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**RESEARCH ARTICLE**

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# Assessment of COVID-19-Related Genes Through Associative Classification Techniques

**ABSTRACT**

**Objective:** This study aims to classify COVID-19 by applying the associative classification method on the gene data set consisting of open access COVID-19 negative and positive patients and revealing the disease relationship with these genes by identifying the genes that cause COVID-19.

**Methods:** In the study, an associative classification model was applied to the gene data set of patients with and without open access COVID-19. In this open-access data set used, 15979 genes are belonging to 234 individuals. Out of 234 people, 141 (60.3%) were COVID-19 negative and 93 (39.7%) were COVID-19 positives. In this study, LASSO, one of the feature selection methods, was performed to choose the relevant predictors. The models' performance was evaluated with accuracy, balanced accuracy, sensitivity, specificity, positive predictive value, negative predictive value, and F1-score.

**Results:** According to the study findings, the performance metrics from the associative classification model were accuracy of 92.70%, balanced accuracy of 91.80%, the sensitivity of 87.10%, the specificity of 96.50%, the positive predictive value of 94.20%, the negative predictive value of 91.90%, and F1-score of 90.50%.

**Conclusions:** The proposed associative classification model achieved very high performances in classifying COVID-19. The extracted association rules related to the genes can help diagnose and treat the disease.

**Keywords:** Association Rules, Associative Classification, COVID-19, Genes, Classification.

# COVID-19 ile İlgili Genlerin İlişkisel Sınıflandırma Teknikleriyle Değerlendirilmesi

**ÖZET**

**Amaç:** Bu çalışma, açık erişimli COVID-19 negatif ve pozitif hastalardan oluşan gen veri seti üzerinde ilişkisel sınıflandırma yöntemini uygulayarak COVID-19'u sınıflandırmayı ve COVID-19'a neden olan genleri tanımlayarak bu genlerle hastalık ilişkisini ortaya çıkarmayı amaçlamaktadır.

**Gereç ve Yöntem:** Bu çalışmada açık erişimli COVID-19 olan ve olmayan hastaların gen veri setine ilişkisel sınıflandırma yöntemi uygulandı. Kullanılan açık erişimli veri setinde 234 kişiye ait 15979 gen bulunmaktadır. 234 kişiden 141'i (%60.3) COVID-19 negatif ve 93'ü (%39.7) COVID-19 pozitifti. Bu çalışmada, ilgili tahmin edici değişkenleri seçmek için değişken seçim yöntemlerinden LASSO gerçekleştirilmiştir. Modelin performansı doğruluk, dengelenmiş doğruluk, duyarlılık, seçicilik, pozitif tahmin değeri, negatif tahmin değeri ve F1 skoru ile değerlendirildi.

**Bulgular:** Çalışmanın bulgularına göre, ilişkisel sınıflandırma yönteminden performans ölçütleri doğruluk %92.70, dengelenmiş doğruluk %91.80, duyarlılık %87.10, seçicilik %96.50, pozitif tahmin değeri %94.20, negatif tahmin değeri %91.90 ve F1 puanı %90.50 olarak elde edilmiştir.

**Sonuç:** Önerilen ilişkisel sınıflandırma yöntemi, COVID-19'u sınıflandırmada çok yüksek performans elde etmiştir. Genlerle ilgili çıkarılan birliktelik kuralları, hastalığın teşhis ve tedavisine yardımcı olabilir.

**Anahtar Kelimeler:** Birliktelik Kuralları, İlişkisel Sınıflandırma, COVID-19, Gen, Sınıflandırma.

## INTRODUCTION

The SARS-CoV-2 virus, which emerged in Wuhan, China's Hubei province on December 31, 2019, quickly spreads to six continents and hundreds of countries, making history the first pandemic caused by coronaviruses (1). This virus has been called SARS-CoV-2 because of its similarity to the Coronavirus (SARS CoV) related to the severe acute respiratory syndrome. The name of the disease it caused has been accepted as COVID-19 worldwide (2). The COVID-19, which has a high contagion property, emanates to the whole world, especially to Europe, in a short time (3). By the World Health Organization (WHO), the COVID-19 outbreak has been declared an International Health Emergency (4).

The COVID-19 pandemic affected the whole world in March, and as of December 2020, 69 million people were reported to be sick in the world. It caused a total of 1,516,516 deaths on six continents around the world (5). The COVID-19 is a highly contagious disease that causes physical, psychological, and widespread systemic to function disorders in patients, especially respiratory disorders (6). The incubation period for COVID-19 is considered within 14 days after exposure, and most cases occur approximately four to five days after exposure (7). The WHO's situation report on February 19 confirmed that the average incubation period is 4-5 days, but it is extended up to 14 days (8). COVID-19 symptoms are not specific. There is no specific clinical feature that can reliably distinguish COVID-19 from other respiratory viral infections. WHO defined common symptoms as fever, fatigue, and dry cough. Other symptoms were reported as shortness of breath, myalgia, sore throat, and very few people diarrhea (9).

According to the data available so far, advanced age (60 years and over), adults with chronic diseases (cardiovascular diseases, hypertension, diabetes, chronic obstructive pulmonary disease, asthma, hypertension, and cancer), obesity, and tobacco use constitute a group at risk for the disease (10).

The related researches have not yet determined the transmission route, diagnosis, clinical features, treatment, and prevention methods of COVID-19. Therefore, it is important to examine genomic sequences for COVID-19 in different clinical studies. Additionally, genomic characterization will help us to describe the origin and evolution of the virus accurately. Demonstrating the mechanism of SARS-CoV-2 replication in various cell-based models can help us understand the pathogenesis and identify specific targets to develop effective antiviral drugs (11).

Data mining can be defined simply as the discovery of useful information hidden in data (12). Data mining enables researchers to make effective and informed decisions with techniques offered by different disciplines such as artificial intelligence, machine learning, statistics, and optimization. It also enables revealing hidden, implicit, beneficial relationships, patterns, relations, or trends that are difficult to reveal with classical methods (13).

Models used in data mining are examined under four headings. These models are; classification, clustering, predictive models, and association rules analysis (14). There is an association rules model under the associative analysis, which is one of the data mining models. Association rules are widely used in data mining due to their easy understanding and usefulness. Methods of data mining that analyze the co-occurrence of events are called the rules of the association. While doing this analysis, association rules express the occurrence of events together with certain probabilities. The association rules' purpose is to give relationships and associations as rules (15, 16).

Associative classification is a classification approach and uses the logic of combining the classification and association rule model, which are among the data mining methods while creating the model. In associative classification, classification models are created with the set of rules obtained by association rule analysis. In the associative classification approach, the response/target variable being on the right side of the obtained rule made it easier to understand and interpret (17).

This study aims to classify COVID-19 by applying the associative classification method on the gene data set consisting of open access COVID-19 negative and positive patients and revealing the disease relationship with these genes by identifying the genes that cause COVID-19.

## MATERIAL AND METHODS

**Dataset:** In the study, an associative classification model was applied to the gene data set of patients with and without open access COVID-19. In this open-access data set used, 15979 genes are belonging to 234 individuals. Out of 234 people, 141 (60.3%) were COVID-19 negative and 93 (39.7%) were COVID-19 positives. Testing for COVID-19 was carried out in the UCSF Clinical Microbiology Laboratory using polymerase chain reaction (PCR) of nasopharyngeal (NP) swab or pooled NP + Oropharyngeal (OP) swab. In all our analyses, we defined patients with COVID-19 as those with a positive SARS-CoV-2 result by PCR. Detailed protocol and sample information are available at the relevant web address (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE156063) (18).

**Feature Selection:** Working with high dimensional data to be analyzed and modeled increases computation time, making interpretation difficult, and leading to computational inefficiency. For this reason, reducing the data size in high dimensional data increases the ease of analysis. The

main purpose of the size-reduction methods is to minimize the loss of data contained by the maximum reduction of data size. Choosing the most suitable data for the purpose is important in increasing the analysis's quality and obtaining meaningful results (19).

The gene data set in this study is a high dimensional data set. For this reason, feature selection has been made in this data set to reduce the calculation time, eliminate calculation inefficiency, and increase the quality of the analysis. LASSO feature selection method was used while performing the feature selection process.

LASSO feature selection was first proposed by Tibshirani (1996) to increase Least Squares (LS) prediction accuracy, and its usage areas have been expanded over time. Today, it is used in many different areas, especially in the field of health. When the areas of use are examined, it can be seen that the studies focus on data sets where the number of variables is higher than the number of observations, especially in very large data sets (20). The LASSO (Least Absolute Shrinkage and Selection Operator) estimator aims to increase the prediction accuracy by getting a certain penalty to the LS estimation equation. In the LASSO estimator, one or more parameters are narrowed to zero in the prediction equation due to penalty constraints. Thus, it allows obtaining models that are easy to interpret, especially in large data sets. LASSO is a widely preferred estimator because it provides ease of operation by enabling feature selection and parameter estimation simultaneously (21).

**Association Rules and Associative Classification**

**Association Rules:** Data mining is a technique that tries to identify previously unknown hidden relationships between data in databases. Data mining uses statistical, mathematical, and machine learning techniques while exploring and revealing these relationships (22). Association rules, which is one of the data mining methods, explain the occurrence of some events in the database with probabilistic expressions (23).

Association rules used to discover hidden relationships in large data sets are unsupervised data mining methods. Association rules define potential relationships of data. The aim is to reveal the rules of events that are likely to occur together. With this method, a series of operations are applied to the records in the databases in bulk, and the rules explaining the relationship between the records are derived (24). Form of association rules, "IF <if certain conditions are met>" is in the form "THEN <estimate the values of some attributes>" and are rules that are measures of support and confidence (25). Confidence and support value are units of measure that show power in the association rule. Rules with high trust and support values are called strong rules. The researcher determined the minimum support threshold value (min_support) and the minimum confidence threshold value (min_confidence) in the association rules. Association rules with higher values than the specified threshold values are taken into consideration (26).

**Associative Classification**: Associative classification is a new, effective supervised learning approach that aims to predict unseen situations. Particularly, an associative classification is an approach that uses rules obtained with association rules to create classification models. Associative classification effectively integrates classification with association rule mining and can produce more accurate results than other traditional data mining classification algorithms. On the right side of association rules in associative classification, it consists only of the class/response / dependent variable categories. The rules of the association are derived using precursor-successor clauses called if-then. Thus, it becomes easier for the user to understand and interpret the output. This situation ensures that associative classification is more advantageous than classical classification approaches (17).

There are many algorithms used and developed in associative classification. Classification based on association rules (CBA) algorithm was used in this study.

**Classification Based on Association Rules (CBA):** Classification based on association rules is an algorithm consisting of two parts that combine the classification and association rules. The first episode, called CBA-RG, is an adaptive version of Apriori used to find CARs (complete set of class association rules). The second part of CBA, called CBA-CB, is an algorithm that builds the classifier based on CARs found using CBA-RG. The classifier is built in three steps. First, the discovered CARs rank in order of priority; if the reliability of r1 is greater than r2 or if the support of r1 is greater than r2 while having the same confidence, the r1 rule is considered to precede r2. In step 2, all rules that correctly classify at least one case, and the majority class of undiscovered data are selected. Finally, in step 3, rules that do not improve the classifier's accuracy are discarded (27).

**Performance Evaluation Criteria**: The classification matrix for the calculation of performance metrics is given in Table 1.

**Table 1.** The classification matrix for calculating performance metrics

| | | **Real** | | |
|---|---|---|---|---|
| | | **Positive** | **Negative** | **Total** |
| **Predicted** | **Positive** | True positive (TP) | False positive (FP) | **TP+FP** |
| | **Negative** | False negative (FN) | True negative (TN) | **FN+TN** |
| | **Total** | **TP+FN** | **FP+TN** | **TP+TN+FP+FN** |

Accuracy = (TP+TN)/(TP+TN+FP+FN)
Balanced accuracy = [[TP/(TP+FN))]+[TN/(FP+TN)]]/2
Sensitivity = TP/(TP+FN)
Specificity = TN/(FP+TN)
Positive predictive value = TP/(TP+FP)
Negative predictive value =TN/(TN+FN)
F1-score = (2*TP)/(2*TP+FP+FN)

**Data Analysis**: Quantitative data are summarized by median (minimum-maximum). Normal distribution was evaluated with the Kolmogorov-Smirnov test. In terms of input variables, the existence of a statistically significant difference and the relationship between the categories of the output variable, "positive " and "negative" groups, were examined using the Mann-Whitney U test. $p<0.05$ values were considered statistically significant. IBM SPSS Statistics 26.0 for the Windows package program was used in the analysis. A web-based application developed by İnönü University Faculty of Medicine Biostatistics and Medical Informatics Department was used (28).

## RESULTS

In this study, 31 genes remained in the data set after the LASSO feature selection method was applied to the data set consisting of 15979 genes. Thirty-one genes obtained by the LASSO trait selection method are given in Table 2.

Descriptive statistics for the variables examined in this data set are given in Table 3.

**Table 2**. Genes obtained as a result of the Lasso variable selection

| Genes | | | | | |
|---|---|---|---|---|---|
| LMO3 | RASL11A | ITGB1BP2 | METRNL | GLTPD2 | TBCE |
| PCSK5 | RTN2 | FAM83A | SIX5 | DCUN1D3 | |
| VSIG1 | LGR6 | AZGP1 | CD163L1 | TPSB2 | |
| BACH2 | TPT1 | SCGB3A1 | PCDHB9 | ERVMER34-1 | |
| PDGFRB | TNS3 | IFI27 | LDLRAD3 | MTRNR2L12 | |
| CR2 | DUSP6 | STK32A | ALOX15B | AC005832.4 | |

**Table 3.** Descriptive statistics for quantitative independent variables

| **Genes** | **Groups** | | **p-value*** |
|---|---|---|---|
| | **Negative** | **Positive** | |
| | **Median(min-max)** | **Median(min-max)** | |
| LMO3 | 8 (0-95) | 7 (0-168) | 0.654 |
| PCSK5 | 311 (0-21818) | 891 (14-63448) | **<0.001** |
| VSIG1 | 12 (0-155) | 6 (0-93) | **0.023** |
| BACH2 | 29 (0-708) | 42 (0-2224) | 0.186 |
| PDGFRB | 11 (0-592) | 2 (0-218) | **<0.001** |
| CR2 | 7 (0-130) | 8 (0-143) | 0.579 |
| RASL11A | 16 (0-214) | 10 (0-119) | 0.046 |
| RTN2 | 32 (0-585) | 15 (0-158) | **0.002** |
| LGR6 | 18 (0-307) | 50 (0-386) | **<0.001** |
| TPT1 | 3438 (322-38186) | 2801 (69-19508) | **0.009** |
| TNS3 | 109 (0-3155) | 94 (1-1677) | 0.085 |
| DUSP6 | 175 (0-2178) | 102 (1-931) | **<0.001** |
| ITGB1BP2 | 6 (0-63) | 4 (0-49) | 0.085 |
| FAM83A | 656 (24-11025) | 1700 (4-28484) | **<0.001** |
| AZGP1 | 31 (0-621) | 26 (0-379) | **0.016** |
| SCGB3A1 | 30 (0-7220) | 13 (0-1008) | **0.012** |
| IFI27 | 254 (3-6763) | 1014 (22-4814) | **<0.001** |
| STK32A | 3 (0-297) | 4 (0-42) | 0.757 |
| METRNL | 148 (6-2373) | 80 (2-518) | **<0.001** |
| SIX5 | 35 (0-438) | 28 (0-271) | 0.227 |
| CD163L1 | 2 (0-184) | 1 (0-84) | 0.168 |
| PCDHB9 | 9 (0-148) | 3 (0-51) | **<0.001** |
| LDLRAD3 | 5 (0-229) | 5 (0-130) | 0.155 |
| ALOX15B | 9 (0-200) | 5 (0-85) | **0.017** |
| GLTPD2 | 21 (0-207) | 12 (0-92) | **0.004** |
| DCUN1D3 | 314 (4-3904) | 117 (3-657) | **<0.001** |
| TPSB2 | 4 (0-339) | 7 (0-2935) | 0.383 |
| ERVMER34-1 | 20 (0-157) | 14 (0-197) | 0.063 |
| MTRNR2L12 | 18 (0-1865) | 15 (0-321) | 0.282 |
| AC005832.4 | 1 (0-100) | 3 (0-50) | 0.954 |
| TBCE | 371 (2-9996) | 280 (0-51630) | 0.548 |

***: Mann-Whitney U test.**

According to the findings obtained; There is a statistically significant difference between the dependent/target variable groups in terms of BPCSK5, VSIG1, PDGFRB, RTN2, LGR6, TPT1, DUSP6, FAM83A, AZGP1, SCGB3A1, IFI27, METRNL, PCDHB9, ALOX15B, GLTPD2, DCUN1D3 variables ($p<0.05$).

The distribution table for the dependent/target variable in the data set obtained with 31 genes is given in Table 4.

**Table 4.** Distribution table of the dependent/target variable

| **Negative** | | **Positive** | |
|---|---|---|---|
| Count | Percentage | Count | Percentage |
| 141 | 60.3 | 93 | 39.7 |

The associative classification model was used to classify the dataset in this study. The classification matrix of this model is given below in Table 5.

**Table 5.** Classification matrix for the associative classification model

| **Prediction** | **Reference** | | |
|---|---|---|---|
| | **Positive** | **Negative** | **Total** |
| **Positive** | 81 | 5 | 86 |
| **Negative** | 12 | 136 | 148 |
| **Total** | 93 | 141 | 234 |

The values for the classification performance metrics for the associative classification model are shown in Table 6. From the associative classification model, the obtained accuracy was 92.70%, balanced accuracy 91.80%, sensitivity 87.10%, Specificity 96.50%, positive predictive value 94.20%, negative predictive value 91.90%, and F1-score 90.50%.

**Table 6.** Values for the classification performance metrics of the associative classification model

| **Metric** | **Value** (%) |
|---|---|
| Accuracy | 92.70 |
| Balanced accuracy | 91.80 |
| Sensitivity | 87.10 |
| Specificity | 96.50 |
| Positive predictive value | 94.20 |
| Negative predictive value | 91.90 |
| F1-score | 90.50 |

Table 7 shows the association rules used by the classification algorithm. As expressed in Table 7, when TPT1=[69,8.14e+03), IFI27=[622,6.76e+03), METRNL=[2,155) and MTRNR2L12=[0,188) are considered, the probability of COVID-19 positive is 100%. Similarly, TPT1=[69,8.14e+03), ITGB1BP2=[0,18.5), IFI27=[622,6.76e+03) and DCUN1D3=[3,254) are taken into account, the probability of COVID-19 positive is 100%, and when ITGB1BP2=[0,18.5), IFI27=[622,6.76e+03), SIX5=[0,112) and DCUN1D3=[3,254) are regarded, the probability of COVID-19 positive is 100%. If FAM83A=[4,1.75e+03), IFI27=[3,622) and PCDHB9=[6.5,148) are considered, the probability of COVID-19 negative is 100%. Similarly, ITGB1BP2=[0,18.5), IFI27=[622,6.76e+03) and DCUN1D3=[3,254) are considered, the probability of COVID-19 positive is 98.1%.The other rules generated from the classification based on association rules model can be interpreted as the rules described earlier (Table 7).

## DISCUSSION

In December 2019, the new COVID-19 outbreak in Wuhan, China's Hubei province, started as an epidemic and turned into a pandemic in a short time. The most important feature that distinguishes COVID-19 from other pandemics is that it is concentrated in underdeveloped countries and developing countries (29). This disease has become the most important health problem of the 21st century due to its high contagious feature, unfavorable clinical prognosis, and lethal effect in almost every age group, especially those aged 65 and above (30).

COVID-19 poses a serious threat to global public health today, and the very high human-to-human transmission capacity raises concerns about the control of the epidemic. It is not known how the pandemic will follow in the next period; It is thought that studies and investments on preventive healthcare services should be increased within health systems (31). Therefore, it is important to develop effective treatments to clarify the virus's source to combat the rapidly advancing COVID-19 pandemic. Therefore, it is necessary to reveal the genome structure of the virus. In the COVID-19 outbreak, host genomic factors cause the disease to manifest with quite different clinical symptoms. As disease-causing host genomic factors are discovered, new strategies that support rapid clinical practice can be put forward to achieve recovery in SARS-CoV-2 infected patients (32).

Due to the large size of the data in medical databases, effective data mining methods are needed. Information obtained from medical data processed using different data mining techniques is valuable for decision making, diagnosis, and predictions. The main challenge in data mining is to create a sensitive and efficient classifier (33).

In recent years, a new approach called associative classification that combines attribution and classification has been proposed. Association rule mining and classification are two important data mining methods, and associative classification combines these two methods. There is evidence that combining classification and association rule mining will give more efficient and more accurate classification performance than traditional classification techniques. Since the result of the rule

**Table 7.** Association rules used by the classification algorithm

| Left-hand side rules | Right-hand side rules | Support | Confidence | Frequency |
|---|---|---|---|---|
| {TPT1=[69, 8.14e+03), IFI27=[622, 6.76e+03), METRNL=[2, 155), MTRNR2l12=[0, 188)} | {grup=positive} | 0.222 | 1 | 52 |
| {TPT1=[69, 8.14e+03), ITGB1BP2=[0, 18.5), IFI27=[622, 6.76e+03), DCUN1D3=[3, 254)} | {grup=positive} | 0.218 | 1 | 51 |
| {ITGB1BP2=[0, 18.5), IFI27=[622, 6.76e+03), SIX5=[0, 112), DCUN1D3=[3, 254)} | {grup=positive} | 0.218 | 1 | 51 |
| {FAM83A=[4, 1.75e+03), IFI27=[3, 622), PCDHB9=[6.5, 148)} | {grup=negative} | 0.205 | 1 | 48 |
| {LMO3=[0, 56), PDGFRB=[15.5, 592), DCUN1D3=[254, 3.9e+03)} | {grup=negative} | 0.239 | 0.982 | 56 |
| {ITGB1BP2=[0, 18.5), IFI27=[622, 6.76e+03), DCUN1D3=[3, 254)} | {grup=positive} | 0.226 | 0.981 | 53 |
| {LMO3=[0, 56), PCDHB9=[6.5, 148), DCUN1D3=[254, 3.9e+03)} | {grup=negative} | 0.226 | 0.981 | 53 |
| {TPT1=[69, 8.14e+03), IFI27=[622, 6.76e+03), METRNL=[2, 155)} | {grup=positive} | 0.222 | 0.981 | 52 |
| {VSIG1=[0, 30.5), TPT1=[69, 8.14e+03), IFI27=[622, 6.76e+03), DCUN1D3=[3, 254)} | {grup=positive} | 0.218 | 0.981 | 51 |
| {VSIG1=[0, 30.5), TNS3=[0, 560), IFI27=[622, 6.76e+03), STK32A=[0.5, 34.5)} | {grup=positive} | 0.209 | 0.98 | 49 |
| {LMO3=[0,56), METRNL=[155,2.37e+03), DCUN1D3=[254,3.9e+03),TBCE=[97.5,2.42e+03)} | {grup=negative} | 0.205 | 0.98 | 48 |
| {LMO3=[0, 56), LGR6=[0, 35.5), DUSP6=[172, 2.18e+03), TBCE=[97.5, 2.42e+03)} | {grup=negative} | 0.205 | 0.98 | 48 |
| {RASL11A=[0, 22.5), TNS3=[0, 560), IFI27=[622, 6.76e+03), MTRNR2L12=[0, 188)} | {grup=positive} | 0.201 | 0.979 | 47 |
| {LMO3=[0, 56), LGR6=[0, 35.5), DCUN1D3=[254, 3.9e+03), TBCE=[97.5, 2.42e+03)} | {grup=negative} | 0.239 | 0.966 | 56 |
| {LMO3=[0, 56), METRNL=[155, 2.37e+03), DCUN1D3=[254, 3.9e+03)} | {grup=negative} | 0.226 | 0.964 | 53 |
| {TPT1=[69, 8.14e+03), TNS3=[0, 560), IFI27=[622, 6.76e+03), ALOX15B=[0, 24)} | {grup=positive} | 0.226 | 0.964 | 53 |
| {TNS3=[0, 560), IFI27=[622, 6.76e+03), SIX5=[0, 112), ALOX15B=[0, 24)} | {grup=positive} | 0.226 | 0.964 | 53 |
| {LMO3=[0, 56), PDGFRB=[15.5, 592), CR2=[0, 102), LGR6=[0, 35.5)} | {grup=negative} | 0.218 | 0.962 | 51 |
| {LMO3=[0, 56), LGR6=[0, 35.5), METRNL=[155, 2.37e+03)} | {grup=negative} | 0.214 | 0.962 | 50 |
| {CR2=[0, 102), SCGB3A1=[41.5, 7.22e+03), IFI27=[3, 622)} | {grup=negative} | 0.214 | 0.962 | 50 |
| {RTN2=[0, 58), ITGB1BP2=[0, 18.5), IFI27=[622, 6.76e+03), ALOX15B=[0, 24)} | {grup=positive} | 0.209 | 0.961 | 49 |
| {IFI27=[3, 622), PCDHB9=[6.5, 148)} | {grup=negative} | 0.244 | 0.95 | 57 |
| {CR2=[0, 102), LGR6=[0, 35.5), FFAM83A=[4, 1.75e+03), DCUN1D3=[254, 3.9e+03)} | {grup=negative} | 0.235 | 0.948 | 55 |
| {SCGB3A1=[41.5, 7.22E+03), IFI27=[3, 622)} | {grup=negative} | 0.214 | 0.943 | 50 |
| {PCSK5=[0, 347), IFI27=[3, 622), TBCE=[97.5, 2.42e+03)} | {grup=negative} | 0.244 | 0.934 | 57 |

obtained in the associative classification method is with the response variable, it is possible to create a more accurate classifier (34, 35).

Associative classification stands out as a new approach that provides easier interpretation for users when applied to medical data sets (34). In this study, an associative classification model was applied to an open-access gene data set. In this context, different factors (explanatory variables) that may be associated with COVID-19 (the dependent variable) positive-negative are estimated with the associative classification model, and rules have been obtained. According to the results of the findings, from the performance metrics obtained

from the associative classification model, the accuracy was 92.70%, balanced accuracy 91.80%, sensitivity 87.10%, specificity 96.50%, positive predictive value 94.20%, negative predictive value 91.90%, and F1-score 90.50%.

The same data set was used in an article where the most significant genes upregulated by SARS-CoV-2 were interferon-inducible, including IFI6, IFI44L, IFI27, and OAS2. Also, IFI27 was induced by SARS-CoV-2 significantly more than by other viruses, even at low viral load(18). In this study, the TPT1, IFI27, METRNL, MTRNR2l12, ITGB1BP2, DCUN1D3, LMO3, SIX5, VSIG1, STK32A, RASL11A, TNS3, ALOX15B, RTN2, ITGB1BP2 genes determine the status of being COVID-19 positives. In this study, the proposed associative classification method achieved very high performances in determining disease-related genes. Besides, this research presents an associative classification model to help researchers diagnose early for COVID-19 prediction.

In this study, the study was completed using an open source data set. If the study could be studied with real data, real experimental results could be reached and the results could be made more general and valuable. In addition, with the relational classification method preferred in the study, the results were obtained by considering the interdependent conditions of the genes associated with the disease, and a set of rules for the conditions causing the disease were obtained.

## CONCLUSION

As a result, genes associated with these rules can help in the early diagnosis and treatment of the disease. The disease can be successfully managed if further research is encouraged to develop this area's prediction system.

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RESEARCH ARTICLE

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# Could Hemogram Parameters Be Useful Biomarkers for The Diagnosis of Impaired Glucose Tolerance?

**ABSTRACT**

**Objective:** Elevated blood glucose may be affect complete blood count parameters. In this study, we compared the hemogram parameters of healthy individuals and cases with impaired glucose tolerance.

**Methods:** We examineted 134 patients with impaired glucose tolerance and 30 healthy cases. Patients files were evaluated retrospectively and the levels of hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, red blood cell, red cell distribution wide, mean platelet volume, platelet distribution wide, leukocycte, neutrophil, lymphocycte, platelet, and hemoglobin A1c were recorded. Control and patient groups were compared in terms of these parameters with student T test.

**Results:** There was no significantly difference between study and control groups in terms of hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, red blood cell count, red cell distrubition wide, leukocycte, neutrophil, lymphocycte, platelet levels. The mean platelet volume and platelet distribution wide values were significantly higher in the patient group than the control group (p value: 0.002, p value: 0.04 respectively).

**Conclusions:** Complete blood count is an easy, inexpensive test that can be performed in most hospitals. If our study results are supported by other studies, some hemogram parameters may be used to diagnose of impaired glucose tolerance.

**Keywords:** Complete Blood Count, Hyperglycemia, Impaired Glucose Tolerance.

# Bozulmuş Glukoz Toleransının Tanısında Hemogram Parametreleri Biyobelirteç Olarak Kullanılabilir mi?

**ÖZET**

**Amaç:** Yüksek kan şekeri, tam kan sayımı parametrelerini etkileyebilir. Biz bu çalışmada sağlıklı bireylerin ve bozulmuş glukoz toleranslı olguların hemogram parametrelerini karşılaştırdık.

**Gereç ve Yöntem:** Biz bozulmuş glukoz toleranslı 134 hasta ve 30 sağlıklı bireyi inceledik. Hasta dosyaları retrospektif olarak değerlendirildi ve hemoglobin, hematokrit, ortalama eritrosit hacmi, ortalama eritrosit hemoglobini, kırmızı kan hücresi, eritrosit dağılım genişliği, ortalama trombosit hacmi, trombosit dağılım genişliği, lökosit, nötrofil, lenfosit, trombosit ve hemoglobin A1c düzeyleri kaydedildi . Kontrol ve hasta grupları bu parametreler açısından student T testi ile karşılaştırıldı.

**Bulgular:** Çalışma ve kontrol grupları arasında hemoglobin, hematokrit, ortalama eritrosit hacmi, ortalama eritrosit hemoglobini, kırmızı kan hücresi sayısı, eritrosit dağılım genişliği, lökosit, nötrofil, lenfosit, trombosit düzeyleri açısından anlamlı fark yoktu. Hasta grubunda ortalama trombosit hacmi ve trombosit dağılım genişliği değerleri kontrol grubuna göre anlamlı olarak yüksekti (sırasıyla p değeri: 0.002, p değeri: 0.04).

**Sonuç:** Tam kan sayımı, çoğu hastanede yapılabilen kolay ve ucuz bir testtir. Çalışma sonuçlarımız başka çalışmalarla desteklenirse, bozulmuş glukoz toleransını teşhis etmek için bazı hemogram parametreleri kullanılabilir.

**Anahtar Kelimeler:** Tam Kan Sayımı, Hiperglisemi, Bozulmuş Glukoz Toleransı.

## INTRODUCTION

Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are prediabetic conditions. Diabetes mellitus develops in approximately 25% over three to five years in prediabetic people (1). Prediabetic conditions are associated with cardiovascular disease (2,3). Also stroke, large-vessel occlusive disease, retinopathy, renal disease and polyneuropathy are more common in prediabetic patients (4). If prediabetes is diagnosed early, patients can be protected from these complications by regulating their diet and lifestyle change. Parameters that showing glycemic control are the levels of blood glucose, fructosamine and hemoglobin A1c (HbA1c). HbA1c level is affected by presence of variant hemoglobin, hemolytic and renal anemia and shows the last 3 months blood glucose status of the patients. But it is an expensive test and cannot be performed in all hospitals. Fructosamine is a glycolyzed protein and shows glycemic status in the last 2-3 weeks. It is affected that serum protein concentration and the levels of plasma bilirubin, hemoglobin, uric acid.

Complete blood count (CBC) is a test performed using an automated instrument. CBC measures the amounts and sizes of leukocyte, platelet and erythrocyte. In some studies, it has been reported that CBC parameters may be used as a biomarker that shows glycemic status. Insulin is an anabolic hormone (5). Therefore, hyperinsulinemia may trigger hematopoiesis in bone marrow in patients with hyperglycemia. As a result, the numbers of erythrocyte and leukocyte increase. However, there are contradictory results in the literature about this topic. Emilia et al. showed that erythrocyte half-life was shortened due to impaired erythrocyte shape and function by increasing erythrocyte caspase 3 activity in diabetic patients (6). Diabetes mellitus affects the number and function of platelets and triggers atherosclerosis and atherothrombosis. Platelet hyperactivity has been demonstrated in diabetic patients in some studies (7,8). Some authors reported that the MPV value was an indicator of the platelet activity. In this study, we aimed to compare the hemogram parameters of healthy cases and patients with impair red glucose tolerance.

## MATERIAL AND METHODS

We determined 134 patients with IGT and 30 healthy cases who were admitted to the Internal Medicine Outpatient Clinic of University of Health Sciences, Erzurum Regional Education and Research Hospital in Turkey. Local ethical committee approval was obtained before starting this study (date:01.07.2019, number: 2019/10-115). This study was in accordance with the Helsinki Declaration of 1964. Informed consent was obtained from all individual participants included in the study. We excluded cases with hematological disease, diabetes mellitus, cardiac failure, chronic renal and hepatic diseases, pregnancy, autoimmune and infectious diseases from this study. The diagnosis of impaired glucose tolerance was made by performing a 75 gram oral glucose tolerance test (OGTT) that was applied to patients in the morning after 12 hours of night hunger. Venous blood sample was drawn for measurement of glucose at fasting and at 120-min after ingestion of the glucose load. In the OGTT test, cases with a glucose level of 140 to 199 mg/dl (7.8 to 11.0 mmol/l) at the second hour were considered impaired glucose tolerance, according to American Diabetes Association Guideline. CBC was performed using a hematology analyzer (model XN-1000; Sysmex) in all cases. We retrospectively examined the patients' files and recorded age, gender, hbA1c, fasting blood glucose, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red blood cell (RBC), red cell distribution wide (RDW), leukocyte, neutrophil, lymphocyte, platelet, mean platelet volume (MPV), platelet distribution wide (PDW) levels of all cases.

Statistical evaluations were made by SPSS-21 windows software. Categorical values were specified as numbers and percentages (%). The Student t-test was used to compare differences between two independent groups. Categorical variables were compared by the Chi-square test or Fisher exact test. Statistical significance was defined as a $p < 0.05$.

## RESULTS

We evaluated 134 IGT patients and 30 control cases. Seventy-one (53%) of the patient group were women and 63 (47%) were men, while the number of men and women in the control group was equal (men: 15 cases, women:15 cases). The mean age was 48.89 ± 13.47 years in patients with IGT and 40.2 ± 15.32 years in control group. There was no statistically significant difference between the study and control groups in terms of erythrocyte indices such as hemoglobin, hematocrit, MCV, MCH, RDW, RBC levels (Table 1).

**Table 1.** Erythrocyte indices in control and patient groups.

| Variables | Patient group | Control group | P value |
|---|---|---|---|
| Hemoglobin (g/dL) | 15.09 ± 1.31 | 15.09 ± 1.20 | 0.90 |
| Hematocrit (%) | 45.57 ± 3.79 | 44.55 ± 3.56 | 0.18 |
| RBC ($10^6$/µL) | 5.25 ± 0.45 | 5.11 ± 0.47 | 0.12 |
| RDW-CV | 12.69 ± 0.99 | 12.78 ± 0.83 | 0.64 |
| MCV (fl) | 85.99 ± 7.80 | 84.72 ± 14.56 | 0.50 |
| MCH (pg) | 28.51 ± 2.37 | 28.83 ± 3.69 | 0.54 |

RBC: Red blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, RDW: Red cell distribution wide

We did not find significant difference between the study and control groups in terms of leukocyte, lymphocyte and neutrophil counts (Table 2). Platelet count was not significantly different in the study and control groups (p value: 0.28).

MPV and PDW values were higher in patients with impaired glucose tolerance than the control group (p value:0.002, p value: 0.04, respectively) (Table 3).

**Table 2.** Leukocyte, lymphocyte and neutrophil counts in patient and control groups.

| Variables | Patient Group | Control Group | p value |
|---|---|---|---|
| Leukocyte count ($10^3$/µL) | 7.54 ± 1.6 | 7.08 ± 1.34 | 0.14 |
| Lymphocyte count ($10^3$/µL) | 2.47 ± 0.71 | 2.46 ± 0.66 | 0.93 |
| Neutrophil count ($10^3$/µL) | 4.28 ± 1.32 | 3.86 ± 1.23 | 0.11 |

**Table 3.** Platelet parameters in patient and control groups.

| Variables | Patient Group | Control Group | P value |
|---|---|---|---|
| Platelet Count ($10^3$/µL) | 290.33 ± 69.89 | 275.66 ± 58.52 | 0.28 |
| MPV (fl) | 10.23 ± 0.93 | 9.22 ± 1.70 | 0.002 |
| PDW | 14.78 ± 4.07 | 12 ± 1.96 | 0.04 |

MPV: Mean platelet volume, PDW: Platelet distrubition wide

## DISCUSSION

The results of studies on the effects of high blood glucose on RBC, hemoglobin and hematocrit levels are contradictory. Increased blood glucose causes to reduce of red blood cell lifespan, reduction in erythrocyte production due to low erythropoietin level in patients with nephropathy (9). Therefore RBC, hemoglobin and hematocrit levels are also low in patients with diabetic nephropathy. It reported that the levels of hemoglobin, hematocrit and RBC in T2DM patients are lower than in the control group (10). This condition was explained by the erythrocyte aging triggered with nonenzymatic glycation due to hyperglycemia. In a study, it wasn't found difference between diabetic patients and the control group in terms of hemoglobin value (11). On the contrary, in some studies reported that high blood glucose causes incresased red blood cell count due to hyperinsulinemia and insulin resistance positively affecting erythropoiesis (12, 13). We did not find statistically significant difference between the impaired glucose tolerance and the control groups in terms of hemoglobin and hematocrit levels.

MCV, MCH levels increase due to nonenzymatic glycolisation of cell membrane proteins in patients with elevated glucose. Alamri et al examinated 1000 type 2 Saudi diabetic patients and they reported RBC, MCV, MCHC levels were high in patients with hyperglisemia and RDW was negatively correlated with poor glycemic control (14). Nada et al reported RDW was high in diabetic patients (15). In contrary, Cakır et al determined that no significant correlation RDW level and diabetes mellitus (16). We did not find difference between the study group and the control group in terms of RBC, MCV,MCH, RDW. This may be due to ethnic and cultural differences.

Nada et al. found that leukocyte count was higher in patients with uncontrolled hyperglycemia (HbA1c > 7%) than in patients with good glycemic control (HbA1c ≤ 7%) (15). Nagareddy et al. declared that high blood glucose level causes leukocytosis by triggering myelopoesis due to oxidative stress (17, 18). Leukocytosis may contribute acute and chronic ischemic vascular diseases that cause to nephropathy, coronary artery disease. It reported that WBC level was positively associated with fasting plasma glucose in female patients with impaired glucose tolerance. In our study, the numbers of leukocyte, lymphocyte and neutrophil were not significantly different in the study and control groups.

High MPV and PDW values are associated with increased platelet activation and function. MPV was higher in patients with diabetes mellitus than in normoglycemic patients (19). Chen et al reported that platelet count and platelet distribution wide (PDW) were not increased in T2DM patients (20). But Zuberi et al. detected that MPV was high in patients with impaired glucose tolerance (7). In addition, Hekimsoy et al. examineted 145 diabetic and 100 nondiabetic patients. They found MPV value was significantly higher in patients with diabetes than non-diabetic patients (8). In our study; we detected MPV and PDW values were higher in the study group compared to the control group.

## CONCLUSION

Complete blood count is a cheap test that can be done in most hospital. CBC parameters can be used to determine blood glucose status if our study results are supported by larger and randomized studies. MPV and PDW were higher in patients with impaired glucose tolerance than in the control group in our study. This may be a cause of complications in impaired glucose tolerant patients. Reference ranges can be determined for MPV and PDW values and cases with high MPV and PDW values can be followed up more closely for the devolepment of IGT.

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RESEARCH ARTICLE

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# Distribution of Microorganisms and Antibiotic Resistance Rates Isolated From Blood Cultures: 5-Year Evaluation in a University Hospital in Northern Cyprus

**ABSTRACT**

**Objective:** Bloodstream infections (BSI) are considered to be the most important cause of morbidity and mortality. The main purpose of this study is to examine the distribution of microorganisms in blood cultures and the rates of antimicrobial resistance.

**Methods:** Microorganisms isolated from 7.866 blood cultures which were sent to our laboratory between January 2016-December 2020 were retrospectively evaluated. Blood culture bottles were incubated in BACTEC 9120 system. Blood samples were taken from the bottles with a sterile syringe and cultured on Eosin-Methylene Blue (EMB) and 5% sheep blood agars if there was a positive signal. The cultivated cultures were left to incubate at 35°C for 24-48 hours. VITEK 2 compact automated system was used for identification and antibiotic susceptibility tests (AST).

**Results:** Growth was detected in 691 (8.8%) of blood cultures. There were 56.7% gram-negative, 42.7% gram-positive bacteria and 0.6% *Candida* species. The most common bacteria isolated are; coagulase negative staphylococci (CNS) (21.1%), *Escherichia coli* (15.2%), *Klebsiella pneumoniae* (11.9%) and *Staphylococcus aureus* (11.4%). The rate of ESBL-*Escherichia coli* increased in 2020, but this was not statistically significant. ESBL-*Klebsiella pneumoniae* species showed a significant increase over the years and reached the highest level (69.6%) in 2019. MDR (multi drug resistance) rate for *Pseudomonas aeruginosa* was determined as 13%. MDR rate for *Acinetobacter baumannii* was determined as 97%. The rate of methicillin resistant *Staphylococcus aureus* among patients is 30.4%.

**Conclusions:** It is critical to determine microorganisms and their antibiotic susceptibilities as soon as possible in BSI. Active surveillance systems help manage the BSI.

**Keywords:** Blood Culture, Infection, Antimictobial Susceptibility, Resistant, Northern Cyprus.

# Kan Kültürlerinden İzole Edilen Mikroorganizmaların Dağılımı ve Antibiyotik Direnç Oranları: Kuzey Kıbrıs'ta Bir Üniversite Hastanesindeki 5 Yıllık Değerlendirme

**ÖZET**

**Amaç:** Kan dolaşımı enfeksiyoları (KDE), morbidite ve mortalitenin en önemli nedeni sayılmaktadır. Bu çalışmada, kan kültürlerinde üreyen mikroorganizmaların dağılımını ve antimikrobiyal direnç oranlarını incelemek amaçlanmıştır.

**Gereç ve Yöntem:** Yakın Doğu Üniversitesi (YDÜ) Hastanesi, Mikrobiyoloji Laboratuvarı'na Ocak 2016-Aralık 2020 tarihleri arasında gönderilen 7.866 kan kültüründen izole edilen mikroorganizmalar retrospektif olarak değerlendirmeye alınmıştır. Kan kültür şişeleri BACTEC 9120 otomatize sisteminde inkübe edilmiştir. İnkübasyon sırasında pozitif sinyal elde edilmesi halinde, steril enjektörle kan kültür şişelerinin içerisinden örnek alınarak Eosin-Methylene Blue (EMB) ve %5 koyun kanlı agarlara ekimleri yapıldı. Ekimi yapılan kültürler 24-48 saat süresince 35°C'de etüvde inkübasyona bırakıldı. Üreme saptanan kültürlerdeki mikroorganizmaların identifikasyon ve antibiyotik duyarlılık testleri (ADT) için VİTEK 2 (Biomerieux) kompakt otomatize sitemi kullanıldı.

**Bulgular:** Kan kültürlerinin 691 (%8,8)'inde üreme saptanmıştır. İzole edilen mikroorganizmaların 392 (%56,7)'sini gram negatif bakteriler, 295 (%42,7)'ini gram pozitif bakteriler ve 4 (%0,6)'ünü ise *Candida* türleri oluşturmaktadır. En sık izole edilen bakteriler sırasıyla; Koagülaz negatif stafilokoklar (KNS) (%21,1), *Escherichia coli* (%15,2), *Klebsiella pneumoniae* (%11,9), *Staphylococccus aureus* (%11,4) idi. *Escherichia coli* dağılımına bakıldığı zaman genişlemiş spektrumlu beta-laktamaz (GSBL) oranı 2020 yılında artış göstermiştir fakat bunun istatistiksel olarak anlamlı olmadığı anlaşılmıştır. GSBL pozitif *K. pneumoniae* türleri ise yıllar içerisinde anlamlı derecede artış göstermiş ve 2019 yılında en yüksek (%69,6) seviyeye ulaşmıştır. *Pseudomonas aeruginosa* için ÇİD (çoklu ilaç dirençli) oranı %13 olarak belirlenmiştir. *Acinetobacter baumannii* için ÇİD oranı ise %97 olarak bulunmuştur. İzole edilen *Staphylococcus aureus* suşlarının %30,4'ü metisilin dirençli *Staphylococccus aureus* (MRSA) idi.

**Sonuç:** KDE'dan izole edilen mikroorganizmaların ve duyarlılıklarının en kısa sürede belirlenmesi kritik öneme sahiptir. Ayrıca, aktif sürveyans sistemleri KDE'nın yönetimine yardımcı olmaktadır.

**Anahtar Kelimeler:** Kan Kültürü, Enfeksiyon, Antimikrobiyal Duyarlılık, Direnç, Kuzey Kıbrıs.

## INTRODUCTION

Bloodstream infections (BSI) can cause serious clinical consequences such as sepsis and multiple organ failure, so it is considered as the most important cause of morbidity and mortality. Therefore, application of rapid and appropriate empirical therapy is critical for patients. Management of these types of infections becomes more complicated when antimicrobial resistance increases (1,2). There is a parallel increase in microorganisms isolated from BSI due to reasons such as the frequent use of invasive interventions, the increase in the use of broad-spectrum antibiotics, cancer surgery, organ transplantation applications and the increase in immunosuppressive treatments (3). Bacteremia caused by bacteria with multiple antibiotic resistance extends the hospitalization period of patients, increases the mortality rate, creates the risk of other infections and increases the cost per patient in hospitals and healthcare institutions (4).

Although the ratio of bacteremia varies between 20-30%, this rate can be up to 50% in patients with sepsis and multiple organ failure. The estimated mortality rate of bacteremia due to hospital is accepted as 15-30%. In addition, bacteria have resistance issues against antibiotics due to the increasing use of antibiotics. There are some resistant bacteria which causes treatment failures and increase in mortality rates. These are; methicillin resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* which produces extended spectrum beta-lactamases (ESBL), and carbapenemase-forming *Enterobacteriaceae* (5-7).

Blood cultures, which are accepted as gold standard in the diagnosis of bacteremia, play an important role in identifying infections, determining antibiotic susceptibility parameters and in the treatment process. The distribution of microorganisms isolated from blood cultures and antibiotic resistance rates vary according to years and geographic regions, as well as different results can occur between hospitals in the same region or even in different departments within the same hospital. Therefore, it is important to know and follow the effective microorganisms and resistance patterns in order to guide appropriate and correct empirical treatment (8-10).

Surveillance systems are used in developed countries to monitor changing infection trends. These surveillance systems provide entire data on microorganisms and provide clues about the clinical significance, etiological trends and antimicrobial resistance patterns of all infections, including BSI. Active surveillance systems are not available in most developing countries where infectious diseases are more prevalent (2).

The main purpose of this study is to examine the distribution of microorganisms grown in blood cultures and their antimicrobial resistance rates in order to guide clinicians in the appropriate and correct empirical treatment of BSI. In addition, it is among our goals to determine infection prevention policies by shedding light on surveillance studies that are not carried out in our country.

