rats with traumatic brain injury: An in vivo study. Ann Med Surg (Lond). 2022;81:104399.
31. Bennett ER, Reuter-Rice K, Laskowitz DT. Genetic influences in traumatic brain injury. In: Laskowitz D, Grant G, editors. Translational research in traumatic brain injury. Boca Raton (FL): CRC Press/Taylor and Francis Group; 2016. Chapter 9.

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Duloxetine-Induced Sleep Bruxism and Tooth Fracture in Fibromyalgia

Fibromiyaljide Duloksetin Kaynaklı Uyku Bruksizmi ve Diş Kırılması

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ABSTRACT

Duloxetine is a serotonin-norepinephrine reuptake inhibitor that is approved for the treatment of fibromyalgia. Duloxetine has many side effects such as nausea, somnolence, insomnia, decreased appetite, dry mouth, and constipation. Sleep bruxism is also a very rare side effect of duloxetine. In this case report, a case of a 35-year-old male patient who developed sleep bruxism in the 4th week after duloxetine use, and fracture of the 1st upper molar tooth which had previously undergone root canal treatment after severe bruxism in the 6th week was presented. Duloxetine treatment was stopped and treatment was continued with amitriptyline. The patient had a significant reduction in both bruxism and fibromyalgia symptoms after the amitriptyline treatment. Bruxism symptoms were not observed after one year of follow-up.
Keywords: Duloxetine; sleep bruxism; tooth fracture; fibromyalgia.

ÖZ

Duloksetin, fibromiyalji tedavisi için onaylanmış bir serotonin-norepinefrin geri alım inhibitörüdür. Duloksetinin bulantı, uyuklama, uykusuzluk, iştah azalması, ağız kuruluğu ve kabızlık gibi birçok yan etkisi vardır. Uyku bruksizmi de duloksetinin çok nadir görülen bir yan etkisidir. Bu olgu sunumunda, duloksetin kullanımından sonra 4. haftada uyku bruksizmi gelişen ve 6. haftada şiddetli bruksizm sonrası daha önce kanal tedavisi görmüş olan 1. üst molar dişinde kırık gelişen 35 yaşında bir erkek hasta sunulmuştur. Kullanmakta olduğu duloksetin tedavisi kesildi ve tedaviye amitriptilin ile devam edildi. Amitriptilin tedavisinden sonra hastanın hem bruksizm hem de fibromiyalji semptomlarında belirgin bir azalma görüldü. Bir yıllık takip sonrasında bruksizm semptomları görülmedi.
Anahtar kelimeler: Duloksetin; uyku bruksizmi; diş kırılması; fibromiyalji.

INTRODUCTION

Sleep bruxism is defined as an involuntary movement of the jaw muscles characterized by jaw clenching, stretching, grinding, and gnashing of teeth (1). Bruxism can be categorized into primary and secondary types. The primary type is usually idiopathic and is not associated with any other medical situation. While, the secondary type can be iatrogenic and is related to medical conditions (e.g. sleep disorders, psychiatric or neurological) or intake of medicines for their treatment (2,3).

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Secondary type sleep bruxism may be common during treatment with antipsychotics and antidepressants (4). Duloxetine is a serotonin-norepinephrine reuptake inhibitor that is approved by the United States Food and Drug Administration (FDA) for the treatment of fibromyalgia. In this case report, severe sleep bruxism and related tooth fracture due to duloxetine used in fibromyalgia patients was presented.

CASE REPORT

A 35-year-old male patient presented with complaints of widespread body pain, joint pains, weakness, morning fatigue, and sleeplessness for more than three months. After the evaluation of the patient, duloxetine 30 mg was started considering fibromyalgia according to FMS ACR 2016 criteria, and the patient was called for control after one month. After 6 weeks, the patient who came to the control stated that bruxism complaints at night started in the 4th week. In the 6th week, he stated that her right upper 1st molar tooth, which had previously undergone root canal treatment, was broken due to sleep bruxism and therefore he came to the control. In the same direction, a partial fracture of the tooth in the lower jaw was observed. The patient's colleagues, who work as firefighters and work in shifts, said that they heard the patient grinding his teeth while he was asleep and woke him up several times a night. At the same time, he stated that he was woken up several times a night by his wife because of the sound of grinding his teeth, but he was not suffering from bruxism during the day. After the tooth fracture, he came to the control, and duloxetine was stopped and complete blood count, biochemistry, and thyroid function tests were evaluated as normal. Amitriptyline 10 mg was started and he was referred to the dentist to use night plasters. He was called for a follow-up one month later. At the next visit, the patient reported a significant reduction in bruxism but did not use the night plate regularly. Amitriptyline was gradually increased to 25 mg and when he came to the control after three months, he stated that his complaints of both fibromyalgia and bruxism had decreased significantly. Bruxism was not observed after one year of follow-up.

DISCUSSION

Duloxetine is a serotonin-noradrenaline reuptake inhibitor used in fibromyalgia syndrome. There are many side effects associated with duloxetine intake. These include nausea, somnolence, insomnia, dry mouth, constipation, decreased appetite and libido (5). Few cases of duloxetine-induced sleep bruxism have been reported. In one case, it was treated with amitriptyline, and in another case duloxetine was discontinued and it was treated with buspirone (6,7).

The etiology of sleep bruxism, which is seen in 8-21% of the population, includes multifactorial causes such as smoking, alcohol, caffeine, diseases, trauma, genetics, and drugs (8,9). Sleep bruxism is a rare side effect of duloxetine use. Sleep bruxism can cause masseter muscle hypertrophy, tongue indentation, decreased salivary flow, abnormal tooth abrasion, tooth fracture, lip or cheek biting and tongue burning with accompanying oral habits, polygraphic observation of jaw muscle activity with grinding sounds (10). In our case, severe bruxism was observed after duloxetine use, and the tooth, which had previously undergone root canal treatment, was fractured.

The neurochemical mechanism of bruxism is not well known, but it has been suggested that the central dopaminergic system controlling muscle activity may play a role. For SSRI-induced bruxism, it has been hypothesized that the mechanism may involve excessive serotonergic action on the mesocortical neurons, which leads to a dopaminergic deficit (4). Tricyclic antidepressants have a suppression effect on the REM phase of the sleep cycle; this may help to cease the bruxism symptoms appearing in that phase of the sleep cycle (11).

In studies, similar side effects after duloxetine use in fibromyalgia patients were treated with amitriptyline and buspirone, and successful results were obtained (7,8). In the study conducted by Şahin Onat and Manas (6), sleep bruxism was resolved after 2 months of amitriptyline treatment, and significant improvement was observed in fibromyalgia.

We used amitriptyline in our patient and observed a significant decrease in both bruxism and fibromyalgia symptoms at the end of 1-year follow-up.

CONCLUSION

Sleep bruxism may be kept in mind when questioning the anamnesis of fibromyalgia patients and medical treatment alternatives may be considered in the foreground. Physicians should be aware of this rare side effect of duloxetine in order to manage treatment successfully.

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

Conflict of Interest: None declared by the authors.

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REFERENCES

1. Minakuchi H, Fujisawa M, Abe Y, Iida T, Oki K, Okura K, et al. Managements of sleep bruxism in adult: A systematic review. Jpn Dent Sci Rev. 2022;58:124-36.
2. de la Hoz-Aizpurua JL, Díaz-Alonso E, LaTouche-Arbizu R, Mesa-Jiménez J. Sleep bruxism. Conceptual review and update. Med Oral Patol Oral Cir Bucal. 2011;16(2):e231-8.
3. Lavigne GJ, Khoury S, Abe S, Yamaguchi T, Raphael K. Bruxism physiology and pathology: an overview for clinicians. J Oral Rehabil. 2008;35(7):476-94.

4. Milanlioglu A. Paroxetine-induced severe sleep bruxism successfully treated with buspirone. Clinics (Sao Paulo). 2012;67(2):191-2.
5. Rynn M, Russell J, Erickson J, Detke MJ, Ball S, Dinkel J, et al. Efficacy and safety of duloxetine in the treatment of generalized anxiety disorder: a flexible-dose, progressive-titration, placebo-controlled trial. Depress Anxiety. 2008;25(3):182-9.
6. Şahin Onat S, Malas FÜ. Duloxetine-induced Sleep bruxism in fibromyalgia successfully treated with amitriptyline. Acta Reumatol Port. 2015;40(4):391-2.
7. Albayrak Y, Ekinci O. Duloxetine-induced nocturnal bruxism resolved by buspirone: case report. Clin Neuropharmacol. 2011;34(4):137-8.
8. Bulanda S, Ilczuk-Rypuła D, Nitecka-Buchta A, Nowak Z, Baron S, Postek-Stefańska L. Sleep bruxism in children: etiology, diagnosis, and treatment-A literature review. Int J Environ Res Public Health. 2021;18(18):9544.
9. Goldstein G, DeSantis L, Goodacre C. Bruxism: Best evidence consensus statement. J Prosthodont. 2021;30(S1):91-101.
10. de Baat C, Verhoeff MC, Ahlberg J, Manfredini D, Winocur E, Zweers P, et al. Medications and addictive substances potentially inducing or attenuating sleep bruxism and/or awake bruxism. J Oral Rehabil. 2021;48(3):343-54.
11. Adams M. Antidepressant effects on REM sleep. Biology and Microbiology Graduate Students Plan B Research Projects. 2020;27:1-22.

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Acute Left Branch Block in Pregnancy: A Case of Peripartum Cardiomyopathy

Gebelikte Akut Sol Dal Bloğu: Bir Peripartum Kardiyomiyopati Olgusu

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ABSTRACT

Peripartum cardiomyopathy is a rare but significant condition occurring during late pregnancy or early postpartum period. Risk factors of peripartum cardiomyopathy include pregnancy, advanced maternal age, multiple pregnancies, and preeclampsia. This case report presented a case of a 32-year-old woman who developed an acute left bundle branch block due to peripartum cardiomyopathy during pregnancy. This case underscores the importance of considering peripartum cardiomyopathy in pregnant patients presenting with cardiac symptoms, especially those with risk factors such as advanced maternal age and multiple pregnancies. Prompt diagnosis and management are crucial to mitigate maternal and fetal risks associated with peripartum cardiomyopathy. Preeclampsia can lead to the development of peripartum cardiomyopathy, a rare but severe condition. Accurate diagnosis and appropriate treatment are essential. Clinicians should consider peripartum cardiomyopathy in patients presenting with preeclampsia symptoms.
Keywords: Peripartum cardiomyopathy; pregnancy; acute left bundle branch block; preeclampsia; twin pregnancy.

ÖZ

Peripartum kardiyomiyopati, gebeliğin sonlarında veya doğum sonrası erken dönemde ortaya çıkan nadir fakat önemli bir durumdur. Peripartum kardiyomiyopatinin risk faktörleri arasında gebelik, ileri anne yaşı, çoğul gebelikler ve preeklampsi bulunur. Bu vaka raporunda gebelik sırasında peripartum kardiyomiyopatiye bağlı olarak akut sol dal bloğu gelişen 32 yaşındaki kadın hasta sunulmaktadır. Bu vaka, kardiyak semptomlarla başvuran, özellikle de ileri anne yaşı ve çoğul gebelik gibi risk faktörleri olan gebe hastalarda peripartum kardiyomiyopatinin dikkate alınmasının önemini vurgulamaktadır. Hızlı teşhis ve tedavi, peripartum kardiyomiyopati ile ilişkili anne ve fetüs risklerini azaltmak için çok önemlidir. Preeklampsi, nadir fakat ciddi bir durum olan peripartum kardiyomiyopatinin gelişmesine yol açabilir. Doğru tanı ve uygun tedavi önemlidir. Klinisyenler preeklampsi semptomlarıyla başvuran hastalarda peripartum kardiyomiyopatiyi düşünmelidir.
Anahtar kelimeler: Peripartum kardiyomiyopati; gebelik; akut sol dal bloğu; preeklampsi; ikiz gebelik.

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INTRODUCTION

Peripartum cardiomyopathy (PC) is a variant of dilated cardiomyopathy that occurs in the late stages of pregnancy or early postpartum (1,2). Although PC is very rare, its mortality is high (3). Risk factors of PC include pregnancy, advanced maternal age, multiple pregnancies and preeclampsia (4,5). While the exact cause of PC remains incompletely understood, factors such as inflammation, selenium deficiency,

genetic predisposition, dysregulation of angiogenic factor, and prolactin metabolites are recognized as significant contributors (6,7). In this particular case, an investigation conducted on an acute left bundle branch block secondary to PC during pregnancy was presented in a 32-year-old woman.

CASE REPORT

A 32-year-old patient was admitted to the emergency department at the 37th week of pregnancy (history of in vitro fertilization (IVF), twin pregnancy). Her complaints were palpitations and dyspnea. Initial vital signs showed blood pressure was 150/100 mmHg, pulse 120 bpm, and other symptoms were normal.

Routine blood and urine tests, including troponin levels, were normal. Obstetric ultrasonography revealed a dichorionic diamniotic twin pregnancy consistent with 36-37 weeks. Umbilical artery Doppler ultrasonography of both fetuses was normal and their estimated fetal weight was measured as 2600-2660 g. The non-stress test showed no uterine contractions, and both fetal heart sounds were regular and rhythmic.

The patient's electrocardiography (ECG) exhibited a QRS duration exceeding 120 milliseconds, a broad S wave in leads V1-3, notching in the QRS complex in lateral leads (DI, aVL, V5, V6), and a monophasic wide R wave in leads DI, DII, and V6. Additionally, there was an absence of the septal Q wave in leads DI-aVL and V6, along with an extended R wave peak time in the left precordial leads DI-aVL-V5-V6 (Figure 1).

The patient was diagnosed with acute left bundle branch block and was referred to cardiology. Echocardiography showed a decrease in ejection fraction (30%) and an enlargement of the left heart chambers, along with moderate regurgitation of both the mitral and tricuspid valves (Figure 2). Based on these findings, a diagnosis of PC was made.

A cesarean section was performed, and two female babies were delivered with 1st- and 5th-minute APGAR scores of 7 and 9, respectively. The patient was transferred to the postoperative intensive care unit. Postoperative ECG indicated a normal sinus rhythm (Figure 3).

Medical treatment included metoprolol, enalapril, enoxaparin, bromocriptine, and a magnesium infusion. After 24 hours of follow-up in the intensive care unit, the patient was transferred to the ward. The patient's blood pressure remained stable at around 120/70 mmHg, and she was discharged with her babies on the 7th postoperative day. Approximately nine months later, follow-up showed improvement in cardiomyopathy findings and mitral-tricuspid valve regurgitation, with an ejection fraction of 50-55%.

DISCUSSION

Preeclampsia is a pregnancy complication that has an impact on both maternal and fetal mortality and morbidity. In this case, the patient presented with high blood pressure and dyspnea, leading to a diagnosis of preeclampsia (5). The patient's history of IVF and multiple pregnancy are known risk factors (6). It's important to recognize that, as illustrated in this case, not every instance of preeclampsia may exhibit impaired proteinuria and liver transaminase levels (8).

Dyspnea and chest pain in a term pregnant should not be attributed solely to preeclampsia; Other possible diseases include acute myocardial infarction (AMI) and PC (1,3). The ECG results in our patient reveal signs of left bundle branch block, and the absence of any elevation in troponin levels in the blood serum rules out AMI in this case. The absence of any prior cardiological complaints in the patient strongly suggests that the current cardiomyopathy is PC. The echocardiogram conducted by the cardiology team revealed features consistent with cardiomyopathy, including

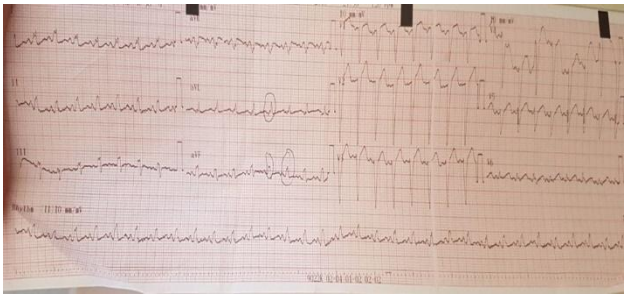

Figure 1. Pre-cesarean electrocardiography: The QRS duration exceeds 120 milliseconds, exhibiting a broad S wave in leads V1-3 and notching in the QRS complex in lateral leads (DI, aVL, V5, V6). Additionally, there is a monophasic wide R wave in leads DI, DII, and V6. Notably, the septal q wave is absent in leads DI-aVL and V6

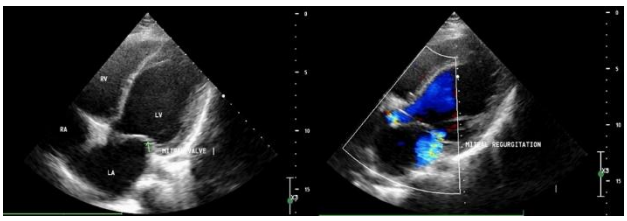

Figure 2. Echocardiography reveals dilated four chambers and a dilated cardiomyopathy with an ejection fraction of 30%, accompanied by mitral and tricuspid regurgitation

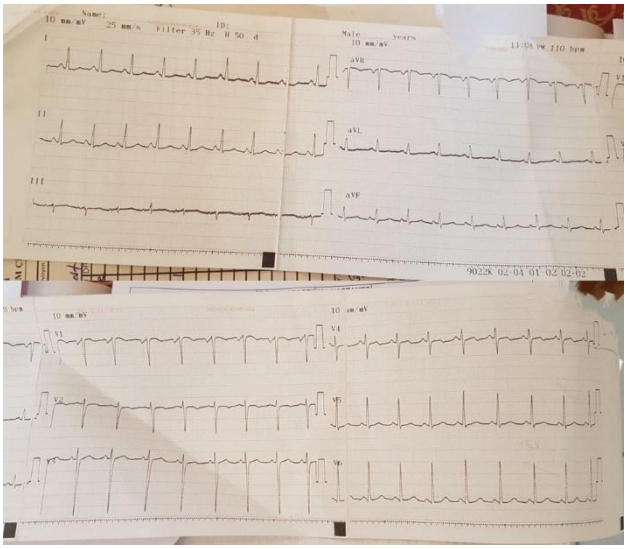

Figure 3. Post-cesarean electrocardiography: Notably, the electrocardiogram obtained approximately 30 minutes after birth demonstrates a normal sinus rhythm

a low ejection fraction of 30%, mitral-tricuspid valve regurgitation grade 2. The patient's diagnosis of PC was established by integrating both the ECG and echocardiography findings with her medical history. As highlighted in a study by Iorgoveanu et al. (5), preeclampsia is identified as a significant risk factor for PC. The patient, diagnosed with left bundle branch block and PC, underwent an emergency cesarean section and was admitted to the intensive care unit for post-operative monitoring and treatment. The initial ECG obtained in the intensive care unit revealed an improvement in the left bundle branch block findings, with the ECG returning to a normal sinus rhythm. In the context of PC, particularly left atrial enlargement, the sensitivity of ECG findings is reported to be low (38%), but the specificity is high (96%) (10). Although the exact etiology of PC remains unclear, factors such as angiogenic factor dysregulation and prolactin metabolites are considered primary contributors (9,10). While there is no defined time interval for the resolution of abnormal ECG findings in PC, they typically normalize within an average of 6-8 months (10). Interestingly, in contrast to the literature, the ECG in our patient returned to a normal rhythm within the first postpartum hour. According to the study by Davis et al. (11), although PC tends to improve, these patients have a high risk of developing cardiovascular diseases in later life.