## MATERIAL AND METHODS

**Study Design:** Microorganisms isolated from 7.866 blood cultures sent to Microbiology Laboratory of Near East University (NEU) Hospital between January 2016 and December 2020 were retrospectively evaluated. Only one result was included in the study if the same bacteria replicated in more than one blood culture of the same patient.

**Bacterial Identification and Antibiotic Susceptibility Test:** BACTEC 9120 (Becton, Dickinson and Company Sparks, USA) automated blood culture system was used to detect growth in blood cultures. Blood samples of 8-10 mL from adult patients and 1-3 mL from infant and pediatric patients were taken and transferred to BD BACTEC Plus Aerobic/F and BD BACTEC Peds Plus/F culture bottles, respectively, and the samples were incubated on the device for seven days. If a positive signal was obtained during the incubation, samples were taken from blood culture bottles with a sterile syringe and cultivated on Eosin-Methylene Blue (EMB) and 5% sheep blood agars. The cultivated cultures were left to incubate at 35°C for 24-48 hours. VITEK 2 (Biomerieux) compact automated system was used for identification and antibiotic susceptibility tests (AST) of microorganisms in cultures with growth. VITEK 2 GN and VITEK 2 GP cards used for identification. Also, VITEK 2 AST-N325, VITEK 2 AST-N327, VITEK 2 AST-N326, VITEK 2 AST-P641 and VITEK 2 AST-P640 cards were used to measure antibiotic susceptibility. ADT was evaluated according to the EUCAST (European Committee on Antimicrobial Susceptibility Testing) criteria and antibiotics detected as intermediate were considered as resistant. Coagulase negative staphylococci (CNS) growth samples which were in a single blood culture bottle and *Micrococcus* spp. growth samples were considered as contamination. Resistance to one antibiotic from at least three different antibiotic groups is accepted as 'Multi Drug Resistant' (MDR), and resistance to all other antibiotics except one or two antibiotics is accepted as 'Extreme Drug Resistant' (XDR).

**Statistical Analysis:** SPSS (Statistical Package of the Social Sciences) Demo Ver 22 (SPSS Inc., Chicago, IL, USA) program was used for all statistical analysis of the data. In order to determine statistical significance, Pearson Chi-square, Fisher's Exact Test and One-Way ANOVA tests were used and $p<0.05$ values were considered significant.

**Ethical Approval:** Ethics committee approval was obtained for our study with the

project number NEU/2021/88-1292 at the meeting held by the NEU Scientific Research Ethics Committee on 25.02.2021.

**RESULTS**

A total of 7.866 blood culture tests were carried out in a 5-year period from January 2016 to December 2020 in our laboratory. There were 4,590 (58.4%) blood samples from male and 3.276 (41.6%) blood samples from female and the age average was 59.25±24.44 (0-100 years old). Growth was detected in 691 (8.8%) of blood cultures which are 367 (53.1%) of these patients were male and 324 (46.9%) female, and their mean age was 66.15±19.82 (0-100 years old). While there was no growth in 6,531 (83%) of the samples, 644 (8.2%) of them were accepted as contamination. It was determined that the mean age of the patients with growth was significantly higher than those without growth (p<0.001). In parallel with this, it can be seen in the Table 1, it was found that the most growth of blood cultures has seen at patients who are aged >60 and the least growth has seen at the patients who are 21-40 years old due to the analysis performed among age groups (p<0.001). Accordingly, it is seen that the risk of BSI may increase with age. In addition, a significant relationship was observed between the growth in blood cultures and gender, and it was found that the growth in female was higher than in male as shown in Table 1 (p=0.002).

**Table 1.** Evaluation of blood culture results

| | Blood Culture Positive | Blood Culture Negative | *P* |
|---|---|---|---|
| **Gender** | | | |
| Male | 367 (%8.7) | 3867 (%91.3) | 0.002 |
| Female | 324 (%10.8) | 2664 (%89.2) | |
| **Age Group** | | | |
| 0-20 | 42 (%5.5) | 726 (%94.5) | <0.001 |
| 21-40 | 17 (%2.5) | 669 (%97.5) | |
| 41-60 | 130 (%10.6) | 1099 (%89.4) | |
| >60 | 502 (%11.1) | 4037 (%88.9) | |
| **Year** | | | |
| 2016 | 89 (%8.2) | 990 (%91.8) | 0.009 |
| 2017 | 162 (%9.5) | 1545 (%90.5) | |
| 2018 | 155 (%9.8) | 1429 (%90.2) | |
| 2019 | 170 (%11.8) | 1275 (%88.2) | |
| 2020 | 115 (%8.2) | 1292 (%91.8) | |
| **Season** | | | |
| Spring | 158 (%8.4) | 1723 (%91.6) | 0.007 |
| Summer | 196 (%11.0) | 1583 (%89.0) | |
| Autumn | 178 (%10.6) | 1500 (%89.4) | |
| Winter | 159 (%8.4) | 1725 (%91.6) | |

It can be seen that from Table 1, the growth in blood cultures was the highest in 2019 (170, 11.8%) and there was a significant decrease in growth (115, 8.2%) in 2020 (p=0.020). In addition, it was observed that BSI were most common in the summer months when compared within the seasons (p=0.005). In blood cultures of patients who have been sent blood samples were isolated the most is shown in Figure 1. These are; CNS (146/691, 21.1%), *Escherichia coli* (105/691, 15.2%), *Klebsiella pneumoniae* (82/691, 11.9%), *Staphylococccus aureus* (79/691, 11.4%), *Pseudomonas aeruginosa* (54/691, 7.8%), *Enterococcus faecalis* (43/691, 6.2%) and *Acinetobacter baumannii* (33/691, 4.8%) respectively.

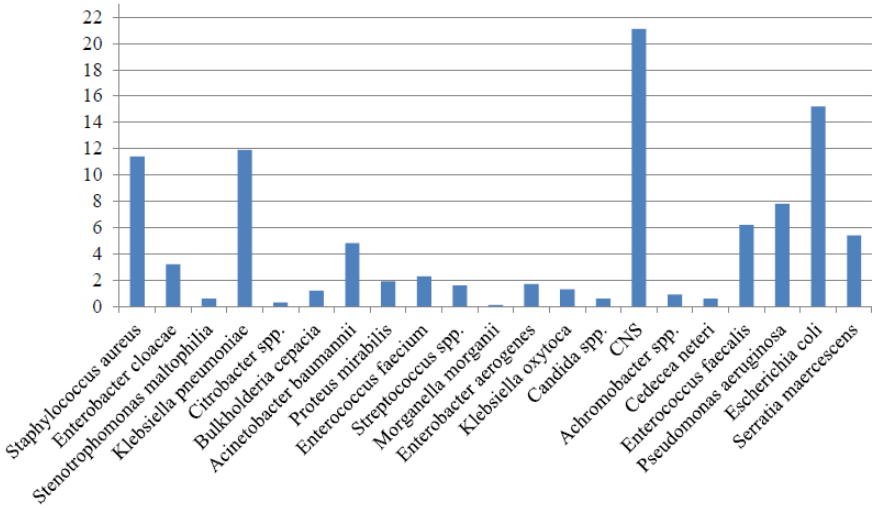


**Figure 1.** Distribution of microorganisms isolated in blood cultures (%)

The species of the microorganisms found from blood cultures are; 392 (56.7%) gram-negative, 295 (42.7%) gram-positive and 4 (0.6%) *Candida* species. 287 (73.2%) were *Enterobacterales* and 105 (26.8%) were non-fermenting gram-negative bacteria among the gram-negative bacteria. When the antibiotic sensitivities in *Escherichia coli* are examined, it is seen that the most resistant antibiotics are ciprofloxacin (64.1%), trimethoprim-sulfamethoxazole (59.8%) and cefepime (58.1%). The most sensitive antibiotics were found as meropenem (97.1%), imipenem (96.7%) and amikacin (96.2%). It was determined that *Klebsiella pneumoniae* isolates were most resistant to cefepime (55.7%), ceftazidime (53.2%) and aztreonam (53.1%) antibiotics. What is more, amikacin (95.1%), imipenem (83.9%) and gentamicin (81.2%) antibiotics were the most effective antibiotics against *Klebsiella pneumoniae* isolates. Table 2 shows the antibiotic resistance rates and ESBL positivity in the most frequently isolated *Enterobacterales*.

**Table 2.** Antibiotic resistance rates in the most frequently isolated in blood cultures, n (%)

| Antibiotic | *Escherichia coli* | *Klebsiella pneumoniae* | *Enterobacter* spp. | *Serratia marcescens* |
|---|---|---|---|---|
| **Amikacin** | 4/105 (3.8) | 4/82 (4.9) | 0/32 (0.0) | 1/37 (2.7) |
| **Aztreonam** | 46/88 (52.3) | 34/64 (53.1) | 8/30 (26.7) | 3/36 (8.3) |
| **Cefepime** | 61/105 (58.1) | 44/79 (55.7) | 14/32 (43.8) | 4/37 (10.8) |
| **Ceftazidime** | 57/102 (55.9) | 42/79 (53.2) | 15/32 (46.9) | 2/36 (5.6) |
| **Ceftriaxone** | 54/97 (55.7) | 40/77 (51.9) | 22/31 (71.0) | 2/22 (9.1) |
| **Ciprofloxacin** | 66/103 (64.1) | 40/79 (50.6) | 9/33 (27.3) | 1/37 (2.7) |
| **Ertapenem** | 6/97 (6.2) | 18/75 (24.0) | 14/31 (45.2) | 1/22 (4.6) |
| **Gentamicin** | 21/103 (20.4) | 15/80 (18.8) | 7/34 (20.6) | 1/37 (2.7) |
| **Imipenem** | 3/92 (3.3) | 10/62 (16.1) | 7/31 (22.6) | 14/36 (38.9) |
| **Meropenem** | 3/105 (2.9) | 16/80 (20.0) | 7/32 (21.9) | 2/37 (5.4) |
| **PTZ** | 18/105 (17.1) | 25/78 (32.1) | 17/32 (53.1) | 2/37 (5.4) |
| **SXT** | 61/102 (59.8) | 39/81 (48.1) | 10/29 (34.5) | 1/37 (2.7) |
| **ESBL** | 59/105 (56.2) | 44/82 (53.7) | - | - |

Abbreviations: PTZ, piperacillin-tazobactam; SXT, trimethoprime-sulfametoxazole; ESBL, extended spectrum beta lactamase

When the ESBL distribution in *Escherichia coli* is examined, it can be seen that it increased in 2020, but this is not statistically meaningful (p=0.188). ESBL positive *Klebsiella pneumoniae* blood samples were found to be at very low rates (1/13, 7.7%) in 2016, but showed a significant increase over the years, reaching the highest level (16/23, 69.6%) in 2019 (p=0.007) (Table 3).

**Table 3.** ESBL positivity by years of the most commonly grown *Enterobacterales* in blood cultures, n (%)

| | **2016** | **2017** | **2018** | **2019** | **2020** | **p** |
|---|---|---|---|---|---|---|
| *Escherichia coli* ESBL + | 12/23 (52.2) | 10/21 (47.6) | 12/19 (63.2) | 14/29 (48.3) | 11/13 (84.6) | 0.188 |
| *Klebsiella pneumoniae* ESBL + | 1/13 (7.7) | 15/24 (62.5) | 3/6 (50.0) | 16/23 (69.6) | 9/16 (56.3) | 0.007 |

Abbreviation: ESBL, extended spectrum beta lactamase

The distribution of non-fermenting *Pseudomonas aeruginosa* and *Acinetobacter baumannii* over the years is shown in Figure 2, which are the most common isolated bacteria in blood cultures. When the resistance rates were examined, it was determined that amikacin (98.1%), meropenem (96.1%), colistin (92%), imipenem (90.2%) and gentamicin (90%) antibiotics were the most effective options in the treatment of *Pseudomonas aeruginosa*. MDR rate for *Pseudomonas aeruginosa* was determined to be 13%. The most sensitive antibiotics for isolated *Acinetobacter baumannii* are tigecycline (94.1%) and colistin (90.9%), respectively. In addition, MDR rate for *Acinetobacter baumannii* was found to be 97% (Table 4).

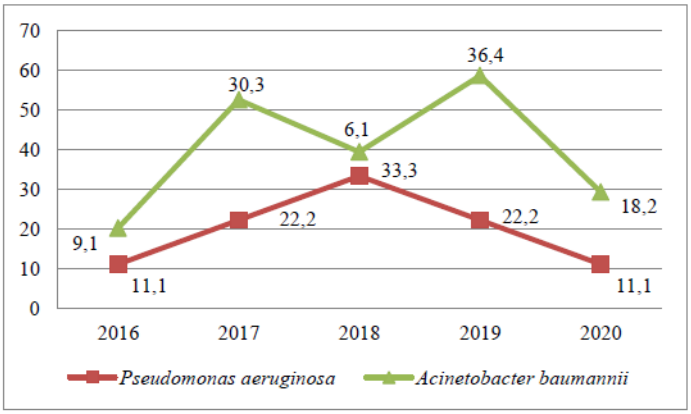


**Figure 2.** Distribution of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* over the years (%)

**Table 4.** Antibiotic resistance rates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

| Antibiotic | *Pseudomonas aeruginosa* n (%) | *Acinetobacter baumannii* n (%) |
|---|---|---|
| **Amikacin** | 1/53 (1.9) | 28/30 (93.3) |
| **Aztreonam** | 28/53 (52.9) | - |
| **Cefepime** | 19/52 (36.6) | - |
| **Ceftazidime** | 15/49 (30.6) | - |
| **Ciprofloxacin** | 9/48 (18.8) | 28/29 (96.6) |
| **Colistin** | 4/50 (8.0) | 3/33 (9.1) |
| **Gentamicin** | 5/50 (10.0) | 26/28 (92.9) |
| **Imipenem** | 5/51 (9.8) | 29/30 (96.7) |
| **Meropenem** | 2/52 (3.9) | 31/32 (96.9) |
| **PZT** | 12/53 (22.6) | - |
| **Tigecycline** | - | 1/17 (5.9) |
| **SXT** | - | 24/28 (85.7) |
| **MDR** | 7/54 (13) | 32/33 (97) |

Abbreviations: PTZ, piperacillin-tazobactam; SXT, trimethoprime-sulfametoxazole; MDR, multiple drug resistant

The gram-positive isolated in this study were CNS (146/295, 45.9%), *Staphylococcus aureus* (79/295, 26.8%), *Enterococcus faecalis* (43/295, 14.6%), *Enterococcus faecium* (16/295, 5.4%) and S*treptococcus* spp. (11/295, 3.7%). The rate of MRSA among this patients is 30.4% (24/79). The Table 5 indicates that MRSA was detected at the highest rate (6/9, 66.7%) in a statistically significant in 2019 when looked by years (p=0.021). The most resistant antibiotics in blood samples with MRSA were erythromycin (15/16, 93.7%), tetracycline (15/23, 65.2%) and clindamycin (13/22, 59.1%).

**Table 5.** Distribution of *Staphylococcus aureus* in blood cultures over the years, n (%)

| | 2016 | 2017 | 2018 | 2019 | 2020 | *P* |
|---|---|---|---|---|---|---|
| **MSSA** | 13 (%72.2) | 8 (%61.5) | 24 (%88.9) | 3 (%33.3) | 7 (%58.3) | 0.021 |
| **MRSA** | 5 (%27.8) | 5 (%38.5) | 3 (%11.1) | 6 (%66.7) | 5 (%41.7) | |

Abbreviations: MSSA, methicillin sensitive *Staphylococcus aureus*; MRSA, methicillin resistant *Staphylococcus aureus*

Despite this, vancomycin and linezolid resistance were not found in any of the MRSA isolates, while resistance to daptomycin, rifampin, and teicoplanin was 5.6%, 8.3%, and 13.0%, respectively. A statistically significant difference was observed between methicillin resistance and clindamycin, daptomycin, erythromycin, teicoplanin, and tetracycline antibiotic resistances in all *Staphylococcus aureus* isolates. This is shown in Table 6 as p=0.000, p=0.025, p=0.000, p=0.027, p=0.000, respectively.

**Table 6.** Resistance rates in MRSA isolates detected in blood cultures, n (%)

| Antibiotic | MRSA | MSSA | *P* |
|---|---|---|---|
| Ciprofloxacin | 6/20 (30.0) | 4/49 (8.2) | 0.029* |
| Clindamycin | 13/22 (59.1) | 2/53 (3.8) | <0.001* |
| Daptomycin | 1/18 (5.6) | 0/51 (0.0) | 0.025* |
| Erythromycin | 15/16 (93.7) | 6/40 (15.0) | <0.001* |
| Linezolid | 0/21 (0.0) | 0/53 (0.0) | - |
| Rifampin | 1/12 (8.3) | 3/38 (7.9) | 0.269 |
| Teicoplanin | 3/23 (13.0) | 0/53 (0.0) | 0.027* |
| Tetracycline | 15/23 (65.2) | 9/52 (17.3) | 0.000* |
| SXT | 4/22 (18.2) | 9/55 (16.4) | 0.094 |
| Vankomycin | 0/24 (0.0) | 0/55 (0.0) | - |

*Statistically significant

Abbreviations: MSSA, methicillin sensitive *Staphylococcus aureus*; MRSA, methicillin resistant *Staphylococcus aureus*; SXT, trimethoprime-sulfametoxazole

In our study, ESBL positive *Escherichia coli* and *Klebsiella pneumoniae* and MRSA isolates were evaluated according to age groups in patients who requested blood culture. Accordingly, it was determined that ESBL positivity increased significantly with age and ESBL positive bacteria were grown most frequently in the age group >60. However, it can be seen that from the Table 7, there was no significant relationship between the growth of MRSA in blood cultures and age groups (p=0.115).

**Table 7.** Distribution of ESBL and MRSA positivity by age groups, n (%)

| | 0-20 | 21-40 | 41-60 | >60 | *P* |
|---|---|---|---|---|---|
| **ESBL positive** | 1/8 (12.5) | 1/2 (50.0) | 17/38 (44.7) | 84/139 (60.4) | 0.028* |
| **ESBL negative** | 7/8 (87.5) | 1/2 (50.0) | 21/38 (55.3) | 55/139 (39.6) | |
| **MRSA** | 1/11 (9.1) | 1/5 (20.0) | 9/18 (50.0) | 13/45 (28.9) | 0.115 |
| **MSSA** | 10/11 (90.9) | 4/5 (80.0) | 9/18 (50.0) | 32/45 (71.1) | |

* Statistically significant

Abbreviations: ESBL, extended spectrum beta lactamase; MSSA, methicillin sensitive *Staphylococcus aureus*; MRSA, methicillin resistant *Staphylococcus aureus*

Furthermore, it was determined that the most samples with any microorganism in blood cultures were sent from the intensive care department.

The distribution of blood cultures with growth detected according to the departments is shown in Table 8.

**Table 8.** Distribution of blood cultures with growth detected according to hospital department, n (%)

| Department | *E. coli* | *K. pneumoniae* | *P. aeruginosa* | *A. baumannii* | *S. aureus* | *E. faecalis* |
|---|---|---|---|---|---|---|
| Emergency | - | 2 (2.4) | - | - | 4 (5.1) | - |
| Neurology | - | - | 1 (1.9) | - | 4 (5.1) | 1 (2.3) |
| Cardiology | 8 (7.6) | 2 (2.4) | - | 1 (3.0) | 9 (11.4) | 2 (4.7) |
| Child Health and Diseases | 1 (1.0) | 1 (1.2) | - | - | 2 (2.5) | - |
| Intensive Care | 45 (42.9) | 53 (64.6) | 37 (68.5) | 29 (87.9) | 25 (31.6) | 28 (65.1) |
| Chest Diseases and Allergy | 5 (4.8) | 1 (1.2) | 5 (9.3) | 2 (6.1) | 2 (2.5) | 3 (7.0) |
| Internal Medicine | 7 (6.7) | 8 (9.8) | 2 (3.7) | - | 13 (16.5) | 3 (7.0) |
| Geriatrics | 7 (6.7) | - | 1 (1.9) | - | 2 (2.5) | - |
| Dialysis | 2 (1.9) | 2 (2.4) | - | - | 2 (2.5) | - |
| Infection Diseases | 18 (17.1) | 10 (12.2) | 2 (3.7) | - | 16 (20.3) | 4 (9.3) |
| Oncology | 5 (4.8) | 2 (2.4) | 4 (7.4) | 1 (3.0) | - | 2 (4.7) |
| Urology | 1 (1.0) | - | - | - | - | - |
| Obstetrics | 1 (1.0) | - | - | - | - | - |
| Gastroenterology | 3 (2.9) | - | - | - | - | - |
| General Surgery | 2 (1.9) | - | - | - | - | - |
| Brain Surgery | - | 1 (1.2) | 1 (1.9) | - | - | - |
| Orthopedics and Traumatology | - | - | 1 (1.9) | - | - | - |

## DISCUSSION

Failure to apply early and appropriate antibiotic treatment in BSI may cause an increase in mortality, morbidity and costs (1). Approximately 30 million people are affected by these infections each year and causes death of 6 million people. In addition, it is important to determine of hospital surveillance for the management of BSI besides detection and identification of the causative pathogen and performing ADT on time (11). According to the researches have been conducted, inappropriate antibiotic treatments significantly increase mortality rates in patients with bacteremia, and the importance of appropriate empirical treatment in such cases has been emphasized (12).

Furthermore, positivity rates detected in blood cultures vary in many of the studies carried out. According to the study of Müderris et al., bacterial growth was detected in 18.3% (8.248/45.071) of the blood samples sent to the laboratory (12). In a study conducted in Izmir, Turkey, it was reported that positive signals were received in 15.2% (327/2.148) of blood cultures (13). In another study conducted by Şafak et al., growth was detected in 24.3% (2.809/11.559) blood culture samples (3). The isolation of bacteria in blood cultures taken from febrile patients was determined as 28% (144/514) (14), whereas, blood culture positivity was reported at a rate of 16% (3.949/24.694) in another research (11). Despite all these studies discussed before with high positivity, the positivity obtained from blood cultures is observed at lower rates in some studies. A study carried out by Prakash et al., a total number of 7.579 blood cultures were examined and 5% (n: 382) positivity was found (15). In addition, only 9.2% (n: 132) of 1.440 blood cultures were found to be positivity in a study conducted by Gohel et al. (16). The observed positive growth of our study was at a relatively low rate (8.8%) compared to the most similar studies in the literature.

It is obvious that, as the age increases, the isolations in blood cultures also increase significantly in this research. Accordingly, it was determined that BSI is most common in patients 41-60 and over 60 years old (10.6% and 11.1% respectively). Reports in the literature indicates that BSI is associated with age groups. The increase in these infections with advanced age may be due to reasons such as malnutrition, immunosuppression, decreased cognitive functions, increased comorbidity and the associated increase in the rate of referrals to health institutions (5). Sepsis and septic shock can be seen in all age groups. However, especially it is more common in elderly patients. In addition, newborns are more susceptible to these infections due to their weak immunological barriers (17). According to a research conducted by

Kante et al., growth in the blood cultures with a rate of 25% has been determined at the patients over 60 years of age (18). However, there are some studies showing that isolation rates in blood cultures decrease as age increases. For example, Sweta et al. found 4.6% blood culture positivity in patients over 64 years old and 38.7% in newborns (19). What is more, Nazir et al. determined the most common rate of blood culture positivity in newborns with a rate of 25.6% and this is followed by 22.1% of blood culture positivity at the age of 60 and above (17). Also, a research carried out by Bolukçu et al. reported that age over 65 is not related with blood culture positivity (20).

The researches about this topic indicates that BSIs are more common in male. According to the studies conducted by Kalın-Ünüvar et al. (5), Akyıldız et al. (8) and Kante et al. (18), it was reported that these infections are more common in male than in female. However, the rate of positivity detected in blood cultures was higher in female in our study.

Climate and seasonal changes can affect the diagnosis of infections in humans and direct infection prevention struggles. Studies about this area have emphasized that gram-negative bacteria cause more frequent infections during the summer months (21). According to a study conducted by Chazan et al., it was shown that BSI caused by *Escherichia coli* increased significantly in the summer months (22). Similarly, in the study carried out by Rodrigues et al., it was indicated that BSI caused by *Klebsiella* spp. and *Acinetobacter baumannii* increased significantly in the summer season (23). Parallel to all these, infections detected in blood cultures were observed to be seen more frequently in the summer season compared to other seasons in our study.

During this study, gram-negative bacteria were reproduced in 392 (56.7%) of the blood culture samples, 295 (42.7%) of them reproduced gram-positive and 4 (0.6%) *Candida* species reproduced. *Escherichia coli* (15.2%) from gram-negative and CNS (21.1%) among gram-positive were the most frequently isolated bacteria. In a study, gram-positive (67.3%) were isolated more frequently than gram-negative (29.4%) in blood cultures (24). Differently in another study, 64.3% of 224 blood samples had gram-negative and 35.7% had gram-positive reproduced. In the same study, *Escherichia coli* (59.7%) was the most frequently isolated bacteria among gram-negatives (25). In a study conducted by Keihanian et al., 225 samples were reproduced in blood cultures which gram-negative and gram-positives were detected at a rate of 64% and 36%, respectively. In the same study, the most frequently isolated bacterium was *Pseudomonas aeruginosa* (29.3%) unlike the others (4).

As stated by the World Health Organization (WHO), there is an increase in *Enterobacteriaceae* species that produce ESBL in parallel with the increasing rates of antimicrobial resistance worldwide (26,27). The ineffectiveness of most antibiotics in infections developed by ESBL-producing bacteria causes increased mortality and serious economic losses (8). In the studies conducted, it has been proven that ESBL positivity in *Escherichia coli* and *Klebsiella pneumoniae* strains is a risk factor that increases mortality (28,29). In a study carried out by Anggraini et al., ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* rates were determined as 62.2% and 66.2%, respectively (30). In another study, the rate of ESBL positive *Escherichia coli* was 80% and the rate of ESBL positive *Klebsiella pneumoniae* was 85% (31). However, ESBL positivity rate in *Escherichia coli* and *Klebsiella pneumoniae* strains was 56.2% and 53.7% in our study, respectively. In addition, it was found that ESBL positive rate was at the highest rate (84.6%) in 2020 when looking at the distribution of ESBL positive *Escherichia coli* over the years, but this was not statistically significant. Despite this, ESBL-positive *Klebsiella pneumoniae* is observed to have progressed increasingly between 2016-2019 and reached the highest level (69.6%) in 2019. The ESBL rates obtained in this study were found to be relatively low compared to similar studies. However, it is important that the frequency of ESBL should be considered in the empirical treatment of ESBL positive *Enterobacteriaceae* infections.

*Pseudomonas aeruginosa* is held responsible for 3-7% of bloodstream infections. In addition, it is known that it progresses with high morbidity and mortality (27-48%) in critically ill patients. MDR is a common feature of hospital-acquired *Pseudomonas aeruginosa* strains (32). In our study, the most resistant antibiotic of *Pseudomonas aeruginosa* isolates was aztreonam (52.9%) and the most sensitive was amikacin (98.1%). In addition, the frequency of MDR in *Pseudomonas aeruginosa* isolates was 13%. In a research conducted by Choi et al., it was found that the most resistant antibiotic was aztreonam (50%), and the most sensitive was colistin (100%) and amikacin (96%) just similar to this research. In the same study, the rate of MDR *Pseudomonas aeruginosa* was 22% (33). In a study carried out by Coşar et al., it was stated that the most resistant antibiotic was aztreonam (51.7%) and the most effective antibiotic was amikacin (89.3%) to *Pseudomonas aeruginosa* (6).

The mortality rate in patients with bacteremia caused by *Acinetobacter baumannii* exceeds 50% (34). The bacteria's high antibiotic resistance and ability to survive on inanimate, dry surfaces lead to epidemics in hospitals. It has now been reported that *Acinetobacter baumannii* is resistant to almost all antibiotics, including colistin, tigecycline and polymylcin B. In recent years, an increase has been detected in the rate of resistance it developed against carbapenem group antibiotics,

which are frequently preferred in the treatment of serious infections (35,36). The rate of *Acinetobacter baumannii* isolated from blood cultures was 4.8% (33/691) in this study. In a study conducted in Turkey, *Acinetobacter baumannii* was the second factor (16.7%) detected in blood cultures (10). In another study, the incidence of *Acinetobacter baumannii* in blood cultures was found to be 11.3% (1). This rate was found to be lower in our study compared to the literature. However, 97% of *Acinetobacter baumannii* strains isolated from blood cultures were found to be MDR and 84.8% were XDR, and this rate considered as high. In a study carried out by Al-Mously et al., the rate of MDR *Acinetobacter baumannii* in BSI was reported as 69%. In the same study, it was emphasized that the most effective antibiotics against *Acinetobacter baumannii* were colistin (99.5%) and tigecycline (96.1%) (37). In the light of the data obtained, it is clear that it is a situation that requires urgent action when it is considered that the bloodstream infection caused by MDR and XDR *Acinetobacter baumannii* may have an effect on mortality.

Gram-positive bacteria (especially *Staphylococcus* spp.) are frequently isolated in BSI. One of the most important factors of bacteremia caused by gram-positive bacteria is *Staphylococcus aureus* (38,39). The effects of MRSA bacteremias on the mortality of patients hospitalized in vital departments such as intensive care must be considered (7). MRSA strains are resistant to all beta-lactam antibiotics (except ceftaroline and ceftobiprol), but also to macrolides, lincosamides, quinolones and aminoglycosides (38). MRSA isolates are considered reservoirs for MDR genes, and limitations in their treatment lead to serious health problems (40). In a study conducted by Kula-Atik et al., 20.367 blood cultures were examined and *Staphylococcus aureus* was isolated in 8.6% (n: 390) of them and 41% (n: 160) of these strains were found to be MRSA (38). Gu et al. determined the incidence of *Staphylococcus aureus* as 7.4% in the blood culture of 2.760 patients, and found that 44.2% of them were MRSA (41). In another study conducted in Turkey, the prevalence of MRSA in blood cultures was determined as 50.8% (40). According to the data of Turkey, the frequency of *Staphylococcus aureus* in blood cultures varies between 4.9-38.3%. The frequency of MRSA is between 12.2% and 71.7% (10). The frequency of *Staphylococcus aureus* and MRSA in this study was 11.4% and 30.4%, respectively. Furthermore, it is obvious that BSI caused by MRSA does not progress at a high level in our hospital when compared with the literature. Despite this, while the rate of MRSA detected in 2019 was 66.7% alarming, thanks to the infection control measures taken, this rate was reduced to 41.7% in 2020.

**Limitations:** The reference methodology for detecting colistin susceptibility according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) criteria is the broth microdilution (BMD) method. While some of the studies conducted that automated systems were insufficient for colistin susceptibility tests, it was reported that these systems were sufficient in some studies (42,43). Despite this, the fact that the BMD method was not applied is considered as a limitation of our study.

## CONCLUSION

Determination of microorganisms isolated from blood cultures and antibiotic resistance conditions guides the clinician for empirical treatment. Although bacteria isolated from blood cultures and antibiotic susceptibility rates that are effective against them vary depending on both geographic characteristics and seasons, they may vary even among different institutions in the same country. These types of studies should be carried out at certain time intervals in each institution and the most common factors of each institution should be determined. Thus, in addition to the detected factors, antibiotic susceptibility patterns against them can also be determined. Since the resistance conditions of the factors causing bacteremia may change over time, it will be easier for the clinician to follow the antibiotic resistance rates of the factors regularly and carefully in order to determine the correct treatment strategy. Active surveillance systems play a important role for monitoring BSIs.

**Author Contributions:** KS, NC and EG designed the study and analyzed the data. UH and LHFA collected and prepared the data. EG and UH wrote the manuscript.

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RESEARCH ARTICLE

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# Traditional and Complementary Medicine Practices Used by Women with Premenstrual Syndrome

**ABSTRACT**

**Objective:** This research was carried out to determine the Traditional and Complementary Medicine (TCM) practices used by women experiencing Premenstrual Syndrome (PMS).

**Methods:** The sample of this descriptive study comprised 357 women who applied to the "Health Practice and Research Center Gynecology and Obstetrics" polyclinic of a university in Turkey. Study data were collected by using the Personal Information Form and the Premenstrual Syndrome Scale (PMSS) to determine the life situation of women with PMS.

**Results:** It was determined that 82.9% of women experienced PMS and that 83.8% of women living with PMS used TCM practices. The most commonly used TCM practices of the women were determined as follows: hot application on abdomen (90.7%), hot shower (85.1%), fennel tea (26.2%), walking (23.8%), massage (21.8%), yoga (11.7%), and music (10.5%). Almost all of the women undertook hot application to the abdomen, hot showers, massages, walking, and yoga (99.1%-99.5%-90.7%-96.6%-93.1%). Overall, 80% of the women who listened to music and 60% of the women who drank fennel tea experienced benefits as a result.

**Conclusions:** PMS is a commonly observed health issue among women, and many frequently use TCM practices to cope. Hot applications were found to be the most commonly practices used TCM.

**Keywords:** Premenstrual Syndrome, Traditional and Complementary Medicine, Women's Health.

# Premenstrual Sendrom Yaşayan Kadınların Kullandıkları Geleneksel ve Tamamlayıcı Tıp Uygulamaları

**ÖZET**

**Amaç:** Araştırma, Premenstrual Sendrom (PMS) yaşayan kadınların kullandıkları Geleneksel ve Tamamlayıcı Tıp (GETAT) uygulamalarının belirlenmesi amacıyla yapılmıştır.

**Gereç ve Yöntem:** Tanımlayıcı olarak yapılan araştırmanın örneklemini Türkiye'de bir üniversitenin "Sağlık Uygulama ve Araştırma Merkezi Kadın Hastalıkları ve Doğum" polikliniğine başvuran 357 kadın oluşturmuştur. Veriler Kişisel Bilgi Formu ve PMS yaşama durumunu belirlemek amacıyla Premenstrual Sendrom Ölçeği (PMSÖ) kullanılarak toplanmıştır.

**Bulgular:** Kadınların %82,9'unun PMS yaşadığı, PMS yaşayan kadınların %83,8'inin GETAT uygulamalarını kullandığı belirlenmiştir. PMS yaşayan kadınların en çok kullandıkları GETAT uygulamaları sırasıyla karına sıcak uygulama (%90.7), sıcak duş (%85.1), rezene çayı (%26.2), yürüyüş (%23.8), masaj (%21.8), yoga (%11.7) ve müzik (%10.5) olarak belirlenmiştir. Karına sıcak uygulama yapan, sıcak duş alan, masaj, yürüyüş ve yoga yapan kadınların tamamına yakını (%99.1-%99.5-%90.7-%96.6-%93.1), müzik dinleyenlerin %80'i, rezene çayı içenlerin %60'ı yarar görmüştür.

**Sonuç:** PMS kadınlarda yaygın görülen bir sağlık sorunudur ve başa çıkmak için sıklıkla GETAT uygulamalarını kullanırlar. Kadınlar tarafından en yaygın kullanılan yöntem sıcak uygulamalardır.

**Anahtar Kelimeler:** Premenstrual Sendrom, Geleneksel ve Tamamlayıcı Tıp, Kadın Sağlığı.

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## INTRODUCTION

Premenstrual Syndrome (PMS) is a combination of recurrent psychological and physical symptoms that occur in the luteal phase of the menstrual cycle, that decrease or end a few days after menstruation starts, and which repeat in most cycles (1). Over 40 million women worldwide experience these symptoms, which negatively affect their quality of life (2, 3). While 90% of women have only mild premenstrual symptoms, 20% have to cope with symptoms that severely disrupt their daily lives (4). Studies conducted in Turkey determine that PMS prevalence ranges between 20.1% and 65% (5-10).

Traditional and Complementary Medicine (TCM) practices are gaining worldwide importance, both as a research subject and as previously used methods. This situation is also valid for developed countries. TCM includes traditional medicine and various commonly unknown practices, products, and medical or healthcare systems (11). TCM practices play fundamental roles in the treatment and management of diseases in many Asian countries, such as China, Korea, Japan, and India (12). Globally, in countries such as the USA, Germany, Switzerland, Cuba, Japan, and Chile, more than 40% of the population uses TCM at least once a year (13). In Turkey, the usage rate of TCM ranges between 60% and 98.3% (14-17).

TCM practices included in the current regulations published by the Turkish Ministry of Health include phytotherapy, maggot therapy, mesotherapy, prolotherapy, music therapy, hypnosis, cup therapy, homeopathy, ozone therapy, leech therapy (hirudotherapy), osteopathy, acupuncture, reflexology, chiropractic, and apitherapy (18). According to *An International Journal of Obstetrics and Gynecology* (BJOG, 2016), TCM practices applied in PMS are; herbal medicines, body therapies (massage, acupuncture and acupressure, reflexology, phytotherapy), and mind and body techniques (yoga, physical exercise, hypnosis, hot application) (19).

Several researches determined that, as well as medication, women also prefer complementary treatment methods in PMS, such as massage (42–76.8%), hot application to the abdomen (67–75.1%) (20,21), exercise (15–69%) (21,22), herbal tea (20–63%) (21,22,23), vitamin supplement (27–34%) (21,22), reflexology (19.9%), chiropractic (15.5%), and yoga (11.0%) treatments (21).

PMS is a non-life-threatening but frequently observed health condition that can negatively affect the quality of life of women, as well as their mental health and daily life activities. In Turkey, it can be seen that most researches on this subject are conducted using students. Although there has been an increasing interest in TCM practices in recent years in Turkey, data obtained from clinical studies are limited and are not robust. There are therefore no sufficient data regarding TCM practices. This study will provide information on, and the benefits of those TCM practices used by women experiencing PMS in Turkey. Through this, it is considered that frequently used and beneficial TCM practices will generate a database for future researches to be conducted on evidence value.

The research was carried out to determine the TCM practices used by women experiencing PMS.

## MATERIAL AND METHODS

**Study Design:** The research was carried out using a descriptive design.

**Sampling:** The study data were collected in the in the Health Practice and Research Center Gynecology and Obstetrics polyclinic of a university in Turkey. The study universe comprised 4973 women who applied to the polyclinic between the dates of January and December 2018. Considering those existing studies that examine PMS frequency among women in Turkey (5-10) the research sample was calculated as 357, using a 50% estimate ratio/incidence/frequency with a 5% error limit and 95% confidence. The formula $n = (N.t^2.p.q)/(d^2(N-1)+ t^2.p.q)$ was used for this calculation.

The inclusion criteria for the study participants were as follows: women who knew how to read and write, who accepted to participate in the study, who were not pregnant or puerperant, who were not being in the premenopausal period, who did not have any chronical disease, and did not have a psychiatric disorder.

**Instruments:** The research data were collected by researchers in accordance with the literature using the Personal Information Form, which comprises 37 questions on the sociodemographic and obstetric characteristics of responders, as well as the PMS and the Premenstrual Syndrome Scale (PMSS). Data were collected using the face-to-face interviewing technique and the researcher conducted all interviews. Prior to the collection of the study data, the aim of the study was explained to all women participants by the researcher, all of whom gave their written consent.

The PMSS was developed by Gencdogan (24) to determine the severity of premenstrual symptoms and comprises 44 items. Responses are given according to a 5-point Likert-type scale (“Never”, “Rarely”, “Sometimes”, “Often”, “Always”). To score the scale, the options are evaluated as follows: “Never” (1 point), “Rarely” (2 points), “Sometimes” (3 points), “Often” (4 points), and “Always” (5 points). A total PMSS score is then obtained from the sum of all nine subscales and the sum of the responses to the Scale. To apply the PMSS, it is necessary to evaluate the individual respondents by “taking into account this condition the week before menstruation”. The lowest possible PMSS score is 44 and the highest possible score is

220. Higher PMSS scores indicate greater symptom severity during PMS. More than 50% of the total PMSS score were classified as 'PMS positive'. The Cronbach's alpha coefficient for the Scale was calculated in the scale's reliability study, and was found to be 0.75. In our study, the Cronbach alpha coefficient was found to be 0.90.

**Data Analysis:** Data were evaluated in a computer environment and $p<0.05$ was accepted as statistically significant. To analyze the data, frequency distributions were used for categorical variables, descriptive statistics (mean+SD) were used for numeric variables, and Chi-Square Test was used for the relationship between two categorical variables.

**Ethical Considerations**: To conduct the study, written permissions were received from Duzce University Non-invasive Health Research Ethics Committee (Decision No: 201886**)**, Duzce University Health Practice and Research Center, and Başaran Gençdoğan, who developed the PMS.

**RESULTS**

In the research, it was found that the study participants received an average PMSS score of 129.67±19.17. It was determined that 82.9% of women experienced PMS (Table 1).

**Table 1.** PMS Experiences of Women

| | n (n=357) | % |
|---|---|---|
| **PMS** | | |
| Have PMS (≥110) | 296 | 82.9 |
| Do not have PMS (<110) | 61 | 17.1 |

The mean age of the women who experienced PMS was found to be 27.94±3.982 years. Of these women, 84.1% were married, 63.5% were high school graduates, and 55.1% were not employed (Table 2).

**Table 2.** Distribution of Sociodemographic Characteristics of Women Experiencing PMS (n=296)

| | Min.–Max. | Mean ± SD |
|---|---|---|
| **Age** | 18–40 | 27.94±3.982 |
| | n | % |
| **Education Status** | | |
| Literate/Primary school | 23 | 7.8 |
| Secondary school | 12 | 4.1 |
| High school | 188 | 63.5 |
| College and higher education | 73 | 24.7 |
| **Employment status in an income generating job** | | |
| Yes | 133 | 44.9 |
| No | 163 | 55.1 |
| **Marital Status** | | |
| Married | 249 | 84.1 |
| Single | 47 | 15.9 |

Min.=Minimum, Max.=Maximum, SD=Standard Deviation.

Overall, 68.9% of the women's mother or sister had also experienced PMS. In our study, it was determined that women who experienced PMS experienced anger (93.6%), stomach pain (56.1%), mild depression (46.3%), and headache (44.6%), breast tenderness (31.1%), and abdominal swelling (20.3%). A total of 62.8% of women experienced their symptoms 2–6 days before menstruation, and they experienced their symptoms for an average of 4.59±1.531 days. Of the women, 99.7% experienced pain during their menstrual period, 98.3% took painkillers to subdue their pain, and 90.2% did something other than taking medicine.

It was found that 83.5% of the women who participated in the current study used TCM practices (Table 3).

**Table 3.** Distribution of the Characteristics of Women Experiencing PMS regarding TCM* Use (n=296)

| | n | % |
|---|---|---|
| **Know TCM practices** | | |
| Yes | 124 | 41.9 |
| No | 25 | 8.4 |
| Some | 147 | 49.7 |
| **Believe TCM practices** | | |
| Yes | 121 | 40.9 |
| No | 35 | 11.8 |
| Some | 140 | 47.3 |
| **Use TCM practices** | | |
| Yes | 248 | 83.8 |
| No | 48 | 16.2 |

*** TCM:** Traditional and Complementary Medicine

Furthermore it was found that, of these practices, fennel tea (26.2%) was used most among all the herbal medicines, with 60% of users experiencing benefits as a result. Women reported that, of those who used body therapies, most used apply massage (21.8%), with 90.7% of massage users reporting that they experienced benefits as a result. Among mind and body techniques, women used walking (23.8%) and yoga (11.7%) the most, with 93.1% of those who used yoga and 96.6% of those who used walking experiencing benefits as a result. Women who apply hypnosis reported that they did not experience any benefits as a result. It was determined that 85.1% of women took showers, 90.7% undertook hot application on their abdomens, and that 99.5% of the women who used hot application experienced benefits as a result (Table 4).

**Table 4.** Uses and Benefits of TCM for Women Experiencing PMS (n=248)

| TCM PRACTICES | USAGE STATUS | | | | BENEFIT STATUS | | | |
|---|---|---|---|---|---|---|---|---|
| | Yes, I use | | No, I do not use | | Yes, I experienced benefits | | No, I experienced no benefits | |
| **Herbal Medicines** | n | % | n | % | n | % | n | % |
| Fennel tea | 65 | **26.2** | 183 | 73.8 | 39 | **60.0** | 26 | 40.0 |
| Rose tea | 8 | 3.2 | 240 | 96.8 | 0 | 0.0 | 8 | 100.0 |
| Applying olive oil on stomach | 8 | 3.2 | 240 | 96.8 | 3 | 37.5 | 5 | 62.5 |
| Taking vitamin supplement | 0 | 0,0 | 248 | 100.0 | - | - | - | - |
| **Body Therapies** | | | | | | | | |
| Massage | 54 | **21.8** | 194 | 78.2 | 49 | **90.7** | 5 | 9.3 |
| Acupuncture | 3 | 1.2 | 245 | 98.8 | 2 | 66.7 | 1 | 33.3 |
| Acupressure | 0 | 0.0 | 248 | 100.0 | - | - | - | - |
| Applying reflexology | 1 | 0.4 | 247 | 99.6 | 1 | 100.0 | 0 | 0.0 |
| Applying phytotherapy | 0 | 0.0 | 248 | 100.0 | - | - | - | - |
| **Mind and Body Techniques** | | | | | | | | |
| Yoga | 29 | **11.7** | 219 | 88.3 | 27 | **93.1** | 2 | 6.9 |
| Physical exercise | 10 | 4.0 | 238 | 96.0 | 8 | 80.0 | 2 | 20.0 |
| Walking | 59 | **23.8** | 189 | 76.2 | 57 | **96.6** | 2 | 3.4 |
| Sport | 4 | 1.6 | 244 | 98.4 | 3 | 75.0 | 1 | 25.0 |
| Praying | 6 | 2.4 | 242 | 97.6 | 4 | 66.7 | 2 | 33.3 |
| Relaxation exercises | 12 | 4.8 | 236 | 95.2 | 9 | 75.0 | 3 | 25.0 |
| Listening to music | 26 | **10.5** | 222 | 89.5 | 21 | **80.8** | 5 | 19.2 |
| Hypnosis application | 4 | 1.6 | 244 | 98.4 | 0 | 0.0 | 4 | 100.0 |
| **Hot application** | | | | | | | | |
| Shower | 211 | **85.1** | 37 | 14.9 | 210 | **99.5** | 1 | 0.5 |
| Hot application on abdomen | 225 | **90.7** | 23 | 9.3 | 223 | **99.1** | 2 | 0.9 |

In the current study, among those women experiencing PMS, a statistically significant relationship was found between age, education, employment status, the experience of similar symptoms among their mothers or sisters before menstruation, and the use of TCM practices (n=248) ($p<0.05$). Accordingly, the use of TCM among women aged 26–30 years (90%) was found to be significantly higher than the use of TCM among those aged 31 and older (73.1%) (Table 5). Concerning education status, the rate of TCM users among literate/primary school/secondary school graduates (45.7%) was found to be significantly lower than the rate of TCM users among high school graduates (85.6%) and those individuals who had graduated from college or higher levels of schooling (97.3%, Table 5). The use rate of TCM among employed women (91%) was found to be significantly higher than the use rate of TCM among non-working women (77.9%) (Table 5). The use rate of TCM among those women whose mothers or sisters had experienced PMS (96.7%) was found to be significantly higher than the use rate of TCM use among those women whose mother or sister had not experienced any PMS (80.5%) (Table 5).

**Table 5.** Examining the Relationship between the Age, Education Status, Employment Status, PMS History among Mothers or Sisters of and TCM Use (n=248)

| | | | Use TCM | Do not use TCM | Total | $x^2$, p |
|---|---|---|---|---|---|---|
| Age Group | 18–25 | N | 64 | 15 | 79 | 10.305, **0.006*** |
| | | **%** | 81.0 | 19.0 | 100.0 | |
| | 26–30 | N | **135** | 15 | 150 | |
| | | % | **90.0** | 10.0 | 100.0 | |
| | 31 and higher | N | **49** | 18 | 67 | |
| | | % | **73.1** | 26.9 | 100.0 | |
| Education Status | Literate- Primary/ Secondary school | N | **16** | 19 | 35 | 47.569, **0.000*** |
| | | % | **45.7** | 54.3 | 100.0 | |
| | High school | N | **161** | 27 | 188 | |
| | | % | **85.6** | 14.4 | 100.0 | |
| | College and higher education | N | **71** | 2 | 73 | |
| | | % | **97.3** | 2.7 | 100.0 | |
| Employment Status | Yes | N | 121 | 12 | 133 | 9.199, **0.002** |
| | | % | 91.0 | 9.0 | 100.0 | |
| | No | N | 127 | 36 | 163 | |
| | | % | 77.9 | 22.1 | 100.0 | |
| Having complaints in the mother or sister prior to the menstruation | Yes | N | 178 | 6 | 184 | 20.156, **0.000*** |
| | | % | 96.7 | 3.3 | 100.0 | |
| | No | N | 70 | 17 | 87 | |
| | | % | 80.5 | 19.5 | 100.0 | |

$x^2$=Chi-Square Test, p=Significance Level ***p<0.001

## DISCUSSION

In this study, which was carried out to determine TCM practices used by women experiencing PMS, it was found that 82.9% of women experience PMS. Those studies conducted in Turkey determined that PMS prevalence ranges between 20.1% and 65% (5-10). The number of questionnaires and scales used to diagnose PMS and determine PMS severity is increasing. In Turkey, a growing number of scales, such as the PMSS, the Premenstrual Assessment Form, and the Menstrual Distress Complaint List (MDQ) are used (25). This is considered to be based on the use of different methods, the age of the studies, and differences and changes concerning the professional group, universe, and samples used.

In our study, it was determined that 83.8% of women who experience PMS use TCM practices. The TCM use rate in Turkey ranges between 60% and 98.3% (14-17). On examination of TCM practices globally, it was found that 42.1% use TCM practices in the USA, 48.2% use them Australia, 49.3% in France, 70.4% in Canada, 40% in Columbia, 71% in Chile, 70% in China, and that 80% use them in African countries (26). In Peltzer et al.'s study conducted across 32 countries, it was determined that 26.4% of the study participants use TCM practices (27). According to these results, it can be interpreted that the transnational and regional differences of the TCM use may differ based on the research method used, universe–sample differences, and sociocultural characteristics of the places in which the research was conducted. The TCM use rate of women in our study was found to be higher when compared with those in the literature. This finding might be because the prevalence of women visiting the doctor for treatment when experiencing PMS is low in Turkey, and also because TCM practices such as hot application to the abdomen, taking a shower, and drinking herbal tea are known and applied by a wide section of society.

The rate of TCM use among women in our study was found to be higher among those who believed TCM practices were effective (47.3%). Based on this finding, it can be considered that the belief in TCM practices have positive effect on TCM use.

The most commonly used TCM practices of the women who experience PMS were determined as follows: hot application on abdomen (90.7%), hot shower (85.1%), fennel tea (26.2%), walking (23.8%), massage (21.8%), yoga (11.7%), and music (10.5%). Almost all of the women who undertook hot application on the abdomen and who took hot showers (99.1–99.5%) experienced benefits as a result. Certain scientific papers have proved the efficacy of hot application (20,28). Among those studies conducted in Turkey, it was found that the prevalence of hot application among women ranges between 32.3% and 75.6% (20,21,29). Our study findings show similarities with those of the literature.

The current study determined that fennel tea was the most used herbal medicine among the TCMs. In their study, Wong et al. determined that 19.4% of the women use herbal/traditional practices (30). Comparatively, among Turkish studies Keskin et al. (21) remark that the use rate of herbal product was 63%, while Gün et al. (20)'s study, which was carried out to prevent dysmenorrhea as one of the PMS symptoms, found that 4.6% of the women consume fennel tea. Jahromi et al. (31) found that fennel tea prevents uterine contraction and reduces of the severity of feelings of dysmenorrhea among the most frequently observed PMS symptoms. In our study, 60% of the women who drank fennel tea reported that it provided benefits. Accordingly, the literature supports our study.

It was determined that, among TCM body therapies, the most commonly used by the women was massage, with a majority of these women reporting that they benefitted as a result (90.7%). Keskin et al. (21) determined that the student participants in their study mostly used massage (76.8%) to reduce their PMS complaints. Furthermore, in their study conducted with students experiencing PMS, Lotfipur-Rafsanjani et al. (28) found that PMS applying massage reduces physical, mental, and psychological symptoms of PMS. Shafeequa (2017) determined that foot massage decreases the symptoms of the women with PMS women with PMS (32). These findings form the literature support those of our current study.

Women stated that they mostly practice walking, yoga, and listening to music among the body and mind techniques of TCM. Of the women who practice yoga, (93.1%) reported that they benefitted as a result. In a study conducted in India, yoga was found to have positive effects in decreasing anxiety, heart rate, and blood pressure of women with PMS (33, 34). Divedi et al. (35) determined that there is a reduction in many PMS symptoms, such as anger, nervousness, worry, depressive mood, and being unable to concentrate, among women practicing yoga as a TCM. In our study, 27 out of the 29 women who practiced yoga reported that they benefitted as a result, a finding that is similar to those in the literature. Women who used the method of listening to music that may be interpreted as the mean age of the group who experiencing PMS is young (27.94±3.982) and they aim to distract their attention to another way.

In the current study, among those women who experience PMS, a statistically significant relationship was found among age, education, employment status, having mothers and sisters with a history of PMS, and the use of TCM practices ($n=248$) ($p<0.05$). Accordingly, the TCM usage rate of among those aged 26–30 (90%) was found to be higher than those aged 31 or older (73.1%). In the

study by Kutlu et al. (36), in the collection of Frass et al. (37), the use rate of TCM was found higher among those women in the middle age group and with a higher education status. In our study, it was found that TCM use increases with increased educational level (primary/secondary school, 45.7%; high school, 85.6%; college and higher education, 97.3%). Buda et al. (38) and Kutlu et al. (36) determined that individuals with higher education status have positive attitudes toward the use of TCM methods. These findings form the literature support those of our study.

The use of TCM practices were also found to be higher among employed women (91%) when compared with women not in employment (77.9%). Güngörmüş et al. (39) found that the use of TCM is significantly high among employed and self-employed women. This information in the literature supports our study. The use rate of TCM was found to be higher in those women whose mothers or sisters have a history of PMS (68.9%) when compared with those women with mothers or sisters do who experienced no negative symptoms in the premenstrual period (31.1%). Keskin et al. (21) found that students who use TCM practices to reduce their complaints during PMS mostly learned TCM practices from their mothers or sisters (52.4%). According to these results it can be stated that the immediate environment is effective in the use of TCM methods.

## CONCLUSION

PMS is a health condition that affects women of every age and reduces their quality of life. It is recommended that affected women are informed about TCM methods used for PMS, as well the benefits of these methods. In addition to informing them about hot application, taking shower, massage, drinking fennel tea, and walking among TCM practices, awareness could also be raised among these women by providing education on other TCM methods such as acupuncture, reflexology, and phytotherapy to reduce PMS symptoms. Finally, nurses should be careful while providing consultancy on TCM practices and presenting effective methods to their patients.

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RESEARCH ARTICLE

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# Extension Block Pinning of Mallet Fractures: Comparison Between Early and Delayed Surgery

**ABSTRACT**

**Objective:** The objective of this study was to compare the outcomes of individuals who had acute mallet fracture treatment early versus those who underwent delayed surgical treatment.

**Methods:** This was a retrospective cohort study in which all patients were skeletally mature and underwent closed extension pinning surgery for Doyle Type 4B mallet fractures. Crawford criteria were used to evaluate extension lag, loss of DIP joint flexion, and pain outcomes following surgery. All patients were evaluated clinically and radiographically.

**Results:** There were 16 females and 32 males with total of 48 patients in this study. The mean age at the time of surgery was 31.50 ± 9.75 years (range, 19-60 years). A total of 30 patients with early surgical treatment of mallet fractures and 18 patients with delayed surgical therapy were included in the study. There was no significant difference in the active flexion of the DIPJ, Crawford criteria (p=0.085, p=0.907, respectively), except for complication rates (p=0.045).

**Conclusions:** When compared to the early closed extension-block pinning technique, the delayed closed extension-block pinning technique yielded a satisfactory functional outcome in the treatment of mallet fractures.

**Keywords:** Mallet Fracture, Closed Surgery, Ishiguro Technique, Delayed Surgery.

# Mallet Kırıklarında Ekstansiyon Blok Pinleme: Erken ve Gecikmeli Cerrahi Karşılaştırması

**ÖZET**

**Amaç:** Bu çalışmanın amacı, erken dönem mallet kırığı cerrahisi yapılan hastalarla gecikmiş dönem cerrahi tedavi uygulanan hastaların sonuçlarını karşılaştırmaktı.

**Gereç ve Yöntem:** Bu retrospektif kohort çalışmaya Doyle Tip 4B mallet kırığı nedeniyle kapalı ekstansiyon-blok pinleme tekniği uygulanan, iskelet matürasyonu tamamlanmış erişkin hastalar dahil edilmiştir. Distal interfalangeal eklem ekstansiyon, fleksiyon kaybı ve cerrahi sonrası ağrı sonuçlarını değerlendirmek için Crawford kriterleri kullanıldı. Tüm hastalar klinik ve radyografik olarak değerlendirildi.

**Bulgular:** Bu çalışmada 48 hastadan 16'sı kadın, 32'si erkekti. Ameliyat sırasındaki ortalama yaş 31.50 ± 9.75 yıl (aralık, 19-60 yıl) idi. Mallet kırığı erken cerrahi tedavisi uygulanan 30 hasta ve cerrahi tedavisi gecikmiş 18 hasta çalışmaya dahil edildi. İki grup arasında distal interfalangeal eklem aktif fleksiyonu ve Crawford kriterleri (sırasıyla p=0.085, p=0.907) açısından anlamlı fark olmamasına rağmen komplikasyon oranları (p=0.045) ve ekstansiyon gecikmesi açısından istatistiksel olarak anlamlı fark saptandı.

**Sonuç:** Gecikmiş dönem kapalı ekstansiyon-blok pinleme tekniği, erken dönem kapalı ekstansiyon-blok pinleme tekniği ile karşılaştırıldığında, mallet kırıklarının tedavisinde tatmin edici fonksiyonel sonuç vermiştir.

**Anahtar Kelimeler:** Mallet Kırığı, Kapalı Cerrahi, Ishiguro Tekniği, Gecikmiş Cerrahi.

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## INTRODUCTION

Mallet fracture is an avulsion fracture of the distal phalanx with a terminal extensor tendon, causing an inability to actively lengthen the distal interphalangeal (DIP) joint (1). There is no gold standard treatment method for mallet fractures, and many alternative treatment methods ranging from conservative to surgical have been described (2). There are several techniques for surgical treatment of mallet fractures reported in the literature. These treatment techniques include extension block, percutaneous direct fragment fixation, external fixator, tension band, pull-out wire technique, open reduction with Kirschner wire (K-wire), and internal fixation with plates and screws (1). Ishiguro et al. described the "extension-block pinning" technique, which is the most frequently utilized technique for mallet fracture treatment (3).

While several studies have been conducted on surgical treatment of acute mallet fractures, only a few have been conducted on surgical treatment of delayed mallet fractures (4). The surgical outcome of delayed mallet fractures is critical since untreated mallet fractures can result in extension lag, distal interphalangeal joint (DIPJ) osteoarthritis, and swan-neck deformity (5).

This study aimed to compare the functional results of patients who underwent early surgical treatment for acute mallet fractures with those who applied 14 days or more after injury and underwent delayed surgical treatment. We hypothesized that delayed operative treatment may adversely affect functional and radiological outcomes.

## MATERIAL AND METHODS

This was a retrospective study that was authorized by the local institutional review board. Inclusion criteria in this study were patients who were skeletally mature and underwent surgical treatment for a mallet fracture with a fracture fragment (Doyle Type 4B) containing 20%–50% articular surface (6). Exclusion criteria included open injury, pure tendinous avulsions, fracture fragments covering more than 50% of the articular surface, open physis, and patients under the age of 18. The early surgical treatment group included patients who were operated on within the first 2 weeks of the injury, while the delayed surgical treatment group included patients who were operated on between the 14th and 28th days after their injury. A total of 30 patients were treated with the extension-block pinning technique in the early period, while 18 patients were treated with the extension-block pinning technique in the delayed period. The patient's age, gender (male or female), side of injury, and time to surgery were all recorded as descriptive data.

This extension-block pinning technique was performed as described by Ishiguro et al. (3) (Figure 1).

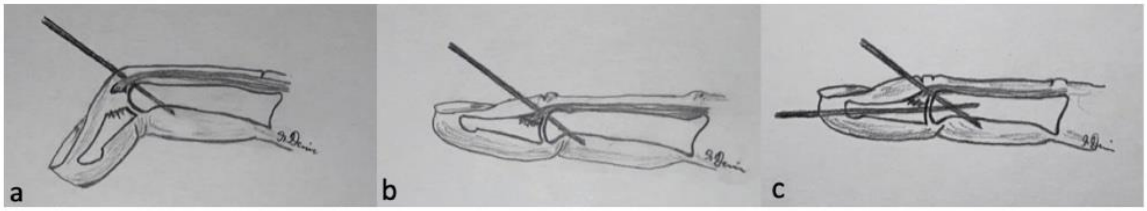


**Figure 1**. An illustration of the extension-block pinning technique. a: Inserting the first pin into the middle phalanx for indirect reduction while keeping the DIPJ flexed; b: DIPJ extension for fracture reduction; c: Insertion of the second fixation K-wire to keep the DIPJ extended.

**Postoperative Management:** Patients were discharged the same day and were seen weekly at the attending surgeon's office. After clinical and radiographic confirmation of union (4-6 weeks after surgery), K-wires were removed and active range of motion exercises started immediately.

**Evaluation:** Clinical examinations and weekly radiographs were used to assess complications and progression due to bone union (Figure 2,3). A goniometer was used to determine the range of motion and extension lag of the DIPJ. Crawford criteria were used to evaluate functional outcomes (Table 2) (7).

**Statistical Analysis:** For statistical analysis, SPSS 15.0 for Windows was utilized. For categorical variables, descriptive statistics were calculated as number and percentage; for numerical variables, mean and standard deviation were calculated, as well as minimum, maximum, and median. The chi-square test was used to compare the rates between groups. When the normal distribution condition was met, the T-test was used to do independent 2-group comparisons of numerical variables, and when the normal distribution condition was not met, the Mann-Whitney U test was utilized. For all analyses, a $p<0.05$ was considered statistically significant..

## RESULTS

Of the 48 patients included in this study, 16 were female and 32 were male. The mean age at the time of surgery was 31.50 ± 9.75 years (range, 19-60 years). The study population was divided into two groups according to the early and delayed treatment of mallet fractures: the early surgically treated group consisted of 30 patients, and the delayed treated group consisted of 18 patients. There was no significant difference in age, gender, side, mechanism of injury, and follow-up time between the two groups (Table 1).

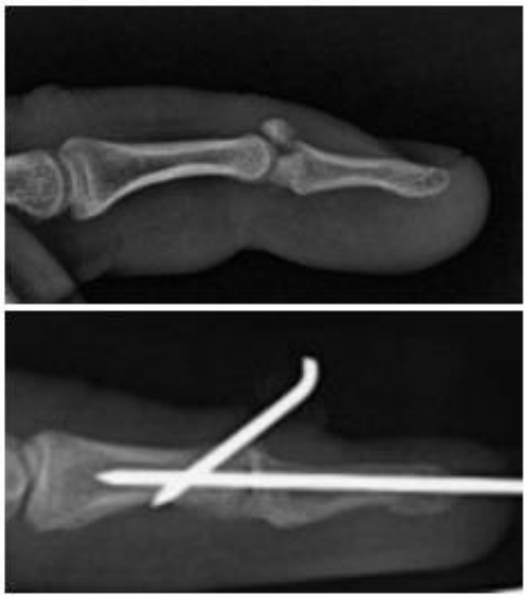

**Figure 2.** Preoperative and postoperative lateral radiographs of the patient who was operated on with the Extension-Block Pinning technique in the early period.

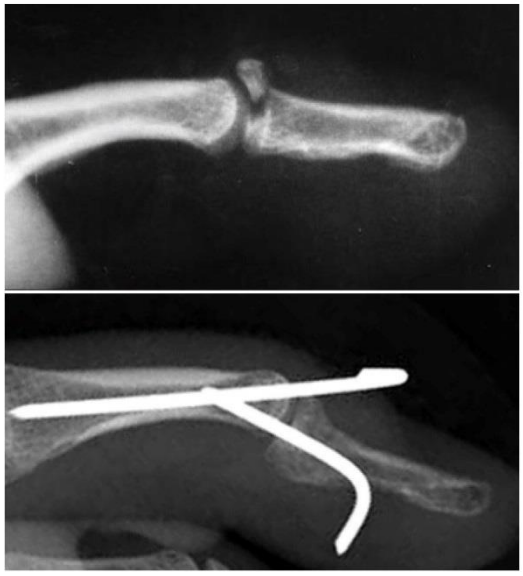

**Figure 3.** Preoperative and postoperative lateral radiographs of the patient who was operated on with the Extension-Block Pinning technique in the delayed period.

**Table 1.** Demographic and clinical parameters of patients in both groups.

| | **Early surgery** | **Delayed surgery** | ***p*-value** |
|---|---|---|---|
| **Age (Years)** | 32.20 ± 9.63 (19–60) | 30.33.23 ± 10.12 (19–56) | 0.52726* |
| **Gender** | | | |
| **Female (%)** | 11 (22.9%) | 5 (10.4%) | 0.379** |
| **Male (%)** | 19 (39.6%) | 13 (27.1%) | |
| **Side** | | | 0.417** |
| **Right (%)** | 18 (37.5%) | 14 (29.2%) | |
| **Left (%)** | 12 (25.0%) | 4 (8.3%) | |
| **Mechanism of Injury** | | | 0.202** |
| **Fall (%)** | 9 (15%) | 9 (18.8%) | |
| **Sports Injury (%)** | 9 (15%) | 6 (12.5%) | |
| **Work Accident (%)** | 12 (20%) | 3 (6.3%) | |
| **Follow-up (months)** | 17.13 ± 3.74 (12–24) | 17.67 ± 3.90 (12–24) | 0.640* |

*****Independent samples t-test.
**Pearson's chi-square test

Postoperative active range of motion, clinical and radiological evaluation are shown in Table 2. The mean extension lag of the DIP joint in the group of patients treated in the early period (5.33°) was statistically significantly better than the other group (10°) (p=0.025). There was no significant difference between the two groups in terms of active flexion of the DIPJ, Crawford criteria, and complication rates (p=0.085, p=0.907, p=0.45, respectively).

**Table 2.** Comparison of postoperative results in both groups.

| | **Early Surgery (n = 30)** | **Delayed Surgery (n = 18)** | ***p*-value** |
|---|---|---|---|
| **DIPJ extension lag** | 5.33° ± 5.56° (0° – 20°) | 10.00° ± 8.40° (0°– 25°) | **0.025*** |
| **DIPJ flexion** | 87.67° ± 4.30° (80°– 90°) | 85.00° ± 6.18° (70°– 90°) | 0.085* |
| **Crawford criteria** | | | 0.907** |
| **Excellent** | 11 (36.6%) | 3 (16.7%) | |
| **Good** | 12 (40%) | 7 (38.8%) | |
| **Fair** | 7 (23.3%) | 7 (38.8%) | |
| **Poor** | 0 | 1 (5.5%) | |
| **Complications (n)** | | | 0.045** |
| **None** | 26 (86.6%) | 10 (55.5%) | |
| **Pin-tract infection** | 2 (6.6%) | 3 (16.6%) | |
| **Nail deformity** | 1 (3.3%) | 2 (11.1%) | |
| **Osteoarthritis** | 1 (3.3%) | 3 (16.6%) | |

DIPJ: distal interphalangeal joint, mean ± standard deviation, bold values indicate significance
*Independent samples t-test; **Pearson's chi-square test

At the last follow-up of the patients who were operated on in the early period, 11 (36.6%) were excellent, 12 (40.0%) were good, and 7 (23.3%) fair results were determined according to Crawford criteria. Additionally, the distal interphalangeal joint's mean flexion angle was 87.67 (80-90) degrees, and the mean extension loss was 5.33 (0-20) degrees. At the last follow-up of delayed-treated patients, 3 (16.6%) were excellent, 7 (38.8%) good, 7 (38.8%) fair, and 1 (5.55%) poor. Additionally, the distal interphalangeal joint's mean flexion angle was 85 (70-90) degrees, and the mean extension loss was 10 (0-25) degrees.

In the early surgery group, there was one osteoarthritis, one nail deformity, and two pin-tract infections; in the late surgery group, there were three osteoarthritis, three pin-tract infections, and two nail deformities.

**DISCUSSION**

In this study, we evaluated the outcomes of patients with Doyle Type 4B mallet fractures who presented to the emergency department or clinic and were treated with the closed extension-block pinning technique described by Ishiguro during the first two weeks or longer in the case of delayed fracture. Although studies on early or delayed surgery for mallet fractures have been published, there are no recent studies comparing the results of both. Additionally, there are studies in the literature comparing early and delayed surgery for various fractures (8,9). This study is noteworthy in that it compares the functional outcomes of early and delayed surgical treatment for mallet fractures utilizing the extension-block pinning technique.

Nonsurgical treatment of mallet fractures with less than one-third joint involvement and no volar displacement of the distal phalanx has resulted in satisfactory pain alleviation and functional extension recovery (10,11). Typically, more than one-third of the articular surface of the distal phalanx is recommended for surgical treatment (13,3). In our study, we used the closed extension pinning technique for a mallet fracture with a fracture fragment (Doyle Type 4B) containing one-third to two-thirds of the articular surface.

Closed extension-block pinning of acute mallet fracture reported excellent functional outcomes and patient satisfaction (14). Agarwal & Akhtar, and Kootstra et al. reported the results of extension-block pinning for mallet fractures that had been delayed for more than three weeks in their study. In comparison to previous studies, their study showed that delaying surgical treatment of bone mallet fingers resulted in a satisfactory functional outcome (4,15). According to the criteria we used, our early and delayed surgery outcomes are consistent with the literature. However, a significant difference in extension lag outcomes was seen between early and delayed treatment. The mean extension lag of the DIPJ was statistically significantly greater in the early treatment group (5.33°) than in the delayed treatment group (10°) ($p=0.025$). Garberman et al. compared extension lag in the treatment of early vs delayed mallet fractures in their research. It has been stated that both groups' extension lags had similar outcomes (10) The greater extension lag in the delayed surgery group is attributed to the anatomical failure of the extensor mechanism to mend as a result of cal tissue developing in the fracture gap two weeks after the injury. Although the extension lag in the delayed surgery group was approximately 10 degrees greater than in the acute surgery group, it had no significant negative impact on the patients' functional outcomes.

In this study, all patients achieved radiologic bone union. The complication rate of early surgery groups was lower (13.3%) compared with delayed surgery groups (44.5%) in this study. While Hofmeister et al. (16) reported a complication rate of 21%, King et al. (17) reported a complication rate of 41%.

The limitations of our study are that it did not include the treatment of mallet fractures that covered more than two-thirds of the joint surface, and the clinical results of the patients were evaluated by the operating physician. Although this situation may cause prejudice on physicians, we believe that our study conducted on a very large patient population will contribute to the literature.

In conclusion, we recommend that the extension-block pinning technique be performed as early as possible in the surgical treatment of Doyle Type 4B mallet fractures. We believe that the extension-block pinning technique is a reliable treatment, even if there is a delay due to unexpected circumstances.

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RESEARCH ARTICLE

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# An Empirical Study on Determining the Communication and Empathy Skill Levels of Healthcare Professionals

**ABSTRACT**

**Objective:** This study was conducted to determine the communication skills and empathy levels of healthcare professionals working in a public hospital in Ankara and to determine the relationship between them.

**Methods:** This study, which is based on the field research method, is carried out in order to determine the communication skills and empathy levels of healthcare professionals working in a public hospital in Ankara, and to reveal the relationship between communication skills and empathy level. The data in the study, which was designed as a descriptive field study, was collected by face-to-face questionnaire technique from 385 healthcare workers in Ankara city center between February and March 2021.

**Results:** Research findings show that the participants have moderate communication skills and empathy. Communication skills and empathy levels of healthcare professionals do not differ according to their gender. As the weekly working time increases, communication skills and empathy levels decrease. There is a positive and significant relationship between communication skill level and empathy.

**Conclusions:** The results of the research indicate that the level of empathy is a positive determinant of communication skills, in other words, as the empathy level increases, the communication skill level increases.

**Keywords:** Communication Skills, Empathy, Healthcare Professionals.

# Sağlık Çalışanlarının İletişim ve Empati Beceri Düzeylerinin Belirlenmesi Üzerine Ampirik Bir Çalışma

**ÖZET**

**Amaç:** Bu çalışma, Ankara'da yer alan bir kamu hastanesinde hizmet veren sağlık çalışanlarının iletişim becerileri ve empati düzeylerini tespit etmek ve aralarındaki ilişkiyi belirlemek için yapılmıştır.

**Gereç ve Yöntem:** Saha araştırması yönteminin esas alındığı bu araştırmada, Ankara merkezindeki bir kamu hastanesinde görevli sağlık çalışanlarının iletişim becerileri ve empati düzeylerini belirlemek, iletişim becerileri ve empati düzeyi arasındaki ilişkiyi ortaya koymak amacıyla yapılmıştır. Betimleyici bir alan araştırması olarak tasarlanan çalışmada veriler, Şubat-Mart 2021 tarihleri arasında Ankara merkezdeki 385 sağlık çalışanından yüz yüze anket tekniği ile toplanmıştır.

**Bulgular:** Araştırma bulguları katılımcıların orta düzeyde iletişim becerileri ve empati durumuna sahip olduklarını göstermektedir. Sağlık çalışanlarının cinsiyetine göre iletişim beceri ve empati düzeyi farklılaşmamaktadır. Haftalık çalışma süresi artıkça iletişim beceri ve empati düzeyi düşmektedir. İletişim beceri düzeyi ile empati durumu arasında pozitif yönde anlamlı ilişki bulunmaktadır.

**Sonuç:** Araştırma sonuçları, empati düzeyinin iletişim becerilerinin pozitif yönde anlamlı belirleyicisi olduğuna, bir başka anlatımla, empati düzeyi arttıkça, iletişim beceri düzeyinde de bir artış yaşandığına işaret etmektedir.

**Anahtar Kelimeler:** İletişim Becerileri, Empati, Sağlık Çalışanı.

## INTRODUCTION

While the fast developments in technology consist a help to communications, on the other hand, the complexities of a globalizing world can sometimes make technology a hindrance to starting communication. Thus, today's communication has made the mastering of communication technologies and skills a requirement. Because the human being is a social entity on his/her own, his/her need for communication never ceases (1). The human tries to communicate from the first beginnings of his/her life. The fundamentals of communication is an interaction between individuals that is based on trading emotions and thoughts (2). Further, communication is a symbolic process that includes the reproduction, maintenance and transformation of a reality (3). In a context of healthcare communication, healthcare related individuals and/or groups communicate with their respective targets and include the transaction of information, views and emotions between healthcare professionals and patients and their relatives(4). Without doubt, an effective and quality healthcare service is related directly to the communication skills of healthcare professionals. Because all healthcare professionals work in a context of face-to-face communication, possessing strong communication skills is of much importance (5). Naturally, communication skills are important for all positions in human life, but they acquire an added importance while tending to the sick individual. These skills are a professional requirement for all healthcare staff and must be used in every interaction with the patient (6).

Generally, also symbols and signs can be used alongside spoken and written words, for the communication of thoughts, views and information. The process of communication starts with the conveying of a first message or information by a sender –a speaker or writer- to the receiver –the audience or reader- through an instrument or channel. The process continues with the sender coding the message –interpreting it-, making a feedback (7). Thus, communication is "an ongoing process where meaning is induced in the minds of the involved persons by the two directional flow of verbal and nonverbal messages" (8). Considered that most of the problems that are encountered in close interaction result from ineffective communications, the importance of acquiring communication skills is obvious. While communication is prevalent throughout everyday life, effective and reliable communication is not to be taken as granted. Effective and reliable communication is possible when the involved persons share a mutual sense of understanding and respect, and the affirmation that they are understanding and are understood. While there is research that stresses the innate and intuitional aspects of communication, many studies show that most aspects of communication are acquirable by learning and training (9).

According to Grover, fundamental communication skills are, listening, asking open ended questions, asking closed questions, explaining, interpreting, using facilitatives, evaluating non-verbal expressions, and silence. ***Listening*** is a part of being sensitive to the other person and requires an interest to the other person. It includes participatory skills, maintaining eye contact, striving to solve or interpret the message. ***Asking open ended questions***; is the key to an effective conversation. These are questions that cannot be answered with a simple "yes", "no" or a short sentence. At routine conversations, closed ended questions are most effective when they are weaved with open ended questions or interpretations. They should not be used as the main conversation technique. ***Asking closed questions;*** while open ended questions are good for getting additional information, getting to the truth requires closed question and they are obligatory in emergencies. ***Explaining*** questions are another kind of open ended question. The aim is to open up the conversation and encourage the partners to give further details of previously shared information and get the most of the interaction. ***Interpretation;*** the person gets the message and transforms it by interpreting, helps detailing the cognitive meaning for the co-workers. ***Using facilitators;*** facilitators are expressions, questions or actions that encourage colleagues or other individuals to continue to share information. Behaviors like nodding, smiling and questions like "how come?", "if it's convenient", "and then?" are examples for communication facilitating actions. ***Evaluating non-verbal expression;*** is among the most important skills to develop. It is described as "the content level of a message". Examples are, facial expressions, eye movements, gestures and postures, and personal space. ***Silence;*** another skill for enhancing the communication is the usage of silence. Short, silent stops of a couple of seconds or longer with accompanying eye contact or a small nod can encourage a speaker to continue (8).

Good communication skills help build respectful, efficient relations between healthcare workers and patients, their relatives, and coworkers and also improve personal leadership qualities, solving conflicts and also in helping to motivate others. They are essential for helping patients to understand their health situations, their problems and their treatment plans. Persons with effective communication skills can also manage unforeseen professional situations better (10). Perfection in communication skills and inter-personal skills are a universal requirement for healthcare workers who work in a multi-faceted work environment. This requirement is a direct result of patient expectations

and forms the prerequisite for the work in an increasingly complex hospital environment (11). In recent years, the aim for improving service quality and patient satisfaction has increased the emphasis on the learning and training of communication. Findings over the negative impact of ineffective and insufficient communication on patients are accumulating with each day. Conversation styles and behaviors of healthcare workers determine the degree of the benefits that patients gain from the communication. Considered that the quality of communication between the doctor and his/her patient affects all related processes, the importance of the issue is obvious (12).

Our ability for understanding others and relating to them is partially a function of empathy. The speed and accuracy of our inferences that we make about the feelings, objectives, attitudes, motives, beliefs, intents and behaviors of others determine to a great extent the contributions we make to a given social situation and also determine the valuation that others make about us. Thus, empathic comprehension is an important determinant of our communication proficiency but also it is a personal characteristic that is helpful in feeling out your way of action or convincing others to adhere to a particular way of action (13). Empathy is for humans a most important quality and for healthcare empathic behavior is fundamental. A patient-centered therapy requires emphatic healthcare practitioners (14).

Empathy in its most basic form, is the human ability to imagine placing yourself into the shoes of another person or to "feel his/her distress" (14). In other words, empathy is a person's ability to accurately perceive and understand the emotions of another person. Empathy is a multi-dimensional concept that includes emotional, cognitive, behavioral, moral and communicative components. The outcome of empathic communication for healthcare workers is the ability to form good interpersonal relations with co-workers and the management. Empathy is the ability to feel how others feel (8). In other words, empathy is a process where someone ties to mirror the viewpoint of another person in order to gain a glimpse into their thoughts and feelings. As a result, by placing us into the shoes of another person, empathy promises to look into his/her mind (15).

Increasing awareness to the advantages of mastering communication skills in the business world have aroused interest into this field and scholarly research has increased as well. The related literature encompasses most studies about healthcare workers (16, 17, 18, 19, 20, 5), university students (21,22), teacher trainees (23) and business people (24).

Our study aims to evaluate the communication skills and empathy levels of healthcare workers by various variables. This paper tried to find out the particular communication skills and empathy levels and their mutual relations of healthcare workers in public hospitals located in Ankara.

Based on the above literature, the hypotheses formed in line with the purpose of the study are shown below:

**$H_1$:** There is a statistically significant relationship between communication skills and empathy levels according to the unit they work in.

**$H_2$:** Communication skills and empathy levels differ significantly according to the weekly working hours.

**$H_3$:** Communication skills differ significantly by gender.

**$H_4$:** The level of empathy differs significantly by gender.

**$H_5$:** There is a positive and significant relationship between the communication skills and empathy tendency levels of the participants.

## MATERIAL AND METHODS

A descriptive survey has been designed to assess the communication skills and empathy levels of healthcare workers working in Ankara.

**Selection of the Sample and the Implementation of the Survey:** The sample consisted of healthcare workers of 19 years and older, who were working in public hospitals in Ankara. Data was collected by conducting a face to face questionnaire from 385 random participants of which 41.3% were men (N=159) and 58.7% were women, thus allowing a gender comparison.

Age distribution in the sample was 19 youngest, oldest 63 years, the average of the sample was 32 years.

74,3% of the participants were university graduates, 15.3% were high school, 8.3% postgraduate, 8% middle school and 1.3% were of elementary school education.

**Data Collection Tools:** The questionnaire consisted of 46 questions in 4 sections. The utilized scales are given below:

***Communication Skills Evaluation Scale:*** As adapted by Korkut, Owen and Demirbaş-Çelik (25), it is a 5 scale Likert-type scale, including 25-statements and developed for the evaluation of communication skills of adults. While early studies had used a 4 points scale, later studies standardized a 5 point scale beginning with "never" (1) rising up to "always" (5). The scale does not include reverse items, high points means that the participants in question evaluated his/her communication skills positively (26). Korkut Owen and Demirbaş-Çelik have found in their study, for the reliability of the scale, the Cronbach alpha coefficient as 0.94 (25). Our study's Cronbach Alpha reliability index has been found as 0.90.

***The Toronto Empathy Scale;*** The Toronto Empathy Questionnaire (TEQ), is a 5-scale Likert-type scale that has been developed to evaluate the empathy levels of individuals, with a self-

declaration style, one dimensional, 16 items (8 items are counted negative, 8 items are counted positive). During the development of the Toronto Empathy Questionnaire, the researchers aimed to evaluate empathy as an emotional process, contrary to existing other questionnaires. The Toronto Empathy Questionnaire has been developed by Spreng and others (2009) and adapted to Turkish by Totan and colleagues, and includes reverse coding. The reverse coded items are: 1-3-5-7-8-9-11-12. Higher points indicate higher levels of empathy. The scale is one dimensional (27). The reliability of the scale has been found as Cronbach's Alpha index 0.83.

**The Tests Used in the Data Analysis:** The survey was conducted between 24.02-12.03.2021 by face to face interviews. The questionnaires were analyzed by statistical software. The points that were obtained by The Communication Skills and Empathy scales were found showing a normal distribution by the Kolmogorov-Smirnov test, thus parametric test were chosen for the data analysis. The demographics of the healthcare workers were shown by *Frequency Analysis*. To find out whether the communication skills levels and the empathy inclination levels would differ in relation to gender, *Independent Sample T-Test* was used, to determine the differences in relation to weekly work hours, *One-way analysis of variance (ANOVA)* was used. *Linear Regression Analysis* was used on the findings of the empathic tendency to predict communication skills. The level and direction of the relationship between communication skill levels and empathic situation was explored by *Correlation Analysis.*

## RESULTS

**Findings and Interpretation:** This caption evaluates first the communication skills level, the empathic tendency situation and the descriptive statistics results of communication skills; then, the relationships between communication skills and empathy tendency situation are investigated thoroughly.

**Communication Skills Level:** The points given by the participants for the communication skills scale, gave totals ranging from 53 to 125 points. The communication skills level average of the participants' is $\bar{X}$=101.84. The Standard Deviation of the distribution was found as 11.42.

**Table 1.** Communication Skills Level Differences by Gender

| | Gender | N | $\bar{X}$ | t-value | Sig. |
|---|---|---|---|---|---|
| **Communication Skills Level** | Women | 226 | 102.16 | 0.66 | .504 |
| | Men | 159 | 101.37 | | |

Communication Skills Levels of the participants did not show any meaningful difference in relation to gender (t= 0.66; p> .05). Participant women (X= 102.16) and men ($\bar{X}$= 101.37) obtained close communication skills points. These results show that Hypothesis 3 is rejected. Korkut, Owen and Demirbaş-Çelik (29), in their study found meaningful differences between men's and women's communication skills scale totals, namely in the dimensions of effective listening and non-verbal communication and adherence to communication principles which made them conclude that women showed better communication skills.

**Table 2.** Differences in the Communication Skills Level in relation to Weekly Work Hours and the Work Unit

| | Weekly Work Hours | N | SD | $\bar{X}$ | F | Sig. |
|---|---|---|---|---|---|---|
| **Communication Skills Level** | 40-45 | 286 | 11.12 | 102.6 | 5.66 | .004 |
| | 46-50 | 68 | 11.87 | 101.3 | | |
| | 51-56 | 31 | 11.52 | 95.5 | | |
| | **Work Unit** | **N** | **SD** | $\bar{X}$ | **F** | **Sig.** |
| **Communication Skills Level** | Surgical Service | 37 | 11.9 | 104.0 | 4.28 | .014 |
| | Internal Diseases Service | 45 | 11.62 | 97.4 | | |
| | Other Service Units | 303 | 11.19 | 102.2 | | |

On the other hand, the communication skills levels show meaningful differences in relation to weekly work hours (F= 5.66; p< .05). As we investigate the 5% Tukey Test results, the differences occur between those who work 51-56 hours a week and those who work 40-45 and also 46-50 hours a week. Those who work 51-56 hours a week show a lower level ($\bar{X}$ = 95.5) of communication skills than those who work 40-45 hours a week ($\bar{X}$ = 102.6), and those who work 46-50 hours a week ($\bar{X}$ = 101.3). Obviously, communication skills levels fall with increasing work hours. A parallel study that was conducted by Akgün, Şahin and colleagues (16), found that the communication skill levels of nurses decreased with increasing work hours. Meaningful differences in communication levels occurred also in relation to the work units (F= 4.28; p< .05). 5% score of the Tukey Test show differences between the wards of Internal Diseases, Surgical Service and also the other units.

The healthcare workers in the Internal Diseases Service show lower communication skills levels ($\bar{\mathbf{X}}$ = 97.4) than those working in the Surgical Service ($\bar{\mathbf{X}}$ = 104.0), and also from those working in other units ($\bar{\mathbf{X}}$ = 101.3).

On the other hand, Akgün, Şahin and friends in their study, did not find any meaningful differences between the communication skills levels of nurses working in different wards (16). The obtained results indicate that Hypothesis 1 was confirmed.

**Empathy Level:** The totals of the empathy scale were ranged from 32 to 65. The communication skills average was $\bar{X}$= 50.85. The Standard deviation for distribution found as 8.32. Participants' Empathy levels did not show any meaningful differences in relation to gender (t= 1.67; p> .05). Women ($\bar{X}$= 51.45) and men ($\bar{X}$= 50.01) showed close values of Empathy level scores. The results show that Hypothesis 4, which predicts that empathy level differs significantly according to gender, was rejected.

**Table 3.** Empathy Levels Differences in Relation to Gender

| | Gender | N | $\bar{X}$ | t-value | Sig. |
|---|---|---|---|---|---|
| **Empathy Levels** | Women | 226 | 51.45 | 1.67 | .094 |
| | Men | 159 | 50.01 | | |

**Table 4**. Empathy Level Differences in Relation to Weekly Working Hours

| | Weekly Working Hours | N | SD | $\bar{X}$ | F | Sig. |
|---|---|---|---|---|---|---|
| **Empathy Levels** | 40-45 | 286 | 7.94 | 52.02 | 12.93 | .000 |
| | 46-50 | 68 | 8.53 | 48.36 | | |
| | 51-56 | 31 | 8.25 | 45.54 | | |

Differences in the weekly working hours showed also meaningful differences in the empathy levels (F= 12.93; p< .000). A Tukey Test of 5% meaningfulness results showed that difference occurred between those who worked 51-56 hours a week and those 40-45 hours and those who worked 46-50 hours a week. Those working 51-56 hours a week showed a lower empathy level ($\bar{X}$ = 45.54) compared to those working 40-45 hours a week ($\bar{X}$ = 52.02) and those working 46-50 hours a week ($\bar{X}$ = 48.36). It is obvious that with increasing work hours, empathy level averages fell. The obtained results indicate that Hypothesis 1 was confirmed.

**The Empathy Situation as the Determinant of Communication Skills Levels:** In this part of the study, the explanatory power of the empathy situation of healthcare workers for their communication skills level was explored by Linear Regression Analysis as shown on *Table 5*.

**Table 5.** Linear Regression Analysis showing Effect of Empathy Level on Communication Skills Level

| | B | Beta (β) | t | Sig. |
|---|---|---|---|---|
| **(Constant) Communication Skills Level (Index)** | 73.085 | | 22.18 | .000 |
| **Empathy Level (Index)** | .565 | .412 | 8.84 | .000 |
| $R^2$= ,170; Adjusted $R^2$ = ,167; F= 78,25; df= 1; p= ,000 | | | | |

As the result of the empathy level taken as an independent variable into the model, the regression index was found as 565. The Empathy level explains for 16.7% of the communication skills total variance. To find out whether communication skills levels would predict empathy levels, β and t values analysis showed (β=.412; p< .001) that indeed empathy levels predicted positively and meaningful the communication skills scores.

**Table 6.** Relationship between Empathy Level and Communication Skills

| | Communication Skills Level |
|---|---|
| **Empathy Level** | .412** |

Note: ** Correlation is significant at the 0.01 level (2-tailed).

To explain the strength and direction of the relationship between empathy level and communication skills, the results of a Correlation Analysis were studied which indicated a medium-level positive meaningful relationship (r= .412; p< .01). With other words, with rising empathy level, communication skills levels also increased. These results show that Hypothesis 5 is confirmed.

## DISCUSSION

Empathy plays a particularly important role in interpersonal relations as a facilitating ability that helps individuals establishing effective communication. While communication skills are important throughout all stations of life, they increase in importance when tending to a sick individual. These skills are required for all members of the healthcare team and should be prevalent throughout all encounters with the patient (6). With another words, as for the whole of society and at all stages of life, communication in healthcare professions is the essence of the processes that aim to reestablish balance in

destabilized systems. Healthcare workers (doctors, nurses, midwifes, and etc.) are using communication techniques in determining problems, solving problems, coping with stress, and at training for healthcare. From this aspect, communication skills represent a very important professional value.

Descriptive statistical findings indicate medium-level communication skills and empathy for healthcare workers in average. The study found that the average of the total scores for communication and empathy skills of healthcare workers was at medium-level. The study also found that with increasing weekly work hours, the average communication and empathy skills score decreased. Similar findings were presented by research in the literature (16, 18).

As we look into the relationship of gender with communication and empathy skills levels, our findings presented close scores which shows no meaningful differences between men and women. These results do not corroborate with some similar studies in the literature that reveal a meaningful relationship between gender and communication skills-empathy tendency (28, 5). Similarly, Korkut, Owen and Demirbaş-Çelik's study indicated in the total score for communication skill scale, men and women showed meaningful differences in the dimensions of effective listening and nonverbal communication and adherence to communication principles. The related study results indicate a difference in favor of women (29).

The most important finding of this study is the affirmation of a meaningful positive relationship between the communication skills and empathy skills of healthcare workers. So, communication skills level rises with the rise in empathy level. The findings prove that in the event of the participants in our study, healthcare workers' communication skills improve with increasing sensibility such as been affected from the misfortunes of others, from disrespectful behaviors to others, or understanding the sadness of others without being said. Regression Analysis results indicated that empathy levels contributed meaningful and positively onto communication skills levels scores.

As a result, while aiming to reveal the relationship between communication skills and empathy level, this study has particular limitations. First, this study has been conducted on healthcare professionals working in a public hospital in Ankara. The implementation of the study coincided with the outbreak of COVID-19 pandemic which increased the importance of healthcare workers and increased the demand for research with a focus on healthcare workers. Future research may target various regions and professions. They may explore the relations of communication skills with different variables. They may include various negative attributes like communication anxiety, stress, depression alongside positive attributes like self-respect, happiness, life satisfaction.

Suggestions may include, in parallel with research results, in order to improve communication and empathy levels of healthcare workers, attendance to in-service training, courses and seminars should be supported, attendance to social and cultural activities should be encouraged, and opportunities for further academic development for healthcare workers of all levels should be increased.

**Contributions of the authors:** N.Ö.: Contributing the study subject, reviewing the existing literature, conducting the study, statistical design and analysis, authoring the paper. E.K.: Contributing the study subject, planning, conducting the study, supervising the authoring. A.G.: planning, gathering the data.

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RESEARCH ARTICLE

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# The Effect of Education with Simulated Patient on the Empathy Attitudes of Medical Students: An Intervention Study

**ABSTRACT**

**Objective:** In this study, it was aimed to investigate the effect of simulated patient and education on the empathy levels of third-year students in medical school.

**Methods:** The study was carried out with 269 volunteer students. As a data collection tool, a short information form and the Jefferson Empathy Scale-Student Form were used. The scale was applied twice before and after the interview. Paired t test and Wilcoxan test were used for comparisons between the two groups.

**Results:** The average age of the students was 21±2.04 and 54.6% (n=147) were female. Total empathy scores of the students was found as 116.63±17.86 before the interview, and 117.35±18.48 after the interview, but this increase was not statistically significant (p>0.05). After the interview, a significant increase in the total empathy scores of female students (p<0.05) was detected. Women's total empathy scores before and after the interviews were significantly higher than male students (p<0.05). The total scores of the students who prefered the medical school due to their ideal/willingness to help people before (117.92±17.15) and after (119.10±17.68) the interview was significantly higher than the students who prefered the medical school due to other reasons (p=0.01). No significant difference was found between the empathy scores of students with and without doctors in their family (p>0.05). Ninety-four percent of the students emphasized that empathy was what they realized as the most important thing in this interview.

**Conclusions:** Education with the simulated patient affects empathy attitudes. After the experience of giving bad news with the simulated patient, nearly all of the students understood the importance of empathy in patient-physician communication.

**Keywords:** Simulated Patient, Bad News, Empathy, Medical Education.

# Simüle Hasta ile Eğitimin Tıp Öğrencilerinin Empati Tutumları Üzerine Etkisi: Bir Müdahale Çalışması

**ÖZET**

**Amaç:** Bu çalışmada, simüle hasta ile eğitimin tıp fakültesi üçüncü sınıf öğrencilerinin empati düzeyleri üzerine etkisinin araştırılması amaçlandı.