CONCLUSION

Preeclampsia can lead to the development of PC, a rare but severe condition. Accurate diagnosis and appropriate treatment are essential. Clinicians should consider PC in patients presenting with preeclampsia symptoms.

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

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REFERENCES

1. Davis MB, Arany Z, McNamara DM, Goland S, Elkayam U. Peripartum cardiomyopathy: JACC state-of-the-art review. J Am Coll Cardiol. 2020;75(2):207-21.
2. Ijaz SH, Jamal S, Minhas AMK, Sheikh AB, Nazir S, Khan MS, et al. Trends in characteristics and outcomes of peripartum cardiomyopathy hospitalizations in the United States between 2004 and 2018. Am J Cardiol. 2022;168:142-50.
3. Hoes MF, Arany Z, Bauersachs J, Hilfiker-Kleiner D, Petrie MC, Sliwa K, et al. Pathophysiology and risk factors of peripartum cardiomyopathy. Nat Rev Cardiol. 2022;19(8):555-65.
4. Karaye KM, Ishaq NA, Sa'idu H, Balarabe SA, Talle MA, Isa MS, et al. Registry investigators. Incidence, clinical characteristics, and risk factors of peripartum cardiomyopathy in Nigeria: results from the PEACE Registry. ESC Heart Fail. 2020;7(1):235-43.
5. Iorgoveanu C, Zaghloul A, Ashwath M. Peripartum cardiomyopathy: a review. Heart Fail Rev. 2021;26(6):1287-96.
6. Jha N, Jha AK. Peripartum cardiomyopathy. Heart Fail Rev. 2021;26(4):781-97.
7. Gorog DA, Vilahur G. Peripartum cardiomyopathy: can the link between prolactin and PAI-1 provide a clue? Cardiovasc Res. 2020;116(11):1791-3.
8. Tanner MS, Davey MA, Mol BW, Rolnik DL. The evolution of the diagnostic criteria of preeclampsia-eclampsia. Am J Obstet Gynecol. 2022;226(2S):835-43.
9. Keukens A, van Wely M, van der Meulen C, Mochtar MH. Pre-eclampsia in pregnancies resulting from oocyte donation, natural conception or IVF: a systematic review and meta-analysis. Hum Reprod. 2022;37(3):586-99.
10. Laksono S, Prawara AS, Halomoan R. Diagnosis and management of severe peripartum cardiomyopathy. Indones J Obstet Gynecol. 2021;9(4):215-8.
11. Davis MB, Jarvie J, Gambahaya E, Lindenfeld J, Kao D. Risk prediction for peripartum cardiomyopathy in delivering mothers: a validated risk model: PPCM risk prediction model. J Card Fail. 2021;27(2):159-67.

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Immune Checkpoint Inhibitor-Associated Transverse Myelitis

İmmün Kontrol Noktası İnhibitörü ile İlişkili Transvers Miyelit

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ABSTRACT

Immune checkpoint inhibitors (ICIs) are highly effective in treating cancer and are increasingly used. Thus, awareness of various complications in the form of immunity-related adverse events is increasing. Transverse myelitis following ICIs is a rare but severe neurological adverse event, and information about this entity is minimal. ICI-associated transverse myelitis should be considered a rapid and comprehensive differential diagnosis after evaluating infective, metabolic, or other inflammatory-autoimmune pathologies. After diagnosis, early immunomodulation is required through intravenous high-dose methylprednisolone, IVIg, or plasmapheresis. It should be kept in mind that different etiologies may coexist or a superimposed condition may cause each other, and concurrent treatment should not be delayed. Further studies are needed to investigate the neurological manifestations that may develop in association with these therapies further and help establish guidelines for their management. In this case report, a rare case of ICI-associated transverse myelitis in a 62-year-old male patient was presented.
Keywords: Immune checkpoint inhibitor; adverse event; transverse myelitis.

ÖZ

İmmün kontrol noktası inhibitörleri (immune checkpoint inhibitors, ICIs) kanser tedavisinde oldukça etkilidir ve giderek daha fazla kullanılmaktadır. Bu nedenle, bağışıklıkla ilişkili advers olaylar şeklinde çeşitli komplikasyonlara ilişkin farkındalık artmaktadır. ICI'ları takiben gelişen transvers miyelit, nadir ancak ciddi bir nörolojik advers olaydır ve bu antite hakkındaki bilgiler çok sınırlıdır. Enfektif, metabolik veya diğer enflamatuar-otoimmün patolojilerin değerlendirilmesinden sonra hızlı ve kapsamlı bir ayırıcı tanı olarak ICI kaynaklı transvers miyelit dikkatle düşünülmelidir. Tanıdan sonra, intravenöz yüksek doz metilprednizolon, IVIg veya plazmaferez şeklinde erken immünomodülasyon gereklidir. Farklı etiyolojilerin bir arada bulunabileceği veya üst üste binen bir durumun birbirine neden olabileceği akılda tutulmalı ve eş zamanlı tedavi geciktirilmemelidir. Bu tedavilere bağlı olarak gelişebilecek nörolojik bulguların daha fazla araştırılması ve bunların yönetimine ilişkin kılavuzların oluşturulmasına yardımcı olmak için daha fazla çalışmaya ihtiyaç vardır. Bu olgu sunumunda, 62 yaşında bir erkek hastada, nadir görülen bir ICI ile ilişkili transvers miyelit vakası sunulmuştur.
Anahtar kelimeler: İmmun kontrol noktası inhibitörü; advers olay; transvers miyelit.

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INTRODUCTION

Immune checkpoint inhibitors (ICIs) may cause immune-related adverse events (1,2). ICI-associated transverse myelitis should be carefully considered as a rapid and comprehensive differential diagnosis after evaluation of infective, metabolic, or other inflammatory-autoimmune pathologies. On the other hand, it should be remembered that different etiologies may coexist, overlapping conditions may cause

each other, and concurrent treatment should not be delayed. This case report aimed to present a rare but confusing case of ICI-associated transverse myelitis.

CASE REPORT

A 62-year-old male was diagnosed with small cell lung cancer (SCLC) with lymphatic and vertebral metastases one year ago. He was treated with seven cycles of etoposide and carboplatin (3 doses daily per cycle) and currently two doses of atezolizumab. One month after the second dose of atezolizumab, he presented with subacute onset bilateral lower extremity weakness, urinary overflow incontinence, numbness in all extremities, and involuntary contractions in upper extremities. On examination, there were proximal (4/5) and distal (3/5) bilateral symmetrical upper extremity and bilateral lower extremity (right; 3/5, left; 2/5) weakness, sensory ataxia, spasticity in all extremities, loss of bilateral deep tendon reflexes and loss of deep sensation (vibration and joint position). He was limited to a wheelchair and self-catheterization.

Cervical and thoracic spine magnetic resonance imaging demonstrated symmetrical T2 signal hyperintensity confined to the lateral tracts without contrast enhancement from C1 to T11 and a T1 hypointense lesion of the T9 and T11 vertebral body with irregular T2 signal and contrast enhancement (Figure 1). EMG demonstrated a polyneuropathy syndrome in the subacute period with axonal damage in which sensory and motor fibers were affected. There was no response in bilateral lower and upper extremities' sensory evoked potentials (SEPs). In visual evoked potentials (VEPs), the P100 wave could not be obtained on the left, and there was a prolongation of P100 wave latency on the right. Routine laboratory examination, hemogram, thyroid function tests, serum cobalamin, folate, and methylmalonate (0.11 nmol/ml, NV 0-0.4) were within the normal range. No lymphocytes or malignant cells were detected in the CSF analysis, and the glucose level was in the normal range proportionally to serum (63/102 mg/dl). A slight increase was in protein (46.2 mg/dl, NV 15-45), and LDH (27 U/L, NV 10-19). Serum antibodies against aquaporin-4 and myelin oligodendrocyte glycoprotein (MOG), serology for neurotropic viruses (including CMV, HIV, EBV, HBV, HCV, and HSV1-2), rheumatological screening tests (including ANA, thyroid antibodies, beta2glycoprotein, p-ANCA, rheumatoid factor, c-ANCA, anti-cardiolipin, celiac antibodies), serology for *Brucella*, *Borrelia burgdorferi*, and *Treponema pallidum*, paraneoplastic panel antibodies (including anti-NMDR, AMPA1, AMPA2, CASPR2, LGI1, Amphiphysin, GABARB1/B2, CV2, PNMA2, Ri, Yo, Hu, Recoverin, Titin, Zic4, GAD65, TRAB, SOX1), and brain MRI were normal. Serum copper level (53 μg/mL, NV 70-140) and ceruloplasmin (193 mg/L, NV 200-600) were slightly below normal limits, and serum zinc concentration mildly exceeded the reference range (159 ug/L, NV 73-127). The copper level (4.2 μg/24h) in 24-hour accumulation urine was average. The patient denied any history of zinc abuse. Total blood count did not reveal anemia or cytopenias.