**Gereç ve Yöntem:** Çalışma gönüllü 269 öğrenci ile gerçekleştirildi. Verilerin toplanmasında kısa bir bilgi formu ile Jefferson Empati Ölçeği-Öğrenci Formu kullanıldı. Ölçek öğrencilere görüşme öncesi ve sonrası iki kez uygulandı. İki grup arasındaki karşılaştırmalarda Paired t testi ve Wilcoxan testi kullanıldı.

**Bulgular:** Öğrencilerin yaş ortalaması 21±2.04, %54.6'sı (n=147) kadındı. Öğrencilerin toplam empati skorları görüşme öncesinde 116,63±17,86, sonrasında ise 117.35±18.48 bulundu ancak bu artış istatistiksel olarak anlamlı değildi (p>0.05). Görüşme sonrası kadın öğrencilerin toplam empati skorlarında anlamlı bir artış saptandı (p<0.05). Kadınların görüşme öncesi ve sonrası empati toplam empati skorları erkek öğrencilerden anlamlı şekilde yüksekti (p<0.05). Tıp fakültesini ideal/insanlara yardım etme isteği nedeniyle tercih eden öğrencilerin hem başlangıç skorları (117.92±17.15) hem de görüşme sonrası toplam skorları (119.10±17.68), diğer nedenlerle tercih yapan öğrencilerden anlamlı şekilde yüksekti (p=0.01). Ailesinde doktor bulunan ve bulunmayan öğrenciler arasında empati skorları açısından anlamlı farklılık saptanmadı (p>0.05).

**Sonuç:** Simüle hasta ile eğitim empati tutumlarını etkilemektedir. Simüle hasta ile eğitimden sonra öğrencilerin tamamına yakını hasta-hekim ilişkisinde empatinin önemini anlamıştır.

**Anahtar Kelimeler:** Simüle Hasta, Kötü Haber, Empati, Tıp Öğrencisi, Tutum, Tıp Eğitimi.

## INTRODUCTION

Empathy is "one's ability to look at things through one's eyes, while putting themselves in someone else's shoes" (1). This feature, also called clinical empathy in health communication, is also defined as "the ability to understand the patient's internal experiences and perspective and communicate this understanding to the other side" (2).

Empathy is one of the most important elements of patient-physician communication and is a psychological process with cognitive and emotional dimensions, not just an attitude (3). While the emotional dimension consists of passive responses of the individual to the other person's emotions, cognitive dimention is accepted as an active, and improvable skill (1, 4).

Research shows that physicians are poorly trained in emotionally charged subjects such as empathy and compassion (5). However, there are many proven benefits of empathic approach in the patient physician relationship.

Empathy is an approach that increases a patient's trust to the doctor (6). Empathy reduces conflict, calms the patient, ensures positive communication and increases the behavior of help. Communication and empathy skills increase patient satisfaction and have a direct positive effect on health outcomes (7). Empathy has also been associated with a reduction in allegations of medical misconduct (3). All those benefits are not only helping to the patient, but also providing a positive work environment and job satisfaction for the doctor (8). Roter et al. has shown that doctors with a positive way of communicating experience less burnout (9).

Although empathy characteristics vary by personality, culture and society, it is known that it can be improved and positive attitudes can be achieved with a planned education program (10, 11).

Empathetic attitudes of medical school students are taking form due to the impacts of personal features, environmental conditions, training programs, standard/simulated patient interviews, reflexion, role play practices and role models (12). In recent years, opinions have been raised that empathy is an important feature for doctors and that communication and empathy skills are evaluated in the criteria of admission to medical school (13).

"Bad news" is all kinds of news that the patient and/or their relatives do not like, that provoke a feeling of helplessness, that destroy their hopes. Bad news is a task that physicians cannot avoid, and it is difficult for to both give and receive the bad news. Giving bad news requires training and a complex communication skills. It is known that bad news which are not given properly has devastating effects on patients/relatives (14).

Giving bad news to the patient/patient's relatives is one of the communication moments when empathy is used the most and its importance is best understood. After such an experience, it is hoped that the party that gives the bad news will be able to better understand the importance of empathy in communicating with patients. The empathy skills of doctors who give bad news have been found to be associated with better coping of the patient (15).

Simulated/standardized patient (SP) are people that trained to act as patients. Interaction with the simulated patient is one of the most effective methods in communication skills training. Simulated patients are used to both teach and evaluate communication and empathy skills (16).

Communication skills which learned under the influence of role models in the past are now an integral part of both undergraduate and postgraduate medical education. One of the stars in the World Health Organization's definition of a "five-star doctor" is the label as "communicative" (17). Communication skills are one of the main physician qualifications determined by the Canadian Council of Medical Educators Experts (CanMEDS) and the Accreditation Council for Graduate Medical Education (ACGME) (18, 19). One of the competencies that physicians should have in the National Core Education Program (UÇEP), which is the minimum education program that medical schools must comply with in our country, is "communication skills" (20).

At the Faculty of Medicine in Atatürk University, the courses about communication skills start in the first year and gradually progress from simple to complex. In the third grade, after the theoretical courses, students give bad news to a SP over five scenarios. This interview is made with the accompaniment of a structured training and evaluation form. Immediate feedback is given to the student by SP's right after the interview. In the analysis session afterwards, the interview is evaluated by both educators and students. This session also offers the student an opportunity of self-evaluation.

This study aims to investigate the impact of "breaking bad news" interaction with SP on the empathy levels of third-grade medical students.

## MATERIAL AND METHODS

**Ethical Consent:** Ethical permissions were taken from the Atatürk University, Medical Faculty Clinical Research Ethics Committee (IRB Number: B.30.2.ATA.0.01.00-10/56, No:38 Date:16.01.2020). The study was carried out per the rules of the Helsinki Declaration.

**Study Setting and Participants:** The study is an intervention study which was conducted on 14-16 February 2020 in a pretest-posttest pattern. Third-grade students who interviewed for "breaking

bad news" with SH, and volunteered were included in the study. Students were informed about the study and their consent was obtained. The questionnaire was applied to the same student twice before interviewing with SP, and right after having a "breaking bad news" interview with SP. The survey took about 10 minutes to answer.

**Study Size:** The universe of the study was created by 335 students in the third year at Faculty of Medicine of Atatürk University. The sample calculation was not made because it was aimed to reach all the students. Full data of 269 students who participated in both surveys were evaluated. 80% of the student universe has been reached.

**Data Collection Tools:** A short sociodemographic information form and the Jefferson Empathy Scale-Student Form (JES-SF) were used as a data collection tool.

**Sociodemographic Information Form:** Students were asked four closed-ended questions about age, gender, the reason for choosing medical school and whether there were doctors in the family, and an open-ended question about what they realized was most important in this interview.

**Jefferson Empathy Scale- Student Form:** It is a 20-point scale which is developed by Hojat et al. in 2001 (21). There are three different versions of the scale developed for medical and health workers, medical students, non-medical health students. In our study, the student version was used. Turkish adaptation of the JES-SF was made by Gönüllü et al. (22). The scale is answered according to the sevens likert system and is rated as I disagree at all (1), fully agree (7). In the scale, there are three dimensions such as 1) Perpective taking (PT), 2) Compassionate care (CC) ve 3) Standing in patient's shoes (SPS). While the lower dimension points were calculating separately, the total score is obtained by collecting all factor points. In the adaptation study, the internal consistency of the scale was found to be 0.83, 0.70, 0.60, respectively, for factors PT, CC, and SPS. In our study, we found cronbach alpha values for s subscales 0.83, 0.92 and 0.88 respectively.

**Statistical Analysis:** Data analyzed by using SPSS 25.0 (SPSS Inc., Chicago, IL, U.S.) statistical package program and presented with numbers, percentages, averages, standard deviations, median, min, max values. Paired t test was used in cases where normal distribution was achieved in comparisons between dependent groups, and Wilcoxan test was used in cases where it was not. The test reliability was estimated by using Cronbach α. A p-value of <0.05 was considered statistically significant.

## RESULTS

The average age of the students was 21±2 and 54.6% (n=147) of them were female. They all interviewed with SP. Sociodemographic features of students presented in Table 1.

**Table 1.** Sociodemographic features

| Variables | Number (n) | Percent (%) |
|---|---|---|
| **Gender** | | |
| Female | 147 | 54.6 |
| Male | 122 | 45.4 |
| **The reason for prefering the medical school** | | |
| Ideal/willingness to help people | 191 | 71 |
| Guidance from parents and teachers | 31 | 11.5 |
| Economic return /dignity | 47 | 17.5 |
| **Presence of doctors in the family** | | |
| There is | 122 | 45 |
| No | 147 | 55 |

Students' empathy scores before and after "breaking bad news" are shown in Table 2. Total empathy scores were 116.63±17.86 in the pre-test and 117.35±18.48 in the post-test, however this increase is not statistically significant (p>0.05), no significant changes were detected in the sub-factor scores (p>0.05).

**Table 2.** Comparison of empathy scores before and after "breaking bad news"

| | Mean ± SD | Med (min-max) | z | p |
|---|---|---|---|---|
| **Total score – before** | 116.63±17.86 | 120 (37-140) | | |
| **Total score – after** | 117.35±18.48 | 122 (64-140) | -0.925 | 0.355 |
| **PT –before** | 55.42±7.16 | 57 (22-63) | | |
| **PT – after** | 55.49±8.35 | 57 (9-63) | -0.728 | 0.467 |
| **CC –before** | 39.41±10.91 | 43 (7-49) | | |
| **CC –after** | 39.59±11.43 | 43 (7-49) | -0.864 | 0.388 |
| **SPS –before** | 11.20±3.32 | 12 (2-14) | | |
| **SPS –after** | 11.20±3.37 | 12 (2-14) | 0.017 | 0.986 |

PT Perpective taking, CC Compassionate care, SPS Standing in patient's shoes

Comparison of empathy scores by gender is presented in Table 3. Female students' TS increased significantly after interview (p<0.05). While there was no change in the PT factor, an increase in SP, and SPS factor scores was detected, however, it was not found statistically significant (p>0.05). After the interview, male students found a decrease in all factor scores and total scores other than SPS but it is not statistically significant (p>0.05). Women's TS after "breaking bad news" (p=0.001), PT scores before and after interview (p=0.19, p=0.16 respectively), CC scores after interview (p=0.08), SPS scores were found to be significantly higher than male students after interview (p=0.02).

**Table 3.** Comparison of empathy scores of male and female students

| | **Gender** | | | | | |
|---|---|---|---|---|---|---|
| | **Male** | | **Female** | | | |
| | **Mean±SD** | **Med (min-max)** | **Mean±SD** | **Med (min-max)** | **z** | **p** |
| **Total**-before | 114.58±19.20 | 119 (37-140) | 118.33±16.54 | 120(77-140) | -1.417 | .156 |
| **Total** -after | 113.52 ±19.33 | 117 (64-140) | 120.52±17.17 | 124 (68-140) | -3.317 | .001 |
| **PT**-before | 54.25 ±7.96 | 56 (22-63) | 56.39±6.28 | 57 (30-63) | -2.353 | .019 |
| **PT** -after | 54.59 ±8.59 | 56 (9-63) | 56.23±8.09 | 58 (9-63) | -2.418 | .016 |
| **CC** -before | 38.67 ±11.15 | 43 (7-49) | 40.01±10.7 | 43 (7-49) | -1.070 | .285 |
| **CC** -after | 37.65 ±12.48 | 42 (7-49) | 41.20±10.26 | 44 (7-49) | -2.654 | .008 |
| **SPS** -before | 11.13 ±3.38 | 12 (2-14) | 11.27±3.28 | 12 (2-14) | -.285 | .776 |
| **SPS**-after | 10.71 ±3.64 | 12 (2-14) | 11.61±3.09 | 13 (2-14) | -2.329 | .020 |

PT Perpective taking, CC Compassionate care, SPS Standing in patient's shoes

After the interview, both all subgroups scores and TS of the students who chose the medical school due to the ideal/willingness for help were found to be significantly higher than the other group (p<0.05, Table 4).

Although the PT and TS of students who were doctors in their family were somewhat high, they were not statistically significant (p>0.05, Table 5).

In the open-ended question, 94% of students stated that "empathy" was the thing they noticed as the most important in the experience of bad news for SP.

**Table 4.** Comparison of empathy scores according to reasons of preference

| | **Reason for preference** | | | | | |
|---|---|---|---|---|---|---|
| | **Ideal/ willingness to help people** | | **Other reasons** | | | |
| | **Mean±SD** | **Med (min-max)** | **Mean±SD** | **Med (min-max)** | **z** | **p** |
| **Total- before** | 117.92 ±17.15 | 121(37-140) | 113.47 ± 19.23 | 119 (60-140) | -1.695 | .090 |
| **Total-after** | 119.10 ±17.68 | 124 (68-140) | 113.06 ± 19.78 | 116 (64-140) | -2.572 | **.010** |
| **PT- before** | 55.61 ± 6.85 | 57 (22-63) | 54.95 ± 7.89 | 57(26-63) | -.274 | .784 |
| **PT-after** | 56.16 ± 8.46 | 58 (9-63) | 53.83 ± 7.87 | 55(24-63) | -2.896 | **.004** |
| **CC-before** | 40.15 ± 10.46 | 43 (7-49) | 37.59 ± 11.83 | 43(7-49) | -1.626 | .104 |
| **CC-after** | 40.29 ± 11.48 | 44 (7-49) | 37.88 ± 11.21 | 41(7-49) | -2.691 | **.007** |
| **SPS-before** | 11.37 ± 3.21 | 12 (2-14) | 10.81 ± 3.56 | 12 (2-14) | -1.035 | .301 |
| **SPS-after** | 11.43 ± 3.28 | 12 (2-14) | 10.63 ± 3.55 | 12 (2-14) | -2.075 | **.038** |

PT Perpective taking, CC Compassionate care, SPS Standing in patient's shoes

**Table 5.** Comparison of empathy scores based on whether there are doctors in the family

| | **Doctor in the family** | | | | | |
|---|---|---|---|---|---|---|
| | **There is not** | | **There is** | | | |
| | **Mean±SD** | **Med (min-max)** | **Mean±SD** | **Med (min-max)** | **z** | **p** |
| **TS-before** | 116.33 ±17.38 | 119 (37-140) | 116.99 ±18.49 | 122 (72-140) | -.811 | .417 |
| **TS-after** | 117.91 ±17.93 | 123 (64-140) | 116.67 ±19.18 | 121 (68-140) | -.274 | .784 |
| **PT- before** | 55.08 ±7.20 | 56 (22-63) | 55.83 ±7.11 | 57 (30-63) | -1.158 | .247 |
| **PT- after** | 55.18 ±8.21 | 57 (9-63) | 55.86 ±8.53 | 58 (9-63) | -1.144 | .253 |
| **CC- before** | 39.39 ±10.06 | 42 (7-49) | 39.42 ±11.90 | 44 (7-49) | -.984 | .325 |
| **CC-after** | 40.46 ±10.89 | 44 (7-49) | 38.55 ±12.02 | 43 (7-49) | -.988 | .323 |
| **SPS-before** | 11.27 ±3.06 | 12 (2-14) | 11.12 ±3.61 | 12 (2-14) | -.661 | .509 |
| **SPS-after** | 11.05 ±3.39 | 12 (2-14) | 11.39 ±3.36 | 12 (2-14) | -1.272 | ,203 |

TS total score, PT perspective taking, CC compassionate care, SPS Standing in patient's shoes

## DISCUSSION

The increase in the lack of communication between physicians and patients can be resolved with trainings in this area. Studies have concluded that communication skills are basic clinical skills

that can be taught and evaluated, and that medical school students should be taught about this issue with the same rigorousness as other clinical skills (23).

Standard surveys and scales are used in the evaluation of empathy, as well as educators, patients, peers, SP's and observer assessments (13).

In our study, JES-student form was used as a measurement tool and empathy scores of the students were found to be good (116 points out of 140 points). Although there was an increase in the total scores of the students after the simulated patient interview, it was not statistically significant ($p>0.05$).

The findings on the subject in the literature are contradictory. After the trainings and patient interviews, different results were reported as the empathy scores of the students increased, decreased and did not change.

Hojat et al.'s studies with third-year students, Rees et al.'s first-year students found a decrease in after training empathy attitudes (24, 25). In the study that help and ark. has made with the third-year students, it was reported that there was a significant decrease in empathy scores of the students after interaction with the standard patient (26). Contrary to these studies, a study with first-year students of theh-tribal department reported a significant increase in post-education empathy score averages (27). In these different results, numerous factors may have influenced the educational program, the characteristics of the trainers, the time of evaluation, and the attitudes of the students towards the course.

In our study, TS of female students increased significantly after interaction with SP ($p<0.05$, Table 3), although not significant in CC and SPS factors ($p>0.05$). There was a significant change in the post-interview scores of male students ($p>0.05$). Female students have significantly higher empathy attitude scores than male students.

Few studies report that empathy scores are higher in women, some in men, while some studies suggest there is no gender difference.

Studies on the subject support our findings (28-31). In the study of Yardım et al, the total empathy scores of female students were found to be higher than that of male students (26). In the other study, made by Cangür et al., women have higher scores but it is not statistically significant (32).

According to these results, it can be concluded that women are more empathetic and more affected by education. High empathy scores in female students have been linked to gender characteristics, women's better understanding of emotion and compassion in relationships and greater success in communication (1, 2, 31).

Contrary to these results, male students' empathy scores were found to be high in a large research sample of 1,074 students from six medical schools in the study of Karaoglu et al. (33). Some studies which are fewer, have reported no difference between men and women in terms of empathy (34, 35).

Considering the decrease in the score for male students in our study, it can be considered that new studies should be carried out that investigate the cause of this decrease and that more effective educational programs should be implemented according to the results.

In our study, the scores of students who chose because of their ideal/willingness to help people were significantly higher than those who preferred for other reasons both before and after the interview. These results suggest that communication skills and empathy training are more effective in students who make their choices consciously due to the wills.

There was no change in the total scores and factor scores of the students who preferred medical school for other reasons both before and after the interview. More attention and effort needs to be put into these students. There are studies in the literature that report that students' reasons for preference affect their empathy levels. In the study of Karaoglu et al. (2012), the empathy scores of students who choose medical school for the desire to help people and with ideals were found to be significantly higher than those who stated that they preferred medical school for economic reasons (33).

In our research, it was not determined whether there is a doctor or not in the family, had a significant effect on the empathy levels of the students.

Empathy is facilitating communication in the patient physician relationship as well as in daily life. It is important to establish training programs aimed at gaining communication and empathy skills that care as much about the human aspects of medicine as it is about the scientific dimension. Educational models should be provided to improve communication and empathy skills, and attitude-enhancing trainings should be started at the earliest stage.

## CONCLUSION

Although the students stated that empathy was the most important thing they realized the importance of after the experience of giving bad news, there was no significant change in empathy attitude scores. More effective programs are needed to improve the empathic attitudes of students.

**Limitations:** Since the study is conducted with third-year students of a single medical school, the results can not be generalized for medical school students. Because the study does not cover different classes, it could not be determined whether there was a difference between class levels. Finally, since the students are in the preclinical stage, the effect of interaction with the real patient could not be evaluated.

**Informed Consent:** Informed consent was obtained from all participants included in the study.

**Declaration of Interest**: The authors declared no conflict of interest.

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RESEARCH ARTICLE

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# Awareness of Patients Applying to a Cancer Research Center About Early Diagnosis of Cancer

**ABSTRACT**

**Objective:** This study was conducted to investigate the level of knowledge, attitudes and behaviors of patients about cancer types, symptoms, and early diagnosis methods.

**Methods:** Individuals over the age of 18 who agreed to participate in the study were included in this descriptive study. Those who had any psychiatric disease and treated for cancer were excluded from the study. In order to collect the data, a questionnaire form was applied by face-to-face interview method. SPSS-24 program was used for statistical analysis.

**Results:** A total of 324 volunteers, 266 women (82.1%) participated in the study. The mean age of the participants was 49.04±6.19, 263 of them were married (81.2%), 71.3% were primary school graduates and 37.3% had cancer in one of their first degree relatives. A statistically significant correlation was found between having cancer in the family and having cancer screening regularly (p=0.038). There was no significant relationship between gender, marital status and regular cancer screening (p>0.05). Of the participants 18.2% thought that cancer screening did not improve health, and 11% thought that we did not need to be screened because we were not at risk.

**Conclusions:** The level of knowledge and awareness of the participants about cancer types, screening methods and screening programs was found to be low. In order to implement national cancer control programs, public knowledge and awareness of cancer should be increased. Further studies should be carried out so that the importance of early diagnosis can be learned by the society and made it an individual responsibility.

**Keywords:** Cancer Screening, Early Diagnosis, Mammography, Fecal Occult Blood Test.

# Kanser Araştırma Merkezine Başvuran Hastaların Kanserin Erken Tanısı Konusunda Farkındalıkları

**ÖZET**

**Amaç:** Kanser, mortalite ve morbiditesinin yüksek olması, tedavi maliyeti, süresi ve yan etkileri nedeniyle önemli halk sağlığı sorunlarından birisidir. Bu nedenle kanserden korunma öncelikli olarak ele alınması gereken bir konudur. Bu çalışma, hastaların kanser türleri, belirtileri, erken tanı yöntemleri hakkındaki bilgi düzeylerini, tutum ve davranışlarını araştırmak amacıyla yapılmıştır.

**Gereç ve Yöntem:** Tanımlayıcı tipteki bu çalışmaya, Kanser Erken Teşhis, Tarama ve Eğitim Merkezi'ne başvuran ve araştırmaya katılmayı kabul eden 18 yaş üstü bireyler dahil edildi. Herhangi bir psikiyatrik hastalığı olanlar ile kanser tanısıyla tedavi görenler çalışma dışı bırakıldı. Verileri toplamak için ilgili literatüre uygun olarak oluşturulmuş bir anket yüz yüze görüşme yöntemi ile uygulandı. Verilerin istatistiksel analizinde SPSS 24 programı kullanıldı.

**Bulgular:** Çalışmaya 266 kadın(%82.1), 58 erkek(%17.9) toplam 324 gönüllü katıldı. Katılımcıların yaş ortalaması 49.04±6.19 olup, 263'ü evli(%81.2), %71.3 'ü ilkokul (n=231) mezunu, %37.3 'ünün (n=121) birinci derece akrabalarından birinde kanser hastalığı mevcuttu. Ailede erken yaşta kanser görülmesi ile düzenli olarak kanser taraması yaptırma arasında istatistiksel olarak anlamlı ilişki saptandı (p=0.038). Cinsiyet ve medeni durum ile düzenli kanser taraması yaptırma arasında anlamlı ilişki saptanmadı (p>0.05). Katılımcıların %18.2'si kanser taramalarının sağlığı geliştirmediğini, %11'i risk altında olmadığımız için tarama yaptırmamıza gerek olmadığını düşünmekteydi.

**Sonuç:** Çalışmamızda katılımcıların, kanser türleri, tarama yöntemleri ve tarama programları hakkında bilgi düzeyi ve farkındalıkları düşük bulunmuştur. Ulusal kanser kontrol programlarının uygulanabilmesi için halkın bilgi düzeyinin ve kanser farkındalığının arttırılması sağlanmalıdır. Erken tanının öneminin toplum tarafından öğrenilerek, bireysel bir sorumluluk haline getirilebilmesi için daha geniş çaplı çalışmalar yapılmalıdır.

**Anahtar Kelimeler:** Kanser Tarama, Erken Tanı, Mamografi, Gaitada Gizli Kan.

## INTRODUCTION

Cancer is one of today's most important public health problems due to its high mortality and morbidity, cost of treatment, duration, and side effects. One out of every five people in the world gets cancer in their lifetime, and 1 in 8 men and 1 in 11 women die of cancer. Worldwide, the total number of people alive within five years of being diagnosed with cancer, called the 5-year prevalence, is estimated to be 50.6 million (1).

According to the latest estimates of the global cancer burden by the International Agency for Research on Cancer (IARC), a subsidiary of the World Health Organization, 19.3 million new cases and 10 million deaths were reported in 2020. In parallel with the world's increasing population, the number of new cases increased to 19.3 million in 2020, and the number of deaths increased to ten million. Cancer incidence in total is 201 per hundred thousand (1).

The top 5 most common cancers in men are lung, prostate, colorectal, bladder, and stomach cancer, respectively. It is estimated that at least 40% of cancers in men are associated with smoking. The top 5 most common cancers in women are breast, thyroid, colorectal, lung, and uterine cancer, respectively. Breast cancer continues to be one of every four women's cancers and is responsible for 1 in 6 cancer-related deaths. Approximately 2.3 million new cases of breast cancer will be reported in 2020. It is seen that 1 out of every eight cancers diagnosed is breast cancer.

For this reason, the prevention of cancer is an issue that should be handled with priority. Risk factors should be evaluated for primary prevention. The five main behavioral risk factors that cause one-third of deaths from cancer are; high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use, and alcohol use. Obesity increases the risk of stomach, colon, kidney, gall bladder, breast, endometrium, ovarian and cervical cancers. It increases the risk of men's colon, rectum, pancreas, stomach, kidney, gall bladder, and prostate cancers. The total number of cancer cases attributed to obesity is estimated to be around 5,896. Cancers caused by obesity primarily affect women. If cancer is detected early, the chance of treatment increases, and its progression can be prevented with simple precautions. Life expectancy and quality of life are higher in early diagnosed cancers. Treatment and maintenance costs of the disease are less than late-diagnosed cancers (2). Secondary prevention is achieved especially by screening the groups that are at risk (3). Cancer Early Diagnosis, Screening and Training Centers (CEDSTC) have been established in our country to detect cancer cases early and reduce cancer deaths. For this purpose, breast, cervical, and colorectal cancers are screened at CEDSCs.

Unless the public is aware of the importance of early diagnosis, screening programs can't be successful. Adult individuals are more open to learning and applying what they have learned when they feel ready and aware of what and why they need to know. Healthcare workers play an essential role in the early diagnosis and prevention of cancer by increasing society's awareness with community-based screening and effective training methods.

This study was conducted to investigate individuals' knowledge levels, attitudes, and behaviors about cancer types, symptoms, and early diagnosis methods. We aimed to evaluate the awareness of cancer screening of the participants.

## MATERIAL AND METHODS

**Study Design and Sample Selection:** Approval of the local noninvasive research ethics committee was obtained before starting the study and informed consents of the participants who agreed to participate were obtained after being given brief information about the purpose of the study.

Individuals over 18 years old who were registered to Cancer Early Diagnosis, Screening and Training Center (CEDSTC) and who agreed to participate in the study between June 2019 and February 2020 were included in this cross-sectional descriptive study. Those with any psychiatric disease and those diagnosed with cancer and treated were excluded from the study. In order to collect the data, a questionnaire created following the relevant literature is used. The questionnaires were applied to the participants by face-to-face interview method. Filling the questionnaire took approximately ten minutes for each participant. Twentyeight participants were excluded because they refused to answer some questions.

**Socio-demographic Characteristics Questionnaire:** The survey consisted of four functional domains: socio-demographic characteristics, knowledge, attitude, and practice related questions. The demographic characteristics form included questions regarding age, gender, marital status, education level, economical situation, chronic illness, whether they have any relatives with cancer, whether they have cancer screening at regular intervals. They were asked which types of cancer they have information about and which types of cancer can be diagnosed early with screening tests. The knowledge level of cancer symptoms, risk factors, and screening methods were questioned. It was also questioned whether they received doctor's advice on cancer screening and whether they followed it if they did.

After questionnaire administration to the participants, verbal information about cancer types, risk factors, prevention methods, and breast self-examination (BSE) were given.

Statistical analysis was performed using the Statistical Package for Social Sciences version 24 (IBM, Armonk, NY) software to evaluate the data. Descriptive statistics were expressed as mean, standard deviation, minimum-maximum values, frequency, and percentile. Kolmogorow-Smirnow test was used to determine the normal distrubution of the data set. Chi-square test was used to compare the knowledge of cancer types, which types of cancer can be diagnosed early by screening and gender, marital status, whether they have any relatives with cancer. A p value of less than 0.05 was considered statistically significant with a 95% confidence level.

## RESULTS

A total of 324 volunteers, 266 women (82.1%), 58 men (17.9%), participated in the study. The mean age of the participants was 49.04 ± 6.19 (min: 30-max: 67) years old and 263 participants were married (81.2%). Of the participants, 71.3% were primary school (n = 231), 7.7% secondary school (n = 25), 11.4% high school (n = 37), 9.6% were university (n = 31) graduates. One of the first-degree relatives of 37.3% (n = 121) participants had cancer or died at an early age due to cancer.

The types of cancer that the participants had information about are summarized in Table 1. Breast cancer was the most known type of cancer and uterine and cervical cancers followed this. The most widely known types of cancer were female cancers. Although prostate and skin cancers are quite common, they were the least known types of cancer. Awareness of cancer types and awareness of whether early detection is possible with screening are shown in Figure 1.

**Table 1.** Awareness of cancer types

| Cancer Type | Aware | | Not aware | |
|---|---|---|---|---|
| | Number | % | Number | % |
| **Breast cancer** | 293 | 90.4 | 31 | 9.6 |
| **Uterine and cervical cancer** | 217 | 67.0 | 107 | 33.0 |
| **Colorectal cancer** | 113 | 34.9 | 211 | 65.1 |
| **Lung cancer** | 187 | 57.7 | 137 | 42.3 |
| **Leukemia** | 124 | 38.3 | 200 | 61.7 |
| **Brain cancer** | 112 | 34.6 | 212 | 65.4 |
| **Thyroid cancer** | 88 | 27.2 | 236 | 72.8 |
| **Prostate cancer** | 107 | 33.0 | 217 | 67.0 |
| **Skin cancer** | 37 | 11.4 | 287 | 88.6 |

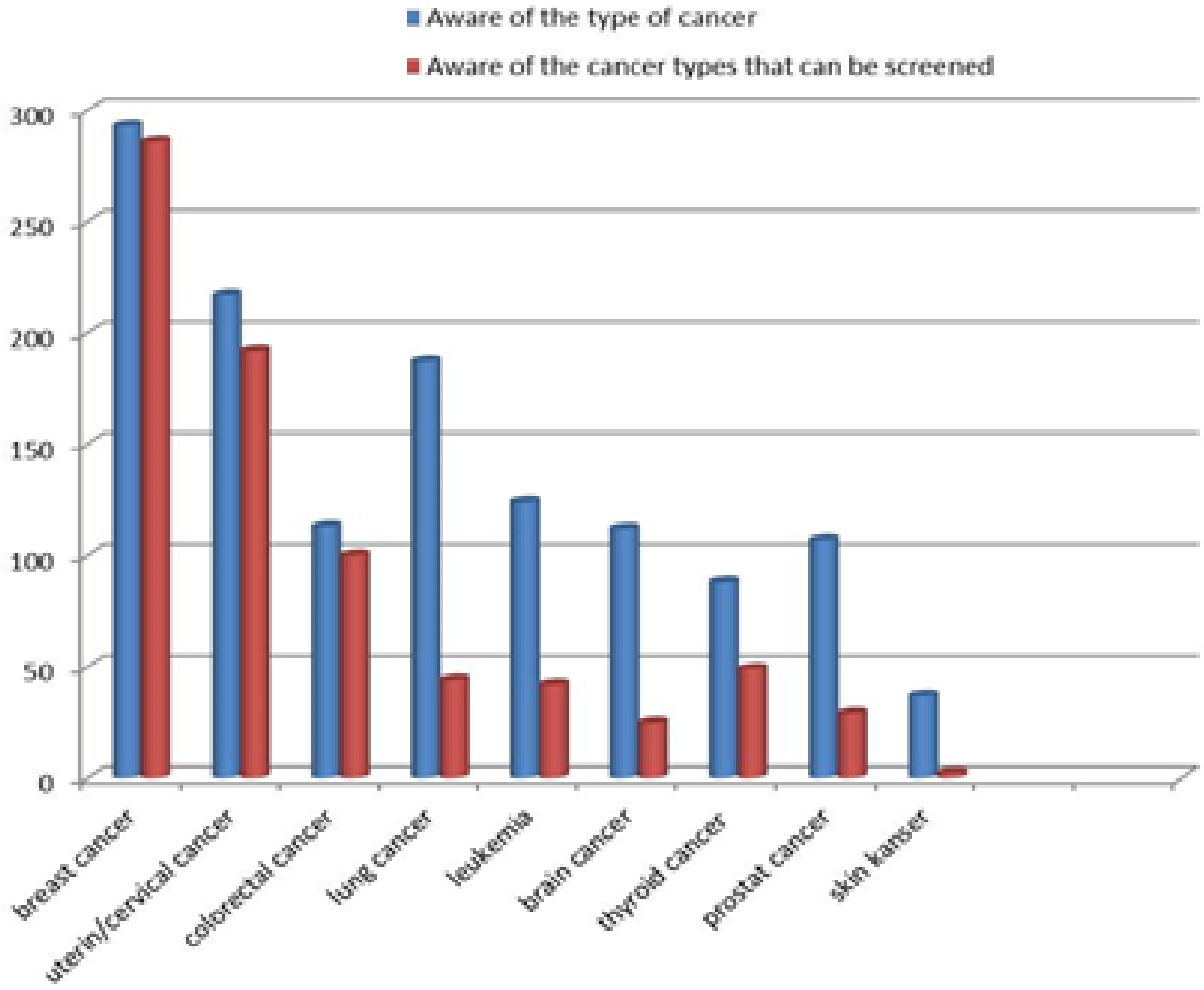


**Figure 1**. Awareness of cancer types and awareness of whether early detection is possible with screening are shown. Breast cancer was the most known type of cancer and uterine and cervical cancers followed this.

The knowledge of participant about which types of cancer could be diagnosed early with screening tests is summarized in Table 2. Breast cancer was in the first place in knowing the types of cancer that can be detected early by screening. Awareness of cancer screening methods is shown in Figure 2. Mammography was the most known cancer screening method.

**Table 2.** Awareness of cancer types that can be screened

| Cancer type that can be screened | Aware | | Not aware | |
|---|---|---|---|---|
| | Number | % | Number | % |
| **Breast cancer** | 286 | 88.3 | 38 | 11.7 |
| **Uterine and cervical cancer** | 192 | 59.3 | 132 | 40.7 |
| **Colorectal cancer** | 100 | 30,8 | 224 | 69,2 |
| **Lung cancer** | 44 | 13.6 | 280 | 86.4 |
| **Leukemia** | 42 | 13.0 | 282 | 87.0 |
| **Brain cancer** | 25 | 7.7 | 299 | 92.3 |
| **Thyroid cancer** | 49 | 15.1 | 275 | 84.9 |
| **Prostate cancer** | 29 | 9.0 | 295 | 91.0 |
| **Skin cancer** | 2 | 0.6 | 322 | 99.4 |

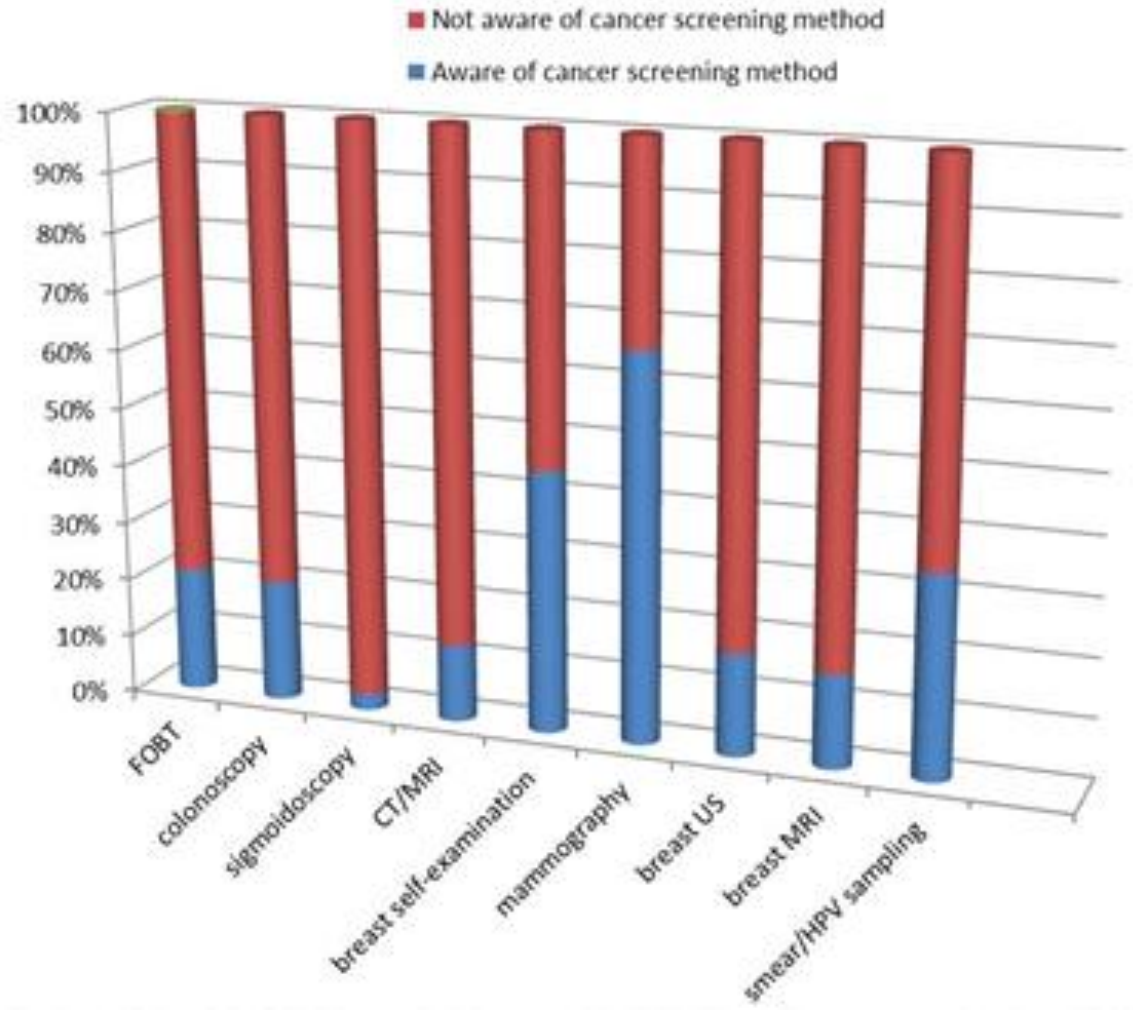


**Figure 2.** Awareness of cancer screening methods is shown. Mammography was the most known cancer screening method. This was followed by breast self-exam and HPV sampling.

When the symptoms of cancer disease were questioned, palpable mass was 78.7% (n = 255), unusual bleeding 37.7% (n = 122), weakness 37% (n = 120), constipation 29.3% (n = 95) and cough was given 21.3% (n = 69) as an answer. Only 2.5% (n=8) believed there was not any symptom suggesting cancer. Of the participants 32.4% (n = 105) stated that they had periodic cancer screening, 84% (n = 272) received a physician's advice on this issue, and 75.9% (n = 246) followed this recommendation. The answers of participants about cancer screening methods are listed in Table 3. The most known cancer screening method was mammography (66.0%).

**Table 3.** Awareness of cancer screening methods

| Cancer Screening method | Aware | | Not aware | |
|---|---|---|---|---|
| | Number | % | Number | % |
| **FOBT** | 69 | 21.3 | 255 | 78.7 |
| **Colonoscopy** | 67 | 20.7 | 257 | 79.3 |
| **Sigmoidoscopy** | 9 | 2.8 | 315 | 97.2 |
| **CT/MR** | 43 | 13.3 | 281 | 86.7 |
| **Breast Self-Examination** | 145 | 44.5 | 179 | 55.2 |
| **Mammography** | 214 | 66.0 | 110 | 34 |
| **Breast US** | 57 | 17.6 | 267 | 82.4 |
| **Breast MR** | 52 | 16.0 | 272 | 84 |
| **Smear / HPV sampling** | 112 | 34.6 | 212 | 65.4 |

FOBT:Fekal occult blood test, CT: Computed tomography, MR: Magnetic resonance, US: Ultrasonography, HPV: Human papilloma virus

Smoking 83.3% (n = 270), alcohol 59.9% (n = 194), fiber-free food 20.1% (n = 65), obesity 34% (n = 110), chemicals 36.7% (n = 119) were specified as cancer-causing factors. Of the participants, 13.6% (n = 44) stated that they smoke, 38.6% (n = 125) of them had a chronic disease, 41% (n = 133) of them stated that they regularly use some drugs.

Unfortunately, 18.2% (n = 59) thought that cancer screening does not improve health and quality of life, and 11.1% (n = 36) thought that they do not need screening because they were not at risk of cancer.

The vast majority of the participants (96.0%) (n = 311) believed that the life expectancy of cancer patients who were diagnosed and treated early will be prolonged and (99.1%) (n = 321) their quality of life is better than delayed diagnosed patients.

There was no statistically significant relationship between gender and their knowledge about cancer types and which types of cancer can be diagnosed early by screening ($p>0.05$). There was no statistically significant relationship between marital status and knowledge of cancer types and screening tests ($p>0.05$). A statistically significant relationship was found between having a cancer patient at an early age in the family and regular cancer screening ($p = 0.038$). There was no statistically significant relationship between gender, education level, marital status and getting regular cancer screening ($p>0.05$).

## DISCUSSION

In the fight against cancer, which is accepted as the disease of the age and is a global problem, the United Nations called on all countries to prepare and implement their own national cancer control programs in 2011 (4). With the effective implementation of well-structured screening programs that can reach the target audience, prevention and early diagnosis of cancers are aimed. Social beliefs, values and insufficient information are among the important factors affecting screening for early cancer detection. Better communication with healthcare professionals and facilitating access to healthcare services will support to increase cancer screening rates (5).

In a study conducted in cancer early diagnosis and screening center, 55.2% of the participants think that cancer is a fatal disease, and 80.9% are afraid of getting cancer. However, 61.3% of the participants do not do anything to prevent cancer, 81.4% have never had a cancer screening before. Scan rates are well below target (6). Contrary to our study, it was found that women were more likely to have information about cancer, concern about cancer, and take precautions and screening for cancer than men.

In a cervical cancer screening study conducted with 409 women, 34.2% of the participants had no idea about cervix cancer, and 96% of them never had screening. Of the participants, 70.8% did not get information about breast and cervix cancer in the last 1 year; 68.6% of them stated that cervix cancer could be a type of cancer that can be diagnosed early with screening. Awareness of breast and cervix cancer is low and there is a lack of supplementary information (7).

In addition to these low awareness rates, the health literacy levels of individuals should also be raised. Individuals with high self-risk perception, who know their health responsibilities, will be more willing to screen for cancer. In addition, it is necessary to increase the delivery capacities of cancer screening services in local health institutions. Just giving the information that cancer can be cured by early diagnosis motivates people to have screening.

In a study conducted with 500 female patients, 90% of the participants knew how to perform breast self-examination (BSE) and 35% applied it. 15% of the participant accept mammography, 19.8% accept clinical breast examination as a screening method. Of the participants 20.6% did not do BSE because they did not know how to do it. The most common obstacle to breast cancer screening is that patients were unaware of screening methods (8).

As a result of the lack of information about the scanning method, the target audience for scans cannot be reached. Social media is considered the main source of information. The rate of reaching healthcare professionals as a source of information is low. The importance of primary health care for

early diagnosis of cancer should be emphasized more intensely. Every patient, applying to the outpatient clinic for any reason, should be evaluated as an opportunity and informed about cancer screenings at regular intervals.

With a group of 600 people over 50 years old, a colorectal cancer screening study was conducted. Of the participants, 65% stated that they had information about colorectal cancer, 40% knew the fecal occult blood test (FOBT) as a screening method, and 95% did not receive medical advice on this issue. It was found that there was a lack of information about the risk factors of colorectal cancer and the importance of early diagnosis. Therefore participation in the screening program was limited. Most of the participants thought that the incidence of this cancer was very low, so that screening could be neglected (9). Good training opportunities should be provided for both physicians and patients, and proper doctor-patient communication should be established to increase awareness of this issue.

For healthcare workers to advise patients about cancer screening, their knowledge and awareness must be high. In a study conducted on this subject, it was found that half of the participants who were healthcare workers had insufficient knowledge about cancer screening methods (50.3% for Pap smear, 57.5% for mammography, 68.4% for colonoscopy, and 54.3% for fecal occult blood). Elderly healthcare workers, those with a family history of cancer, and those with more than 11 years of work experience applied cancer screening tests more frequently than others. Our study found that the rate of participation in cancer screening programs was statistically significantly higher in patients with a family history of cancer. In addition, it was found that there was no statistically significant difference in being a health worker in the case of having a cancer screening test or not (10).

In a study questioning common female cancers, the participants knew 60% cervix, 24% breast, 4% ovarian cancers, and 12% did not know. When the early symptoms of breast cancer are questioned, 66% answered breast mass. Similarly, in our study, the answer was a small palpable mass (78.6%). Based on this awareness, the importance of breast self-examination can be emphasized more clearly (11).

While the majority (60%) answered the question of the causes of cancer as having no idea, the most frequent answers in our study were smoking and alcohol intake (83.7%, 60.4%). The rate of those who did not have any idea about breast cancer screening methods was 72%. A group of 30% was not sure whether it could be early diagnosed by screening. These results show that there is an apparent lack of information. In addition, 40% of the participants stated that they did not receive any doctor's advice about cancer screening. In our study, the rate of those who received physician advice was 84.4% (11).

Participation in breast cancer screening programs and BSE rates were higher among those with a high level of education (12). In another study, the frequency of breast self-examination was higher in women with a family history of breast cancer (13). Family physicians working especially in low socioeconomic regions and lower education levels have to tell the importance of early breast cancer diagnosis and adequately explain breast self-examination and other screening tools.

In the United States, cities, where colorectal cancer screening programs were provided by family physicians and not provided, are compared. The rate of awareness and application was higher in the group receiving service on this subject by family physicians (14).

Cancer screening services are one of the leading service areas in family medicine in our country. Family physicians take the necessary care to carry out our national screening program. In our study, a significant relationship was found between receiving a doctor's advice and having screening, but its effect on regular screening was not found to be statistically significant. To ensure the continuity of cancer screening services, physicians should advise their patients at regular intervals.

In a study conducted with people over the age of 50, it was found that patients preferred to focus on primary prevention methods such as developing a healthy lifestyle rather than screening to prevent colorectal cancer (15). It was found that those who knew that colorectal cancer is one of the most common types of cancer were screened more (16).

The sources of motivation for cancer screening are symptoms, fear of getting cancer, feeling obliged to be healthy for the family, and obtaining a doctor's advice. The barriers to screening are the absence of symptoms, uncomfortable screening test procedures, lack of information, low perceived risk of cancer, insecurity in healthcare providers, fear of being diagnosed with cancer, and embarrassment. Increasing motivation and increasing barriers will only be possible with reaching sufficient awareness and knowledge level. The sense of health promotion leads to the need to take responsibility for one's health. People who have adequate information about scans are aware of their responsibilities and volunteer for scans.

This study has some limitations. It was performed on a limited number of participants who applied to a cancer screening center. Nevertheless, their level of knowledge was found to be relatively low. If this study was done in a rural area, their level of knowledge and awareness would probably be lower. Another limitation was that the study was conducted by focusing only on knowledge levels about common types of cancer. If other rare types

of cancer were included, the knowledge level would probably be much lower. A study involving a more comprehensive patient series and knowledge levels of rare cancer types may be the subject of future research.

In conclusion, the level of knowledge and awareness about cancer types, screening methods and screening programs were found to be quite low in our study. In order for the national cancer control programs to be implemented properly, the public's level of knowledge and cancer awareness should be increased. The role of family physicians, who are the first medical contact point of the individual with the health system, informing and encouraging patients about cancer early diagnosis and screening programs in primary care is very valuable. Identifying target populations and implementing screening methods that can be done in family health centers is a very effective and practical step to increase screening rates. Large-scale studies should be conducted to learn the importance of early diagnosis by society and make it an individual responsibility.

**Conflict of Interest:** Authors declared no conflict of interest.

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RESEARCH ARTICLE

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# Impact of Rational Laboratory Practice on Hospital Procedure Costs Based on Evidence-Based Medicine: Case Study in a University Hospital

**ABSTRACT**

**Objective:** By integrating the rational laboratory system into hospital processes with evidence-based medicine applications, it is ensured that hospital resources are used more effectively and efficiently by preventing unnecessary test requests and reducing laboratory operation costs.

**Methods:** The data in this qualitative study are the primary data and were obtained through document review and focus group (physician) interviews. The data in question were analyzed comparatively before the Rational Laboratory Application (RLA) (between 01.06.2018-31.12.2018) and after the RLA (between 01.06.2019-31.12.2019). The universe of the study consists of all the data in the laboratory of Düzce University Health Application and Research Center Hospital (DUHARCH). In this universe, the data evaluated for rational laboratory application constitute the sample of the research.

**Results:** Before the RLA, a total of 446,300 test requests were made in the seven months (in 2018) and the cost of these tests was determined to be 1,591,063 ₺ ($ 330,782.33). After the RLA, a total of 475,585 test requests were made in the seven months (in 2019), and the cost of these tests was determined to be 1,537,903 ₺ ($ 271,235.10). It was found that after RLA, there was a 6.56% increase in the number of examination requests compared to before RLA, but as an amount, costs fell by 3.34% on a ₺ basis and 22% on a $ basis. In the unit-based analysis, it was seen that successful units were surgical, and unsuccessful units were internal units that requested more tests.

**Conclusions:** It was concluded that the rational laboratory system based on evidence-based medicine reduces hospital processing costs, provided that patient safety is protected, so this method can be a tool for more effective and efficient use of hospital resources.

**Keywords:** Evidence-Based Medicine, Rational Laboratory Application, Hospital Costs, Cost Management.

# Kanıta Dayalı Tıp Ekseninde Akılcı Laboratuvar Uygulamasının Hastane İşlem Maliyetleri Üzerindeki Etkisi: Bir Üniversite Hastanesinde Vaka Çalışması

**ÖZET**

**Amaç:** Kanıta dayalı tıp uygulamalarıyla akılcı laboratuvar sistemini hastane süreçlerine entegre ederek gereksiz tetkik istemlerini engelleyip laboratuvar işlem maliyetlerini düşürerek hastane kaynaklarının daha etkin ve verimli kullanılmasını sağlamaktır.

**Gereç ve Yöntem:** Nitel bir araştırma olan bu çalışmadaki veriler birincil veri niteliğini taşımakta olup doküman incelemesi ve odak grup (hekim) görüşmeleriyle elde edilmiştir. Söz konusu veriler, Akılcı Laboratuvar Uygulaması (ALU) öncesi (01.06.2018-31.12.2018 tarihleri arası) ve ALU sonrası (01.06.2019-31.12.2019 tarihleri arası) karşılaştırmalı olarak analiz edilmiştir. Çalışmanın evreni Düzce Üniversitesi Sağlık Uygulama ve Araştırma Merkezi (Hastanesi)'nin laboratuvardaki tüm verilerden oluşurken bu evren içerisinde akılcı laboratuvar uygulaması için değerlendirilen veriler ise araştırmanın örneklemini oluşturmaktadır.

**Bulgular:** ALU öncesi 2018/7 aylık dönemde toplam 446.300 adet tetkik istemi yapılmış ve bu tetkiklerin maliyeti 1.591.063 ₺ ($ 330,782.33) olarak tespit edilmiştir. ALU sonrası 2019/7 aylık dönemde ise toplam 475.585 adet tetkik istemi yapılmış ve bu tetkiklerin maliyeti 1.537.903 ₺ ($ 271,235.10) olarak tespit edilmiştir. ALU sonrası, ALU öncesine göre tetkik istem sayılarında %6.56 artışın olduğu, fakat tutar olarak ise ₺ bazında %3.34, $ bazında %22 oranında maliyetlerin düştüğü tespit edilmiştir. Birim bazında yapılan analizde başarılı birimlerin cerrahi, başarısız birimlerin ise daha çok tetkik isteminde bulunan dâhili birimlerin olduğu görülmüştür.

**Sonuç:** Hasta güvenliğini korumak şartıyla kanıta dayalı tıp ekseninde uygulanan akılcı laboratuvar sisteminin hastane işlem maliyetlerini azalttığı bu nedenle bu yöntemin hastane kaynaklarının daha etkin ve verimli kullanılabilmesinde bir araç olabileceği sonucuna varılmıştır.

**Anahtar Kelimeler:** Kanıta Dayalı Tıp, Akılcı Laboratuvar Uygulaması, Hastane Maliyetleri, Maliyet Yönetimi.

## INTRODUCTION

Rational use applications, which have become increasingly important especially in recent years, have become a system that encourages the presence of more logical, reliable, reasonable, and conscientious medical systems. Rational laboratory use practice based on evidence aims to reduce the high health expenses by preventing unnecessary test requests. In this respect, rational laboratory use means the rational use of laboratory services to provide an accurate diagnosis, to prevent unnecessary tests, and to reduce costs (1).

When a physician requests any test, he or she must evaluate the suitability, effectiveness, reliability, and cost of the test in advance. If the test is necessary, it is useful to show scientific evidence and to demonstrate the necessity in the light of this scientific evidence (2).

Evidence-based medicine is quickly making clinical application more scientifically and empirically based to provide practical evidence depended on the best evidence research summaries. Evidence-based medicine thus involves building implementation strategies that are committed to providing safer, more consistent, and less costly care (3-4).

Rational laboratory use can be defined as effective and correct laboratory use by making the most accurate test request in the light of correct clinical questions and evidence-based information, taking into account patient-employee safety and cost-effectiveness (5).

In general, non-rational drug, laboratory, radiology, pathology, biochemistry, tissue typing, and microbiology laboratories applications, and rational billing procedures are among the most fundamental problems of health organizations. Such problems reach greater dimensions especially in developing countries and underdeveloped countries. Rational applications have started to show their effectiveness gradually to eliminate these problems, which are a huge financial burden for health organizations.

To increase the clinical usefulness of test results in our country, to ensure the correct diagnosis of the patient, to prevent unnecessary test requests, to ensure that test requests can be maintained cost-effectively, the "Rational Laboratory Use Project", which covers medical microbiology, medical biochemistry, tissue typing, and medical pathology laboratories were first handled by Ministry of Health, General Directorate of Health Services (Inspection and Diagnostic Services Department) in 2016 (6-7-8).

Unnecessary requests, unnecessary intervention, unnecessary tests, and unnecessary treatments are the most important factors that threaten the health of patients in healthcare and cause an increase in care costs. In this respect, the necessity of rational use is needed (9).

The main purpose of this study is to integrate the rational laboratory system into the hospital processes, to protect patient safety and to prevent unnecessary test requests, to reduce procedures and costs by developing rational strategies, and to ensure effective and efficient management of health expenditures.

For this purpose, the transition process to the application related to the recommendations of the Ministry of Health has been started at the (DUHARCH). The effect of this process on hospital costs was analyzed following the problem and purpose of this study.

## MATERIAL AND METHODS

To start RLA, it was decided to establish a commission consisting of representatives of microbiology, medical biochemistry, infection industry experts, information processing, and hospital administration by DUHARCH. In the studies carried out by the commission, within the scope of the Rational Laboratory Use Project, the use of microbiology and medical biochemistry tests in outpatient applications was determined as the first step. At the same time, departments were asked by the commission to make suggestions for the RLA system. By the scientific evidence-based suggestions from the departments, it was decided to make arrangements in the tests that are not within the scope of rational laboratories. As a result, it was put into practice with the decision to be implemented and to observe the process in March 2019.

In March 2019, it was decided that some changes should be made to the Hospital Information Management System (HIMS) by The Rational Laboratory Commission to start implementation. To initiate the application under its purpose, software improvements were made in HIMS for obstructive restrictions such as test repeat constraint, branch constraint, day constraint, request number constraint, and not re-requesting the same test without the result of the previous request, and the arrangement made was announced to all hospital staff.

In this context, a change would be revealed before and after RLA by analyzing the data available in the hospital database for test requests in-hospital processes.

This research, which aims to adopt a cost-effective approach to practice by avoiding unnecessary test requests in the process of integrating a rational laboratory system into hospital processes, is a descriptive type of research with a screening model. The reason why this research is with a screening model is that the data is not reproduced by the researcher as in the trial model. The data from the study was obtained from the database and attended meetings like the screening model.

DUHARCH's entire database and healthcare professionals constitute the general universe of the research. The study universe of the study is composed of the rational laboratory data available in the DUHARCH database and the healthcare professionals involved in this study. As a result of the information provided, the universe of this study was determined in one category. This is formed according to the document analysis technique, which is the method of collecting research data. Accordingly, DUHARCH's database formed the universe of work on document analysis techniques. In this universe, the data evaluated for rational laboratory application constitute the sample of the research.

## RESULTS

The evidence-based medicine pyramid levels of the studies related to the analyzed tests were determined as follows (Table 1), taking into account the codes in the Health Implementation Communique (HIC).

**Table 1.** Evidence-based medicine pyramid levels of studies related to the tests analyzed

| Evidence-Based Medicine Pyramid Levels of Studies Related to Analyzed Investigations | | | | | | | |
|---|---|---|---|---|---|---|---|
| **R.Nu** | **Assay Name** | **HIC Code** | **Request Unit Constraint (On)** | **Request Unit Constraint (Closed)** | **Scientific Evidence Level** | **Source** | **RLA Test Request Time (Days)** |
| 1 | 25-Hydroxy Vitamin D | 900130 | Child Health and Diseases, Physical Medicine and Rehabilitation, Pediatric Endocrinology, Orthopedics and Traumatology, Neonatology, Pediatric Nephrology, Pediatric Surgery, Gynecology and Obstetrics, Pediatric Cardiology, Nephrology, Child and Adolescent Mental Health and Diseases, Pediatric Endocrinology, Neurology, Internal Medicine | Forensic Medicine, Family Medicine, Anesthesiology and Reanimation, Brain and Nerve Surgery, Skin and Venereal Diseases, Endocrinology and Metabolism Diseases, Infectious Diseases, Gastroenterology, General Surgery, Thoracic Surgery, Chest Diseases, Ophthalmology, Hematology, Hemodialysis, Cardiovascular Surgery , Cardiology, Ear-Nose-Throat Diseases, Mental Health and Diseases. | Article: Retrospective analysis KP: Case control studies Level 3 | (10) | 30 |
| 2 | Folate (Serum / Plasma) | 901240 | Child Health and Diseases, Physical Medicine and Rehabilitation, Pediatric Endocrinology and Metabolic Diseases, Anesthesiology and Reanimation, Mental Health and Diseases, Medical Oncology, Orthopedics and Traumatology, Neonatology, Pediatric Nephrology, Pediatric Surgery, Gynecology and Obstetrics, Pediatric Cardiology, Gastroenterology, , Nephrology, Child and Adolescent Mental Health and Diseases, Endocrinology and Metabolic Diseases, Dermatology and Venereal Diseases, Hematology, Hemodialysis, Neurology, Internal Medicine. | Forensic Medicine, Family Medicine, Brain and Nerve Surgery, Infectious Diseases, General Surgery, Thoracic Surgery, Chest Diseases, Eye Diseases, Cardiovascular Surgery, Cardiology, Ear-Nose-Throat Diseases, Urology. | Article: Retrospective analysis KP: Case control studies Level 3 | (11-12-13-14) | 42 |
| 3 | PSA (Prostate specific antigen) | 903220 | Anesthesiology and Reanimation, Medical Oncology, Family Medicine, Gastroenterology, Nephrology, Endocrinology and Metabolic Diseases, Dermatology and Venereal Diseases, Urology, Internal Medicine | Brain and Nerve Surgery, Pediatric Surgery, Pediatric Endocrinology, Pediatric Cardiology, Pediatric Nephrology, Infectious Diseases, Physical Medicine and Rehabilitation, General Surgery, Thoracic Surgery, Chest Diseases, Ophthalmology, Hematology, Hemodialysis, Gynecology and Obstetrics, Cardiovascular Surgery , Cardiology, Ear-Nose-Throat Diseases, Neonatology, Neurology, Orthopedics and Traumatology, Mental Health and Diseases. | Article: Retrospective analysis KP: Case control studies Level 3 | (15) | 28 |
| 4 | Free T3 | 903470 | Child Health and Diseases, Pediatric Endocrinology, Medical Oncology, Neonatology, Pediatric Nephrology, Pediatric Surgery, Gynecology and Obstetrics, Family Medicine, Pediatric Cardiology, Gastroenterology, General Surgery, Nephrology, Pediatric and Adolescent Mental Health and Diseases, Forensic Medicine, Pediatric Endocrinology , Endocrinology and Metabolic Diseases, Hematology, Hemodialysis, Neurology, Internal Medicine. | Anesthesiology and Reanimation, Brain and Nerve Surgery, Skin and Venereal Diseases, Infectious Diseases, Physical Medicine and Rehabilitation, Thoracic Surgery, Chest Diseases, Eye Diseases, Cardiovascular Surgery, Cardiology, Ear-Nose-Throat Diseases, Orthopedics and Traumatology, Mental Health and Diseases, Urology. | Article: Retrospective analysis KP: Case control studies Level 3 | (16-17-18-19) | 13 |
| 5 | Free T4 | 903480 | Child Health and Diseases, Medical Oncology, Neonatology, Pediatric Nephrology, Pediatric Surgery, Gynecology and Obstetrics, Family Medicine, Pediatric Cardiology, Gastroenterology, General Surgery, Nephrology, Child and Adolescent Mental Health and Diseases, Forensic Medicine, Pediatric Endocrinology, Endocrinology and Metabolic Diseases, Hemodialysis, Neurology, Internal Medicine. | Anesthesiology and Reanimation, Brain and Nerve Surgery, Skin and Venereal Diseases, Infectious Diseases, Physical Medicine and Rehabilitation, Thoracic Surgery, Chest Diseases, Eye Diseases, Cardiovascular Surgery, Cardiology, Ear-Nose-Throat Diseases, Orthopedics and Traumatology, Mental Health and Diseases, Urology, | 1.2.3.4. Article: Retrospective analysis KP: Case control studies Level 3 | (16-17-18-19) | 13 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 6 | Vitamin B12 | 904150 | Child Health and Diseases, Physical Medicine and Rehabilitation, Pediatric Endocrinology and Metabolic Diseases, Anesthesiology and Reanimation, Mental Health and Diseases, Medical Oncology, Orthopedics and Traumatology, Neonatology, Pediatric Nephrology, Pediatric Surgery, Gynecology and Obstetrics, Pediatric Cardiology, Gastroenterology, Nephrology, Child and Adolescent Mental Health and Diseases, Child Endocrinology, Endocrinology and Metabolic Diseases, Dermatology and Venereal Diseases, Hematology, Hemodialysis, Neurology, Internal Medicine. | Forensic Medicine, Family Medicine, Brain and Nerve Surgery, Infectious Diseases, General Surgery, Thoracic Surgery, Chest Diseases, Eye Diseases, Cardiovascular Surgery, Cardiology, Ear-Nose-Throat Diseases, Urology. | Article 1: Retrospective analysis 1.KP: Case control studies Level 3 | (14-20) | 30 |
| 7 | Anti CMV IgG (Microparticle immune assay-MEIA or similar) | 906360 | Child Health and Diseases, Pediatric Endocrinology and Metabolic Diseases, Neonatology, Pediatric Nephrology, Pediatric Surgery, Pediatric Cardiology, Child and Adolescent Mental Health and Diseases, Pediatric Endocrinology, Infectious Diseases, Internal Medicine | Forensic Medicine, Family Medicine, Anesthesiology and Reanimation, Brain and Nerve Surgery, Skin and Venereal Diseases, Endocrinology and Metabolic Diseases, Physical Medicine and Rehabilitation, Gastroenterology, General Surgery, Thoracic Surgery, Chest Diseases, Ophthalmology, Hematology, Hemodialysis, Gynecology and Obstetrics, Cardiovascular Surgery, Cardiology, Ear-Nose-Throat Diseases, Nephrology, Neurology, Orthopedics and Traumatology, Mental Health and Diseases, Medical Oncology, Urology. | Article: Retrospective analysis KP: Case control studies KP: Systematic Review (23) Level 1, Level 3 | (21-22-23-24-25) | On the same day the test is not repeated. |
| 8 | Anti CMV IgM (Microparticle immune assay-MEIA or similar) | 906370 | Child Health and Diseases, Pediatric Endocrinology and Metabolic Diseases, Neonatology, Pediatric Nephrology, Pediatric Surgery, Pediatric Cardiology, Gastroenterology, Pediatric Endocrinology, Infectious Diseases, Internal Medicine. | Forensic Medicine, Family Medicine, Anesthesiology and Reanimation, Brain and Nerve Surgery, Child and Adolescent Mental Health, Dermatology and Venereal Diseases, Endocrinology and Metabolism Diseases, Physical Medicine and Rehabilitation, General Surgery, Thoracic Surgery, Chest Diseases, Eye Diseases, Hematology, Hemodialysis, Gynecology and Obstetrics, Cardiovascular Surgery, Cardiology, Ear-Nose-Throat Diseases, Nephrology, Neurology, Orthopedics and Traumatology, Mental Health and Diseases, Medical Oncology, Medical Urology. | Article: Retrospective analysis KP: Case control studies KP: Systematic Review (23) Level 1, Level 3 | (21-22-23-24-25) | On the same day the test is not repeated. |
| 9 | Anti HAV IgG (Microparticle immune assay-MEIA or similar) | 906510 | Pediatric Health and Diseases, Pediatric Endocrinology and Metabolic Diseases, Anesthesiology and Reanimation, Neonatology, Pediatric Nephrology, Pediatric Surgery, Gastroenterology, Pediatric Cardiology, Nephrology, Pediatric Endocrinology, Dermatology and Venereal Diseases, Hematology, Infectious Diseases and Clinical Microbiology, Internal Medicine | Forensic Medicine, Family Medicine, Brain and Nerve Surgery, Child and Adolescent Mental Health, Endocrinology and Metabolic Diseases, Physical Medicine and Rehabilitation, General Surgery, Thoracic Surgery, Chest Diseases, Eye Diseases, Hemodialysis, Gynecology and Obstetrics, Cardiovascular Surgery , Cardiology, Ear-Nose-Throat Diseases, Neurology, Orthopedics and Traumatology, Mental Health and Diseases, Medical Oncology, Urology. | Article: Retrospective analysis KP: Case control studies KP: Systematic Review (23) Level 1, Level 3 | (21-22-23-24-25) | On the same day the test is not repeated |
| 10 | Anti HAV IgM (Microparticle immune assay-MEIA or similar) | 906530 | Pediatric Health and Diseases, Pediatric Endocrinology and Metabolic Diseases, Anesthesiology and Reanimation, Neonatology, Pediatric Nephrology, Pediatric Surgery, Pediatric Cardiology, Gastroenterology, Nephrology, Pediatric Endocrinology, Dermatology and Venereal Diseases, Hematology, Infectious Diseases and Clinical Microbiology, Internal Medicine | Forensic Medicine, Family Medicine, Brain and Nerve Surgery, nChild and Adolescent Mental Health, Endocrinology and Metabolic Diseases, Physical Medicine and Rehabilitation, General Surgery, Thoracic Surgery, Chest Diseases, Eye Diseases, Hemodialysis, Gynecology and Obstetrics, Cardiovascular Surgery , Cardiology, Ear-Nose-Throat Diseases, Neurology, Orthopedics and Traumatology, Mental Health and Diseases, Medical Oncology, Urology. | Article: Retrospective analysis KP: Case control studies KP: Systematic Review (23) Level 1, Level 3 | (21-22-23-24-25) | On the same day the test is not repeated. |
| 11 | Anti Hbc IgG (Microparticle immune assay-MEIA or similar) | 906560 | Pediatric Health and Diseases, Pediatric Endocrinology and Metabolic Diseases, Anesthesiology and Reanimation, Medical Oncology, Neonatology, Pediatric Nephrology, Pediatric Surgery, Pediatric Cardiology, Gastroenterology, Nephrology, Child and Adolescent Mental Health and Diseases, Pediatric Endocrinology, Pediatric, Endocrinology and Metabolism Diseases, Skin and Venereal Diseases, Hematology, Infectious Diseases and Clinical Microbiology, Internal Medicine. | Forensic Medicine, Family Medicine, Brain and Nerve Surgery, Physical Medicine and Rehabilitation, General Surgery, Thoracic Surgery, Chest Diseases, Eye Diseases, Hemodialysis, Gynecology and Obstetrics, Cardiovascular Surgery, Cardiology, Ear-Nose-Throat Diseases, Neurology Orthopedics and Traumatology, Mental Health and Diseases, Urology. | Article: Retrospective analysis KP: Case control studies KP: Systematic Review (23) Level 1, Level 3 | (21-22-23-24-25) | 365 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 12 | Anti HBc IgM (Microparticle immune assay-MEIA or similar) | 906580 | Pediatric Health and Diseases, Pediatric Endocrinology and Metabolic Diseases, Anesthesiology and Reanimation, Medical Oncology, Neonatology, Pediatric Nephrology, Pediatric Surgery, Pediatric Cardiology, Gastroenterology, Nephrology, Pediatric Endocrinology, Endocrinology and Metabolic Diseases, Dermatology and Venereal Diseases, Hematology and Clinical Microbiology, Internal Medicine. | Forensic Medicine, Family Medicine, Brain and Nerve Surgery, Child and Adolescent Mental Health, Physical Medicine and Rehabilitation, General Surgery, Thoracic Surgery, Chest Diseases, Eye Diseases, Hemodialysis, Gynecology and Obstetrics, Cardiovascular Surgery, Cardiology, Ear-Nose and Throat Diseases, Neurology, Orthopedics and Traumatology, Mental Health and Diseases, Urology, | Article: Retrospective analysis KP: Case control studies KP: Systematic Review (23) Level 1, Level 3 | (21-22-23-24-25) | 365 |
| 13 | Anti HBe (Microparticle immune assay-MEIA or similar) | 906600 | Child Health and Diseases, Pediatric Endocrinology and Metabolic Diseases, Anesthesiology and Reanimation, Neonatology, Pediatric Nephrology, Pediatric Surgery, Pediatric Cardiology, Gastroenterology, Nephrology, Pediatric Endocrinology, Endocrinology and Metabolic Diseases, Hematology, Infectious Diseases and Clinical Microbiology | Forensic Medicine, Family Medicine, Brain and Nerve Surgery, Child and Adolescent Mental Health, Skin and Venereal Diseases, Physical Medicine and Rehabilitation, General Surgery, Thoracic Surgery, Chest Diseases, Ophthalmology, Hemodialysis, Gynecology and Obstetrics, Cardiovascular Surgery , Cardiology, Ear-Nose-Throat Diseases, Neurology, Orthopedics and Traumatology, Mental Health and Diseases, Medical Oncology, Urology. | Article: Retrospective analysis KP: Case control studies KP: Systematic Review (23) Level 1, Level 3 | (21-22-23-24-25) | 365 |
| 14 | Anti rubella IgG (Chemiluminescence or similar) | 906820 | Pediatric Health and Diseases, Pediatric Endocrinology and Metabolic Diseases, Neonatology, Pediatric Nephrology, Pediatric Surgery, Gastroenterology, Pediatric Cardiology, Pediatric Endocrinology, Infectious Diseases and Clinical Microbiology, Internal Medicine. | Forensic Medicine, Family Medicine, Anesthesiology and Reanimation, Brain and Nerve Surgery, Child and Adolescent Mental Health, Dermatology and Venereal Diseases, Endocrinology and Metabolism Diseases, Physical Medicine and Rehabilitation, General Surgery, Thoracic Surgery, Chest Diseases, Eye Diseases, Hematology, Hemodialysis, Gynecology and Obstetrics, Cardiovascular Surgery, Cardiology, Ear-Nose-Throat Diseases, Nephrology, Neurology, Orthopedics and Traumatology, Mental Health and Diseases, Medical Oncology, Urology. | Article: Retrospective analysis KP: Case control studies KP: Systematic Review (23) Level 1, Level 3 | (21-22-23-24-25) | On the same day the test is not repeated |
| 15 | Anti rubella IgM (Chemiluminescence or similar) | 906840 | Pediatric Health and Diseases, Pediatric Endocrinology and Metabolic Diseases, Neonatology, Pediatric Nephrology, Pediatric Surgery, Pediatric Cardiology, Gastroenterology, Pediatric Endocrinology, Infectious Diseases and Clinical Microbiology, Internal Medicine. | Forensic Medicine, Family Medicine, Anesthesiology and Reanimation, Brain and Nerve Surgery, Child and Adolescent Mental Health, Dermatology and Venereal Diseases, Endocrinology and Metabolism Diseases, Physical Medicine and Rehabilitation, Hematology, Hemodialysis, Gynecology and Obstetrics, Cardiovascular Surgery, Cardiology, Ear-Nose-Throat Diseases, Nephrology, Neurology, Orthopedics and Traumatology, Mental Health and Diseases, Medical Oncology, Urology. | Article: Retrospective analysis KP: Case control studies KP: Systematic Review (23) Level 1, Level 3 | (21-22-23-24-25) | On the same day the test is not repeated |
| 16 | Anti toxoplasma IgG (Chemiluminescence or similar) | 906910 | Pediatric Health and Diseases, Pediatric Endocrinology and Metabolic Diseases, Neonatology, Pediatric Nephrology, Pediatric Surgery, Pediatric Cardiology, Gastroenterology, Pediatric Endocrinology, Infectious Diseases and Clinical Microbiology, Internal Medicine. | Forensic Medicine, Family Medicine, Anesthesiology and Reanimation, Brain and Nerve Surgery, Child and Adolescent Mental Health, Dermatology and Venereal Diseases, Endocrinology and Metabolism Diseases, Physical Medicine and Rehabilitation, General Surgery, Thoracic Surgery, Chest Diseases, Eye Diseases,, Hematology Hemodialysis, Gynecology and Obstetrics, Cardiovascular Surgery, Cardiology, Ear-Nose-Throat Diseases, Nephrology, Neurology, Orthopedics and Traumatology, Mental Health and Diseases, Medical Oncology, Urology. | Article: Retrospective analysis KP: Case control studies KP: Systematic Review (23) Level 1, Level 3 | (21-22-23-24-25) | On the same day the test is not repeated. |
| 17 | Anti toxoplasma IgM (Chemiluminescence or similar) | 906930 | Child Health and Diseases, Neonatology, Pediatric Nephrology, Gastroenterology, Infectious Diseases and Clinical Microbiology. | Forensic Medicine, Family Medicine, Anesthesiology and Reanimation, Brain and Nerve Surgery, Pediatric Surgery, Pediatric Endocrinology, Pediatric Endocrinology, Pediatric Cardiology, Child and Adolescent Mental Health, Dermatology and Venereal Diseases, Endocrinology and Metabolic Diseases, Physical Medicine and Rehabilitation, General Surgery , Thoracic Surgery, Chest Diseases, Eye Diseases, Hematology, Hemodialysis, Internal Diseases, Gynecology and Obstetrics, Cardiovascular Surgery, Cardiology, Ear-Nose-Throat Diseases, Nephrology, Neurology, Orthopedics and Traumatology, Mental Health and Diseases, Medical Oncology, Urology. | Article: Retrospective analysis KP: Case control studies KP: Systematic Review (23) Level 1, Level 3 | (21-22-23-24-25) | On the same day the test is not repeated |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 18 | HBeAg Qual (Chemoluminescence or similar) | 907420 | Pediatric Health and Diseases, Pediatric Endocrinology and Metabolic Diseases, Anesthesiology and Reanimation, Neonatology, Pediatric Nephrology, Pediatric Surgery, Pediatric Cardiology, Gastroenterology, Nephrology, Pediatric Endocrinology, Endocrinology and Metabolic Diseases, Hematology, Hemodialysis, Infectious Diseases and Clinical Microbiology . | Forensic Medicine, Family Medicine, Brain and Nerve Surgery, Child and Adolescent Mental Health, Skin and Venereal Diseases, Physical Medicine and Rehabilitation, General Surgery, Thoracic Surgery, Chest Diseases, Eye Diseases, Gynecology and Obstetrics, Cardiovascular Surgery, Cardiology , Ear-Nose-Throat Diseases, Neurology, Orthopedics and Traumatology, Mental Health and Diseases, Medical Oncology, Urology. | Article: Retrospective analysis KP: Case control studies KP: Systematic Review (23) Level 1, Level 3 | (21-22-23-24-25) | 365 |

Evidence-based levels (Table 1) were determined for 18 tests, which are the most demand and cost included in the Ministry of Health's rational laboratory test request procedure. Thus, a rational approach was developed for the units that resist the branch constraint. In other words, the scientific basis of the restriction was supported by the most up-to-date scientific studies for the interlocutors. Thus, it was shown that the objectors are contrary to current scientific literature. It was observed that those who supported their objections with evidence-based medical articles removed the restriction in the process. Therefore, the process is liberated from the naturalistic structure. At the end of the dynamic and interactive process, cost analyzes were made for 18 tests. It was observed that both the cost and the number of test requests of the 18 analysis of which evidence levels were determined decreased (Table 3).

At the unit-based findings stage of the RLA's created as a result of studies on evidence-based medicine, analyzes (Table 2) for each polyclinic were made as follows.

**Table 2.** Pre and post-RLA change rates for units

| **Outpatient Application** | **Before RLA (01.06.2018 - 31.12.2018)** | | | **After RLA (01.06.2019 - 31.12.2019)** | | | **(a-d)/a *100** | **(b-e)/b *100** | **f-c** | **((a/b)* 100) - ((d/e) *100)** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Units** | **(a) Application Numb. (Piece)** | **(b) RLA Number of Assays (Piece)** | **(c) RLA Assay Amount (₺)** | **(d) Application Numb. (Piece)** | **(e) RLA Number of Assays (Piece)** | **(f) RLA Assay Amount (₺)** | **Application Number of Change Rate (%)** | **RLA Number of Test Change Rate (%)** | **RLA Assay Amount Difference (₺)** | **Applic. Average Analysis Request.Differ.** |
| Forensic Medicine | 45 | 140 | 789 | 58 | 163 | 821 | 28,89 | 16,43 | 33 | 3,44 |
| Family Medicine | 4.047 | 34.883 | 126.822 | 4.563 | 32.220 | 74.396 | 12,75 | -7,63 | -52.426 | 2,56 |
| Anesthesiology and Reanimation | 121 | 474 | 1.640 | 164 | 590 | 2.039 | 35,54 | 24,47 | 399 | 2,27 |
| Brain and Nerve Surgery | 370 | 3.050 | 11.198 | 342 | 1.943 | 5.880 | -7,57 | -36,30 | -5.317 | 5,47 |
| Pediatric Surgery | 105 | 252 | 898 | 92 | 274 | 910 | -12,38 | 8,73 | 12 | -8,09 |
| Children's Endocrinology | 2.768 | 15.012 | 58.548 | 3.059 | 18.405 | 76.547 | 10,51 | 22,60 | 17.999 | -1,82 |
| Child Nephrology | 0 | 0 | 0 | 2.113 | 12.050 | 29.910 | - | - | 29.910 | 0,00 |
| Child Neurology | 0 | 0 | 0 | 172 | 1.235 | 6.383 | - | - | 6.383 | 0,00 |
| Child Health and Diseases | 7.148 | 41.188 | 147.032 | 4.330 | 26.437 | 96.743 | -39,42 | -35,81 | -50.289 | -0,98 |
| Child and Adolescent Mental Health and Diseases | 537 | 4.518 | 15.708 | 598 | 5.873 | 20.958 | 11,36 | 29,99 | 5.250 | -1,70 |
| Skin and Venereal Diseases | 1.643 | 8.613 | 27.782 | 2.295 | 13.011 | 43.929 | 39,68 | 51,06 | 16.147 | -1,44 |
| Endocrinology and Metabolic Diseases | 0 | 0 | 0 | 1 | 12 | 28 | - | - | 28 | 0,00 |
| Infectious Diseases and Clinical Microbiology | 2.545 | 16.191 | 76.396 | 3.200 | 18.140 | 74.148 | 25,74 | 12,04 | -2.248 | 1,92 |
| Physical Medicine and Rehabilitation | 1.992 | 15.279 | 66.474 | 3.715 | 29.945 | 109.767 | 86,50 | 95,99 | 43.292 | -0,63 |
| Gastroenterology | 1.820 | 14.056 | 49.699 | 1.386 | 9.761 | 34.475 | -23,85 | -30,56 | -15.224 | 1,25 |
| General Surgery | 2.329 | 15.469 | 58.469 | 3.056 | 18.355 | 56.820 | 31,22 | 18,66 | -1.649 | 1,59 |
| Thoracic Surgery | 511 | 1.382 | 4.804 | 534 | 1.230 | 3.615 | 4,50 | -11,00 | -1.188 | 6,44 |
| Chest Diseases | 2.993 | 8.495 | 22.416 | 2.586 | 8.631 | 17.949 | -13,60 | 1,60 | -4.468 | -5,27 |
| Eye diseases | 452 | 1.477 | 8.623 | 198 | 731 | 2.744 | -56,19 | -50,51 | -5.880 | -3,52 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Hematology | 2.155 | 17.571 | 58.913 | 272 | 1.829 | 5.343 | -87,38 | -89,59 | -53.570 | 2,61 |
| Internal diseases | 8.059 | 81.448 | 294.512 | 9.721 | 93.669 | 330.898 | 20,62 | 15,00 | 36.386 | 0,48 |
| Gynecology and Obstetrics | 6.339 | 31.509 | 125.490 | 8.532 | 38.973 | 130.523 | 34,60 | 23,69 | 5.033 | 1,77 |
| Cardiac surgery | 97 | 431 | 1.042 | 90 | 440 | 859 | -7,22 | 2,09 | -183 | -2,05 |
| Cardiology | 3.750 | 24.363 | 56.378 | 4.562 | 26.209 | 43.393 | 21,65 | 7,58 | -12.985 | 2,01 |
| Ear-Nose-Throat Diseases | 950 | 8.376 | 38.805 | 727 | 4.767 | 12.224 | -23,47 | -43,09 | -26.582 | 3,91 |
| Nephrology | 4.171 | 44.618 | 135.029 | 6.213 | 67.352 | 232.864 | 48,96 | 50,95 | 97.836 | -0,12 |
| Neonatology | 254 | 857 | 1.016 | 181 | 628 | 809 | -28,74 | -26,72 | -208 | -0,82 |
| neurology | 2.118 | 19.320 | 74.921 | 1.580 | 12.233 | 34.611 | -25,40 | -36,68 | -40.310 | 1,95 |
| Orthopedics and Traumatology | 1.339 | 9.517 | 37.304 | 1.310 | 6.398 | 19.075 | -2,17 | -32,77 | -18.229 | 6,41 |
| Mental Health and Diseases | 199 | 1.373 | 5.763 | 93 | 566 | 1.565 | -53,27 | -58,78 | -4.198 | 1,94 |
| Medical Oncology | 2.187 | 10.678 | 29.618 | 2.260 | 11.901 | 31.331 | 3,34 | 11,45 | 1.713 | -1,49 |
| Urology | 4.498 | 15.760 | 54.974 | 3.524 | 11.614 | 36.348 | -21,65 | -26,31 | -18.626 | 1,80 |
| **Total** | **65,542** | **446,300** | **1,591,063** | **71,527** | **475,585** | **1,537,903** | **-9,13** | **-6,56** | **-53.160** | **-0,35** |

Among the 33 units evaluated within the scope of RLA, when the number of applications is listed in ascending order in terms of change rate, it is seen that 14 units succeeded in reducing the number of applications. Among these units, the first three units that reduce the number of applicants the most are hematology (87.38%), eye diseases (56.19), and mental health and diseases (53.27%). The first three units that reduced the minimum number of applications were found to be brain and neurosurgery (7.5%), cardiovascular surgery (7.2%), and orthopedics and traumatology (2.17%) (Table 2).

As a result of studies conducted based on evidence-based medicine, 75 different tests were evaluated within the scope of RLA, and the request amounts and prices of these tests before and after RLA are shown (Table 3).

**Table 3.** Pre-and post-RLA findings based on test

| R.Nu | HIC price | RLA Assay Name | Before RLA (01.06.2018-31.12.2018) | | After RLA (01.06.2019-31.12.2019) | | Assay Difference (Piece) (d-b) (e ) | Amount Difference (₺) (c-d) (f) | Assay Change Rate e/b*100 or f/c*100 |
|---|---|---|---|---|---|---|---|---|---|
| | | | RLA Test amount (₺) (HIC) (a) | RLA Number of tests (pieces) (b) | RLA Test amount (₺) (HIC) (c) | RLA Number of tests (pieces) (d) | | | |
| 1 | 19,13 | 25-Hydroxy Vitamin D | 220.511,25 | 11.530 | 197.025,75 | 10.302 | -1.228 | -23.485,50 | -10,65 |
| 2 | 14,25 | Aldosterone | 99,75 | 7 | 0,00 | 0 | -7 | -99,75 | -100,00 |
| 3 | 6,18 | Alpha-Fetoprotein (AFP) (Serum / Plasma) (Triple Scan) (D) | 8.892,00 | 1.440 | 9.077,25 | 1.470 | 30 | 185,25 | 2,08 |
| 4 | 9,69 | Copper, urine (24 Hours) | 19,38 | 2 | 0,00 | 0 | -2 | -19,38 | -100,00 |
| 5 | 8,08 | Growth Hormone 0, 30, 60, 90, 120 min | 1.340,45 | 166 | 1.485,80 | 184 | 18 | 145,35 | 10,84 |
| 6 | 7,60 | CA 125 (Serum / Plasma) | 5.867,20 | 772 | 9.652,00 | 1.270 | 498 | 3.784,80 | 64,51 |
| 7 | 7,60 | CA 15-3 (Serum / Plasma) | 5.867,20 | 772 | 10.374,00 | 1.365 | 593 | 4.506,80 | 76,81 |
| 8 | 7,60 | CA 19-9 (Serum / Plasma) | 6.581,60 | 866 | 10.640,00 | 1.400 | 534 | 4.058,40 | 61,66 |
| 9 | 2,38 | CRP (Turbidimetric) | 0,00 | 0 | 8.298,25 | 3.494 | 3.494 | 8.298,25 | - |
| 10 | 4,28 | CRP (Nephelometric) | 83.443,73 | 19.519 | 79.959,60 | 18.704 | -815 | -3.484,13 | -4,18 |
| 11 | 9,69 | Zinc | 48,45 | 5 | 0,00 | 0 | -5 | -48,45 | -100,00 |
| 12 | 1,05 | Iron (Serum / Plasma) | 18.064,92 | 17.287 | 18.522,63 | 17.725 | 438 | 457,71 | 2,53 |
| 13 | 5,70 | Estradiol (E2) (Serum / Plasma) | 11.628,00 | 2.040 | 14.677,50 | 2.575 | 535 | 3.049,50 | 26,23 |
| 14 | 4,75 | Ferritin (Serum / Plasma) | 90.539,75 | 19.061 | 84.056,00 | 17.696 | -1.365 | -6.483,75 | -7,16 |
| 15 | 6,18 | Folate (Serum / Plasma) | 95.101,18 | 15.401 | 81.102,45 | 13.134 | -2.267 | -13.998,73 | -14,72 |
| 16 | 5,70 | FSH | 13.583,10 | 2.383 | 17.288,10 | 3.033 | 650 | 3.705,00 | 27,28 |
| 17 | 1,52 | HDL cholesterol | 19.650,56 | 12.928 | 25.669,76 | 16.888 | 3.960 | 6.019,20 | 30,63 |
| 18 | 19,00 | Homocysteine | 114,00 | 6 | 0,00 | 0 | -6 | -114,00 | -100,00 |
| 19 | 4,75 | Complete Urine Examination | 88.996,00 | 18.736 | 119.823,50 | 25.226 | 6.490 | 30.827,50 | 34,64 |
| 20 | 1,05 | Urea + BUN (Peritoneal Fluid / 24 hours / Spot urine / Serum / Plasma) | 85.136,15 | 81.470 | 95.452,39 | 91.342 | 9.872 | 10.316,24 | 12,12 |
| 21 | 6,65 | Carcinoembryonic antigen (CEA) (Serum / Plasma) | 6.098,05 | 917 | 10.906,00 | 1.640 | 723 | 4.807,95 | 78,84 |
| 22 | 1,05 | Cholesterol (Serum / Plasma) | 14.403,24 | 13.783 | 19.005,42 | 18.187 | 4.404 | 4.602,18 | 31,95 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 23 | 1,05 | Creatinine (Peritoneal Fluid / Serum / Plasma / Spot urine) | 56.355,81 | 53.929 | 64.127,47 | 61.366 | 7.437 | 7.771,66 | 13,79 |
| 24 | 2,38 | LDL Cholesterol | 32.516,13 | 13.691 | 33.544,50 | 14.124 | 433 | 1.028,38 | 3,16 |
| 25 | 4,85 | Lipoprotein a | 29,07 | 6 | 0,00 | 0 | -6 | -29,07 | -100,00 |
| 26 | 5,70 | LH | 13.167,00 | 2.310 | 17.082,90 | 2.997 | 687 | 3.915,90 | 29,74 |
| 27 | 6,65 | Parathormone (PTH) (Serum / Plasma) | 17.662,40 | 2.656 | 23.507,75 | 3.535 | 879 | 5.845,35 | 33,09 |
| 28 | 6,65 | Progesterone | 1.589,35 | 239 | 1.562,75 | 235 | -4 | -26,60 | -1,67 |
| 29 | 6,65 | Prolactin | 15.501,15 | 2.331 | 19.218,50 | 2.890 | 559 | 3.717,35 | 23,98 |
| 30 | 6,18 | PSA (Prostate specific antigen) | 11.510,20 | 1.864 | 9.392,18 | 1.521 | -343 | -2.118,03 | -18,40 |
| 31 | 10,55 | Protein Electrophoresis (Serum and Body Fluids / urine / spot urine) | 369,08 | 35 | 0,00 | 0 | -35 | -369,08 | -100,00 |
| 32 | 12,16 | Renin | 109,44 | 9 | 0,00 | 0 | -9 | -109,44 | -100,00 |
| 33 | 2,38 | Rheumatoid factor (RF) (Turbidimetric) | 0,00 | 0 | 361,00 | 152 | 152 | 361,00 | - |
| 34 | 4,28 | Rheumatoid factor (RF) (Nephelometric) | 4.420,35 | 1.034 | 7.618,05 | 1.782 | 748 | 3.197,70 | 72,34 |
| 35 | 4,28 | Free T3 | 43.220,25 | 10.110 | 38.923,88 | 9.105 | -1.005 | -4.296,38 | -9,94 |
| 36 | 4,28 | Free T4 | 106.263,68 | 24.857 | 91.104,53 | 21.311 | -3.546 | -15.159,15 | -14,27 |
| 37 | 8,08 | Serum ACE level | 32,30 | 4 | 0,00 | 0 | -4 | -32,30 | -100,00 |
| 38 | 8,55 | Total IgE | 6.327,00 | 740 | 4.847,85 | 567 | -173 | -1.479,15 | -23,38 |
| 39 | 4,75 | Total Testosterone | 4.137,25 | 871 | 4.830,75 | 1.017 | 146 | 693,50 | 16,76 |
| 40 | 1,14 | Triglyceride (Peritoneal Fluid / Serum / Plasma) | 15.604,32 | 13.688 | 20.634,00 | 18.100 | 4.412 | 5.029,68 | 32,23 |
| 41 | 4,28 | TSH | 128.899,80 | 30.152 | 141.335,78 | 33.061 | 2.909 | 12.435,98 | 9,65 |
| 42 | 1,05 | Uric acid (24-hour urine / Serum / Plasma / Synovial Fluid / Spot urine) | 9.673,57 | 9.257 | 16.065,83 | 15.374 | 6.117 | 6.392,27 | 66,08 |
| 43 | 4,75 | Vitamin B12 | 104.001,25 | 21.895 | 86.397,75 | 18.189 | -3.706 | -17.603,50 | -16,93 |
| 44 | 36,29 | Protein C | 435,48 | 12 | 0,00 | 0 | -12 | -435,48 | -100,00 |
| 45 | 36,29 | Protein S | 471,77 | 13 | 0,00 | 0 | -13 | -471,77 | -100,00 |
| 46 | 2,38 | Urine Culture | 14.744,00 | 6.208 | 16.411,25 | 6.910 | 702 | 1.667,25 | 11,31 |
| 47 | 2,85 | Stool Culture | 1.861,05 | 653 | 1.883,85 | 661 | 8 | 22,80 | 1,23 |
| 48 | 2,38 | Sputum Culture | 584,25 | 246 | 370,50 | 156 | -90 | -213,75 | -36,59 |
| 49 | 7,60 | Anti CMV IgG (Microparticle immune assay-MEIA or similar) | 3.389,60 | 446 | 1.831,60 | 241 | -205 | -1.558,00 | -45,96 |
| 50 | 7,60 | Anti CMV IgM (Microparticle immune assay-MEIA or similar) | 3.496,00 | 460 | 2.143,20 | 282 | -178 | -1.352,80 | -38,70 |
| 51 | 7,60 | Anti HAV IgG (Microparticle immune assay-MEIA or similar) | 10.434,80 | 1.373 | 6.232,00 | 820 | -553 | -4.202,80 | -40,28 |
| 52 | 7,60 | Anti HAV IgM (Microparticle immune assay-MEIA or similar) | 3.541,60 | 466 | 1.915,20 | 252 | -214 | -1.626,40 | -45,92 |
| 53 | 7,60 | Anti Hbc IgG (Microparticle immune assay-MEIA or similar) | 25.672,80 | 3.378 | 15.899,20 | 2.092 | -1.286 | -9.773,60 | -38,07 |
| 54 | 7,60 | Anti HBc IgM (Microparticle immune assay-MEIA or similar) | 15.450,80 | 2.033 | 6.642,40 | 874 | -1.159 | -8.808,40 | -57,01 |
| 55 | 7,60 | Anti HBe (Microparticle immune assay-MEIA or similar) | 9.963,60 | 1.311 | 6.916,00 | 910 | -401 | -3.047,60 | -30,59 |
| 56 | 7,60 | Anti HBs (Microparticle immune assay-MEIA or similar) | 53.314,00 | 7.015 | 34.131,60 | 4.491 | -2.524 | -19.182,40 | -35,98 |
| 57 | 7,60 | Anti HCV (Microparticle immune assay-MEIA or similar) | 67.108,00 | 8.830 | 29.982,00 | 3.945 | -4.885 | -37.126,00 | -55,32 |
| 58 | 7,13 | Anti rubella IgG (Chemiluminescence or similar) | 7.972,88 | 1.119 | 2.921,25 | 410 | -709 | -5.051,63 | -63,36 |
| 59 | 7,13 | Anti rubella IgM (Chemiluminescence or similar) | 2.657,63 | 373 | 1.524,75 | 214 | -159 | -1.132,88 | -42,63 |
| 60 | 7,13 | Anti toxoplasma IgG (Chemiluminescence or similar) | 2.493,75 | 350 | 1.581,75 | 222 | -128 | -912,00 | -36,57 |
| 61 | 7,13 | Anti toxoplasma IgM (Chemiluminescence or similar) | 2.614,88 | 367 | 1.104,38 | 155 | -212 | -1.510,50 | -57,77 |
| 62 | 15,39 | CMV IgG avidity | 230,85 | 15 | 323,19 | 21 | 6 | 92,34 | 40,00 |
| 63 | 8,08 | Delta Antibody | 444,13 | 55 | 2.067,20 | 256 | 201 | 1.623,08 | 365,45 |
| 64 | 7,13 | HBeAg Qual (Chemoluminescence or similar) | 7.823,25 | 1.098 | 4.332,00 | 608 | -490 | -3.491,25 | -44,63 |
| 65 | 11,31 | Herpes simplex type 1 IgG | 192,19 | 17 | 0,00 | 0 | -17 | -192,19 | -100,00 |
| 66 | 11,31 | Herpes Simplex Type 2 IgG | 192,19 | 17 | 0,00 | 0 | -17 | -192,19 | -100,00 |
| 67 | 4,75 | IgA (Nephelometric) | 2.892,75 | 609 | 1.615,00 | 340 | -269 | -1.277,75 | -44,17 |
| 68 | 2,38 | IgA (Turbidimetric) | 0,00 | 0 | 87,88 | 37 | 37 | 87,88 | - |
| 69 | 4,75 | IgG (Nephelometric) | 2.289,50 | 482 | 1.401,25 | 295 | -187 | -888,25 | -38,80 |
| 70 | 2,38 | IgG (Turbidimetric) | 0,00 | 0 | 78,38 | 33 | 33 | 78,38 | - |
| 71 | 4,75 | Immunglobulin IgM (Nephelometric) | 2.256,25 | 475 | 959,50 | 202 | -273 | -1.296,75 | -57,47 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 72 | 2,38 | IgM (Turbidimetric) | 0,00 | 0 | 73,63 | 31 | 31 | 73,63 | - |
| 73 | 14,54 | Rubella IgG avidity | 29,07 | 2 | 377,91 | 26 | 24 | 348,84 | 1.200,00 |
| 74 | 16,15 | Toxoplasma IgG avidity | 209,95 | 13 | 419,90 | 26 | 13 | 209,95 | 100,00 |
| 75 | 9,69 | Treponema pallidum hemagglutination (TPHA) | 920,55 | 95 | 1.104,66 | 114 | 19 | 184,11 | 20,00 |
| **TOTAL** | | | **1,591,062.86** | **446,300** | **1,537,903.25** | **475,585** | **29,285** | **-53,159.61** | |

Among the 75 tests conducted based on evidence-based medicine and evaluated within the framework of RLA, 40 types of tests that were successful in reducing the number of requests. The first three tests with the highest number of requests are Anti HCV (Microparticle immune assay-MEIA or similar) (4.885 piece), Vitamin B12 (3.706 piece), and Free T4 (3.546 piece). The least successful tests are Serum ACE level (4 piece), Progesterone (4 piece), and copper urine test (24-hour) (2 piece) (Table 3).