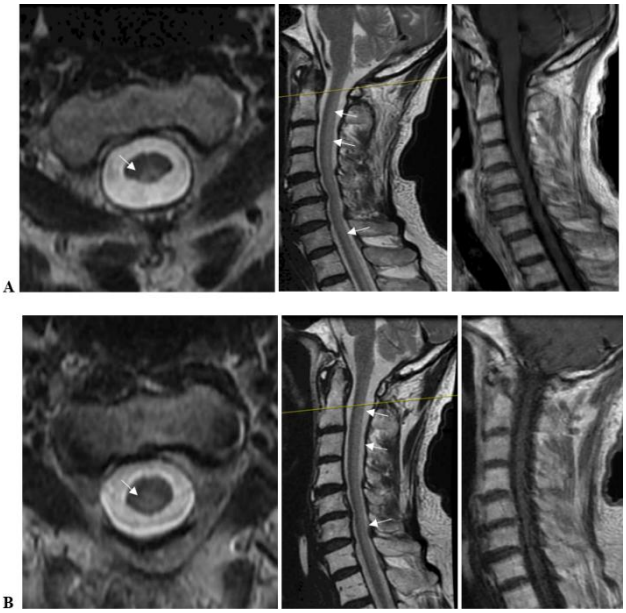

Figure 1. A) Cervical spinal magnetic resonance imaging (MRI) at initial presentation (first month of complaint onset), axial T2, sagittal T2, and sagittal contrast-enhanced (C+) T1, respectively. arrows; T2 hyperintense longitudinally extensive transverse myelitis (LETM); **B)** Spinal MRI repeat at 8th month, axial T2, sagittal T2, and sagittal C+ T1, respectively. arrows; LETM without change

There was minimal improvement in neurological examination (10% improvement in upper limb muscle strength loss) after five cycles of every other day therapeutic plasma exchange for transverse myelitis at initial presentation. Although the amount of copper excreted in urine was normal, serum copper and ceruloplasmin levels were borderline low. So, 8 mg/day oral copper supplementation and zinc-poor diet were administered for eight months. Routine laboratory tests, thyroid function tests, serum cobalamin, folate, and methylmalonate were within the normal range at month eight of replacement therapy. Electrophysiological tests were repeated. In VEP, in addition to prolonged P100 latency on the right side, prolonged conduction could be obtained on the left side (amplitude; 5.28/4.99 μv, latency; 131/133 ms, respectively). While there was no conduction on SEP eight months ago, bilateral prolonged P40 wave latencies (44 and 49.2 ms) and normal p60 waves in the lower extremities, and prolonged N20 and P25 wave latencies (20.8 and 24.6 ms) in the upper extremities on the left side, while no response was obtained on the right side. No change was observed in the control EMG results, neurological examination, and control MRI. Ceruloplasmin, serum copper, and urinary copper excretion in 24 hours were within normal limits (277 mg/L, 110 μg/mL, 4.8 μg/24h, respectively). Serum zinc level decreased after dietary administration (145.4 ug/L), and 24-hour urinary zinc excretion was within normal limits (910 μg/24h, NV 200-1300). With these results, exogenous zinc intake was ruled out. After all exclusion, despite the improvement in laboratory values and electrophysiological tests, the absence of difference in neurological examination and spinal MRI concluded that ICI-associated transverse myelitis was superimposed with possible copper deficiency.

DISCUSSION

The risk of neurological complications is high with the use of ICI. Neurological side effects constitute 1-5% of the side effects related to ICIs. Adverse events may develop with the impact of other organ systems, as they show their effect by increasing the endogenous immune response. In addition to myelitis, hypophysitis, myasthenia gravis,

encephalitis, meningitis, neuromyelitis optica spectrum disorder, vasculitis, and polyneuropathy have been reported as neurological adverse effects (1,2). They are usually severe and carry a risk of long-term disability or death (3). Symptoms typically occur within 6 to 12 weeks of the onset of ICI but can sometimes occur immediately after initiation of the drug or months (2-17 months) after discontinuation of ICI (4-6). Myelitis associated with ICPs is usually in the long, lower cervical, and thoracic segments, with an expansive, punctate, or patchy enhanced or unenhanced appearance (6). It has been reported in the literature after durvalumab, nivolumab, pembrolizumab, and atezolizumab in patients with SCLC, non-SCLC, melanoma, and hepatocellular carcinoma (7,8). A slight increase in isolated bos protein is generally detected (6,8). Although an excellent response to steroid or plasmapheresis treatments has been reported, it is clinically variable, poor response to treatment, and permanent disability may occur (6,7,9). The case of myelitis after atezolizumab is rarely mentioned in the literature. Transverse myelitis has been reported after atezolizumab in one issue of metastatic SCLC and after its combination with bevacizumab in one case of hepatocellular carcinoma (5,10).

The two main manifestations of copper deficiency are bone marrow and the neurological system. Gastrointestinal surgery, zinc overload, malabsorption, long-term parenteral nutrition, and alcohol use are the main risk factors, respectively (11). Copper deficiency myelopathy usually presents as chronic progressive myelopathy (12). Initially, most patients (78%) had anemia/cytopenia (13,14). In a clinically compatible case, one or more risk factors and cytopenia may suggest copper deficiency myelopathy. Copper levels may fall below the normal range with plasmapheresis treatment or in the presence of malignancy; required for tumor growth or development of metastases (15,16).

Subacute onset, absence of bone marrow involvement, lack of risk factors, and serum copper and ceruloplasmin levels close to the minimal limit suggested that this was ICI-associated myelitis rather than metabolic myelopathy or polyneuropathy. This was confirmed by partial clinical improvement with plasmapheresis treatment in the early phase and no clinical change with eight months of copper replacement in the late phase. In conclusion, in this case, in which atezolizumab treatment was not continued during the follow-up period, it was concluded that the leading cause was ICI-associated subacute transverse myelitis clinic and mild copper deficiency secondary to malignancy overlapped.

CONCLUSION

Immune checkpoint inhibitors (ICIs) are highly effective in treating cancer and are increasingly used. Thus, awareness of various complications in the form of immunity-related adverse events is increasing. Transverse myelitis following ICIs is a rare but severe neurological adverse event, and information about this entity is minimal. ICI-associated transverse myelitis should be carefully considered as a rapid and comprehensive differential diagnosis after evaluation of infective, metabolic, or other inflammatory-autoimmune pathologies. After diagnosis, early immunomodulation is required through intravenous high-dose methylprednisolone, IVIg, or plasmapheresis. On the other hand, it should be kept in mind that different aetiologies may coexist or a superimposed condition may cause each other, and concurrent treatment should not be delayed. Further studies are needed to investigate the neurological manifestations that may develop in association with these therapies further and help establish guidelines for their management.

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

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Author Contributions: Idea/Concept: FS, ERK; Design: FS, FH; Data Collection/Processing: FS, FH; Analysis/Interpretation: FS, ERK, ÖFT; Literature Review: FS, FH; Drafting/Writing: FS, FH; Critical Review: ERK, ÖFT.

REFERENCES

1. Mikami T, Liaw B, Asada M, Niimura T, Zamami Y, Green-LaRoche D, et al. Neuroimmunological adverse events associated with immune checkpoint inhibitor: a retrospective, pharmacovigilance study using FAERS database. J Neurooncol. 2021;152(1):135-44.
2. Narumi Y, Yoshida R, Minami Y, Yamamoto Y, Takeguchi S, Kano K, et al. Neuromyelitis optica spectrum disorder secondary to treatment with anti-PD-1 antibody nivolumab: the first report. BMC Cancer. 2018;18(1):95.
3. Farina A, Villagrán-García M, Honnorat J. Neurological adverse events of immune checkpoint inhibitors: An update of clinical presentations, diagnosis, and management. Rev Neurol (Paris). 2023;179(5):506-15.
4. Williams TJ, Benavides DR, Patrice KA, Dalmau JO, de Ávila AL, Le DT, et al. Association of autoimmune encephalitis with combined immune checkpoint inhibitor treatment for metastatic cancer. JAMA Neurol. 2016;73(8):928-33.
5. Kim KH, Baek YH, Kang YW, Yoon BA, Moon SY. A case of transverse myelitis following treatment with atezolizumab for advanced hepatocellular carcinoma. Korean J Gastroenterol. 2023;82(1):35-9.
6. Chatterton S, Xi S, Jia JX, Krause M, Long GV, Atkinson V, et al. Case series: Immune checkpoint inhibitor-induced transverse myelitis. Front Neurol. 2023;14:1130313.
7. Moodie T, Alshaqi O, Alchaki A. Longitudinal extensive transverse myelitis after chemoradiation therapy with durvalumab, a rare complication: case report. BMC Neurol. 2022;22(1):107.

8. Picca A, Berzero G, Bihan K, Jachiet V, Januel E, Coustans M, et al. Longitudinally extensive myelitis associated with immune checkpoint inhibitors. Neurol Neuroimmunol Neuroinflamm. 2021;8(3):e967.
9. Bolz S, Ramakrishnan T, Fleischer M, Livingstone E, Stolte B, Thimm A, et al. Detect it so you can treat it: A case series and proposed checklist to detect neurotoxicity in checkpoint therapy. eNeurologicalSci. 2021;22:100324.
10. Esechie A, Fang X, Banerjee P, Rai P, Thottempudi N. A case report of longitudinal extensive transverse myelitis: immunotherapy related adverse effect vs. COVID-19 related immunization complications. Int J Neurosci. 2023;133(10):1120-3.
11. Shibazaki S, Uchiyama S, Tsuda K, Taniuchi N. Copper deficiency caused by excessive alcohol consumption. BMJ Case Rep. 2017;2017:bcr2017220921.
12. Kumar N, Ahlskog JE, Klein CJ, Port JD. Imaging features of copper deficiency myelopathy: a study of 25 cases. Neuroradiology. 2006;48(2):78-83.
13. Jaiser SR, Winston GP. Copper deficiency myelopathy. J Neurol. 2010;257(6):869-81.
14. Halfdanarson TR, Kumar N, Li CY, Phyliky RL, Hogan WJ. Hematological manifestations of copper deficiency: a retrospective review. Eur J Haematol. 2008;80(6):523-31.
15. Yan C, Sheehan M, Wilson R. Copper levels in maintenance plasmapheresis (250). Neurology. 2020;94(15 Suppl):250.
16. Ge EJ, Bush AI, Casini A, Cobine PA, Cross JR, DeNicola GM, et al. Connecting copper and cancer: from transition metal signalling to metalloplasia. Nat Rev Cancer. 2022;22(2):102-13.