Numerically, the first three tests are Urea + BUN (Peritoneal Fluid / 24-hour / Spot urine / Serum / Plasma) (9.876 piece), Creatinine (Peritoneal Fluid / Serum / Plasma / Spot urine) (7.437 piece) and Complete Urine Test (6.490 piece) that are against RLA by increasing more test requests compared to the pre-RLA period. The first three tests that increase the number of requests at least are Toxoplasma IgG avidity (13 piece), Stool Culture (8 piece), and CMV IgG avidity (6 piece) (Table 3).

In terms of price differences, the first three test types that cause the most cost reduction are Anti HCV (Microparticle Immune Assay-MEIA or similar) (37.126 ₺), 25-Hydroxy Vitamin D (23.485 ₺) and Anti HBs (Microparticle Immune Assay-MEIA or similar) (19.182 ₺). The least cost-saving assay types were determined as Lipoproteina (29,07 ₺), Progesterone (26,6 ₺), and copper urine test (24 Hours) (19,38 ₺ pieces)(Table 3).

In terms of price differences, the most contrary to the RLA are the first three types of test: Full Urine Test (30.827 ₺), TSH (12.435 (₺)) and Urea + BUN (Peritoneal Fluid / 24 hours / Spot urine / Serum / Plasma) (10.316 (₺)). The first three tests that caused the least cost increase were determined as IgG (Turbidimetric) (78.37 ₺), IgM (Turbidimetric) (73.62 ₺), and Stool Culture (22.8 ₺) (Table 3).

40 tests were identified that can provide cost savings as required by RLA. The remaining 35 tests, on the other hand, obtained results that were not suitable for RLA.

**DISCUSSION**

June-December 2018 is the period when there is no RLA and June-December 2019 is the period when RLA began and was used. In this process, the decrease in the number of some of the tests was evaluated in the sense that RLA was successful.

In the study titled "Reducing unnecessary laboratory testing using health informatics applications: a case study on a tertiary care hospital" conducted by Khalifa and Khalid (26), it was determined that more than 11% of the requested tests were repeated as the frequency of requests for laboratory tests and that they were overused.

In the study titled "An automated minimum retest interval rejection rule reduces repeat C-reactive protein (CRP) workload and expenditure, and influences clinician-requesting behavior" by Waldron et al. (27), a decrease of 7.0% and 12.3% was achieved in CRP demands for 1 year. Annual savings of £ 10,500 in general costs and £ 3,000 in consumable costs were reported.

In the study titled "An Educational and Administrative Intervention to Promote rational laboratory test ordering on an academic general medicine service" by Wertheim et al. (28), some rules were developed to reduce the number of laboratory test requests in a controlled manner. It was stated that as a result of the interventions, a 9% reduction in total laboratory use was achieved.

In this study, it was determined that the total amount of tests in June-December 2018 before RLA (HIC price x number of tests) was 1,591,063 ₺ ($ 330,782.32). After RLA, the total test amount (HIC price x number of tests) for June-December 2019 was 1.537.903 ₺ ($ 271,235.10) (Table 2 and Table 3). In other words, the economic gain provided by the Rational laboratory application to the hospital was determined to be 50,163.00 ₺ (3.34%).

Since the hospital where the study was conducted was established in 2006 and is a relatively new university hospital, the constantly growing laboratory facilities, newly opened branches and the acceptance of more applications by the physicians who started working for these branches result in the demand for more tests. As of the end of 2018, there were 109 academic personnel and 177 research assistants. As of the end of 2019, the number of academic personnel increased by 8% to 118, and the number of research assistants increased by 6% to 188. In the last 5 years, an average of 10% of the number of hospital admissions is observed. For all these reasons, between 2018 and 2019, when the study was conducted, the number of patient applications already increased by 10% of the number of laboratory tests. Consequently, the decrease in the number of tests and even the demand increase of less than 10% between 2018 and 2019 can be assumed that RLA is successful.

Tests performed in lower quantities are routine analyzes that are not specific to any

particular branch and can be requested by all doctors. Therefore, the increase in the number of personnel and newly opened branches were effective in the increase in the number of routine tests. It was also observed that the average number of requests per application before RLA was 6.81, and the average number of requests per application after RLA was 6.65.

While evaluating thyroid functions, Free T4 and Free T3 are frequently requested in addition to TSH analysis, which is a screening test. To prevent this situation with RLA, it is aimed to request only TSH first and then T3 and T4 if there is an abnormality in TSH. As expected in the results, the expected reduction in Free T4 was achieved without a significant reduction in TSH. While the increase in TSH was minimal, the decrease in the amount of Free T4 was much more prominent. This shows that unnecessary demands targeted in thyroid function tests are avoided (Table 3).

When the units are examined in terms of the number of test requests, it is seen that 13 out of 32 units (40.63%) are successful by decreasing the number of requests. The number of test requests increased contrary to expectations and the number of unsuccessful units was 17 (53.13%). The number of units without pre-RLA test request was determined as 2 (6.25%) (Table 1). When the pre- and post-RLA periods were compared in terms of the number of tests, the rate of successful units (13/30) was 43.33% and the rate of unsuccessful units (17/30) was 56.67%.

When the units are evaluated in terms of test costs, it is seen that 18 out of 32 units (56.25%) are successful by decreasing costs. The number of unsuccessful units by increasing their costs contrary to expectations is 12 (37.50%). The number of units without pre-RLA test request was determined as 2 (6.25%) (Table 1). When the pre-RLA and post-RLA periods were compared in terms of costs, it was concluded that the ratio of successful units (18/30) was 60%, and the rate of unsuccessful units (12/30) was 40%.

## CONCLUSION

In the study, a total of 446,300 test requests were made in the 7 months of 2018 before RLA, and the cost of these tests was found to be 1,591,063 ₺ ($ 330,782.32). In the 7 months after RLA, 475,585 test requests were made in total and the cost of these tests was determined to be 1,537,903 ₺ ($ 271,235.10). When the number of test requests before and after RLA was compared, it was seen that there was an increase of 6.56%. Although the number of patients increased by 10% compared to the previous period, the rate of test requests decreased. On the other hand, it was determined that the costs decreased by 3.34% in ₺ and 22% in terms of $. Although the number of test requests increased, the reason for the decrease in the test amount was found to be that the tests with an increased number of requests have low prices and the prices of tests with a decreased number of requests are high. Considering that the Central Bank Exchange Rate was an average of $ 1 = 4.81 ₺ in 2018, an average of $ 1 = 5.67 ₺ in 2019, and an 18% increase in foreign currency, it was determined that the costs decreased by 22%.

According to the findings in this study, it was concluded that the successful units are mainly surgical, and the unsuccessful units are internal units that require more analysis due to the nature of the work.

According to the results of the study, the following recommendations were made:

Diagnostic algorithms should be established together with stakeholders such as the Ministry of Health (HM), Social Security Institution (SSI), Clinical Specialist Associations. Physicians should be ensured to comply with diagnostic algorithms. The order of priority of tests should be determined and remuneration should be made to those who comply with this order. The tests and prescriptions that do not go through the diagnostic process should not be paid, and costs should be reduced by preventing unnecessary test requests with diagnostic algorithms.

HM and SSI should cooperate in evidence-based medicine and RLA's. Test request time and branch restrictions should be made by the HM and it should be ensured that the reimbursement should be controlled by the SSI if these restrictions are exceeded. Hospitals that have switched to RLA should be given a greater share of the global budget set by the SSI. Additional points should be given to hospitals that have switched to RLA in the quality assessment process every year.

RLA should be further developed by healthcare professionals and put into daily practice. Healthcare professionals should be given feedback and the process should be dynamic. Awareness training should be given to the person who requests the test. Planning should be made about the kits. For the sustainability of RLA processes, the importance of support in the process should be explained by the hospital management. Besides, the evidence-based Audit levels of all existing tests should be determined by the SB. The number of tests within the scope of rational laboratories should be increased and leading work must be done with quality control cards to decide whether the process can be interfered with.

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RESEARCH ARTICLE

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# Clinical Characteristics in Patients Presenting with Red Eye

**ABSTRACT**

**Objective:** Red eye, a frequent cause of presentations to ophthalmology clinics, is an important indicator of ocular inflammation. Although the prognosis is generally good and self-limiting, it is possible to distinguish possible serious conditions and prevent important situations such as blindness, with detailed examination and correct treatment approach. The purpose of this study was to evaluate patients with red eye presenting to the eye diseases clinic in terms of clinical and sociodemographic characteristics.

**Methods:** Diseases causing red eye were classified according to the International Classification of Diseases (ICD 10) coding system. Demographic characteristics such as age and sex and clinical findings were examined. Data were evaluated using number and percentage tests.

**Results:** A total of 2625 patients, 1775 males (67.61%) and 850 females (32.38%), who presented with red eyes, were evaluated. The mean age of the patients was 36.46±18.24 years. The incidence of viral conjunctivitis, the most frequently observed condition in patients presenting due to red eye, was 15.08% (n=396). The most common cause of red eye resulting in decreased vision and increased intraocular pressure (IOP) was acute angle closure glaucoma (AACG). The most common symptom was stinging-burning (70.36%), and the most frequent finding was follicular hyperplasia (74.17%). Five hundred and seventy-one (21.75%) patients who applied to the clinic with red eye had previously applied to a family physician and 289 patients (11.0%) to an emergency physician.

**Conclusions:** Although prognosis is usually good in red eye, and the condition is self-limiting, the detection of serious conditions through a detailed history, examination, and therapeutic approach can be enhanced with early and appropriate intervention. In addition to family physicians and emergency physicians, the first to examine patients with red eye, important morbidities such as blindness can also be prevented by increasing the awareness of ophthalmologists and cooperation between these.

**Keywords:** Red Eye, Glaucoma, Conjunctivitis, Uveitis.

# Kırmızı Gözle Başvuran Hastalarda Klinik Özellikler

**ÖZET**

**Amaç:** Göz hastalıkları polikliniklerine sık başvuru sebeplerinden biri olan kırmızı göz, oküler inflamasyonun en önemli belirtilerindendir. Çoğunlukla prognozu iyi ve kendi kendini sınırlayıcı olmakla beraber olası ciddi durumların ayırt edilmesi ve körlük gibi önemli durumların önüne geçilmesi, detaylı muayene ve doğru tedavi yaklaşımı ile mümkündür. Bu çalışmada kırmızı göz şikâyeti ile göz hastalıkları polikliniğine başvuran hastaların klinik ve sosyodemografik yönden değerlendirilmesi amaçlanmıştır.

**Gereç ve Yöntem:** Kırmızı göze sebep olan hastalıkların tanıları, International Classification of Diseases (ICD 10) kod sistemine göre sınıflandırıldı. Hastaların yaş, cinsiyet gibi demografik özellikleri ve klinik bulguları incelendi. Veriler sayı ve yüzdelik testi kullanılarak değerlendirildi.

**Bulgular:** Çalışmamızda kırmızı göz nedeni ile başvuran 2625 hastanın 1775'i (%67,61) erkek, 850'si (%32,38) kadın'dır. Hastaların yaş ortalaması 36,46±18,24 idi. Yapılan değerlendirmede kırmızı göz nedeniyle başvuranlarda en sık görüldüğü belirlenen viral konjonktivitli hastaların oranı %15,08 (n=396) idi. En sık görme azlığı ve göz içi basınç (GİB) artışı yapan kırmızı göz nedeninin akut açı kapanması glokomu (AAKG) olduğu tespit edilmiştir. En sık semptomun (%70,36) batma-yanma, en sık bulgunun (%74,17) foliküler hiperplazi olduğu görülmüştür. Kırmızı göz nedeni ile polikliniğe başvuran hastalardan 571 (%21,75)'i önceden aile hekimine, 289 (%11,0)'u acil hekimine başvurmuştu.

**Sonuç:** Kırmızı göz varlığında, prognoz çoğunlukla iyi olup, klinik tablo kendi kendini sınırlayabilse de detaylı anamnez, muayene ve tedavi yaklaşımı ile ciddi durumlar fark edilerek erken ve doğru müdahale ile tedavi başarısı arttırılabilir. Kırmızı gözü olan hastaları ilk karşılayan aile hekimleri ve acil hekimlerinin yanı sıra oftalmologların farkındalıklarının ve iş birliğinin arttırılması ile körlük gibi önemli morbiditelerin önüne geçilebilecektir.

**Anahtar Kelimeler:** Kırmızı Göz, Glokom, Konjonktivit, Üveit.

## INTRODUCTION

Red eye, one of the most common reasons for presentations to ophthalmology clinics, is in fact a signal mechanism in the orbit and an important indicator of ocular inflammation. While the clinical manifestation in red eye can be self-limiting, depending on the etiology, visual acuity can also be permanently and adversely affected in some types requiring emergency intervention. Detailed history and detailed examination by an ophthalmologists from time of initial presentation are therefore required in order to determine the etiology (1, 2). Conjunctivitis is the most important reported cause in the development of red eye. Conjunctivitis emerges through both viral and bacterial agents, although viral conjunctivitis is more common viral (1, 3). The clinical manifestation in viral conjunctivitis exhibits a broad spectrum, from mild to progression to evisceration and permanently decreased vision. Among the viral agents, adenoviruses are known to lead to epidemic keratoconjunctivitis. A detailed history taken before contact with the patients in outbreaks is an important protecting the physician against contamination with the infectious agent and the development of red eye (4). In case of bacterial conjunctivitis, onset is sudden, and prognosis may be poorer. Bacterial conjunctivitis is estimated to be seen in 135 out of every 10,000 people annually in the USA (5).

Allergic conjunctivitis, another cause of red eye, is also frequently accompanied by itching and watery eyes. Allergic conjunctivitis affects at least 20% of the population annually. Conjunctival edema and marked chemosis may be seen in severe cases. It is typically reported not to threaten vision and to cause no severe ocular side-effects (6).

Corneal opacities caused by microbial keratitis are the fourth leading cause of blindness globally. The most common findings are acute eye pain and redness. Infectious keratitis generally develops in association with contact lens use in developed countries, while in developing countries it is reported to develop due to trauma and fungal infections among agricultural workers. Vision disorder occurring due to microbial keratitis can generally be prevented by adopting an appropriate approach (7, 8).

In addition to causing red eye, acute angle closure glaucoma (AACG) also results in patients presenting to physicians due to severe ocular pain, reduced vision, and nausea and vomiting. Decreased vision, corneal edema, and mid-dilated pupils are important findings accompanying AACG. Rapid diagnosis and treatment are highly important since the condition can result in permanent damage to the optic nerve and permanent loss of vision. The presence of AACG should always be considered when evaluating patients with migraine-related headache (9).

Episcleritis, one of the causes of red eye, characterized by infection of the sclera and involving only superficial episcleral tissue, is idiopathic in the majority of cases, while 50% of cases of scleritis capable of involving the cornea, episcleral, and uvea are reported to be associated with a systemic disease (10).

Management of red eye relies on collaboration between and sensitivity on the part of family physicians, emergency physicians, and ophthalmologists, the first health professionals to encounter red eye, in terms of differential diagnosis and identification of emergency conditions (11).

The purpose of the present study examining the clinical and demographic characteristics of patients presenting to our eye diseases clinic due to red eye is to describe our own clinical experience and increase awareness of red eye, a condition frequently encountered in the community, among physicians.

## MATERIAL AND METHODS

The present study involved 2625 patients presenting to the Harran University Medical Faculty Ophthalmology Clinic, Turkey, with red eye between 1 May 2016 and 25 March 2020. Approval for the study was granted by the Harran University Institutional Assessment Committee and Ethical Committee (No. E.18161 dated 13/05/2020).

Diseases causing red eye were classified according to the International Classification of Diseases (ICD 10) coding system. A data form containing detailed history and examination findings recorded from patients' files was employed during data collection. Recorded data such as age, sex, red eyes, sensations of stinging, burning, or foreign bodies, eyelid edema, conjunctival edema, corneal involvement, photophobia, blurred vision, presence in environments such as swimming pools, the sea, or thermal spas and other public areas, history of contact with patients with conjunctivitis, and whether or not patients had presented to another health institution or physician with the existing symptoms were all noted. Patients included in the study underwent complete ophthalmological examinations including best corrected visual acuity (BCVA) examination, intraocular pressure (IOP) measurement using an i-care tonometer (Tiolat Oy, Helsinki, Finland), and dilated fundus examination or orbital ultrasonography for diagnostic purposes during presentation. Elevated IOP was defined as values exceeding 30 mmHg (12). Cases with conjunctivitis were examined in isolation from other patients against the risk of contagion in hospital as a routine requirement.

Statistical analyses were performed on IBM SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). Normally distributed numerical variables were expressed as mean +/- standard deviation (SD), and

non-normally distributed numerical variables as median (min-max), while categorical variables were expressed as frequencies (percentages).

**RESULTS**

Males represented 1775 (67.61%) of the patients in this study, and females 850 (32.38%). The ages of the 2625 participants ranged between 0 and 95 years (36.46±18.24). In terms of age distributions, 19.1% (n=500) of patients were aged 0-20 years, 49.2% (n =1291) 21-40 years, 17.8% (n=467) 41-60 years, and 13.9% (n=366) over 61 years.

The most common cause of red eye was viral conjunctivitis as 15.08% (n=396), followed by bacterial conjunctivitis at 13.56% (n=356), allergic conjunctivitis at 12.87% (n=338), keratitis at 10.78% (n=283), corneal / conjunctival foreign body at 9.98% (n=262), episcleritis at 8.0% (n=210), scleritis at 1.75% (n=46 ), uveitis at 8.57% (n=225), acute angle closure glaucoma (AACG) at 7.08% (n=186), subconjunctival hemorrhage at 6.28% (n=165), and dry eye at 6.01% (n=158) (Table 1).

**Table 1.** Patients' demographic and clinical characteristics

| | Age | Sex (F/M) (n) | Number of patients n/% |
|---|---|---|---|
| **Viral conjunctivitis** | 35.46±11.49 | 90/302 | 396 (15.08%) |
| **Bacterial conjunctivitis** | 33.15±13.14 | 54/302 | 356 (13.56%) |
| **Allergic conjunctivitis** | 17.83±6.94 | 136/202 | 338 (12.87%) |
| **Keratitis** | 32.78±12.52 | 81/202 | 283 (10.78%) |
| **Corneal / conjunctival foreign body** | 29.89±10.08 | 64/198 | 262 (9.98%) |
| **Episcleritis** | 31.26±9.05 | 29/181 | 210 (8.0%) |
| **Scleritis** | 30.22±5.63 | 15/31 | 46 (1.75%) |
| **Uveitis** | 26.33±9.52 | 125/100 | 225 (8.57%) |
| **AACG** | 63.43±11.69 | 103/83 | 186 (7.08%) |
| **Subconjunctival hemorrhage** | 66.41±11.15 | 75/90 | 165 (6.28%) |
| **Dry eye** | 63.83±10.96 | 78/80 | 158 (6.01%) |

AACG: acute angle closure glaucoma.

The most frequently reported symptoms were a sensation of stinging or burning at 70.36% (n=1847), photosensitivity at 55.77% (1464), itching at 55.16% (n=1448), drying at 53.83% (n=1413), pain in the eye at 26.97% (n=708), and decreased vision at 21.91% (n=575) (Figure 1).

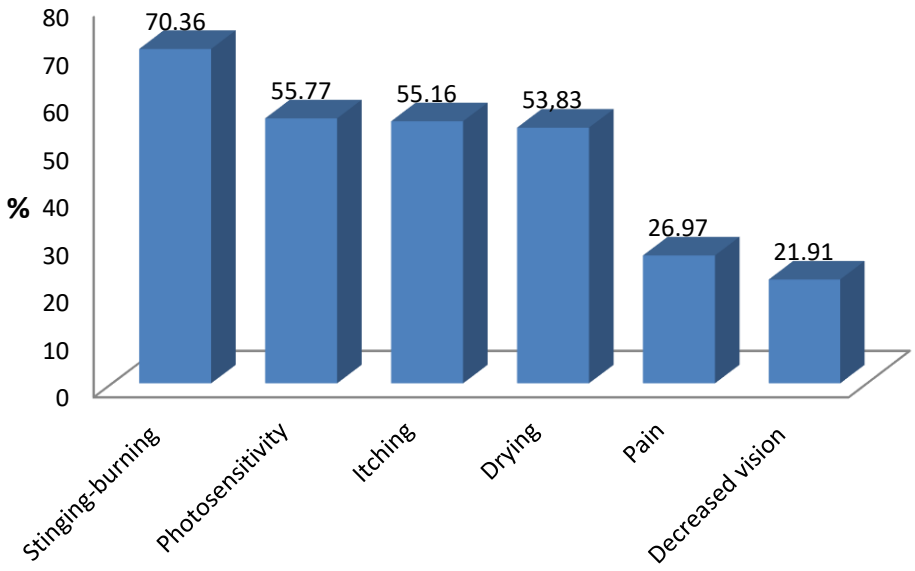


**Figure 1.** Symptoms of patients presenting due to red eye

The most common finding was follicular hyperplasia at 74.17% (n=1947), followed by conjunctival hyperemia at 69.14% (n=1815), discharge at 53.83% (n=1413), loss of corneal transparency at 34.17% (n=897), chemosis at 33.79% (n=887), and increased IOP at 8.46% (n=222). Fundus findings (+ 2, + 3 cells in the vitreous 13 patients; macular edema 8 patients; perivascular encasement 5 patients; 4 patients with active focus of chorioretinitis) were present in 0.76% (n=20) of patients (Figure 2).

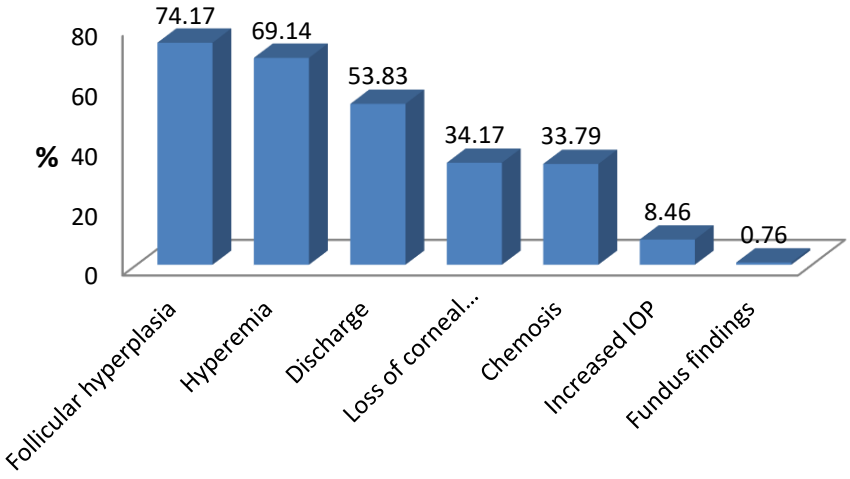


**Figure 2.** Findings of patients presenting with red eye.

Flare and cells in the anterior chamber were observed in patients with uveitis, and corneal edema and mid-dilated pupils in patients with AACG. Seen in 186 patients, AACG was the most common cause of decreased visual acuity, which was also seen in cases of uveitis, corneal foreign body, keratitis, scleritis, bacterial conjunctivitis and dry eye (Table 2).

The condition in which IOP most frequently exceeded 30 mmHg was AACG, followed by episcleritis/scleritis, keratitis, uveitis, and allergic conjunctivitis. (Table 3).

**Table 2.** Visual acuity values of red eye patients

| | P+P+ / HM + | 1/200- 19/200 | 20/200-20/50 | ≤20/40 |
|---|---|---|---|---|
| **Viral conjunctivitis** | | | | 396 |
| **Bacterial conjunctivitis** | | 9 | 10 | 337 |
| **Allergic conjunctivitis** | | | | 338 |
| **Keratitis** | | 30 | 48 | 205 |
| **Corneal / conjunctival foreign body** | | 42 | 46 | 174 |
| **Episcleritis** | | | | 210 |
| **Scleritis** | | 3 | 5 | 38 |
| **Uveitis** | | 69 | 88 | 68 |
| **AACG** | 48 | 128 | 6 | 2 |
| **Subconjunctival hemorrhage** | | | | 165 |
| **Dry eye** | | 2 | 10 | 146 |

HM: Hand movement, P: Projection of rays, AACG: acute angle closure glaucoma.

**Table 3.** Average ıntraocular pressure values

| | Average IOP (mmHg) | Number of patients with IOP above 30 mmHg |
|---|---|---|
| **Viral conjunctivitis** | 15 | 0 |
| **Bacterial conjunctivitis** | 16 | 0 |
| **Allergic conjunctivitis** | 18 | 4 |
| **Keratitis** | 19 | 11 |
| **Corneal / conjunctival foreign body** | 17 | 0 |
| **Episcleritis** | 19 | 5 |
| **Scleritis** | 20 | 7 |
| **Uveitis** | 22 | 9 |
| **AACG** | 44 | 186 |
| **Subconjunctival hemorrhage** | 16 | 0 |
| **Dry eye** | 17 | 0 |

AACG: acute angle closure glaucoma, IOP: intraocular pressure

Five hundred seventy-one (21.75%) patients presenting to our clinic with red eye had previously presented to a family physician, and 289 (11.0%) to an emergency physician. Conjunctivitis was the most common etiological cause in presentations to family physicians, and foreign bodies in the cornea or conjunctiva in case of presentations to emergency physicians.

## DISCUSSION

Red eye, one of the most common reasons for presentations to eye clinics, exhibits a wide course, from mild manifestations to blindness. The present study considered red eye in the community and our own region.

Analysis of the demographic and clinic characteristics of patients presenting to our clinic with red eye identified viral conjunctivitis as the

most common etiological cause. Other frequently observed causes were, in descending order, allergic conjunctivitis, bacterial conjunctivitis, keratitis, foreign bodies in the cornea and conjunctiva, episklerit, scleritis, uveitis, AACG, subconjunctival hemorrhage, and dry eye. Sanchez et al. investigated approximately 3000 patients presenting with ocular emergencies, and reported that approximately half of presentations were due to inflammatory conditions involving the cornea, conjunctiva, and eyelids. Distribution by age and sex in that study was also compatible with the findings of the present study, with male patients representing the majority of presentations, and presentations being more common in the 21-50 age range (35.4%). Presentations in the present study were most frequent in the 21-40 age range (13).

In their retrospective study of 20,822 emergency ocular presentations, Galindo-Ferreiro et al. cited acute conjunctivitis as the most frequent cause of red eye, in agreement with the present study. Presentations were more frequent among male patients than females in the present study, and were most common in the 21-40 age range, while Galindo et al. reported that the proportion of female patients presenting was close to that of men, and that presentations were more frequent in the 45-65 age range (14).

Similarly to the present study, Sridhar et al. described viral conjunctivitis (8.7%) as the most common cause of presentation, followed by dry eye (6.6%) and corneal injury (6.6%). The most frequent other causes of presentation in the present study, in descending order, were bacterial conjunctivitis, allergic conjunctivitis, keratitis, and corneal and conjunctival foreign bodies (15). In their study of approximately 1000 patients, Fitch et al. reported that no definite diagnosis was possible in 24% of cases, similar to our rates, they found viral etiology in 36% of the patients and a higher rate (40%) of bacterial etiology (16).

Yis et al. performed a retrospective investigation of 3199 emergency department presentations due to red eye. Male presentations in that study were similarly to those in the present research, but in contrast to our study, pediatric presentations were more frequent (30.6%). Also consistent with the present study, those authors also described conjunctivitis as the most frequent cause of presentation. In contrast to our study, however, the next most common causes of presentation were foreign bodies in the eye, conjunctival hemorrhage, and hyphema. In the present study, episcleritis was diagnosed in 8% of patients presenting due to red eye, and scleritis in 1.75%, while no patients were diagnosed with these conditions in Yis et al.'s study (17).

Marinos et al. reported that 68% of patients with viral conjunctivitis received antibiotherapy, and that 78% presented to their clinic within one week (18). In the present study, 21.75% of patients, particularly those with conjunctivitis, had presented to family physicians, subsequently presenting to our eye diseases clinic due to referral because of persistence of symptoms or of their own individual volition.

Consistent with the previous literature, the majority of patients developing keratitis in the present study were aged 21-40 years and were men (7, 8). In a study of 857 patients with red eye disease, Ahmad et al. also reported more diagnoses of keratitis among men. These findings may be attributable to the ability to work actively outdoors and a greater likelihood of experiencing ocular trauma in this age group, and to use of contact lenses (19). It is particularly important for individuals using contact lenses to be informed of the need for greater care over use and hygiene rules, and for agricultural workers to be told to use protective equipment to protect their eyes in order to prevent keratitis. Presentation were more frequent between the ages of 40 and 60 in that study. However, in the present study, the distribution was more frequent between 21 and 40. Microbial keratitis was diagnosed in 3.7% of patients presenting due to red eye in that study, considerably lower than the comparable figure in the present study (10.78%) (19). Tena et al. described contact lens use as the most common risk factor (33.2%) for the development of keratitis. Other causes in that study, in descending order of frequency, were blepharitis (19.7%), trauma (13.2%), and immunosuppression (6.8%) (7). Upadhyay et al. reported that the incidence and etiology of microbial keratitis varied depending on the geographical region, and that the annual incidence of microbial keratitis in the USA was 11.0-27.6:100,000, compared to 799:100,000 in Nepal, a developing country (20, 21). In the present study, performed in a region with a high presence of agricultural workers in epidemiological terms, the incidence of trauma-related infectious keratitis (10.7%) was considerably higher than those figures. In another study, the most frequent cause of microbial keratitis, at 43.75%, was trauma due to accidents, significantly higher than the equivalent figure in the present study (19).

Farahni et al. reported that visual acuity in keratitis is always affected in line with the position of the lesion and the degree of intraocular infection, and that IOP elevation should always be considered, particularly in the presence of trabeculitis-related HSV infection (22). Similarly in the present study, visual acuity was adversely affected in 27.56% of patients diagnosed with keratitis, and increased IOP was seen in 11 patients. In a study of 268 pediatric eye trauma patients, Puodžiuvienė et al. reported a lower proportion of individuals with decreased visual acuity among patients with keratitis (18.4%) than in the present study (23). Furlanetto et al. reported pre-treatment visual acuity lower than 20/400 in 53.85% of

patients (24). In the present study, a decrease in vision levels was observed in 78 (27.5%) patients with keratitis, while no decrease was observed in patients with viral conjunctivitis, allergic conjunctivitis, episcleritis, or subconjunctival hemorrhage.

Similarly to the present study, Şahinoğlu et al. found that spontaneous subconjunctival hemorrhages or those developing with Valsalva had histories of systemic disease, while traumatic subconjunctival hemorrhages were more widespread in young men engaged in heavy work and activities (25). A male/female ratio of 0.8 has been reported in the incidence of non-traumatic subconjunctival hemorrhage, rising with age (26). Kaimbo et al. reported that subconjunctival hemorrhage was diagnosed in 0.8% of 6843 patient for whom ocular consultations were requested, with a higher rate being determined in women compared to men (59%/41%), and a mean age of 31. Those authors reported traumatic subconjunctival hemorrhage in 52% of patients and spontaneous subconjunctival hemorrhage in 48%, the most frequently associated condition being hypertension (27). Hu et al. reported that subconjunctival hemorrhage developed in one out of 167 individuals in the East Asian population that it was more common in women than in men, that the most frequent agent was hypertension, and that the incidence was low in childhood, but rose to 136 in 10,000 individuals in the 60-69 age group (28). The mean age of our patients was similar to that in Kaimbo et al., but considerably lower than that in Hu et al. Subconjunctival hemorrhage was also not a cause of decreased visual acuity in any patient (27, 28).

A study of patients with scleritis reported decreased vision in 37% of participants, anterior uveitis in 42%, peripheral ulcerative keratitis in 14%, glaucoma in 13%, and cataract in 17%, while the rate of glaucoma in anterior scleritis ranged between 9% and 22% (29). Thong et al. investigated the epidemiology of episcleritis and scleritis in Australia and reported an incidence of episcleritis of four in 100,000 and of scleritis of 1.03 in 100,000. Both scleritis and episcleritis were more common in women than in men, and patients with scleritis were older. The authors reported that different ethnic origins, environmental factors, and sex may affect the variations in these figures (30). The rates of episcleritis and scleritis among all the patients in the present study were higher than in previous publications, rates of both were higher in women than in men, with both men and women more frequently being in their 30s. No decrease in visual acuity occurred in any patient with episcleritis in the present study, but decreases were observed in 8 patients with scleritis. Increased IOP was also detected in five patients with episcleritis and seven with scleritis. Diaz et al. reported that episcleritis is rare in childhood, the number of adult patients also exceeding that of children in the present study (10).

In a recent systematic review, Miserocchi et al. reported a global incidence of uveitis of 17-52/100,000 (31). Previous research shows that uveitis is one of the five most common causes of blindness in the developed world, representing 10% of all cases in the USA. Some studies have reported equal gender distribution or slight female predominance among patients with uveitis, that decreased visual acuity is a common symptom, and that an increase in IOP may also be seen, consistent with the findings of the present study (32, 33).

Hart et al. reported an estimated incidence in Australia of 21.54/100,000, that the disease affected young Australians of working age (80.8%), and that it exhibits no general gender preference. Those authors reported a distribution of cases of diffuse uveitis of anterior uveitis in 75%, intermediate in 6%, posterior in 15%, and panuveitis in 4% (34). In the present study, uveitis was present in 8.5% of patients with red eye, the incidence being similar between the genders, although slightly higher in women (56%). Consistent with Hart et al., our findings also showed a higher incidence of patients with anterior-type uveitis. In a study examining the epidemiology of uveitis in the Philippines, Abaño et al. reported a mean age at presentation of 38 ± 18.4 years, that anterior uveitis was more common, consistent with the present study, and that 54% case of cases were idiopathic while specific diagnoses were determined in 46%. In addition, they found that children (13%) and the elderly (15%) were less affected (35). In our study, uveitis was seen less in the elderly and children.

Studies have generally reported severe ocular pain, redness, and decreased vision in AACG. Similarly in the present study, the basic symptoms were reduced vision and pain (9). Park et al. investigated approximately 11,000 patients with AACG (73% female), and reported that the incidence increased sharply with age, peaking at the ages of 75-79, and being 2.5-fold more common in women than men (36). Studies have also reported ethnic variation in AACG. These studies have also shown that the incidence of AACG increases significantly with age, peaking in the 75-79 age group (37, 38). AACG was also more common at advanced age and in women in the present study.

Although dry eye is generally regarded as a simple entity, it is also a condition that can cause pain in the eye and blurred vision under severe conditions. The discomfort it causes directly impacts on accurate diagnosis, treatment, and quality of life through its adverse impacts on activities such as reading, watching television, and social activities such as driving, and can result in delayed diagnosis (39). Similarly in the present study, decreased visual acuity was observed in 12 patients presenting with dry eye and diagnosed with dry eye. Tan et al. reported that the prevalence of

dry eye increased with age, affecting 18% of women and 11% of men in the USA, and between 5% and 34% of the adult population. Studies have also shown that symptomatic dry eye disease is significantly associated with contact lens use (40). The prevalence of dry eye among patients with red eye in the present study was 6%, being equally distributed among men and women, the most frequent symptoms, in agreement with previous research, being stinging, burning, and photosensitivity. The most frequent finding was follicular hyperplasia. Ahmad et al. similarly reported a prevalence of dry eye of 6.75% (19).

Frings et al. reported that the duration, laterality (unilateral) of symptoms in red eye, and the severity of pain were the main factors for a family physician to refer to an ophthalmologist (39). Narayana et al. reported that findings of anisocoria and pupillary constriction indicate severe ocular disease in red eye patients (41). In the present study, 21.75% of patients had previously presented to a family physician and 11% to an emergency physician. The most common etiological reason for presentations to family physicians was conjunctivitis, while the most common reason for presentations to emergency physicians was foreign bodies in the cornea or conjunctiva. The limitations of our study are that it is not prospective, it is conducted in the city we are in.

## CONCLUSION

In conclusion, red eye is a condition widely seen in the community and a frequent cause of presentations to family physicians, emergency departments, and ophthalmology clinics. In order to prevent the development of red eye, it is important for patients and physicians to be informed of the importance of negative domestic conditions capable of causing ocular traumas being corrected, of glasses and headgear being worn, particularly when working out of doors, for protection against foreign body, and of hygiene in contact lens use. Physicians should be aware of clinical conditions accompanying red eye, such as hemorrhage, watery eyes, decreased vision and pain in order to prevent permanent damage, and patients should be referred to ophthalmologists without loss of time when necessary. Increasing cooperation between family physicians and relevant branches of medicine and raising awareness is also important in terms of both preventive medicine and early diagnosis and treatment of existing diseases.

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RESEARCH ARTICLE

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# Comparison of in Vitro Antimicrobial Efficacy of Ceftolozane-Tazobactam and Ceftazidime-Avibactam Combination Against Carbapenem-Resistant *Enterobacteriaceae* Species Isolated from Various Clinical Specimens

**ABSTRACT**

**Objective:** The increase in resistant gram-negative bacteria is a major concern and has led to difficulties in the treatment of infections. The aim of this study was to compare the in vitro efficacy of CLZ-TAZ and CAZ-AVB combinations against carbapenem-resistant *Enterobacteriaceae* strains.

**Methods:** Eighty, carbapenem-resistant *Enterobacteriaceae* species isolated from various samples sent to our laboratory were included in the study. Identification and antimicrobial susceptibility of strains were performed using automated systems. The presence of carbapenemases in all isolates was tested using the CarbaNP test and the carbapenem inactivation method. The presence of carbapenemase genes was tested by multiplex PCR.

**Results:** The presence of carbapenemases was detected in 60 % *E. coli* isolates and in 78.5% *K. pneumoniae* isolates via phenotypic tests. OXA-48 enzyme was found in 73.7% of isolates containing carbapenemase. The second most common enzyme was NDM. The assessment of the efficacy of the β-lactam/β-lactamase inhibitor combinations against CRE isolates revealed that the activity of CAZ-AVB (77%) was higher than CLZ-TAZ (48%).

**Conclusions:** Our findings suggest that CAZ-AVB and CLZ-TAZ may be promising in the treatment of infections caused by CRE strains. Sensitivity rates were higher with ceftazidime-avibactam than with ceftolozane-tazobactam. The data obtained in this study will contribute to the clinical use of these agents in our country.

**Keywords:** Carbapenem-Resistant *Enterobacteriaceae*, Ceftolozane/Tazobactam Ceftazidime/Avibactam, *bla*OXA-48, *bla* NDM.

# Çeşitli Klinik Örneklerden İzole Edilen Karbapenem Dirençli *Enterobacteriaceae* İzolatlarına Karşı Seftolozan-Tazobaktam ve Seftazidim-Avibaktam Kombinasyonlarının In Vitro Antimikrobiyal Etkinliğinin Karşılaştırılması

**ÖZET**

**Amaç:** Dirençli gram negatif bakterilerdeki artış önemli bir endişe kaynağıdır ve enfeksiyonların tedavisinde zorluklara yol açmıştır. Bu çalışmanın amacı, carbapenem-resistant *Enterobacteriaceae* suşlarına karşı CLZ-TAZ ve CAZ-AVB kombinasyonlarının in vitro etkinliğini karşılaştırmaktır.

**Gereç ve Yöntem:** Çalışmaya, laboratuvarımıza gönderilen çeşitli örneklerden izole edilen, karbapenemlere dirençli 80 *Enterobacteriaceae* türü dahil edildi. İzolatların tanımlanması ve antimikrobiyal duyarlılıkları otomatize sistemler kullanılarak gerçekleştirildi. Tüm izolatlarda karbapenemazların varlığı, CarbaNP testi ve karbapenem inaktivasyon yöntemi kullanılarak test edildi. Karbapenemaz genlerinin varlığı multipleks PCR ile test edildi.

**Bulgular:** Fenotipik testler ile karbapenemazların varlığı %60 *E. coli* izolatında ve %78.5 *K. pneumoniae* izolatında tespit edildi. Karbapenemaz içeren izolatların %73.7'sinde OXA-48 enzimi bulundu. İkinci en yaygın enzim NDM idi. β-laktam/β-laktamaz inhibitör kombinasyonlarının CRE izolatlarına karşı etkinliğinin değerlendirilmesinde ise, CAZ-AVB'nin (%77) aktivitesinin CLZ-TAZ'dan (%48) daha yüksek olduğu tespit edildi.

**Sonuç:** Bulgularımız, CRE suşlarının neden olduğu enfeksiyonların tedavisinde CAZ-AVB ve CLZ-TAZ'ın umut verici olabileceğini düşündürmektedir. Seftazidim-avibaktam ile duyarlılık oranları, seftolozan-tazobaktamınkinden daha yüksekti. Bu çalışmada elde edilen veriler ülkemizde de bu ajanların klinik kullanımına katkı sağlayacaktır.

**Anahtar Kelimeler:** Karbapenem dirençli *Enterobacteriaceae*, Seftolozan/Tazobaktam, Seftazidim/Avibaktam, *bla*OXA-48, *bla* NDM.

## INTRODUCTION

Antibiotic resistance in gram-negative bacteria has increased over time and led to treatment failures by limiting clinical treatment options. Antibiotic resistance rates can vary significantly between countries and regions. Multidrug-resistant (MDR) gram-negative bacteria-related infections are very difficult to treat and they represent a serious health emergency, especially in patients having comorbid diseases (1,2,3). Because carbapenems are highly effective, they are frequently used as first-line antibiotics for the treatment of infections caused by microorganisms that produce extended-spectrum beta-lactamases (ESBL). However, the increased rates of infections caused by ESBL-producing *Enterobacteriaceae* members have caused a rise in the frequency of use of carbapenems over the years, contributing to carbapenem resistance rates (1,3). Nosocomial infections caused by *Enterobacteriaceae,* which produce different types of carbapenemases, are now common in many countries posing major limitations for antimicrobial therapy. Over the last decade, carbapenem-resistant *Enterobacteriaceae* has been found to be spreading worldwide (3-5). The high morbidity and mortality of infections caused by MDR gram-negative bacteria result from the unavailability of safe and effective antibacterial treatment options.

This necessitates further research to develop new antibiotics. For this reason, the World Health Organization has published the global priority list of antibiotic-resistant bacteria to guide the research, discovery, and development of new antibiotics. The global priority list of antibiotic-resistant bacteria includes carbapenem-resistant *Enterobacteriaceae* (CRE), *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* in the category1 as the critical priority list of pathogens in urgent need of new antibiotics (6,7). Among the newest agents developed to combat antimicrobial resistance, β-lactam/β-lactamase inhibitor combinations have attracted considerable interest with promising results through *in vitro* activity against MDR gram-negative bacteria (6). Ceftazidime/avibactam (CAZ-AVB) is a third generation cephalosporin and a new β-lactamase inhibitor combination. Ceftolozane/tazobactam (CLZ-TAZ) is a fourth generation cephalosporin and β-lactamase inhibitor combination. Both drugs are often used as salvage therapy when the infectious agent is resistant to all other antibiotics available (8).

The aim of this study was to investigate the *in vitro* antimicrobial efficacy of these two new combinations of β-lactam-β-lactamase inhibitors against carbapenemase gene-containing carbapenem-resistant *Enterobacteriaceae* isolate that were isolated from various clinical specimens.

## MATERIAL AND METHODS

**Bacterial Isolates and Antimicrobial Sensitivity Tests:** Various clinical specimens (tracheal aspirates, urine and blood samples, and wound swabs) submitted to our laboratory in the period between August 2017 and October 2017 were examined for eligibility. A total of 80 isolates from the *Enterobacterales* family were included in the study. The included isolates were found to be resistant to carbapenems in antimicrobial sensitivity tests and carbapenemase positive in phenotypic tests. Only one isolate from one patient was included in the study. The BD Phoenix™ (Becton Dickinson, US) automated identification system was used to identify the isolates. The antimicrobial sensitivity of the isolates was tested using the BD Phoenix™ (Becton Dickinson, US) automated identification and antibiogram analysis system and through the gradient test method. The minimum inhibitory concentrations (MIC) of CAZ-AVB and CLZ-TAZ were tested using the gradient test method (Liofilchem, Italy). The results were interpreted in accordance with the EUCAST 2017 criteria.

**Phenotypic Tests to Discover the Presence of Carbapenemases:** The presence of carbapenemases in *K. pneumoniae* and *E. coli* isolates resistant to at least one of the following antibiotics imipenem, meropenem, and ertapenem was tested using the CarbaNP test and carbapenem inactivation method (CIM).

**CarbaNP Test**: A loopful of bacterial colonies from a blood agar plate was suspended in a 200 µl Tris-HCl 20 mmol/L lysis buffer (B-PERII, Bacterial Protein Extraction Reagent; Thermo Scientific, USA). After incubating at room temperature for 30 minutes, 100µl of the suspension was taken and mixed with a phenol red solution containing 6 mg/L imipenem monohydrate (Sigma, France). The bacterial suspension was examined to detect any color changes after a 2-hour incubation period at 35 °C. A color change from red to yellow at the end of the incubation period was considered a positive result. When no changes were observed in the color, the result was accepted as negative.

**Carbapenem Inactivation Method (CIM):** A 10 µL loopful of the bacterial colonies to be tested was suspended in 400 µL sterile distilled water. A 10 µg BD BBL™ Sensi-Disc™ meropenem disc (Becton Dickinson, ABD) is added to the suspension and incubated at 35±2°C for 2 hours. The *E. coli* ATCC 25922 suspension prepared at 0.5 McFarland turbidity was streaked onto Mueller-Hinton agar plates. The discs incubated in the suspension were placed onto the plate as they were placed in the disc diffusion test. The results of the CIM test were evaluated after the incubation. When the efficacy of the meropenem disc was preserved and an inhibition zone occurred

within the limits of susceptibility, the CIM test result was accepted negative. The test result of the CIM test was interpreted as positive when the efficacy of the meropenem disc was not preserved due to carbapenemase activity.

**Examination of the Presence of Carbapenemases through Molecular Methods:** Total DNA extraction from the isolates was performed by the 'sand method' (8). The presence of carbapenemase genes (blaVIM, blaIMP, blaKPC, blaOXA48, and blaNDM) was tested by multiplex PCR. The primer sequences used in the molecular determination of the presence of carbapenemase and the band sizes obtained are shown in Table 1.

**Table 1.** Primer sequences used in the molecular determination of carbapenemase presence and obtained band sizes

| Primer | Sequence 5'-3' | Product size(bp) |
|---|---|---|
| ***bla* KPC** | 5'-TGTCACTGTATCGCGGTC-3'<br>5'-CTCAGTGCTCTACAGAAAAAC-3' | 900 |
| ***bla* NDM** | 5'-CTCAGTGCTCTACAGAAAAAC-3'<br>5'-GCAGCTTGTCGGCCATGCGGGC-3' | 782 |
| ***bla* VIM** | 5'-GATGGTGTTTGGTCGCATA-3'<br>5'-CGAATGCGCAGCACCAG-3' | 390 |
| ***bla* IMP** | 5'-GGAATAGAGTGGCTTAAYTCT-3'<br>5'-CCAAACYACTASGTTATCT-3' | 188 |
| ***bla*OXA-48** | 5'-GCGTGGTTAAGGATGAACAC-3'<br>5'-CATCAAGTTCAACCCAACCG-3'5'- | 438 |

## RESULTS

Of the species isolated during the study period, 1221 were *E. coli* and 378 were *K. pneumoniae*. Of these isolates, 80 were carbapenem resistant isolates. Of these isolates, 70 were *K. pneumoniae* and 10 were *E. coli*. Of the isolates; 43 (51%) were recovered from urine samples, 17 (24%) were recovered from blood samples, 12 (15%) were recovered from wound swab samples, and 8 (10%) were recovered from tracheal aspirates. Of the 80 isolates suspected to have carbapenemases, 61 (76.2%) had at least one carbapenemase gene that was identified via genotypic tests. The presence of carbapenemases was detected in 6 out of 10 (60 %) suspected *E. coli* isolates and in 55 out of 70 (78.5%) *K. pneumoniae* isolates via phenotypic tests. The presence of carbapenemases detected in phenotypic tests was confirmed by the multiplex PCR analysis. It was observed that phenotypic tests were 100% compatible with molecular methods to detect the presence of carbapenemases. Of the isolates with carbapenemases, 45 (73.7%) were found to have the OXA-48 enzyme. The second most common enzyme was NDM. The distribution of identified carbapenemases by species is presented in Table 2.

**Table 2.** Distribution of detected carbapenemase enzymes by species

| Carbapenemase gene | *K.pneumoniae* | *E. coli* | Total |
|---|---|---|---|
| Gene undetectable | 15 | 4 | 19 |
| OXA-48 | 40 | 5 | 45 |
| VIM | 4 | _ | 4 |
| NDM | 6 | _ | 6 |
| KPC | _ | _ | _ |
| OXA-48 + VIM | 3 | 1 | 4 |
| OXA-48 + NDM | 2 | _ | 2 |
| **Total** | 70 | 10 | 80 |

MIC values of imipenem, meropenem, and ertapenem were tested in the isolates via the microdilution method. All isolates (100%) were found to be resistant to ertapenem. The assessment of the efficacy of the β-lactam/β-lactamase inhibitor combinations against CRE isolates revealed that the activity of CAZ-AVB (77%) was higher than CLZ-TAZ (48%). The sensitivity rates to CAZ-AVB were 76% (0.094-256 μg/ml) and 78% (0.094-16 μg / ml) for *K. pneumoniae* and *E. coli*, respectively. Sensitivity rates to CLZ-TAZ were 52% (1-256 μg / ml) for *K. pneumoniae* and 44% (0.38-16 μg / ml) for *E. coli*.

## DISCUSSION

*Enterobacteriaceae* are common pathogens causing a variety of severe infections. Carbapenemase-producing *Enterobacteriaceae* strains cause infections that are treated with combined antibiotherapy regimens but the mortality is high (2). Many carbapenem hydrolyzing enzymes have been identified in gram-negative bacilli. The most common ones that are responsible for resistance are Ambler class A (KPC type), class B (VIM, NDM, and IMP types), and class D (OXA-48-like) enzymes (4).

Of a total of 80 carbapenemase-producing *Enterobacteriaceae* species included in our study, 52 (73.2%) had at least one carbapenemase gene identified through genotypic methods. The most common species was *Klebsiella* spp. having carbapenemase genes at a rate of 87.3%. The results of studies in the literature are compatible with our study results. Those studies reported carbapenemase production most commonly in *K. pneumoniae* followed by *E. coli* (4,5,10,11). Çaycı et al. reported that carbapenem-resistant Klebsiella spp. and *E. coli* were found at rates of 71.43% and

1.54% in their study, respectively (4). A multi-center study conducted in Turkey reported rates of carbapenem-resistant *Klebsiella* spp. and *E. coli* as 86.5% and 13.5%, respectively (5). Similar to other studies, the rates of carbapenem-resistant *Klebsiella* spp. and *E. coli* were 87.3% and 12.6% in our study, respectively.

When we evaluated the distribution of the clinical samples, we observed that carbapenemase-producing *Enterobacteriaceae* strains were found in urine, blood, wound swab, and tracheal aspirate cultures in the decreasing order of frequency at rates of 51%, 24%, 15%, and 10%, respectively. Similar to our study results, other studies reported that urine and blood samples were the leading sample types with the highest frequencies (4,12-14).

OXA-48 has remained to be an enzyme locally found only in our country, however, isolates with OXA-48 have started to cause outbreaks in many countries with increasing prevalence rates. The spread of bacteria with acquired carbapenemases constitutes a major public health issue. Therefore, the identification of such isolates is critical to control infections (5,12). In our study, the blaOXA-48 gene was found in 73.7% of CRE in total. The blaOXA-48 gene was found in 88.8% of *K. pneumoniae* isolates and 11.1% of *E. coli* isolates. In a study, Çelikbilek et al. found out that more than 90% of carbapenem-resistant *K. pneumoniae* isolates were blaOXA-48 positive (13). A multicenter study that included patients from various regions of Turkey reported a high prevalence rate (83%) for the presence of the blaOXA-48 gene in CRE. The blaOXA-48 gene was found in 85.1% of *K. pneumoniae* isolates and 14.8% of *E. coli* isolates (4). In the study conducted by Üsküdar et al., of the 130 samples, 121 (78%) were positive for the blaOXA-48 gene. The blaOXA-48 gene was found in 103 *K. pneumoniae* samples and in 18 *E. coli* samples in that study (14). Another study reported that, out of 181 clinical samples, 88 (%47.5) were positive for the blaOXA-48 gene with rates of 38.1% and 7.1% for *K. pneumoniae* and *E. coli*, respectively (12).

In our study, 6 isolates were positive for the blaNDM-1gene, 4 isolates were positive for the blaVIM gene, and 3 isolates were positive for the blaNDM1 and blaOXA-48 genes concomitantly. A multicenter study reported that 9 (6.3%) isolates were positive for the blaNDM-1gene, 4 (2.8%) isolates were positive for the blaVIM gene, and 3 (2.1%) isolates were positive for the blaNDM1 and blaOXA-48 genes concomitantly (4). A study by Irmak et al. reported that, of the CRE isolates, 6 (3.3%) were positive for the blaNDM-1 gene, 1 (1.45%) isolate was positive for the blaVIM gene, and 3 (1.6%) blaNDM-1 positive isolates had the blaOXA-48 gene concomitantly (12). Another study reported the presence of blaNDM-1in 6 (6.5%) isolates but the concomitant presence of the blaNDM1 and blaOXA-48 genes was not detected in any of the isolates (13). A study by Üsküdar et al. reported that 9 (7.1%) isolates were positive for the blaNDM-1gene but no isolates had the blaVIM resistance genes. No isolates had the blaNDM1 and blaOXA-48 genes concomitantly (14).

Carbapenems are considered the last resort in the treatment of infections caused by MDR-gram-negative bacteria (3,4). The emergence of carbapenem-resistant pathogens in parallel to the increasing use of carbapenems in clinical practice poses a major threat to human health. Therefore, antibiotic resistance of such bacteria is associated with critical clinical and socioeconomic effects (3,15). Because of the limited availability of therapeutic options, colistin, administered alone or in combination, has become the main antimicrobial agent for the treatment of such infections (15). However, previous studies have reported high rates of treatment failure in association with colistin therapy (16). New combinations of β-lactams, including CAZ–AVB and CLZ–TAZ, have strong activity *in vitro* against CPE and have the potential to replace colistin (15,16).

Viala et al. found the sensitivity rates of OXA-48-beta-lactamase-producing *Enterobacteriaceae* as 26/27 (96%) to CAZ–AVB and 8/27 (30%) to CLZ–TAZ. The study reported that CAZ–AVB was more effective than CLZ–TAZ in OXA-48-beta-lactamase-producing Enterobacteriaceae infections. Viala et al. demonstrated the benefits of CAZ–AVB in the empirical treatment of suspected bacterial infections (17). In another study, CAZ–AVB (MIC 50/90, 1/2 mg/L) exhibited 97.5% activity while CLZ–TAZ (MIC50/90, 0.25/2 mg/L) showed 86.9% activity against CRE according to EUCAST criteria (18).

Alatoom et al. reported 45% sensitivity to CAZ–AVB and 10% to CLZ–TAZ in their study, in which 60 CRE isolates (49 *K. pneumoniae* and 11 *E. coli*) were tested (19). Yin et al., in their study on 372 CRE isolates, found that CAZ-AVB (75%) had better activity than CLZ -TAZ (6.2%) when efficacy against all CRE isolates was evaluated. On the other hand, CAZ-AVB had much higher antibacterial activity against carbapenem-resistant *K. pneumoniae* (85%) than that against carbapenem-resistant *E. coli* (28.6%). While 28.6% of *E. coli* isolates and 85% of *K. pneumoniae* isolates were susceptible to CAZ-AVB, only 7.1% and 1.9% of them were susceptible to CLZ-TAZ, respectively (20). Shortridge et al. found that, against *Enterobacteriaceae*, CLZ-TAZ showed favorable activity with 87.5% sensitivity of non-CRE phenotype strains but lacked activity against CRE with 2.4% sensitivity (21). Zhang et al found that CAZ -AVB showed high antibacterial activity with 96.3% sensitivity in their study on a total of 872 carbapenemase-positive *Klebsiella* isolates (22). In our study, the sensitivity rate of the CAZ-AVB combination for carbapenem-resistant

*Enterobacteriaceae* strains isolated from various clinical samples was higher than that of CLZ-TAZ. CAZ-AVB sensitivity rates were 76% (0.094-256 μg /ml) for *K. pneumoniae* and 78% (0.094-16 μg / ml) for *E. coli*. CLZ-TAZ sensitivity rates were 52% (1-256 μg / ml) for *K. pneumoniae* and 44% (0.38-16 μg / ml) for *E. coli*.

**CONCLUSION**

The development of novel agents for the treatment of highly resistant gram-negative pathogens is critical for the availability of therapeutic options. The sensitivity rates with ceftazidime-avibactam were higher than those of ceftolozane-tazobactam. It has been found that ceftazidime-avibactam exhibits better activity against other carbapenem-resistant isolates, except those carrying the NDM-1 enzyme. The data obtained in this study will soon be used in our country to guide the clinical use of these agents. Our findings suggest that CAZ-AVB and CLZ-TAZ may be promising for the treatment of infections caused by CRE strains.

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RESEARCH ARTICLE

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# Post-Traumatic Stress and Associated Factors among Healthcare Workers in the Early Stage Following the 2020 Malatya-Elazig Earthquake

**ABSTRACT**

**Objective:** An earthquake is a natural disaster that seriously affects individuals physically and psychologically. Although there has been a great deal of research on the psychological effects of earthquakes, few have focused on local health workers and its early effects. In this study, it is aimed to determine the levels and predictors of early post-earthquake trauma of the local health workers working in the affected area in the earthquake that occurred on January 24, 2020, Malatya-Elazig.

**Methods:** This cross-sectional descriptive study was carried out on a sample of 201 healthcare workers after three weeks from of the earthquake. In order to determine the factors that may affect the trauma response in the participants, a questionnaire was applied to question demographic variables, previous traumatic experiences, concerns and losses at the time of the earthquake, and institutional and social expectations. Post-Earthquake Trauma Level Determining Scale was used to record post-earthquake trauma levels, and TEMPS-A was used to determine dominant temperament characteristics.

**Results:** Severe trauma level was detected in 25.8% of the participants. Trauma scores were higher in women, those who were married, those who had children, those who experienced the earthquake for the first time and those who had anxiety about losing their own life or their relatives life during the earthquake. In the logistic regression analysis, it was determined that anxious temperament and fear of losing a loved one during an earthquake increased the severity of trauma, whereas a previous earthquake history decreased it.

**Conclusions:** Detection of the factors associated with the trauma response is important both in terms of protecting the mental health of health workers and ensuring the continuity of health services in disasters such as earthquakes that affect millions of people.

**Keywords:** Healthcare Worker, Earthquake, Trauma, Sociodemographic Characteristics, Temperament, Related Factors.

# 2020 Malatya-Elazığ Depremi Sonrası Erken Dönemde Sağlık Çalışanları Arasında Travma Sonrası Stres ve İlişkili Faktörler

**ÖZET**

**Amaç:** Deprem fiziksel ve psikolojik olarak bireyleri ciddi şekilde etkileyen bir doğa felaketidir. Depremin oluşturduğu psikolojik etkiler üzerine yürütülen çok sayıda araştırma olmasına rağmen, bunların çok azı yerel sağlık çalışanlarına ve erken dönem etkilerine odaklanmıştır. Bu çalışmada, 24 Ocak 2020 Malatya-Elazığ depreminde, etkilenen bölgede çalışan yerel sağlık çalışanlarındaki erken dönem travma düzeylerinin ve yordayıcılarının tespit edilmesi amaçlanmıştır.

**Gereç ve Yöntem:** Kesitsel-tanımlayıcı nitelikteki bu çalışma, depremden üç hafta sonra 201 sağlık çalışanından oluşan bir örneklem üzerinde yürütülmüştür. Katılımcılara travma yanıtını etkileyebilecek faktörleri tespit etmek için demografik değişkenleri, önceki travmatik yaşantıları, deprem anındaki kaygı ve kayıpları, kurumsal ve sosyal beklentileri araştıran bir anket uygulandı. Deprem sonrası travma düzeylerinin belirlenmesinde "Deprem Sonrası Travma Düzey Belirleme Ölçeği" ve baskın mizaç özelliklerinin belirlenmesinde TEMPS-A kullanıldı.

**Bulgular:** Katılımcıların %25.8'inde şiddetli travma düzeyi tespit edildi. Kadınlarda, evli olanlarda, çocuğu olanlarda, ilk kez deprem yaşamış olanlarda deprem sırasında yakınları ve kendi hayatını kaybetmekle ilgili kaygı yaşayanlarda travma puanları yüksekti. Yapılan lojistik regresyon analizinde anksiyöz mizaç özelliği ve deprem sırasında bir yakınını kaybetme korkusu yaşamanın travma şiddetini arttırdığı tespit edilirken, daha önce deprem yaşama öyküsünün azalttığı belirlendi.

**Sonuç:** Travma yanıtıyla ilişkili faktörlerin tespiti hem sağlık çalışanlarının ruhsal sağlığını korumak hem de milyonlarca insanı etkileyen deprem gibi afetlerde sağlık hizmetlerinin devamlılığını sağlamak açısından önemlidir.

**Anahtar Kelimeler:** Sağlık Çalışanı, Deprem, Travma, Sosyodemografik Özellikler, Mizaç, İlişkili Faktörler.

## INTRODUCTION

Traumatic experiences occur during the normal course of life and disrupt people's adaptation to life by deactivating their coping mechanisms (1). Unlike ordinary adversities, traumatic events involve threat, violence or death risk to the lives or integrity of the individuals (2). Traumatic experiences are generally common, one study showed that more than two-thirds of the general population may experience a significant traumatic event at some point in their lives (3). War, violence, terrorist attack etc. and natural and man-made disasters are among the important traumatic events (4).

Earthquakes are one of the most studied natural disasters because they occur frequently and affect people's lives profoundly and cause significant psychological damage (3,5). Most research studies on the psychological impact of earthquakes concluded that survivors display different levels of post-traumatic stress symptoms (6). If these symptoms are not properly treated promptly, they can develop into depression, post-traumatic stress disorder (PTSD) etc. (7). PTSD is an important disease that is common after an earthquake, causes serious disability and persists for a long time (3,5). For this reason, it is important to recognize the early psychological stress after the earthquake and to determine the related factors in terms of protecting both personal and public health (8-12).

The probability of developing PTSD after an earthquake is related to the magnitude of the earthquake, preparedness, location during the earthquake, injuries or near-death experiences, dead or missing family members, material losses, reactions to the trauma (dissociation, coping and other reactions), work-related stress factors (lack of communication, heavy workload, etc.) and post-traumatic factors (symptoms, social support and other daily-life sources of stress) (13,14). However, exposure to disaster trauma might not be enough on its own to explain how individuals develop PTSD. Sex, age, education, family history, psychiatric disorders, genetic and neuroendocrine factors, temperament traits and early and late-life traumas might increase individual vulnerability to developing PTSD (15,16).

In recent years, although a large number of studies focused on victims and “traditional” first responders such as police officers, firefighters, emergency medical technicians and military personnel (17-22), body of research on general healthcare professionals is rather limited. Most of these studies have focused on the resistance and reaction levels of healthcare professionals and especially on the mild and long-term psychological impacts of earthquakes, neglecting early-term effects (23,24).

Therefore, targeting this gap concerning healthcare professionals is vital. This is because these individuals, in addition to being survivors of the disaster, play an important role in terms of maintaining the continuation of healthcare services during the earthquake, participating in rescue efforts when necessary, and restoring the psychological health of society (25).

On January 24, 2020, a large earthquake with a magnitude of 6.8 on the Richter scale, where the epicenter is in Sivrice district of Elazig, occurred in Eastern Turkey, lasting approximately 22 seconds on 20.55.pm. Thousands of people were exposed to a life-threatening situation due to the earthquake and forty one people died while 1607 people were injured. More than 1000 aftershocks that occurred within the weeks following the earthquake in the region, which has not experienced any major earthquakes for nearly 150 years, caused fear and anxiety in the entire population (26).

In this study, it was aimed to determine the levels and predictors of early post-earthquake trauma of the local health workers in the 3rd week after the 2020 Malatya-Elazig earthquake.

## MATERIAL AND METHODS

The study was designed as a cross-sectional study and approved by the Ethical Committee of Malatya Inonu University (2020/499). Three weeks after the earthquake, 201 people selected from the health care workers working in the community health center, family health center, and 112 health centers affiliated to Malatya Provincial Health Directorate were included in the study. Since participation in the study was voluntary, patients who refused to participate and whose data were missing were excluded from the study.

**Data Form:** The researchers handed out a survey form consisting of fourth parts to the participants. The first part of the survey included questions on sociodemographic characteristics concerning age, sex, education level, marital status, parental status, family type (nuclear: parents and their children, or extended: parents and their children as well as other relatives) monthly income, history of psychiatric disorders, and history of chronic medical disease (diabetes, malignancy, cardiovascular diseases etc). The second part consisted of questions assessing traumatic incidents occurring during any life stage of the participants (childhood trauma, accident, assault, other natural disasters, etc.). The third part of the survey included questions about the earthquake which were prepared by the authors inspired from previous studies. These questions are given in Table 2. In the fourth part, there were questions about the scales investigating trauma level and temperament characteristics.

The survey forms were given to all participants after the earthquake within two consecutive days to minimize temporal differences in trauma response.

**Post-Earthquake Trauma Level Determining Scale:** The scale was developed by Tanhan and Kayri following the Van earthquake in Turkey to measure levels of post-traumatic stress (27). The scale analyzes 5 factors indicating trauma response, namely "Behavior Problems" (appetite loss, becoming more angry/aggressive, nightmares, claustrophobia arising from the fear of another earthquake), "Emotive Limitedness" (loss of hopes for the future, loss of a sense of the meaning of life, decrease in the will to live, feeling helpless/weak), "Affective Response" (feeling abashed because of needing help, increased attention on behaviors/relationships after the earthquake, appreciating life more, becoming more emotional/crying without any reason), "Cognitive Structure" (worries about children/parents/acquaintances/friends, constant anxiety about another earthquake, reliving the instances of the earthquake, worries about the future), and "Sleep Problems" (waking up suddenly, having trouble falling asleep, sleeping less). The lowest score in the scale of 20 items is 20 while the highest possible score is 100. The increase in the scores indicates the rise in the extent to which individuals are affected by the earthquake. As the original scale stated that the score range of 52.385±5.051 (mean±SD) indicates a threshold at which individuals are traumatized. Above and below this threshold value points out low and high levels of traumatization. In this study, the threshold value was accepted as 52.385 (27). The measured Cronbach alpha internal consistency coefficient of the scale was 0.66 for the 'Behaviour Problems' sub-category, 0.70 for 'Emotive Limitedness', 0.72 for 'Affective Response', 0.59 for 'Cognitive Structure', 0.89 for 'Sleep Problems' and 0.93 for all items.

**Temperament Evaluation of Memphis, Pisa, Paris, San Diego-Auto-Questionnaire (TEMPS-A):** Developed by Akiskal and colleagues in 1997 to evaluate the dominant affective temperament (28), the scale was translated into Turkish by Vahip and colleagues in 2005, who then tested its validity and reliability (29). The scale consists of 5 sub-sections and a total of 99 items to identify depressive, hyperthymic, cyclothymic, irritable, and anxious temperaments.

In this study, the Cronbach alpha internal consistency coefficient of the scale was calculated as 0.762 for the depressive subgroup, 0.875 for the cyclothymic, 0.837 for the hyperthymic and 0.870 for the anxious in the reliability analysis of the scale. The internal reliability coefficient, which includes all subgroups of the Temperament Evaluation of Memphis, Pisa, Paris, San Diego-Auto-Questionnaire (TEMPS-A), was 0.910.

**Statistical Analysis:** In all analyses, the SPSS 23.0 software was used. Continuous variables are expressed as mean±standard (mean±SD) deviation. Categorical variables were compared using Chi-square tests and expressed as numbers and percentages. The suitability of the data for normal distribution was determined by the Kolmogorov-Smirnov test. Continuous variables with normal distribution were compared between groups using Student's t-test. Categorical comparisons were made using the chi-square test. One-way ANOVA tests were used to evaluate the difference between more than two independent groups. Multiple comparisons were carried out by Bonferroni test. Pearson correlation test was used in the correlation analysis. Logistic regression analysis was used to identify independent predictors of trauma response severity. A value of $p<0.05$ was considered statistically significant.

## RESULTS

In this study, 201 healthcare workers participated, of whom 143 (71.1%) were female and the remaining 58 (28.9%) were male. The mean age of the participating healthcare professionals was 35.9 ± 8.9 years. Seventy-five (37.4%) of the participating healthcare professionals were physicians, 70 (34.9%) were nurses or midwives, 31 (15.3%) were health clerks and 25 (12.4%) were emergency medical technicians.

The PETS total score was 41.32 ± 16.24. The sub-scores were as follows: 'Behaviour Problems', 6.27 ± 3.1; 'Emotive Limitedness', 8.49 ± 4.72; 'Affective', 8.68 ± 3.51; 'Cognitive Structures', 10.61 ± 4.63; and 'Sleep Problems', 6.80 ± 3.78.

None of the participants included in the study had a history of earthquake injury. Six participants (3%) had relatives with a history of injury in an earthquake, of whom two (1%) lost one relative in the earthquake. After the earthquake, only one person, who was a health worker, received psychological support.

The analysis of the relationship between sociodemographic variables and trauma scores of healthcare professionals revealed that female participants had statistically higher total scores and sub-scores on the PETS scale compared to their male colleagues ($p<0.001$ for all domains). In terms of marital status, married participants had statistically higher PETS sub-scores for the cognitive dimension of the scale ($p=0.048$) with no significant differences for other sub-scores ($p>0.05$). The PETS total, emotional, affective and cognitive scores were significantly higher in those with at least one child compared to those without children ($p=0.002$, $p=0.001$, $p=0.015$, $p=0.002$, respectively). The participants living within a nuclear family had higher cognitive PETS sub-scores ($p=0.028$) with no other differences when compared to those living alone or within an extended family. Those with a history of psychiatric disorders had a higher emotive PETS sub-score than those without such a history ($p=0.047$) (Table 1).

**Table 1.** The relationship between the socio-demographic characteristics and the scores of the PETS of healthcare professionals

| Socio-demographic characteristics | n (%) | PETS total score mean±SD | *p value* | Behavior Problems score mean±SD | *p value* | Emotive Limitedness score mean±SD | *p value* | Affective score mean±SD | *p value* | Cognitive Structures score mean±SD | *p value* | Sleep Problems score mean±SD | *p value* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gender** | | | | | | | | | | | | | |
| Male | 143(71.1) | 44.7±16.5 | **<0.001**** | 6.8±3.3 | **<0.001**** | 9.1±4.9 | **<0.001**** | 11.6±4.4 | **<0.001**** | 9.3±3.3 | **<0.001**** | 7.6±3.7 | **<0.001**** |
| Female | 58(28.9) | 32.8±11.7 | | 4.9±1.6 | | 7.1±3.3 | | 8.1±3.6 | | 7.3±3 | | 5 ±2.9 | |
| **Marital Status** | | | | | | | | | | | | | |
| Married | 152(75.6) | 42.4±16.4 | 0.064 | 6.4±3 | 0,187 | 8.8±4.7 | 0.161 | 11±4.5 | 0.137 | 8.9±3.4 | **0.048*** | 7±3.8 | 0.278 |
| Single/Widow/Divorced | 49(24.4) | 37.7±15.1 | | 5.9±3.2 | | 7.6±4 | | 9.6±4.5 | | 8.1±3.2 | | 6.3±3.3 | |
| **Having Children** | | | | | | | | | | | | | |
| Yes | 133(66.1) | 43.7±16.5 | **0.002*** | 6.5±3.1 | 0.05 | 9.2±4.8 | **0.001*** | 11.3±4.5 | **0.015*** | 9.2±3.3 | **0.002*** | 7.1±3.9 | 0.192 |
| No | 68(33.9) | 36.6±14.6 | | 5.7±2.8 | | 7.2±3.7 | | 9.2±4.3 | | 8±3.2 | | 6.3±3.2 | |
| **Family Type** | | | | | | | | | | | | | |
| Nuclear family | 153(76.1) | 42.6±16.3 | | 6.4±3.0 | | 8.8±4.7 | | 9.0±3.4 | | 11.0±4.4 | | 7.0±3.9 | |
| Extended family | 29(14.4) | 36.2±14.2 | 0.110 | 5.2±2.3 | 0.139 | 6.7±3.7 | 0.077 | 7.5±3.3 | 0.050 | 9.6±5.1 | **0.028*** | 5.7±3.3 | 0.262 |
| Alone | 19(9.5) | 38.5±17.1 | | 6.5±3.4 | | 8.0±5.3 | | 7.7±3.9 | | 8.4±4.7 | | 6.7±3.7 | |
| **History of psychiatric disorder** | | | | | | | | | | | | | |
| Yes | 7(3.5) | 45.7±17.7 | 0.500 | 6.4±2.5 | 0.721 | 13±7 | **0.047*** | 11±3.9 | 0.487 | 7.8±3.2 | 0.796 | 7±3.9 | 0.885 |
| No | 194(96.5) | 41.1±16.2 | | 6.3±3 | | 8.4±4.4 | | 10.6±4.5 | | 8.8±3.3 | | 6.8±3.7 | |
| **History of chronic medical disease** | | | | | | | | | | | | | |
| Yes | 28 (14) | 39.8±14.6 | 0.734 | 5.6±2.2 | 0.349 | 7.9±4 | 0.483 | 11.1±4.4 | 0.720 | 8.4±3.1 | 0.561 | 6.5±3.7 | 0.675 |
| No | 173 (86) | 41.5±16.5 | | 6.4±3.1 | | 8.6±4.7 | | 10.5±4.5 | | 8.8±3.4 | | 6.9±3.7 | |
| **Family history of psychiatric disorder** | | | | | | | | | | | | | |
| Yes | 14 (7) | 45±17.1 | 0.363 | 6.9±3 | 0.239 | 10.3±6.4 | 0.330 | 11±3.8 | 0.739 | 9±3 | 0.718 | 7.7±3.8 | 0.357 |
| No | 187 (93) | 41±16.1 | | 6.2±3.0 | | 8.4±4.4 | | 10.6±4.6 | | 8.7±3.4 | | 6.8±3.7 | |

*** p<0.05, **p<0.001**

**Table 2.** Relationship between the history of past traumas/earthquake experiences and the data on the Elazıg-Malatya earthquake and PETS scores

| | n (%) | PETS total score mean±SD | *p value* | Behavior Problems score mean±SD | *p value* | Emotive Limitedness score mean±SD | *p value* | Affective score mean±SD | *p value* | Cognitive Structures mean ±SD | *p value* | Sleep Problems score mean±SD | *p value* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **History of traumatic experience** | | | | | | | | | | | | | |
| Yes | 21 (10.5) | 40.1±11.7 | 0.780 | 6.1±1.9 | 0.546 | 7.5±3.9 | 0.257 | 10.8±3.9 | 0.273 | 7.7±2.7 | 0.625 | 7.8±3.4 | 0.140 |
| No | 180(89.5) | 41.4±16.5 | | 6.3±3.1 | | 8.6±4.6 | | 10.6±4.6 | | 8.8±3.4 | | 6.8±3.7 | |
| **History of experience of Earthquake** | | | | | | | | | | | | | |
| Yes | 75 (37.4) | 36.2±12.2 | **0.002*** | 5.3±1.9 | **0.005*** | 7.3±3 | **0.016*** | 9.6±4.3 | **0.001*** | 7.7±3 | **0.014*** | 6.1±3.1 | 0.061 |
| No | 126(62.6) | 44.2±17.6 | | 6.8±3.4 | | 9.3±5.2 | | 11.2±4.6 | | 9.3±3.4 | | 7.3±3.9 | |
| **Which province were you in during the earthquake?** | | | | | | | | | | | | | |
| Elazığ/Malatya | 181(90) | 42±16.5 | 0.069 | 6.3±3.1 | 0.356 | 8.7±4.7 | 0.137 | 10.9±4.5 | **0.031*** | 8.9±3.4 | **0.016*** | 6.9±3.7 | 0.234 |
| Other | 20(10) | 34.8(±11.6) | | 5.8±2.4 | | 7.3±3.4 | | 8.3±3.4 | | 7.3±2.7 | | 6±3.3 | |
| **Were you alone during the earthquake?** | | | | | | | | | | | | | |
| Yes | 12(6) | 40.8±15.9 | 0.961 | 6±2.8 | 0.840 | 7.3±4.7 | 0.126 | 9.3±2.8 | 0.457 | 11±4.7 | 0.847 | 7±3.6 | 0.783 |
| No | 189 (94) | 41.3±16.3 | | 6.3±3 | | 8.6±4.6 | | 8.7±3.4 | | 10.6(±4.5) | | 6.8±3.7 | |
| **Did you ever think that you would die during the earthquake?** | | | | | | | | | | | | | |
| Yes | 105(52.2) | 46.8±17.1 | **<0.001**** | 7.1±3.5 | **<0.001**** | 9.5±5.1 | **0.001*** | 9.7±3.3 | **<0.001**** | 11.9±4.4 | **<0.001**** | 8.2±3.8 | **<0.001**** |
| No | 96(47.8) | 35.3±12.7 | | 5.4±2.1 | | 7.5±3.7 | | 7.7±3.1 | | 9.2±4.2 | | 5.3±2.9 | |
| **Did you ever think that a family member of you would die during the earthquake?** | | | | | | | | | | | | | |
| Yes | 124(61.6) | 45.5±16.8 | **<0.001**** | 6.8±3.3 | **0.001*** | 9.2±5) | **0.021*** | 9.5±3.3 | **<0.001**** | 11.8±4.5 | **<0.001**** | 7.8±3.8 | **<0.001**** |
| No | 77(38.4) | 34.4±12.6 | | 5.4±2.3 | | 7.5±3.5 | | 7.5±2.9 | | 8.8±3.9 | | 5.2±2.8 | |
| **Have you ever seen someone died or injured during the earthquake?** | | | | | | | | | | | | | |
| Yes | 29(14.5) | 41.4±16.8 | 0.986 | 6.6±3.3 | 0.764 | 7.6±3.6 | 0.462 | 9.1±3.5 | 0.621 | 10.9±4.8 | 0.756 | 7.1±4.2 | 0.950 |
| No | 172(85.5) | 41.2±16.1 | | 6.2±3 | | 8.7±4.7 | | 8.7±3.3 | | 10.6±4.5 | | 6.8(±3.6) | |
| **Was your home damaged?** | | | | | | | | | | | | | |
| Yes | 33(16.5) | 43.2±16.4 | 0.455 | 6.2±3.2 | 0.665 | 9.3±5.2 | 0.435 | 8.6±3.2 | 0.867 | 11.8±4.8 | 0.125 | 7±4 | 0.877 |
| No | 168(83.5) | 40.9±16.2 | | 6.3±3 | | 8.4±4.4 | | 8.8±3.4 | | 10.4±4.4 | | 6.8±3.6 | |
| **Have you experienced material loss?** | | | | | | | | | | | | | |
| Yes | 22(11) | 42.1±17.5 | 0.941 | 7.3±3.6 | 0.115 | 9.2±4.8 | 0.486 | 8.7±4.1 | 0.792 | 10.5±4.5 | 0.943 | 6.2±3.6 | 0.391 |
| No | 179(89) | 41.2±16.1 | | 6.1±2.9 | | 8.5±4.6 | | 8.8±3.2 | | 10.6±4.5 | | 6.9±3.7 | |
| **Have you received help from official institutions after the earthquake?** | | | | | | | | | | | | | |
| Yes | 19(9.5) | 43±17.4 | 0.729 | 6.8±3.8 | 0.656 | 8.5±4.2 | 0.951 | 9.4±3.7 | 0.349 | 11.1±5.2 | 0.754 | 6.9±4.1 | 0.878 |
| No | 182(90.5) | 41.1±16.1 | | 6.2±2.9 | | 8.5±4.6 | | 8.7±3.3 | | 10.6±4.4 | | 6.8±3.6 | |
| **Have you received support from your family or friends, after the earthquake?** | | | | | | | | | | | | | |
| Yes | 128(63.6) | 41.7±16.3 | 0.568 | 6.4±3.2 | 0.791 | 8.7±4.5 | 0.286 | 8.8±3.2 | 0.808 | 10.7±4.5 | 0.563 | 6.8±3.6 | 0.924 |
| No | 73(36.4) | 40.5±16.1 | | 6±2.6 | | 8.3±4.7 | | 8.6±3.5 | | 10.4±4.6 | | 6.9±3.7 | |
| **Can anything be done in order to be prepared and decrease the damages of the earthquake?** | | | | | | | | | | | | | |
| Many things can be done | 108(53.7) | 41.7±17.7 | 0.835 | 6.4±3.4 | 0.471 | 8.8±4.9 | 0.587 | 8.8±3.4 | 0.999 | 10.7±4.7 | 0.969 | 6.9±3.9 | 0.829 |
| Some/A little things can be done | 93(46.3) | 40.8±14.4 | | 6.2±2.6 | | 8.2±4.2 | | 8.7±3.3 | | 10.5±4.2 | | 6.8±3.4 | |

*** p<0.05, **p<0.001**

The analysis of the relationship between the history of past traumas and earthquake experiences and the trauma levels of healthcare professionals in Elazig-Malatya earthquake showed no statistically significant differences between participants with and without a history of past traumas (p=0.780). Those experiencing a fear of death during the earthquake had higher trauma scores when compared to those who did not have such experience (p<0.001). Individuals experiencing the fear of losing loved ones during the earthquake had higher trauma score when compared to those who did not feel such a fear (p<0.001). (Table 2)

To analyze the factors underlying severe trauma response of healthcare professionals, the participants were divided into two groups according to trauma response severity. One group consisted of 52 subjects (25.8%) who displayed severe trauma response and another group consisted of the remaining 149 subjects (74.2%) who demonstrated low trauma response. Of the men and women, 10.6% and 89.4% displayed severe trauma response, respectively (p=0.02). The analysis of the TEMPS scores revealed significantly higher cyclothymic (p<0.035), depressive (p<0.007) and anxious temperament (p<0.001) levels among those with a severer trauma level.

Upon examining the relationship between trauma levels and sociodemographic variables, we found that being female, being married, having children, not having a history of earthquake experience, being at the epicenter during the earthquake, experiencing the fear of dying and having the fear of losing a relative during earthquake were the most frequent factors among those with higher trauma levels (p=0.002, p=0.034, p=0.015, p<0.001, p=0.028, p<0.001 and p<0.001, respectively). No correlation was found between the other variables and trauma levels (p > 0.05). (Table3)

**Table 3.** The factors determining trauma levels among healthcare professionals.

| Variable | | High level n(%) | Low level n(%) | p value |
|---|---|---|---|---|
| **Gender** | Female | 42(89.4) | 101(65.6) | **0.002*** |
| | Male | 5(10.6) | 53(34.4) | |
| **Marital status** | Married | 41(87.2) | 111(72.1) | **0.034*** |
| | Single/Widow/Divorced | 6(12.8) | 43(27.9) | |
| **Having children** | Yes | 38(80.9) | 95(61.7) | **0.015*** |
| | No | 9(19.1) | 59(38.3) | |
| **History of earthquake experience** | No | 40(85.1) | 87(56.5) | **<0.001**** |
| | Yes | 7(14.9) | 67(43.5) | |
| **Which province were you in during the earthquake?** | Elazığ/Malatya | 46(97.9) | 135(87.7) | **0.028*** |
| | Other | 1(2.1) | 19(12.3) | |
| **Did you ever think that you would die during the earthquake?** | No | 11(23.4) | 85(55.2) | **<0.001**** |
| | Yes | 36(76.6) | 69(44.8) | |
| **Did you ever think that a family member of you would die during the earthquake?** | No | 7(14.9) | 70(45.5) | **<0.001**** |
| | Yes | 40(85.1) | 84(55.5) | |
| **TEMPS-A** | Depressive | 6.2±3.4 | 4.6±3 | **0.007*** |
| | Cyclothymic | 5.7±3.9 | 4.5±3.9 | **0.035*** |
| | Anxious | 8±5.4 | 4.5±4.4 | **<0.001**** |

*** p<0.05, **p<0.001** (Only significant correlations are listed on the table)

According to the logistic regression analysis, a significant relationship was found between the previous earthquake experience, the fear of death of a family member, and the level of anxiety and trauma. It was observed that the previous earthquake experience was protective from the high trauma response, and the fear of losing a relative during an earthquake was 3.383 (1.081-10.591) times more risky. It was determined that a one-point increase in the anxiety score caused a 1.137 (1.030-1.254) fold increase in the trauma level. (Table 4)

**Table 4.** Logistic regression analysis results of factors affecting trauma severity

| Variables | | OR (95% C.I.) | *p* |
|---|---|---|---|
| **Gender** | Female | 1 | 0.091 |
| | Male | 2.591 (0.859-7.814) | |
| **Marital status** | Married | 1 | 0.846 |
| | Single/Widow/Divorced | 1.153 (0.276-4.807) | |
| **Having children** | Yes | 1 | 0.162 |
| | No | 2.377 (0.705-8.015) | |
| **History of earthquake experience** | No | 1 | **0.002*** |
| | Yes | 0.190 (0.067-0.537) | |
| **Did you ever think that you would die during the earthquake?** | No | 1 | 0.427 |
| | Yes | 1.518 (0.542-4.249) | |
| **Did you ever think that a family member of you would die during the earthquake?** | No | 1 | **0.036*** |
| | Yes | 3.383 (1.081-10.591) | |
| **Depressive** | | 1.098 (0.938-1.286) | 0.246 |
| **Cyclothymic** | | 0.950 (0.842-1.073) | 0.410 |
| **Anxious** | | 1.137 (1.030-1.254) | **0.011*** |

*** p<0.05**

In the correlation analysis between the TEMPS and PETS subgroups and total scores, a significant positive correlation was found between the PETS total score and depressive, cyclothymic, irritable and anxious sub-scores and a negative correlation was found between hyperthymic and depressive temperaments (Table 5).

**Table 5.** Correlation between TEMPS and PETS scores

| | **Behavior prob.** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| **1. Emotive Limitedness** | ,638** | | | | | | | | | |
| **2. Affective Score** | ,625** | ,561** | | | | | | | | |
| **3. Cognitive Structures** | ,666** | ,615** | ,697** | | | | | | | |
| **4. Sleep Problems** | ,717** | ,511** | ,614** | ,695** | | | | | | |
| **5. PETS Total Score** | ,847** | ,809** | ,816** | ,879** | ,835** | | | | | |
| **6. Depressive** | ,125 | ,227** | ,166* | ,183** | ,165* | ,212** | | | | |
| **7. Cyclothymic** | ,232** | ,189** | ,193** | ,234** | ,213** | ,252** | ,584** | | | |
| **8. Hyperthymic** | -,038 | ,016 | ,096 | ,025 | -,073 | ,009 | -,238** | -,038 | | |
| **9. Irritable** | ,116 | ,158* | ,035 | ,146* | ,084 | ,142* | ,316** | ,360** | ,152* | |
| **10. Anxious** | ,332** | ,349** | ,245** | ,379** | ,375** | ,408** | ,536** | ,443** | -,087 | ,530** |

***p<0.05, **p<0.01**

## DISCUSSION

In the present study, early-stage trauma levels in healthcare professionals in Malatya on the third week following the earthquake and the risk factors that might be effective in the consequent trauma response were identified.

High trauma response rate was found in one-fourth of the sampled healthcare workers. This result supported earlier studies on health workers in other traumatic settings, such as war and local earthquakes (24, 30-32).

In the literature, it has been emphasized that both demographic variables such as gender, marital status, occupation, age, and comorbid mental illness affect the trauma response (15,16,33). In terms of these factors, our results did not provide different data to distinguish healthcare professionals from the general public. Similar to previous studies (32,34), our study detected a marked correlation between the increase in the PETS scores and being female. This result was not surprising since women more easily express their emotions. On the other hand, the high stress levels in women can be attributed to gender differences in neuroendocrine response or excessive threat perceptions and concerns about losing control (35,36).

Some studies that examined the correlations between marital status and stress response claimed that being married reduces the risk, whereas other studies indicated the opposite (4,37). Nonetheless, the present study reported equal trauma scores among married and unmarried persons. However, the cognitive sub-score of the PETS scale (worries about children/parents/acquaintances/friends, worry about a sudden earthquake, reliving the earthquake experience and worries about the future) was higher among the married subjects. This result was probably due to the cognitive effects created by the sense of responsibility towards family members. The higher trauma scores of participants with children compared to those without supported this view. This impact could also be inferred from being parents who fear losing their children. Changes in psychological and biological mechanisms caused by being a parent may also have caused these people to internalize the fear their children have experienced and thus became more affected (38-40).With a similar thought, the lower trauma scores of the unmarried participants and those living alone or living with elders can be attributed to the less responsibility and alleviation of the fear by sharing it with older family members.

In the regression analysis, it was also found that the thought of losing a family member was a particular factor determining trauma severity than one's own fear of death. Within the traditional structure of Turkish society, this result was not surprising. In Turkish culture, the family is considered as an important source of social support and emotional development for both parents and children (41). Therefore, worrying more about losing this structure than dying seems to be a response against the erosion of many values. This result may be a reason why the participants who were not at the epicenter of the earthquake also experienced post-traumatic symptoms. Although far away, these people may have been traumatized by learning that their families had been exposed to a traumatic event (42). In this context, our study proposes the view that disasters affect not only those who are exposed to them directly but also other people for different reasons.

This study found a linear correlation between the history of psychiatric disorders and trauma response, similar to previous research (43,44). This was especially significant for the sub-score of emotive limitedness indicating that the individual may be more depressive. Increasing depressive symptoms may lead to weakened coping mechanism, aggravation of the existing psychiatric disease and, perhaps, to the development of other psychiatric diseases such as PTSD in the future. Therefore, providing post-disaster support to

individuals with existing psychiatric disorders can provide significant benefits.

Although the previous studies indicate that past traumatic experiences increase trauma response level (4,14,44,45), this study did not support this outcome. In our study, neither being nor not being traumatized before did not show an effect on the level of trauma, except for previous earthquake experiences. A lower PETS score was found among those with a previous earthquake experience than those who did not. The results of the regression analysis showed that one of the three basic independent variables that influenced the PETS score was the history of earthquake experiences. This outcome is consistent with the finding underlined in the study by Nishi et al. claiming that individuals who have experienced natural disasters at some point in their lives were less affected by later disasters (46). Although this study did not focus on these factors, we can assume that survivors might be less traumatized because of developing some coping strategies and taking precautions as a result of their disaster experiences when the earthquake occurred (12,47,48).

Unlike society-based studies that found correlation between trauma level and many various variables such as seeing someone dead or injured in the earthquake, having one's house damaged, material losses, receiving assistance and support from official institutions, family and/or friends, the belief in the possibility of reducing earthquake damages and being prepared for potential earthquakes (19-49), this study did not detect any. This result may be related to participants' low material damages, as well as a reflection of witnessing and intervening in many traumatic events due to their profession. Perhaps, these experiences may have contributed to their ability to accept possible environmental changes more easily and show fewer symptoms after the earthquake (50).