Case Report / Olgu Sunumu
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An Uncommon Occurrence of Posterior Fossa Ependymoma in Elderly Patient: A Case Report

Posterior Fossa Ependimomunun Yaşlı Hastada Nadir Bir Prezentasyonu: Olgu Sunumu

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ABSTRACT

Cerebellar ependymomas are rare neoplasms, even more so in elderly patients. In this case report, a 75-year-old male patient admitted with a one-year history of progressive general decline, urinary retention, left-sided weakness, memory loss, and insomnia was presented. Physical examination revealed left-sided hemiparesis and mild confusion. Cranial magnetic resonance imaging (MRI) showed a heterogeneously enhancing lesion measuring 22x15x20 mm originating from the fourth ventricle, with signs of hydrocephalus. The patient underwent tumor resection and placement of a ventriculoperitoneal (VP) shunt to address hydrocephalus. Postoperative MRI revealed no residual tumor, and the patient experienced gradual clinical improvement. Hemiparesis showed partial recovery, the overall condition stabilized, and he was subsequently discharged. Pathological examination confirmed a diagnosis of World Health Organization (WHO) grade II ependymoma. The patient is currently on a three-month follow-up.
Keywords: Aged; ependymoma; infratentorial neoplasms; case reports.

ÖZ

Serebellar ependimomlar nadir görülen neoplazmlardır ve yaşlı hastalarda daha da nadirdir. Bu olgu sunumunda, bir yıl süresince ilerleyici genel durum bozukluğu, idrar yapamama, sol tarafta güçsüzlük, hafıza kaybı ve uykusuzluk öyküsü ile başvuran 75 yaşında bir erkek hasta sunulmuştur. Fizik muayenede sol taraflı hemiparezi ve hafif konfüzyon görüldü. Kranial manyetik rezonans görüntüleme (MRG), dördüncü ventrikülden kaynaklanan, 22x15x20 mm boyutlarında heterojen olarak kontrastlanan bir lezyon ve hidrosefali bulguları gösterdi. Hastaya hidrosefaliyi tedavi etmek için tümör rezeksiyonu ve ventriküloperitoneal (VP) şant yerleştirildi. Ameliyat sonrası MRG'de, rezidüel tümör görülmedi ve hasta kademeli olarak klinik iyileşme gösterdi. Hemiparezi kısmi iyileşme gösteren ve genel durumu stabilize olan hasta bunun ardından taburcu edildi. Patolojik inceleme, Dünya Sağlık Örgütü (DSÖ) evre II ependimom tanısını doğruladı. Hasta şu anda üç aylık takiptedir.
Anahtar kelimeler: Yaşlı; ependimom; infratentorial neoplaziler; olgu sunumları.

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INTRODUCTION

Cerebellar ependymomas are an uncommon subtype of ependymomas, accounting for a small percentage of intracranial tumors. These neoplasms, arising from ependymal cells lining the ventricular system, are particularly rare in the elderly, where the differential diagnosis often prioritizes more prevalent conditions such as gliomas or metastases (1-5). The clinical presentation of cerebellar ependymomas can

be insidious and nonspecific, typically manifesting as progressive neurological deficits, hydrocephalus, and symptoms related to increased intracranial pressure. Given their rarity and the often subtle onset of symptoms, cerebellar ependymomas pose a significant diagnostic challenge, particularly in elderly patients who may also suffer from concurrent degenerative or vascular conditions.

This case report highlights the clinical, radiological, and pathological features of a cerebellar ependymoma in a 75-year-old male, emphasizing the importance of considering this diagnosis in the differential workup of progressive neurological decline in elderly patients.

CASE REPORT

A 75-year-old male presented with a one-year history of progressive worsening in general condition, including urinary retention, left-sided weakness, memory loss, and insomnia that started six months before admission. The patient had a history of treatment for presumed Parkinson's disease and dementia but showed no improvement. He was admitted to our tertiary care facility for an advanced diagnostic workup.

The physical examination revealed hemiparesis on the left side and mild confusion. The patient scored 4/6/3 on the Glasgow coma scale (GCS), indicating moderate to severe impairment. Initial laboratory investigations, including blood biochemistry and complete blood count, were within normal ranges. However, magnetic resonance imaging (MRI) of the brain showed a heterogeneously enhancing lesion measuring 22x15x20 mm originating from the fourth ventricle, along with signs of hydrocephalus (Figure 1).

Given the imaging findings, the patient underwent a tumor resection and placement of a ventriculoperitoneal (VP) shunt to address the hydrocephalus. Intraoperatively, the lesion appeared as a well-circumscribed, moderately vascular tumor. Postoperatively, the patient was monitored in the intensive care unit for 48 hours before being transferred to the neurosurgical ward.

Histopathological examination confirmed the diagnosis of World Health Organization (WHO) grade II ependymoma. The tumor exhibited characteristic ependymal rosettes and perivascular pseudorosettes with a low proliferative index (Ki-67 labeling index of 4%). Immunohistochemical staining was positive for GFAP and S-100, supporting the ependymal origin of the tumor.

Postoperative MRI showed no residual tumor (Figure 2). The patient’s hemiparesis showed partial recovery, and his overall condition stabilized. He was discharged home in stable condition with plans for regular follow-up. At his three-month follow-up, the patient reported continued improvement in strength and cognitive function, although he remained dependent on Foley catheterization due to persistent urinary retention.

DISCUSSION

Cerebellar ependymomas are rare, particularly in the elderly (1-9). The majority of intracranial neoplasms in this demographic are metastatic lesions, making primary tumors like ependymomas an unusual finding (10). This case report adds to the limited literature on cerebellar ependymomas in older adults, highlighting the necessity for a high index of suspicion when encountering atypical intracranial masses in this age group.

Diagnosing cerebellar ependymomas in the elderly can be particularly challenging due to the non-specific nature of symptoms and the common presence of comorbid conditions such as dementia and Parkinson’s disease. These overlapping clinical features often lead to initial misdiagnosis, as seen in our patient, who was initially treated for neurodegenerative disorders. Advanced imaging techniques, such as MRI with contrast, are crucial in differentiating ependymomas from other common pathologies like metastatic lesions, meningiomas, or gliomas (11).

Treatment often requires surgical intervention followed by adjuvant therapies (6,7). Although studies have investigated

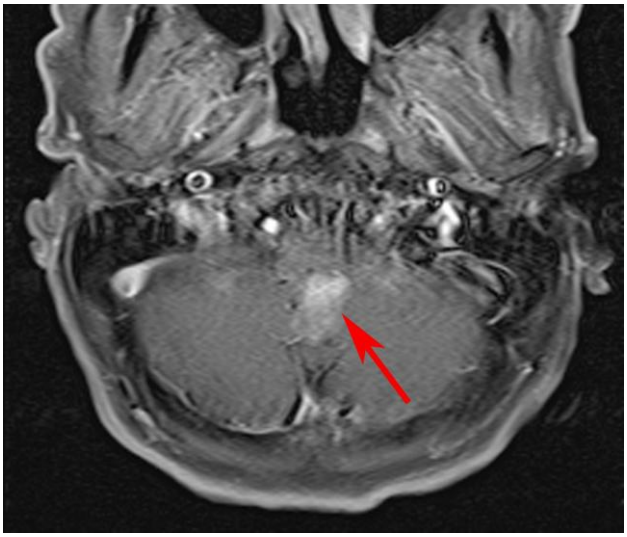

Figure 1. Preoperative magnetic resonance imaging, T1 gadolinium-enhanced sequence, note the contrast-enhancing lesion on the posterior fossa's fourth ventricle wall, marked with the red arrow

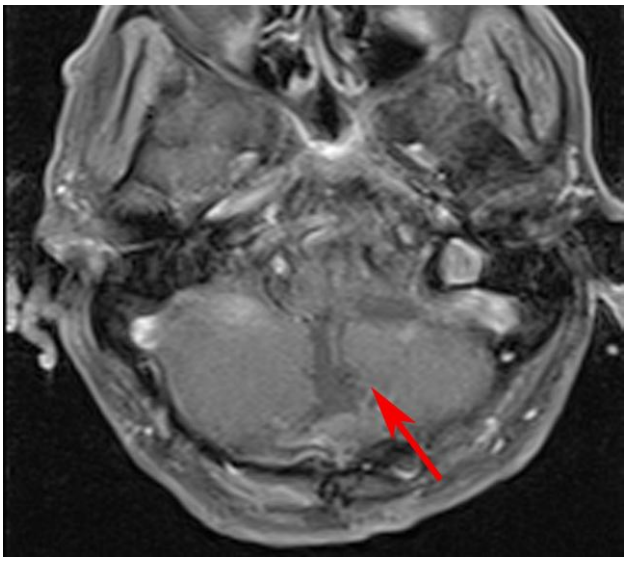

Figure 2. Postoperative magnetic resonance imaging, T1 gadolinium-enhanced sequence, the tumor is entirely resected, and the cavity is marked with the red arrow

the effectiveness of stereotactic radiosurgery with some success, this is particularly important in the elderly population due to their frailty (12). Thus, multidisciplinary collaboration is essential in these cases, involving neurosurgeons, oncologists, radiologists, and rehabilitation specialists to optimize patient outcomes.

This case underscores the need for further research into the pathophysiology, optimal treatment approaches, and long-term prognosis of cerebellar ependymomas in the elderly to inform clinical practice better and improve patient care.

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

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REFERENCES

1. Hsu HI, Hsu SS, Chung WY, Yip CM, Liu SH, Liao WC. Adult posterior fossa anaplastic ependymoma, case series and literature review. World Neurosurg. 2022;158:205-9.
2. Thompson YY, Ramaswamy V, Diamandis P, Daniels C, Taylor MD. Posterior fossa ependymoma: current insights. Childs Nerv Syst. 2015;31(10):1699-706.
3. Kawano N, Yagishita S, Komatsu K, Suwa T, Oka H, Utsuki S, et al. Cerebellar clear cell ependymoma mimicking hemangioblastoma: its clinical and pathological features. Surg Neurol. 1999;51(3):281-7.
4. Hayashi T, Miyazaki H, Ishiyamai N, Kameyama K. Immunohistochemical study is helpful for the diagnosis of cerebellar clear cell ependymoma with atypical radiological findings--case report. No Shinkei Geka. 2005;33(11):1113-7. Japanese.
5. Hayashi T, Inamasu J, Kanai R, Sasaki H, Shinoda J, Hirose Y. Clinical, histological, and genetic features of fourth ventricle ependymoma in the elderly. Case report. Neurol Med Chir (Tokyo). 2012;52(8):611-6.
6. Lampros M, Vlachos N, Alexiou GA. Ependymomas in children and adults. Adv Exp Med Biol. 2023;1405:99-116.
7. Schaff LR, Mellinghoff IK. Glioblastoma and other primary brain malignancies in adults: A review. JAMA. 2023;329(7):574-87.
8. İş M, Gezen F, Yıldız KH, Akyüz F, Can A. Radiation necrosis that is diagnosed by magnetic resonance spektroscopy in an anaplastic ependymoma case: Case report. Duzce Med J. 2005;7(2):23-8.
9. Calıs MD. Intracranial ependymoma with extremely rare extraneural metastasis. J Cancer Res Ther. 2024;20(1):460-3.
10. Bondy ML, Scheurer ME, Malmer B, Barnholtz-Sloan JS, Davis FG, Il'yasova D, et al. Brain tumor epidemiology: consensus from the Brain Tumor Epidemiology Consortium. Cancer. 2008;113(7 Suppl):1953-68.
11. Deng J, Xue C, Liu X, Li S, Zhou J. Differentiating between adult intracranial medulloblastoma and ependymoma using MRI. Clin Radiol. 2023;78(3):e288-93.
12. Yoo KH, Marianayagam NJ, Park DJ, Persad A, Zamarud A, Shaghaghian E, et al. Stereotactic radiosurgery for ependymoma in pediatric and adult patients: A single-institution experience. Neurosurgery. 2024;95(2):456-68.

Case Report / Olgu Sunumu
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Virtual Reality in Vestibular Rehabilitation for Mal de Débarquement Syndrome: A Case Report

Mal de Debarquement Sendromunda Sanal Gerçeklik ile Vestibüler Rehabilitasyon: Olgu Sunumu

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ABSTRACT

Mal de débarquement syndrome (MdDS) is a condition characterized by symptoms of imbalance and a sensation of swaying, particularly after sea travel, that occur following the cessation of movement. It typically appears after long sea voyages but can also be seen after airplane, train, or car travel. The exact cause of MdDS is unknown, but it is thought to involve a disruption in the systems in the brain that govern balance and motion perception. While there is no definitive treatment, therapy, and medications can sometimes alleviate symptoms. The aim of this case report was to demonstrate the success of using virtual reality in the treatment process of a 51-year-old male patient diagnosed with MdDS, and thus to raise awareness among clinicians to effectively manage the not well-known MdDS.
Keywords: Mal de débarquement syndrome; dizziness; virtual reality; vestibular rehabilitation.

ÖZ

Mal de débarquement sendromu (MdDS), özellikle deniz yolculuğundan sonra ortaya çıkan ve hareketin durmasının ardından dengesizlik ve sallanma hissi gibi belirtilerle karakterize edilen bir hastalıktır. Genellikle uzun deniz yolculuklarından sonra ortaya çıkmakla birlikte, uçak, tren veya araba yolculuklarından sonra da görülebilmektedir. MdDS'nin nedeni tam olarak bilinmemektedir, ancak beyindeki denge ve hareket algısını yöneten sistemlerde bir bozulma olduğu düşünülmektedir. Kesin bir tedavi yöntemi bulunmamakla birlikte, bazı durumlarda terapi ve ilaçlar belirtileri hafifletebilir. Bu vaka raporunun amacı, MdDS tanısı konulan 51 yaşında bir erkek hastanın tedavi sürecinde sanal gerçekliğin kullanılmasının başarısını göstermek ve böylece yeterince iyi tanınmayan MdDS'yi etkili bir şekilde yönetebilmek için klinisyenler arasındaki farkındalığı artırmaktır.
Anahtar kelimeler: Mal de débarquement sendromu; baş dönmesi; sanal gerçeklik; vestibüler rehabilitasyon.

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INTRODUCTION

Mal de débarquement syndrome (MdDS) is a movement-induced oscillatory vertigo disorder that persists even after the movement has stopped. MdDS is a persistent oscillatory vertigo illness following passive movements such as sea, air, or land travel (1). Although MdDS involves a sensation of phantom motion, it is not described as genuine rotational vertigo, and patients usually do not report symptoms

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of motion sickness (2). MdDS is classified into two as motion-triggered (MT-MdDS) and non-motion-triggered MdDS (non-MT MdDS), which start spontaneously or in other ways (3).

Unlike dizziness from vestibular disorders or motion sickness, MdDS symptoms typically improve when the person is re-exposed to motion (4). Although various treatment options such as pharmacological therapies, neuromodulation, and vestibulo-ocular reflex (VOR) rehabilitation are available, access to these methods is limited, and they often focus on addressing secondary symptoms rather than resolving the underlying issue. Therefore, finding effective treatment options that can alleviate MdDS symptoms is of great importance (5).

Symptoms are typically triggered by sea, air, and car travel (6). Other symptoms include disorientation, postural instability, imbalance, fatigue, cognitive impairments, and kinesiophobia (7). Visual sensitivity has been reported in MdDS patients (8). MdDS patients may exhibit mild vestibular imbalance with rotation during the Unterberger test (9).

Vestibular rehabilitation is a therapeutic approach used to treat dizziness and balance disorders. It is based on central neuroplasticity mechanisms that facilitate vestibular compensation, including habituation, adaptation, and substitution. Vestibular rehabilitation is known to be effective for many vestibular disorders (10). Virtual reality systems have recently been used as an effective therapy method in the field of vestibular rehabilitation (11). The purpose of using virtual reality technology in vestibular rehabilitation is to reduce symptoms, use a realistic visual environment that causes retinal slip and habituation, increase VOR gain and optokinetic responses, and improve postural stability (12).

The diagnostic criteria for MdDS are as follows:

1. A sensation of non-spinning vertigo (e.g., rocking, bobbing, or swaying) that begins after passive movements such as sea, air, or land travel, or exposure to virtual reality.
2. Occurs within 48 hours of disembarking from a moving vehicle such as a boat, plane, or car.
3. Symptoms temporarily reduce with passive movement exposure (e.g., driving).
4. Symptoms persist for more than 48 hours.

MdDS can be termed 'developing' if observed for less than a month while symptoms persist; 'transient' if symptoms resolve within a month or sooner and are observed at least until the resolution point; or 'persistent' if symptoms last longer than a month. Individuals with MdDS may develop accompanying symptoms such as spatial disorientation, visual motion intolerance, fatigue, and exacerbation of headache or anxiety (1).

This case report aimed to present using virtual reality as a treatment option for MdDS in a 51-year-old male patient.

CASE REPORT

A 51-year-old male military helicopter pilot presented to our clinic with a complaint of a sensation of swaying for the past 7 months. His medical history included benign paroxysmal positional vertigo (BPPV), which had resolved with maneuvers. He was examined by a neurologist, cardiologist, and otolaryngologist at an external center, but no diagnosis was made. Our patient underwent anamnesis, physical examination, and audio-vestibular evaluation. For auditory assessment, an AC40 model audiometer and an AT235H model tympanometer (Interacoustics, Denmark) were used, and for vestibular evaluation, a VE525B model VNG device and a vHIT device (Interacoustics, Denmark) were utilized. Neurological examination and radiological assessment were conducted to rule out central pathologies. Magnetic resonance imaging (MRI) results were normal. A video head impulse test (v-HIT) was performed to evaluate the semicircular canals, and the results were normal. Audiometry tests showed bilateral normal hearing. Acoustic reflex measurements revealed bilateral type A tympanogram and normal immittance measurements. Romberg and tandem Romberg tests were normal. Unterberger test showed a 30-degree turn to the right. Dysmetria and dysdiadochokinesia were normal. Videonystagmography (VNG) test was normal. The dizziness handicap inventory score was 56, the Beck anxiety inventory score was 20, and the visual vertigo analog scale score was 44. Based on the absence of any pathological findings in the tests and the patient's history, the patient was diagnosed with MdDS.

The Interacoustic Virtualis BalanceVR device was used for treatment. As a treatment, virtual reality vestibular rehabilitation was performed once a week for a total of 12 sessions, with each session lasting 1 hour. The subjective visual vertical (SVV), optokinetic (horizontal, rotatory, and vertical) tests, optical flow, car, sea, elevator, escalator, target tracking, metro, city, and shopping mall simulations available in the Virtualis BalanceVR device were used. Among these rehabilitation modules, those that could provoke the patient's condition were individually selected. This selection was made by having the patient experience simulations of daily activities and environments that caused discomfort. The patient's discomfort thresholds were determined, and vestibular rehabilitation was initiated at one level below these thresholds. In the sessions, no higher-level parameters were introduced until sufficient adaptation had developed between the parameters and variables, and the patient was able to tolerate their symptoms. The difficulty level was increased with each session. The patient was standing. In the first session, he struggled with all of them, experiencing a sensation of swaying and dizziness. By the end of the 12th session, he was able to perform all of them. In the evaluation conducted after the sessions, the dizziness handicap inventory score was found to be 18, the Beck anxiety inventory score was 14, and the visual vertigo analog scale score was 6. The patient reported that his complaints had been resolved.

DISCUSSION

Individuals with MdDS describe swaying symptoms after sea, air, or land travel. These symptoms may occur due to the VOR failing to adapt to head rotation. Dai et al. (13) developed an optokinetic stimulation (OKS) paradigm for the treatment of MdDS and suggested that a treatment protocol based on the readaptation of the VOR alleviates the perception of self-motion. They treated a total of 24 MdDS patients who were asked to watch a full-field rotating visual stimulus inside an OKS cabin while turning their heads from side to side.

In a study by Yakushin et al. (14), the effectiveness of virtual reality glasses with a limited visual field in

simulating a laboratory environment for MdDS treatment was examined in 5 patients. All five patients in this study responded positively to treatment with limited-area horizontal optokinetic nystagmus (OKN) stimulation. Therefore, limited-area OKN stimulation could be an effective stimulus for the activation of velocity storage, which can be used in the treatment of MdDS.

In a study by Hoppes et al. (15), a virtual reality city scene and optokinetic stripes were used in a computer-assisted rehabilitation environment for the treatment of MdDS. During the treatment, the patient was seated in a chair. Due to the patient's symptoms disappearing after the second session, they did not attend the third session. In our study, the virtual reality treatment consisted of 12 sessions, each lasting 1 hour, once a week, with the patient standing. The SVV, optokinetic (horizontal, rotatory, and vertical) tests, optic flow, car, sea, elevator, escalator, target tracking, metro, city, and shopping mall simulations were used.

There is no definitive treatment for MdDS. However, it can be controlled with benzodiazepines, antiemetics, selective serotonin reuptake inhibitors, tricyclic antidepressants, beta-blockers, or anticonvulsants. In most patients, antiemetics are tried as a first-line treatment, but in many cases, this is not sufficient, and additional treatment is required (16). In our study, we found that virtual reality vestibular rehabilitation could be effective in treatment.

It has also been explained several times in studies that re-exposure to passive movement can cause up to 80% temporary reduction in MdDS symptoms (7). Therefore, by providing a realistic environment with virtual reality, we aimed to improve postural stability by increasing VOR gain and optokinetic responses in our patient.

CONCLUSION

Virtual reality simulations provided an exact representation of the environments in which the patient felt discomfort during daily activities, supporting the adaptation of the vestibular system and contributing to the control of symptoms. These findings suggest that virtual reality vestibular rehabilitation could be an effective method for the treatment of MdDS. Further studies involving larger patient groups and long-term follow-up are needed to support the results. Due to its rarity, more studies are needed to understand the therapeutic methods for treatment.

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

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REFERENCES

1. Cha YH, Baloh RW, Cho C, Magnusson M, Song JJ, Strupp M, et al. Mal de débarquement syndrome diagnostic criteria: Consensus document of the classification committee of the Bárány society. J Vestib Res. 2020;30(5):285-93.
2. Cha YH. Mal de debarquement. Semin Neurol. 2009;29(5):520-7.
3. Schoenmaekers C, De Smet D, Deblieck C, Van Riel J, Zarowski A, Wuyts FL. Virtual reality application matches the most established treatment for Mal de debarquement syndrome: A non-inferiority, randomized, open clinical trial. Neurotherapeutics. 2024;21(5):e00390.
4. Hain TC, Cherchi M. Mal de débarquement syndrome. Handb Clin Neurol. 2016;137:391-5.
5. Schoenmaekers C, Jillings S, De Laet C, Zarowski A, Wuyts FL. Guideline for standardized approach in the treatment of the mal de debarquement syndrome. Front Neurol. 2024;15:1359116.
6. Ramesh S, Ben-Dov T, April MM, Cho C. Mal de débarquement syndrome in children: a case series. J Pediatr. 2023;259:113435.
7. Van Ombergen A, Van Rompaey V, Maes LK, Van de Heyning PH, Wuyts FL. Mal de debarquement syndrome: a systematic review. J Neurol. 2016;263(5):843-54.
8. Cha YH, Brodsky J, Ishiyama G, Sabatti C, Baloh RW. Clinical features and associated syndromes of mal de debarquement. J Neurol. 2008;255(7):1038-44.
9. Saha K, Cha YH. Mal de debarquement syndrome. Semin Neurol. 2020;40(1):160-4.
10. Alghadir AH, Anwer S. Effects of vestibular rehabilitation in the management of a vestibular migraine: a review. Front Neurol. 2018;9:440.
11. Chen PY, Hsieh WL, Wei SH, Kao CL. Interactive wiimote gaze stabilization exercise training system for patients with vestibular hypofunction. J Neuroeng Rehabil. 2012;9:77.
12. McDonnell MN, Hillier SL. Vestibular rehabilitation for unilateral peripheral vestibular dysfunction. Cochrane Database Syst Rev. 2015;1(1):CD005397.
13. Dai M, Cohen B, Smouha E, Cho C. Readaptation of the vestibulo-ocular reflex relieves the mal de debarquement syndrome. Front Neurol. 2014;5:124.
14. Yakushin SB, Zink R, Clark BC, Liu C. Readaptation treatment of mal de debarquement syndrome with a virtual reality app: a pilot study. Front Neurol. 2020;11:814.
15. Hoppes CW, Vernon M, Morrell RL, Whitney SL. Treatment of mal de debarquement syndrome in a computer-assisted rehabilitation environment. Mil Med. 2022;187(7-8):e1011-5.
16. Memon SF, Khan AA, Mohiuddin O, Memon SA. Mal de debarquement syndrome: a rare entity. Cureus. 2020;12(2):e6837.

Letter to the Editor / Editöre Mektup
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Comment on "Neuraxial Block in A Post-Hemorrhagic Stroke Pregnant Patient"

"Hemorajik İnme Sonrası Gebe Bir Hastada Nöroaksiyel Blok" Başlıklı Yazı Üzerine

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Dear Editor,

We read with profound interest the case report titled "Neuraxial Block in A Post-Hemorrhagic Stroke Pregnant Patient" by Iswara et al. (1) published on pages 85-87 of the 26(1) issue of the Duzce Medical Journal in 2024, and describing the management of a post-hemorrhagic stroke patient for cesarean section under spinal anesthesia. We compliment the authors for managing this challenging case meticulously with multidisciplinary involvement and would like to add a few more discussions.

Although this case was managed successfully, there is no mention of any specific neurological assessment or investigation in the postoperative follow-up. Also, while the optic nerve sheath diameter (ONSD) was measured preoperatively, there is no mention of whether it was performed after the surgery. This is because a constant vigil regarding neurological status is a must in the postoperative period as there is a potential possibility of cerebral re-bleeding. Notably, spinal anesthesia in a patient with undiagnosed chronic subdural hematoma following a mild head trauma resulted in subdural re-bleeding (2).

Secondly, we would like to highlight that the title of that case report does not end with the term "A Case Report". This is imperative as per the guideline for reporting a case report (CARE guideline).

Additionally, a few sentences require clarity in that case report. For instance, the statement "The recent magnetic resonance imaging (MRI) was initially planned to be done, but the patient was then premedicated with paracetamol IV 1000 mg, ondansetron 4 mg, and midazolam 2 mg IV" (1) in the "Case Report" section contradicts with another statement "The computed tomography (CT) scan was not done due to patient refusal due to fetal radiation exposure, and magnetic resonance imaging (MRI) was not able to be done because the patient was already in labor" (1) in the "Case Report" section. While we agree that an MRI could not be done as the patient was in labor as it would take a long time and thus could potentially increase the intracranial pressure, the administration of premedication cannot be the reason.

Besides, in the "Discussion" section, the authors stated that "In this patient, the onset was right at 14 days and the patient was in an emergency situation because…" (1). We

hope that they probably meant that this patient had presented for the surgery (emergency cesarean section) 14 days after the onset of the stroke. Similarly, the last sentence "In the case in which high intracranial pressure and neurological symptoms can be excluded with stable hemodynamics, neuraxial anesthesia is a safe choice in post-hemorrhagic stroke patient" also needs correction. The authors have measured ONSD which is also an important point here, hence; it could have been included to make it more precise: "high intracranial pressure and neurological symptoms can be excluded with stable hemodynamics, as well as the normal ONSD".

Lastly, the statement "Ten mg dose of spinal is enough to reach T8 level block" (1) in the "Discussion" section, needs corrections. Although the drug is not specified here, we understand that it was bupivacaine as mentioned in the "Case Report" section. Ideally, a sensory level of T6 is required for this surgery. Also, it is a common practice to add adjuvant such as fentanyl to reduce the dose of local anesthetic thus resulting in better hemodynamic stability and prolongation of the intraoperative and postoperative analgesia. Although an experienced surgeon performed the surgery in that case (1), it is still better to add fentanyl because of these benefits.

Ethics Committee Approval: Since our study was not an experimental study including human or animal subject, ethics committee approval was not required.

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Author Contributions: Idea/Concept: RMS; Design: RMS; Data Collection/Processing: RMS, PK, SM, SV; Analysis/Interpretation: RMS, PK, SM, SV; Literature Review: RMS, PK, SM, SV; Drafting/Writing: RMS, PK, SM, SV; Critical Review: RMS.

REFERENCES

1. Iswara IBRN, Jeanne B, Suranadi IW. Neuraxial block in a post-hemorrhagic stroke pregnant patient. Duzce Med J. 2024;26(1):85-7.
2. Zeidan A, Chaaban M, Farhat O, Baraka A. Cerebral rebleeding by spinal anesthesia in a patient with undiagnosed chronic subdural hematoma. Anesthesiology. 2006;104(3):613-4.

AUTHOR GUIDELINES

SCIENTIFIC RESPONSIBILITY

In terms of scientific publishing standards, articles to be submitted should be prepared in accordance with the criteria of the International Committee of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME) and the Committee of Publication Ethics (COPE).

- All articles must be complied with the research and publication ethics. The responsibility of the articles belongs to the authors.
- Articles are required to have not been published in anywhere previously, and/or are not in the evaluation process for publication.
- Articles must be submitted with the Copyright Transfer Form signed by all authors to begin the evaluation process. For authors' order, the signature order in the Copyright Transfer Form is based on.
- The corresponding author is responsible for the final version of the article on behalf of all authors.

ETHICAL RESPONSIBILITY

- Compliance with The Principles of Helsinki Declaration (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) is required in all studies including "human" factor. In this kind of studies, authors must state that they perform the study in compliance with these principles, they have taken the approval from ethics committee of their institution and the "informed consent" from people participating the study, in the MATERIAL AND METHODS section.
- If "animal" factor was used in the study, authors must state that they have protected the animal rights in line with the principles of 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) and they have taken the approval from ethics committee of their institution, in the MATERIAL AND METHODS section.
- In case reports, informed consent must be taken from patients.
- The information of the ethics committee approval should be indicated together with the name of the committee, approval date and number, in the MATERIAL AND METHODS section.
- If there is a direct-indirect commercial relation or an institution giving financial support in the study, authors must state that they have no commercial relationship with the commercial product, medicine, company etc. used, or if any, what kind of a relationship they have (consultant, other agreements), in the cover letter to the editor.
- The authors are responsible for reporting all personal and financial relationships that may be related with the study. It is necessary to state clearly whether there is any conflict of interest related to the submission and/or evaluation of the article.
- Compliance of the articles with the scientific and ethical rules is responsibility of authors.

SUBMISSION FILES

Articles must be uploaded to the system as separate files as described below.

Copyright Transfer Form: The Copyright Transfer Form to be obtained from the system during the submission must be signed by all authors in accordance with the authorship order in the article.

Cover Letter: Type of the article, the statement that has not been published previously in anywhere before, and/or not in the evaluation process for publication, if any, the people and institutions supporting the study financially and the relationship of these institutions with authors (if not, there is no relationship) must be stated. The names, academic titles, institutions, contact information and e-mail addresses of at least two reviewers suggested in relation to the subject of the article and not related to the authors and their institutions should be written. Editors' right to choose the reviewers are reserved.

Title Page: It must include the title of article (English and Turkish), short title not exceeding 40 characters, names, academic titles, ORCID® numbers, institutions, e-mail addresses of all authors, and also name, correspondence address, phone number, email address of the corresponding author. If the article has been presented previously in a scientific meeting; the name, date and place of the meeting (if not, not presented) should be stated.

Main Text: The title of the article (English and Turkish), short title not exceeding 40 characters, Abstract (English and Turkish), Keywords (English and Turkish), Main Text (sectioned according to the type of article submitted), References, Tables and Figures should be included.

Ethics Committee Approval Document: Ethics Committee Approval Document should be uploaded as a separate file for all research articles.

Note: If there are figures, pictures or photographs in the article, each of them must be uploaded as separate files.

SECTIONS THAT SHOULD BE USED ACCORDING TO THE TYPE OF ARTICLE

Research Article

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, MATERIAL AND METHODS, RESULTS, DISCUSSION, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 200-250 words.
ABSTRACT should be structured as "Aim, Material and Methods, Results, Conclusion".
ÖZ, should be structured as "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç".

Review (Invited Only)

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, Subtitles Related to the Subject, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 150-200 words.

Case Report

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, CASE REPORT, DISCUSSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 100-150 words.

Other

The general writing rules are applied for the preparation of the writings (letter to the editor, editorial comment/discussion, etc.) except these three basic types of article. There is no title and abstract sections in these writings. The number of references is limited to 5. The dedicated article should be specified by giving the number and date. The name, institution and address of the author should be included at the end of writing. Answer to the letter is given by the editor, or authors of the dedicated article, by publishing again in the journal.

AUTHOR GUIDELINES

WRITING RULES

- Articles should be prepared as Microsoft Word® document.
- The required margins are 2.5 cm on all sides.
- Page numbers should be placed to bottom right corner of pages.
- All texts must be typed with double-space as left-aligned using 12 point Times New Roman font.

KEYWORDS

- Number of the keywords must be at least 2, words should be separated from each other by a semicolon (;).
- Keywords in Turkish must be given in accordance with Türkiye Bilim Terimleri (TBT) (http://www.bilimterimleri.com), and keywords in English must be given in accordance with Medical Subject Headings (MESH) (http://www.nlm.nih.gov/mesh/MBrowser.html).

STATISTICAL METHODS

- All research articles should be assessed in terms of biostatistics and indicated with appropriate plan, analysis and report. In these articles last subtitle of the MATERIAL and METHODS section should be the "Statistical Analysis".
- In this section, the statistical methods used in the study should be written by indicating the purpose of use, package programs and versions used for statistical analysis should be specified.
- p values should be given in three decimal digits (p=0.038; p=0.810 etc.).
- Further information to control the convenience of articles in terms of biostatistics, can obtained from www.icmje.org.

ABBREVIATIONS

- The term should be written in full words with the abbreviation in parenthesis where first mentioned, and the same abbreviation should be used throughout the entire text.
- Abbreviations used internationally should be used in accordance with the Scientific Writing Rules.

TABLES AND FIGURES

- Should be indicated at the end of the relevant sentence in the text as (Table 1) and/or (Figure 1).
- Tables (with headings) and figures (with captions) must be added after references at the end of the text as each to be on a separate page.
- The table headings should be written at top of the table (Table 1. Table heading) and the figure captions should be written below the figure (Figure 1. Figure caption) as their first letters being upper case.
- If any abbreviation or symbol is used in tables and figures, it should be explained as a footnote below.
- The figures and photographs should be upload as separate files in .png, .jpg, etc. format and at least 300 dpi resolution.
- Captions of figure and photograph should be given on a separate page respectively, after the page including last table.
- If figure, picture, table, graphic etc. which have been published before is used, written permission must be taken and it should be stated in the explanation of figures, pictures, tables, graphics. The legal responsibility in this regard belongs the authors.

ACKNOWLEDGEMENT

- If any conflict of interest, financial support, donation and other editorial (English/Turkish evaluation) and/or technical support, it must be stated in this section before the REFERENCES section.

REFERENCES

- References should be numbered according to the order of use and stated with numbers in parentheses as (1) or (1,2) or (3-5) at the end of the relevant sentence in the text.
- Reference list should be formed according to the reference order used in the text.
- If the number of authors are 6 or less, all authors should be specified, if there are 7 or more "et al." should be added after the first 6 authors are specified.
- The conference papers, personal experiences, unpublished papers, theses and internet addresses should not be used as references.
- DOI is the only acceptable online reference.

Article:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Book:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Book Chapter:

Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.

YAZARLARA BİLGİLENDİRME

BİLİMSEL SORUMLULUK

Bilimsel yayıncılık standartları açısından, gönderilecek makaleler, Uluslararası Tıbbi Dergi Editörler Kurulu (ICMJE), Dünya Tıbbi Editörler Birliği (WAME) ve Yayın Etik Kurulu (COPE) kriterlerine uygun olarak hazırlanmalıdır.

- Gönderilecek makalelerde araştırma ve yayın etiğine uyulması zorunludur. Makalelerin sorumluluğu yazarlarına aittir.
- Makalelerin daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmaması gerekir.
- Değerlendirme sürecinin başlaması için makaleler, tüm yazarlar tarafından imzalanmış Telif Hakkı Devir Formu ile birlikte gönderilmelidir. Yazar sıralaması için Telif Hakkı Devir Formu'ndaki imza sırası dikkate alınır.
- Sorumlu yazar, tüm yazarlar adına makalenin son halinin sorumluluğunu taşır.

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.

Makale:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Aho M, Irshad B, Ackerman SJ, Lewis M, Leddy R, Pope T, et al. Correlation of sonographic features of invasive ductal mammary carcinoma with age, tumor grade, and hormone-receptor status. J Clin Ultrasound. 2013;41(1):10-7.

Kitap:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Kitap Bölümü:

Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.

DÜZCE
ÜNİVERSİTESİ

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