Temperament is best described as the trait emotional reactivity of an individual which appears to be stable across life and has strong genetic underpinnings (51,52). Recently, increasing evidence suggests that temperament plays a role in predisposing individuals to many mental disorders (53). In a few studies conducted independently of the nature of the traumatic event, especially anxious temperament was reported to be a predisposing factor to the development of PTSD, while hyperthymic temperament was a protective factor. In a study conducted by Fauerbach et al. in burn-injured patients, a positive relationship was found between anxious temperament and the development of PTSD, while a negative relationship was reported with extraversion (54). In a meta-analysis that examined the relationship between PTSD and temperament, all the temperament characteristics were found to have a moderate-to-weak relationship with PTSD symptoms, regardless of sex, trauma type and time elapsed after trauma (55).

The present study also showed that there was a linear relationship between depressive, cyclothymic, and anxious temperament characteristics and trauma levels. Especially anxious temperament has been identified as one of the main determining factors of trauma severity. Indeed, it was not surprising that people with an anxious temperament exhibited an exaggerated tendency to distressing events, had difficulty adapting to daily changes, and had a more severe trauma response, given that they chose less effective coping strategies (56,57). On the other hand, unlike previous studies, there was no relationship between hyperthymic temperament and trauma level. This difference can be attributed to the fact that these people with hyperthymic temperament tend to show themselves better as a characteristic of their temperament and claim that they are not affected by trauma for the same reason (51).

The examination of the relationship between trauma response and temperament may provide important benefits in determining the recruitment of people at high risk of exposure to trauma and in adjusting the appropriate therapy process for those exposed to trauma especially when trauma-focused therapies are considered ineffective in people with high emotional reactivity. Determining the decisions in job recruitment or distribution of duties according to the temperament profiles of individuals will reduce the development of psychiatric problems (55,58).

Even though this study presents important data concerning the factors determining trauma responses, it has certain limitations. First of all, the exclusion of healthcare workers in other provinces prevents the generalization of outcomes to all healthcare professionals affected by the earthquake. Second, the lack of a sub-analysis regarding the duties of healthcare professionals prevented the researchers from identifying the potential impact of the earthquake among different professional groups. The use of a self-report questionnaire in the study might have reduced reliability of the data. But, despite all these limitations, to our knowledge this study is the first to investigate temperament traits and early trauma response in healthcare professionals. These results regarding the early trauma response could provide pilot data for future studies investigating PTSD prevalence.

## CONCLUSION

This study showed that one-fourth of the health workers had severe trauma levels in the early stages of the earthquake. The trauma scores of women, those who were married and/or had children, those who were worried about losing their own life or their relatives life during the earthquake

were higher. There was a positive correlation between trauma severity and anxious temperament and fear of losing a family member, and a negative correlation between the presence of a previous earthquake experience. As healthcare professionals are an important part of the activities to combat disaster, recognition of the risky groups may be beneficial in the direction of the assistance to be provided and in the planning of future services. In this way, the prevalence of long-lasting and debilitating mental illnesses can be reduced.

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# The Relationship between COVID-19 Suspected Patient's Coagulation and Platelet Parameters and Polymerase Chain Reaction Results

**ABSTRACT**

**Objective:** This study aims to investigate the relationship between prothrombin time (PT), activated partial thromboplastin time (aPTT), INR (International Normalized Ratio), and D-dimer levels, platelet (PLT) levels at hospital admission, and positivity or negativity of Polymerase Chain Reaction (PCR) test results in patients with suspected coronavirus disease-19 (COVID-19) followed at COVID-19 services.

**Methods:** This study was performed on 238 patients with the prediagnosis of COVID-19, all patients are hospitalised in Samsun city at our hospital between 11 March 2020-30 May 2020. According to COVID-19 PCR test results, PCR test negative 119 individua and PCR test positive 119 patients were included in the study. PT, aPTT, D-dimer, INR, and PLT levels were examined.

**Results:** While PCR test negative individuals had a mean PT value of 11.46±0.86 sec, PCR test positive patients had a mean PT value of 12.97±3.65 sec ($p<0.001$). There was no significant difference in mean aPTT values of PCR test positive and negative patients. Whereas INR, D-dimer increased significantly in PCR test positive patients. PLT value decreased from a mean value of 266.75±71.36*$10^9$/L in PCR test negative patients to 241.18±96.64*$10^9$/L in PCR test positive patients ($p=0.002$).

**Conclusions:** In our study, it was found that in patients who were admitted to hospital with COVID-19 suspicion and followed up in COVID-19 services, PT, D-dimer, INR, PLT values were important in detecting coagulopathy and thrombocytopenia in the group who were PCR positivity.

**Keywords:** COVID-19, PT, aPTT, D-dimer, INR, PLT, PCR Test.

# COVID-19 Şüphesi Taşıyan Hastaların Koagülasyon ve Trombosit Parametreleri ile Polimeraz Zincirleme Tepkimesi Sonuçları Arasındaki İlişki

**ÖZET**

**Amaç:** COVID-19 şüphesi ile COVID-19 servislerinde takip edilen hastaların, hastaneye başvuru sırasında yapılan Protrombin Zamanı (PT), aktive Parsiyel Tromboplastin Zamanı (aPTT), INR (International Normalized Ratio), D-Dimer düzeyleri, Trombosit (PLT) düzeyleri ile Polimeraz Zincirleme Tepkimesi (PCR) sonucunun pozitif veya negatif durumu arasındaki ilişkiyi araştırmak amaçlanmaktadır.

**Gereç ve Yöntem:** Bu çalışma 11 Mart 2020- 30 Mayıs 2020 tarihleri arasında, Samsun ilinde, hastanemizde COVID-19 şüphesi ile yatan 238 hasta ile yapılmıştır. COVID-19 sonuçlarına göre PCR testi negatif 119 kişi ve PCR testi pozitif 119 hasta çalışmaya dahil edilmiştir. PT, aPTT, D-dimer, INRve PLT düzeylerine bakılmıştır.

**Bulgular:** PT; PCR testi negatif katılımcılarda ortalama 11,46±0,86 sn değeri bulunurken, PCR testi pozitif hastalarda 12,97±3,65 sn, ($p<0,01$), aPTT; PCR negatif katılımcılarda ortalama 24,38±3,40 sn, PCR pozitif hastalarda ise 24,18±4,83 sn,($p>0,05$) olarak bulunmuştur. D-Dimer, PCR testi negatif katılımcılarda ortalama 0,23±0,12 µg/ML, PCR testi pozitif hastalarda ise 2,10±5,60 µg/ML, ($p<0,01$) olarak tespit edilmiştir. INR; PCR negative katılımcılarda 0,99±0,12 değeri, PCR pozitif hastalarda ise 1,14±0,35, ($p<0,01$) değeri bulunmuştur. PLT; PCR testi negative katılımcılarda 266,75±71,36 ($10^9$/L), PCR testi pozitif hastalarda ise 241,18±96,64 ($10^9$/L) olarak saptanmıştır ($p=0,002$).

**Sonuç:** Çalışmamızda, hastaneye COVID-19 şüphesi ile başvuran, COVID-19 servislerinde takip edilen, PCR pozitif olan hastalarda, PT, D-Dimer, INR, PLT değerlerinin, koagülopati ve trombositopeni tespitinde önemli olduğu saptandı.

**Anahtar Kelimeler:** COVID-19, PT, aPTT D-dimer, INR, PLT, PCR Test.

## INTRODUCTION

COVID-19 which shares similarities with SARS (Severe Acute Respiratory Syndrome) and Middle East Respiratory Syndrome (MERS) viruses responsible for endemic diseases in 2003 and 2012, is a novel beta coronavirus (1,2). In addition to being a critical disease for health, coronavirus 2019 (COVID-19) poses a global threat (3).

First, pneumonia cases of unknown etiology occurred in Wuhan, Hubei province of China; the cases were reported to WHO (World Health Organization) in December 2019, and in March 2020, WHO declared this new infection a pandemic (4,5).

COVID-19 is described as a new betacoronavirus. It is closely related to SARS. It is a new infectious disease in which the virus is the causative pathogen 5. Despite pulmonary pathophysiology during the disease, severe COVID-19 infection, which is not fully understood, is associated with pronounced alveolar inflammatory cell infiltration and is accompanied by systemic cytokine storm (6). One of the essential poor prognosis indicators is the development of coagulopathy in patients (7,8,9). Increased plasma levels of fibrin degradation D-dimers with COVID-19 infection constitute an independent biomarker for poor prognosis (9). In the COVID-19 pathogenesis of coagulation activation, significant pathological changes involving lung microvasculature including widespread microthrombus and significant hemorrhagic necrosis have been highlighted, particularly in line with post-mortem studies (10,11).

Severe COVID-19 increases the risk of developing significantly associated deep vein thrombosis and pulmonary embolism (12,13). For estimating the severity of COVID-19, monitoring functional screening parameters for PLT, PT, aPTT, and D-D (D-dimer) and daily changes in coagulation function is essential in patients with COVID-19 (14).

The poor prognosis that continues with COVID- 19 and continuation of D-dimer increase are precursors of multiorgan failure and development of DIC (Disseminated Intravascular Coagulation) (15). Significantly, a high D-dimer level is associated with increased mortality. In patients who lost their lives, the increase is evident from the fourth day of hospitalization. Despite coagulopathy, hemorrhage findings are not a common biomarker (15,16,17).

## MATERIAL AND METHODS

**Study Design and Participants:** The study was carried out in the COVID-19 services of the Samsun University Samsun Training and Research Hospital between March 11, 2020 and May 30, 2020. Patients with COVID-19 symptoms were included in the study. The PCR results were divided into two groups as PCR positive and PCR negative, in line with the World Health Organization's guideline titled "Laboratory Testing for 2019 Novel Coronavirus (2019-nCoV) in Suspected Human Cases" published on March 2, 2020.

All samples were studied with the SARS CoV-2 Double Gene RT-q PCR Kit (BioSpeedy, Turkey) following the manufacturer's instructions. Briefly, after nucleic acid isolation in nasopharyngeal lavage/aspirate, bronchoalveolar lavage, nasopharyngeal suture, oropharyngeal swab and sputum samples, detection by single-step reverse transcription (RT) and Real-Time PCR targeting the ORF1ab and N gene regions were performed.

Extract ion and inhibition control were checked by targeting the human RNase P gene as an internal control. Nucleic acid extraction was validated with the vNAT buffer, and this process was conducted without any additional work during sample transfer. Nasopharyngeal or oropharyngeal swab samples taken with swabs (dacronor polyester flock) were placed in a sterile transport solution containing vNAT solution and transferred. Reaction components 2X Prime Script mix 10µL, CVD Di Oligo mix 5µL, template nucleic acid 5µL total reaction amount of 20µLvolume was created. Qiagen Rotor-Gene (Germany) Real-Time PCR instrument was run on sigmoidal curves under 38 cycles and were evaluated as positive.

Prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimer, International Normalized Ratio (INR), and platelet (PLT) levels of 119 patients followed in COVID-19 services were taken from the hospital information management system. In addition, PT, aPTT, D-dimer, INR, and PLT levels of 119 individuals with COVID-19 PCR test negative were taken.

D-dimer test was worked on a Beckman Coulter Au 680 device, PT, aPTT, and INR tests were worked at Siemens Ca-7000 device, and platelet level was worked in Beckman Coulter Dx-800 device by using suitable tubes and kits.

**Ethical Approval:** Ethical approval to conduct this study was granted by the hospital's ethics committee on September 30, 2020 (decision number GOKA/2020/9/10).

**Data Analysis:** Statistical Package for the Social Sciences (SPSS) version 22.0 software (IBM Corp., Armonk, NY, USA) was used for the data analysis in the present study. One-Sample Kolmogorov – Smirnov test and the Shapiro-Wilks test were used to evaluate the data. Mann–Whitney U-test was used to compare parameters between the groups. A $p < 0.05$ was considered to be statistically significant. G*Power 3.1.9.7 program was used to calculate the sample size. One hundred ten sample size results were obtained for each group, in this calculation (test=t-test (Wilcoxon-Mann-Whitney test (two groups)), Analysis: A priori: Compute required sample size, Tail(s) =Two, Parent distribution=Normal, Effect size

d=0.5, α err prob=0.05, Power (1-β err prob)=0.95, Allocation ratio N2/N1=1).

## RESULTS

According to the results of combined oropharyngeal/ nasopharyngeal swab test performed, 119 patients were PCR-positive and 119 patients were PCR-negative. The mean age of the PCR positive patients was 58.29 (SD, 10.10) years. Some 50.4% (n=60) of the PCR positive patients were women. Complaints of the PCR positive patients with suspicious contact admitted to our hospital were respiratuar distres (48.2%), loss of smell (16.8%), loss of taste (12%) (Table 1).

**Tablo 1.** Descriptive characteristics of individuals (n=238)

| **Categorical variables** | | **COVID-19(+)** | **COVID-19 (-)** |
|---|---|---|---|
| **n (%)** | | 119 | 119 |
| **Age±SD** | | 58.29±10.10 | 41.08±9.71 |
| **Hospitalization day** | | 15.92 | 6.84 |
| **Gender (n, %)** | Male | 59 (49.6) | 84 (70.6) |
| | Female | 60 (50.4) | 35 (29.4) |
| **Intensive care unit (n, %)** | Yes | 44 (63.0) | - |
| | No | 75 (37.0) | 119 |
| **Ex-Discharge** | Ex | 28 (23.5) | - |
| | Discharge | 91 (76.5) | 119 |
| **Comorbidity (n,%)** | Cancer | 7 (5.9) | 3 (2.5) |
| | DiabetesMellitus | 22 (18.5) | 24 (20.2) |
| | Hypertension | 21(17.6) | 18 (15.1) |
| | Cardiovascular disease | 11 (9.2) | 4 (3.4) |
| | Chronicrenalfailure | 9 (7.6) | 2 (1.7) |
| | Pneumonia | 15 (12.6) | 8 (6.7) |
| | COPD | 6 (5.1) | 7 (5.9) |
| | No | 28 (23.5) | 53 (44.5) |
| **Signs and symptoms (n,%)** | Respiratory Distress | 34 (28.6) | 0 |
| | Loss of Smell | 20 (16.8) | 3 (2.5) |
| | Loss of Taste | 12 (10.0) | 0 |
| | Fever | 38 (32.0) | 17 (14.3) |
| | Headache | 28 (23.5) | 34 (28.6) |
| | Joint Pain | 29 (24.4) | 17 (14.3) |
| | Weakness | 36 (30.3) | 50 (40.0) |
| | Cough | 15 (12.6) | 17 (14.3) |
| | Anorexia | 5 (4.2) | 2 (1.7) |
| | Nausea | 3 (2.5) | 0 |
| | Diarrhea | 2 (1.68) | 0 |

The test reference range of the samples analyzed in Biochemistry Laboratory was taken as 10-14 seconds (sec) for prothrombin time (PT). While the mean of PT value was found to be 11.46±0.86 sec, in PCR negative individuals, the mean of PT value was found to be 12.97±3.65 sec. in PCR positive patients. A statistically difference was observed between the mean of PT values and the gender variable (p<0.001) (Table 2).

**Table 2.** The association between PT, aPTT, D-dimer, INR, PLT levels by gender and COVID-19 results.

| **Gender** | **PCR (n)** / **Statistical values** | **PT±SD (sec)** | **aPTT±SD (sec)** | **D-dimer±SD (µg/ml)** | **INR±SD** | **PLT±SD (*10$^9$/L)** |
|---|---|---|---|---|---|---|
| **Male** | **Negative (84)** | 11.53±0.93 | 24.75±3.52 | 0.22±0.10 | 0.99±0.13 | 253.36±64.15 |
| | **Positive (59)** | 13.19±3.25 | 25.40±5.52 | 2.29±6.27 | 1.16±0.30 | 228.07±91.60 |
| | **U** | **1027.000** | **2449.000** | **378.500** | **1035.000** | **1893.500** |
| | **P** | **<0.001** | **0.905** | **<0.001** | **<0.001** | **0.017** |
| | **Effect size** | **0.50** | **0.01** | **0.72** | **0.50** | **0.20** |
| **Female** | **Negative (35)** | 11.29±0.65 | 23.49±2.96 | 0.27±0.12 | 0.99±0.06 | 298.89±78.22 |
| | **Positive (60)** | 12.75±4.02 | 22.98±3.72 | 1.91±4.91 | 1.13±0.39 | 254.08±100.45 |
| | **U** | **605.500** | **962.000** | **322.000** | **589.500** | **641.500** |
| | **P** | **0.001** | **0.497** | **<0.001** | **<0.001** | **0.002** |
| | **Effect size** | **0.35** | **0.07** | **0.58** | **0.37** | **0.32** |
| **Total** | **Negative(119)** | 11.46±0.86 | 24.38±3.40 | 0.23±0.12 | 0.99±0.12 | 266.75±71.36 |
| | **Positive (119)** | 12.97±3.65 | 24.18±4.83 | 2.10±5.60 | 1.14±0.35 | 241.18±96.64 |
| | **U** | **3765.500** | **6446.000** | **1457.500** | **3616.000** | **5435.500** |
| | **P** | **<0.001** | **0.232** | **<0.001** | **<0.001** | **0.002** |
| | **Effect size** | **0.40** | **0.07** | **0.69** | **0.42** | **0.20** |

**U=Mann-Whitney U value. p =AsymptoticSig. (2-tailed test).**

The test reference range of the samples analyzed in Biochemistry Laboratory was taken as 18-36 seconds (sec) for activated partial thromboplastin (aPTT). While the mean of aPTT value was found to be 24.38±3.40 sec. in PCR negative individuals, the mean of aPTT value was found to be 24.18±4.83 sec in PCR positive patients. No statistically significant difference was observed between the two groups (Table 2). The test reference range of the samples analyzed in Biochemistry Laboratory was 0-0.5 µg/ml for D-dimer. While the mean of D-dimer value was found to be 0.23±0.12 µg/ml in PCR test negative individuals, the mean of D-dimer value was found to be 2.10±5.60 µg/ml in PCR positive patients. There was a significant difference between D-dimer, INR, PLT values of the two groups (p values were <0.001,<0.001,0.002, respectively) (Table 2). In our study, the relationship between PT, aPTT, D-dimer, INR, PLT levels according to the PCR positive group and gender was evaluated. There was a significant difference between the mean of PT values and the gender variable (p<0.001) (Figure 1).

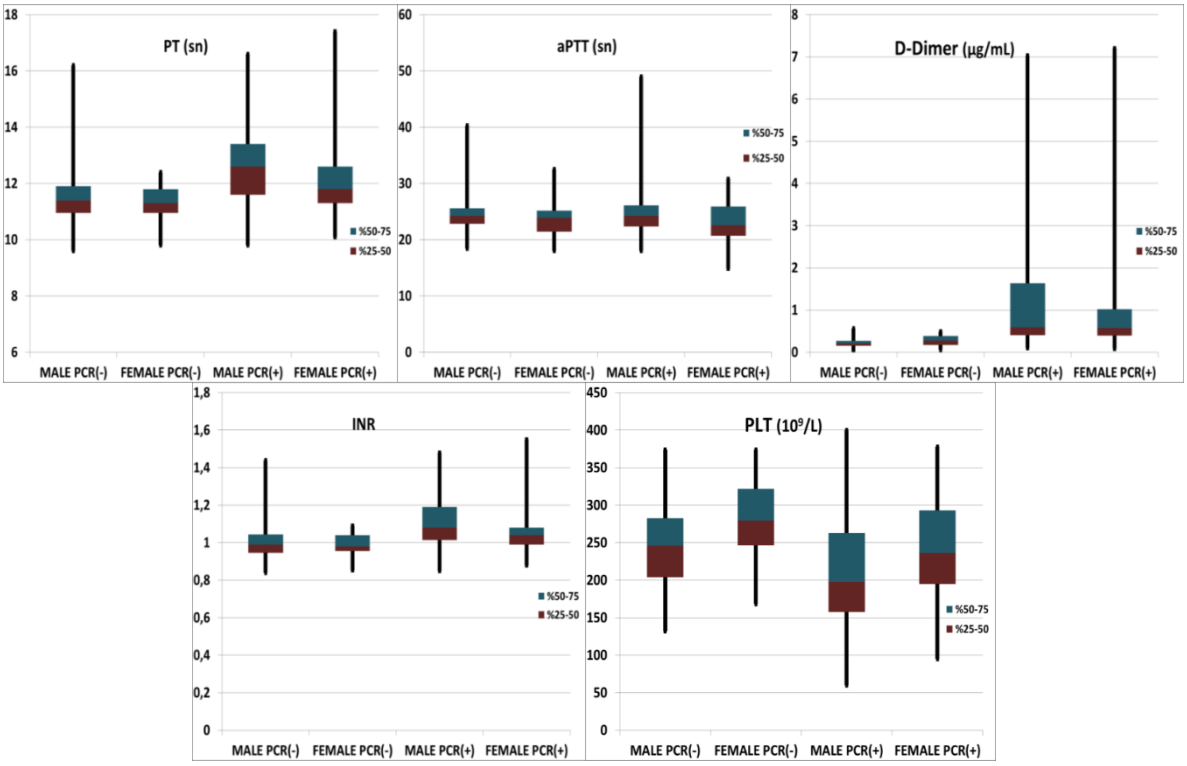


**Figure 1.** The association between PT, aPTT, D-DIMER, INR, PLT levels according to gender COVID-19 results in boxplots

Forty four patients in our hospital's COVID-19 intensive care unit included in the study, these patients were admitted to the intensive care unit for reasons such as respiratory failure, shock or the need for mechanical ventilation support while being monitored. During the period of inpatients, Metilprednisolon and Enoksaparin sodyum were given in the treatment of anticoagulants based on the guidance published by the Ministry of Health called COVID-19.

**DISCUSSION**

This study, which we conducted in our research hospital in the first months of the pandemic, showed that PT, D-dimer, INR, and PLT values between positive and negative groups with PCR tests were statistically significant between groups, especially in terms of COVID-19 results.

Coagulopathy becomes evident with increased D-dimer and fibrinogen levels and minimal change in prothrombin time (PT), active partial thromboplastin time (aPTT), and platelet count (15). Hematological laboratory results can be used to determine the severity and prognosis of COVID-19 infection. Thrombocytopenia is associated with an increased risk of severe disease and mortality associated with COVID-19 (18). Platelet count has been accepted as a potential marker for COVID-19 since it is a simple, inexpensive, and readily available hematological marker and since it is independent of disease severity and morbidity risk in the intensive care unit (19).

In a study conducted on thrombocytopenia with patients with COVID-19, mild thrombocytopenia was observed in approximately 5% of patients who had the mild disease; thrombocytopenia was observed in 70-95% of patients who had severe disease (5,19). In a meta-analysis, when platelet count was compared, a significant difference was found between the COVID-19-negative group and the group that had severe COVID-19 in terms of platelet count and the

individuals who had the severe disease were found to have lower platelet count (18). In another study conducted on 1476 patients, a direct correlation was found in patients with COVID-19 between the decrease in platelet count and mortality (20). In another study conducted on patients with COVID-19, it was reported that low platelet count was associated with increased severe disease and death risk, and it could serve as an indicator of worsening of the disease during the hospital stay COVID-19 (18). It has been reported that platelet count decreases significantly in patients with COVID-19 (21,22), and it is lower in patients who do not survive compared with those who survive (23).

Our study was found to be in parallel with the literature, a significant difference was found between groups, and platelet count was found to be lower in the COVID-19 (+) group.

D-dimer is a fragment produced by the cleavage of fibrin by plasmin during clot breakdown (24). One of the most common laboratory findings in patients with COVID-19 who require hospitalization is the apparent elevation in D-dimer. A high D-dimer value has been reported as a poor prognostic marker associated with the consistent critical course and higher mortality in patients with COVID-19 (25,26). In a study conducted on 1099 patients with COVID-19 in China, high D-dimer levels were found in almost half of the patients (27). In an observational study conducted on 183 patients in China, a statistically significant difference was found in the mean D-dimer concentration at admission between patients with COVID-19 who survived and those who did not (8). In another study conducted, patients with COVID-19 treated in ICU (Intensive Care Unit) were found to have higher D-dimer levels than patients with COVID-19 who were not treated in ICU (5). Finally, in another study involving 5279 patients with COVID-19, the COVID-19 (+) group was compared with the COVID-19 (-) group. The D-dimer level of the COVID-19 (+) group was found to be four times higher (28).

Our study was found to be consistent with the literature. The difference between groups was found to be significant. D-dimer levels were found to be higher in the COVID-19 (+) group, while they were found to be lower in the COVID-19 (-) group.

Among coagulation parameters, PT is another laboratory parameter with varying consequences in COVID-19. PT and aPTT are exogenous and endogenous coagulation system factors that can be used to diagnose DIC early (Disseminated Intravascular Coagulation). In another observational study conducted on 183 patients in China, a mild prolongation was found in the mean PT concentration at admission between patients with COVID-19 who survived and those who did not, and a statistically significant difference was found between the groups (8). In another study conducted in China, the patients receiving treatment in the ICU were found to have higher PT prolongation than patients who were not receiving treatment in ICU, and a significant difference was found between the groups (5). In another study conducted on 187 patients diagnosed with COVID-19 and treated in the hospital, patients with high troponin-T levels were found to have prolonged PT and aPTT levels (29). More pronounced PT and APTT parameters prolongation indicates that patients transition from a high coagulation state to a fibrinolytic state due to excessive coagulation factor consumption.

Our study was found to be consistent with the literature; a significant difference was found between the groups in terms of PT levels, and prolongation was found to be higher in the PCR (+) group.

**Limitations**

There are a number of limitations to this study. First, the research was conducted in a single center. Second, our research was its retrospective design and the data being obtained from files.

In conclusion, the relationship between PT, aPTT, D-dimer, INR, PLT levels were evaluated according to COVID-19 results and gender. Our study showed statistical significance between groups in PT, D-dimer, INR, PLT values between PCR test positive and negative groups in terms of especially COVID-19 results ($p<0.01$). It is crucial to find out coagulopathy and thrombocytopenia in Covid-19 patients. These parameters are also important biomarkers for the prognosis of the disease in COVID-19.

The results of this study have shown that hypercoagulation exists in patients with COVID-19 at an early stage, and hypercoagulation is closely associated with disease progression and clinical outcome. For this reason, coagulation indicators such as D-dimer and PT should be monitored as early as possible to determine thrombotic complications. It is imperative to take preventive treatment to decrease thromboembolism and DIC risk secondary to coagulation disorder and thus to reduce the morbidity and mortality of patients infected with COVID-19.

**Conflict of Interest Statement:** There is no conflict of interest between the authors.

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RESEARCH ARTICLE

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# Etiology and Mortality Investigation in Neonates that Underwent Surgery

**ABSTRACT**

**Objective:** We aimed to examine the demographic characteristics, etiology and postoperative mortality rates of neonates operated on in our hospital.

**Methods:** The records of neonates who were operated in our tertiary care level university hospital between 2013 and 2019 were reviewed retrospectively. Patients with a diagnosis requiring surgical procedure, age (days), gender, anatomical region where the surgical procedure was performed, length of hospital stay, and mortality rates were investigated.

**Results:** 329 neonates were included in the study of which 213 (64.75%) were male and 116 (35.25%) were female. Gastrointestinal system surgery was performed in 214 patients (65.04%), respiratory system surgery in 95 (28.87%) patients and urogenital system surgery in 17 (5.16%) patients. The mortality rate of 156 patients that were followed up in our neonatal unit in the postoperative period was 16.6%. The mortality rate of female babies (21.3%) was higher than that of male babies (13%). Considering the mortality rates of the patients according to the surgical areas, the highest mortality rate was found in respiratory system surgery (38.46%) which was trailed by gastrointestinal system surgery (12.28%) and urogenital system surgery (7.69%), respectively.

**Conclusions:** Despite advances in neonatal care and surgical techniques, immaturity and congenital anomalies still have an important place among the most common causes of death in infants. In particular, the detection of organ dysfunctions due to congenital anomalies which are considered among the preventable causes of death in infants, efforts to correct them will be beneficial to a certain extent in reducing the mortality rates of neonates.

**Keywords:** Neonatal, Surgical Procedure, Mortality.

# Ameliyat Olan Yenidoğanlarda Etiyoloji ve Mortalite Araştırması

**ÖZET**

**Amaç:** Hastanemizde ameliyat edilen yenidoğanların demografik özelliklerini, etiyolojilerini ve postoperatif mortalite oranlarını incelemeyi amaçladık.

**Gereç ve Yöntem:** Üçüncü basamak üniversite hastanemizde 2013-2019 yılları arasında ameliyat edilen yenidoğanların kayıtları geriye dönük olarak incelendi. Cerrahi işlem gerektiren tanısı olan hastaların yaş (gün), cinsiyet, cerrahi işlemin yapıldığı anatomik bölge, hastanede kalış süreleri ve mortalite oranları araştırıldı.

**Bulgular:** Çalışmaya 213'ü (%64,75) erkek, 116'sı (%35,25) kız olmak üzere 329 yenidoğan alındı. 214 hastada (%65.04) gastrointestinal sistem cerrahisi, 95 (%28,87) hastada solunum sistemi cerrahisi ve 17 (%5.16) hastada ürogenital sistem cerrahisi uygulandı. Yenidoğan ünitemizde postoperatif dönemde takip edilen 156 hastanın mortalite oranı %16,6 idi. Kız bebeklerin ölüm oranı (%21,3) erkek bebeklerden (%13) daha yüksekti. Hastaların cerrahi alanlara göre ölüm oranlarına bakıldığında, en yüksek ölüm oranı solunum sistemi cerrahisinde (%38,46) bulunurken, bunu sırasıyla gastrointestinal sistem cerrahisi (%12,28) ve ürogenital sistem cerrahisi (%7,69) izledi.

**Sonuç:** Yenidoğan bakımı ve cerrahi tekniklerdeki gelişmelere rağmen, immatürite ve konjenital anomaliler, bebeklerde en sık ölüm nedenleri arasında hala önemli bir yer tutmaktadır. Özellikle bebeklerde önlenebilir ölüm nedenleri arasında sayılan doğumsal anomalilere bağlı organ işlev bozukluklarının saptanması, düzeltilmesine yönelik çabalar yenidoğan bebek ölüm oranlarının azaltılmasında bir ölçüde faydalı olacaktır.

**Anahtar Kelimeler:** Yenidoğan, Cerrahi Prosedür, Mortalite.

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## INTRODUCTION

Neonatal period refers to the first 28 postnatal days after birth. The most important cause of neonatal death in developed world regions is reported as prematurity (1). The rate of respiratory distress syndrome, necrotizing enterocolitis, congenital anomaly, and sepsis due to a prolonged hospital stay is encountered higher in low-birthweight and preterm neonates than in term neonates (2). The most important causes of infant deaths in developed countries are congenital anomalies, morbidities associated with prematurity and low birth weight, sudden infant death syndrome, morbidities associated with maternal diseases and accidents (3,4). The most common and the most important basic cause of infant deaths in our country is morbidity associated with prematurity, which is also the most important cause of neonatal deaths (5). Although congenital anomalies are responsible for approximately one third of infant deaths in developed countries, it has been observed that these mortality rates are decreasing. One of the reasons for this alleviation is the increased survival rate of many major congenital anomalies, -especially those in cardiovascular, gastrointestinal, or genitourinary nature, with corrective surgical treatments (6,7).

Surgical interventions performed by pediatric surgeons in the neonatal period, most if not all are aimed at congenital anomalies such as congenital diaphragmatic hernia, anal atresia, esophageal atresia, and the rest can be counted as complication management like necrotizing enterocolitis due to prematurity (8,9). These congenital anomalies and complications are surgical diseases that often stand in need of long-term care and an out-patient follow-up. Almost the entirety of the cases operated in the neonatal period should be followed up under neonatal intensive care conditions (9). Due to the technical developments in neonatal intensive care units in recent years, there is an increase in the number of surviving premature neonates and so does the frequency of subsequent surgical intervention (10).

The infant's prenatal follow-up and transfer, physical facilities of the neonatal intensive care unit and operating theatre, post-operative care quality and the experience of the personnel employed hereabouts affect mortality rates of neonatal operations either in the pre-operative or in the post-operative period (8). In this study, the records of 329 neonates who were hospitalized in the tertiary neonatal intensive care unit after being operated on by the pediatric surgery clinic between 2013 and 2019 were analyzed. To provide appropriate and safe anesthesia practices and effective respiratory support during the surgery for all patients' surgical procedures, the anesthesia team worked in coordination with surgeons and neonatal intensivists both before and after the surgery. The etiology of the surgical procedure and the mortality rates that developed in the post-operative period were investigated.

## MATERIAL AND METHODS

This study was conducted on 329 neonates who were hospitalized and subsequently operated on in the tertiary neonatal intensive care unit of Harran University hospital between 2013 and 2019. The data of the cases were obtained retrospectively by scanning the inpatient files within the hospital's software data system. The patients' age (days), gender, anatomical region where the surgical procedure was performed, length of hospital stays, and mortality rates were investigated.

General anesthesia was performed in all cases. Sevoflurane was used for inhalation anesthesia. After the operation, the patients were managed in the neonatal intensive care unit. Institutional permission from the hospital management and ethical approval from the Harran University Clinical Research Ethics Committee (dated 22/02/2019 and decision number HRU/19.02.36) were obtained for the use of patients' data before the study.

**Statistics**: Data analysis and evaluation were performed using SPSS version 24.0 (SPSS Inc., Chicago, IL). Data were expressed as mean ± standard deviation, number of cases and (%). Results were evaluated using Chi-square test, t-test, and Kruskal-Wallis. P value < 0.05 was considered significant.

## RESULTS

329 neonates were included in the study comprising of 213 (64.75%) boys and 116 (35.25%) girls. When the ages of the neonate cases were examined based on the day they were operated on, the mean age was 7.46±7.97 days; the mean age in girls was 6.56±7.58 days, and the mean age in boys was 7.95±8.15 days. 217 (65.95%) of our neonate cases were operated on the first 7 day of their lives. This was followed by 43 patients on 8-14$^{th}$ days (13.06%), 38 patients between 15-21$^{st}$ days (11.55%) and 31 patients between 22-28$^{th}$ days consecutively. The classification of the surgeries performed was sorted according to the surgical fields. Gastrointestinal system surgery was performed in 214 patients (65.04%), respiratory system surgery in 95 (28.87%) patients, and urogenital system surgery in 17 (5.16%) patients. (Table 1).

**Table 1.** Demographic data of patients and distribution of surgeries by anatomical regions

| **Gender** | | **Female (n,%)** | **Male(n,%)** | **Total** |
|---|---|---|---|---|
| | | 116 (%35.25) | 213 (%64.75) | 329 |
| **Age (Days)** | 1-7 | 82 (%24.92) | 135(%41.03) | 217 (%65.95) |
| | 8-14 | 12(%3.64) | 31(%9.42) | 43 (%13.06) |
| | 15-21 | 14(%4.25) | 24(%7.29) | 38 (%11.55) |
| | 22-28 | 8(%2.43) | 23(%6.99) | 31 (%9.42) |
| | Mean | 6.56±7.58 | 7.95±8.15 | 7.46±7.97 |
| **Surgical field** | Respiratory System | 44(%13.37) | 51(%15.50) | 95 (%28.87) |
| | Gastrointestinal system | 71(%21.58) | 143(%43.46) | 214 (%65.04) |
| | Urogenital system | 0(%0) | 17(%5.16) | 17 (%5.16) |
| | Musculoskeletal System | 1(%0.30) | 2(%0.60) | 3 (%0.91) |

Since 173 of the patients were referred to our hospital for surgical procedure from an external center, they were transferred back to the relevant hospital under appropriate conditions after the surgical procedure. Postoperative treatments of 156 patients, who were born in our hospital or referred to our hospital before the surgery, were continued in our neonatal intensive care unit. Therefore, mortality rates and length of hospital stay were calculated on 156 patients who were hospitalized in our hospital in the postoperative period. Although the mortality rate of female infants (21.3%) was higher than that of male infants (13.8%), this difference was not statistically significant (p=0.240). The mortality rate of all patients managed in the postoperative period was 16.6%. Considering the mortality rates of these patients as per to the surgical areas, the highest mortality rate was found in the respiratory system surgery (38.46%) which was followed by gastrointestinal system surgery (12.28%) and genitourinary system surgery (7.69%), respectively (p=0.007) (Table 2).

**Table 2.** Comparison of patients' mortality rates according to gender and surgical fields

| | | **Mortality (n,%)** | | **Hospitalization duration (days)** | |
|---|---|---|---|---|---|
| | | | p value | | p value |
| **Gender** | Female | 10(%21.3) | 0.240 [a] | 18.53±22.27 | **0,018** [b] |
| | Male | 15(%13.8) | | 11.38±13.04 | |
| **Surgical field** | Respiratory System | 10 (%38.46) | **0.007** [a] | 17.53±10.19 | **0,00** [c] |
| | Gastrointestinal system | 14 (%12.28) | | 13.78±18.31 | |
| | Urogenital System | 1 (%7.69) | | 6.00±8.38 | |
| | Musculoskeletal System | 0 | | 2.33±2.30 | |

*a: chi-square; b: t test; c: Kruskal-Wallis*

The mean hospital stay of the patients was 13.53±16.63 days. It was determined that the mean hospitalization span of female babies (18.53±22.27 days) was longer than that of male babies (11.38±13.04 days) ($p<0.05$). Considering the hospitalization times of the patients according to their surgical fields, it was found that the longest hospitalization was in respiratory system surgeries (17.53±10.19 days), and the shortest was in musculoskeletal surgeries (2.33±2.30 days) ($p< 0.05$) (Table 2).

Glancing at the complications of the peri- and postoperative period; a patient who underwent tracheoesophageal fistula repair was lost due to an intraoperative cardiac arrest. It was also detected that recovery from anesthesia was delayed in 2 patients who were operated on for testicular torsion and inguinal hernia. While 13 patients died in the first 24 hours postoperatively, 11 patients died after 24 hours (Table 3).

**Table 3**. Distribution of patients according to their exitus period and gender

| **Mortality** | **Female** | **Male** | **Total** |
|---|---|---|---|
| **Postoperative first 24 hours** | 6 | 7 | 13 |
| **Intraoperative** | 0 | 1 | 1 |
| **Postoperative after 24 hours** | 4 | 7 | 11 |
| **Total** | 10 | 15 | 25 |

## DISCUSSION

In recent years, the rate of survival for premature neonates has increased due to advances in medicine. Parallel to this situation, the number of neonates who underwent surgical intervention by pediatric surgeons has also risen. Neonatal surgery is one of the most specialized areas of pediatric surgery and requires a momentous cooperation of the pediatric surgeon with the neonatal specialist, anesthesiologist, and neonatology nurses (11,12) Çevik et al. reported that they performed surgical procedures mostly because of esophageal atresia (13). In another study, this surgical procedure was given as the most frequently performed procedure (11). In the current study, our frequency of the operated surgical systems was compatible with the literature as gastrointestinal system (65.04%), respiratory system (28.87%), and urogenital system surgery (5.16%), consecutively.

The causes of death in neonates who underwent surgical intervention are multifactorial (14). These reasons can be listed as suitable transfer conditions for the patient, appropriate operating room, safe anesthetic agents, physical conditions of the intensive care unit, training and experience of the employees, prenatal follow-up and risks arising from the severity of the cases themselves. Although it has been reported that the mortality rate in

neonates undergoing surgical procedures is not different between genders (14), Çevik et al. reported that mortality in female cases was statistically higher than in males (13). Similar results have been reported in this regard, depending on the region of residence and socioeconomic structure, and it has been determined that the incidence of mortality is high in female neonates in rural areas (15). In our study, although the mortality rate of female infants (21.3%) was higher than that of male infants (13.8%) which is consistent with the current literature, it was not statistically significant ($p>0.05$).

In a study examining the patients in the surgical neonatal intensive care unit, Siddharth et all found that the mortality rates in the surgical neonatal intensive care units may vary according to countries and regions, the mortality rates are between 20-60% in developing countries and this rate is below 5% in developed countries. They also added that their mortality rate was 14.5%. In the same study, the duration of hospitalization was reported as 16.41±11 (1-101 days) and mentioning similar results in terms of gender. The authors reported that 80.7% of the patients were admitted in the early neonatal period and all deaths occurred in this period. (16). In our study, we found that 65.95% of the patients who underwent surgery were operated within the first 7 days and 80% of all deaths occurred in this first week period. The mortality rate of the patients followed up in the postoperative period was 16.02%. We found the mean hospitalization period of the patients were 13.53±16.63 (1-115 days), and this period was significantly longer in female infants than in males ($p<0.05$). In addition, although the mortality rate was found to be higher in girls which was similar to the length of hospital stay in our study, it was not statistically significant ($p>0.05$).

In the neonatal period, patients requiring intervention by pediatric surgery are frequently of low-birth-weight patients with a possible late diagnosis (2,9,15). Early detection of congenital anomalies and taking precautions for a congenital anomaly in the early postnatal period greatly reduce the risk of neonatal mortality and morbidity (17). The incidence of mortality was reported to be high in patients with necrotizing enterocolitis (NEC), diaphragmatic hernia, and esophageal atresia (13). In the retrospective evaluation of 114 cases with jejunoileal atresia, the mortality rate was 11%, while the mortality rate in cases with NEC could reach 25% (18,19). NEC is one of the leading causes of gastrointestinal mortality in premature infants with an incidence of 1-5 per 1000 neonates (20). In multicenter studies, it is estimated that 7-13% of neonates born before 33 weeks of gestation and weighing less than 1000 g have NEC. Mortality is expected approximately as 50%, especially in those requiring surgical treatment. While most cases are premature infants, the incidence of NEC in term infants is quite low with the overall mortality rate ranging from 15% to 63% (21,22).

In the current study, the mortality rate in all patients that were managed in the postoperative period was 16.6%. The highest mortality was detected in respiratory system surgery (38.46%) which was followed by gastrointestinal system surgery (12.28%) and genitourinary system surgery (7.69%), respectively. Since the organ systems were considered as a whole, no distinction was made according to the diagnoses.

In a study by Puri A et al which comprised of 150 neonates, 30-day mortality rate was found 56.81% in 44 patients who had thoracic surgery and 24.17% who had abdominal surgery. This is roughly consistent with our results (23). Respiratory system surgeries carry a higher burden of risk from the anesthesiologic point of view. Alveolar maturation in the neonatal period is still a proceeding process at birth achieving its peak until 8-10 years of age. Surgery and anesthesia together decrease the durability of an already unstable physiology of the sick neonates (24,25). Therefore, we attribute the high mortality rate of respiratory system surgery to insufficient alveolar development of neonates, further curtailment in respiratory capacity during surgery, and negative effects of anesthetic agents on respiratory parameters such as functional residual capacity.

In our study, we examined patient records through the hospital automation system. Therefore, we completed our study with the data we could obtain. When examining the mortality data of the patients, we could not include those transferred to another center. Since the perioperative anesthesia records of the patients were not recorded on the hospital automation system at the time of the examination, we stated the anesthesia complications in the epicrisis reports. Due to limited data, we interpreted our study according to demographic data, mortality, and surgical field information.

## CONCLUSION

To reduce the incidence of mortality of cases undergoing surgical procedure, we believe that it can be possible with appropriate prenatal and postnatal follow-up, easy access to health services countrywide, appropriate transport procedures, employment of physicians and nurses experienced in neonatal care and surgery, appropriate anesthesia settings and conditions along with the improvement of neonatal intensive care unit environment.

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RESEARCH ARTICLE

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# An Evaluation of Damages Caused by Doxorubicin in Liver Tissue and Potential Protective Effect of Propolis on These Damages

**ABSTRACT**

**Objective:** Doxorubicin (DOX), one of the chemotherapeutic drugs utilized in cancer treatment, has limited clinical use due to its serious toxic effects on non-target organs. The purpose of this study is to reveal the harmful effects of DOX in rat liver and the possible protective effect of propolis (PRPLS), a mixture of various herbal products collected by honeybees, on these damages by Attenuated Total Reflection-Fourier Transformation Infrared (ATR-FTIR) spectroscopy.

**Methods:** *Sprague dawley* rats were separated into 4 groups; control, DOX (cumulative dose: 15 mg/kg), PRPLS (200 mg/kg) and DOX + PRPLS. The rats were given 200 mg/kg PRPLS by oral gavage daily for 20 consecutive days and 2.5 mg/kg DOX intraperitoneally on days 10, 12, 14, 16, 18 and 20 of the experiment. 24 hrs after the last administrations, liver samples were collected and examined by ATR-FTIR spectroscopy.

**Results:** DOX caused a decrease in the amount of glycogen and nucleic acids, an increase in the amount of lipids and proteins and some important changes in the metabolism, structure and conformation of these molecules in the liver. DOX also induced lipid peroxidation, an increase in membrane fluidity, a decrease in membrane order and protein denaturation. PRPLS did not induce any toxic effect on the liver when it was given alone and PRPLS administered before DOX was not effective to eliminate these harmful effects of DOX.

**Conclusions:** DOX caused significant structural and compositional changes in liver tissue and PRPLS was inadequate to prevent these changes at the dose and time used here.

**Keywords:** Doxorubicin, Propolis, ATR-FTIR Spectroscopy, Liver, Chemotherapy.

# Doksorubisinin Karaciğer Dokusunda Oluşturduğu Hasarın ve Propolisin Bu Hasar Üzerindeki Potansiyel Koruyucu Etkisinin Değerlendirilmesi

**ÖZET**

**Amaç:** Kanser tedavisinde kullanılan kemoterapötik ilaçlardan birisi olan doksorubisin (DOX), hedef dışı organlar üzerindeki ciddi toksik etkileri nedeniyle sınırlı klinik kullanıma sahiptir. Bu çalışmanın amacı, DOX'un sıçan karaciğerindeki zararlı etkilerini ve bal arıları tarafından toplanan çeşitli bitkisel ürünlerin bir karışımı olan propolis (PRPLS)'in bu zararlar üzerindeki olası koruyucu etkisini Azaltılmış Toplam Yansıma-Fourier Dönüşüm Kızılötesi (ATR-FTIR) spektroskopisi ile ortaya çıkarmaktır.

**Gereç ve Yöntem:** *Sprague dawley* sıçanlar 4 gruba ayrılmıştır; kontrol, DOX (kümülatif doz: 15 mg/kg), PRPLS (200 mg/kg) ve DOX + PRPLS. Sıçanlara ardışık 20 gün boyunca oral gavaj yoluyla günlük 200 mg/kg PRPLS ve 10, 12, 14, 16, 18 ve 20. günlerde intraperitoneal olarak 2,5 mg/kg DOX verilmiştir. Son uygulamalardan 24 saat sonra karaciğer örnekleri alınmış ve ATR-FTIR spektroskopisi ile incelenmiştir.

**Bulgular:** DOX karaciğerde glikojen ve nükleik asit miktarında azalmaya, lipid ve protein miktarında artışa ve bu moleküllerin metabolizması, yapısı ve konformasyonlarında bazı önemli değişikliklere sebep olmuştur. DOX, ayrıca lipid peroksidasyonuna, membran akışkanlığında bir artışa, membran düzeninde bir azalmaya ve protein denatürasyonuna neden olmuştur. PRPLS tek başına verildiğinde karaciğer üzerinde herhangi bir toksik etki oluşturmamış ve DOX'tan önce verilen PRPLS, DOX'un zararlı etkilerini ortadan kaldırmada etkili olamamıştır.

**Sonuç:** DOX, karaciğer dokusunda önemli yapısal ve kompozisyonel değişikliklere neden olmuş ve PRPLS bu çalışmada kullanılan doz ve zamanda bu değişiklikleri önlemekte yetersiz kalmıştır.

**Anahtar Kelimeler:** Doksorubisin, Propolis, ATR-FTIR Spektroskopisi, Karaciğer, Kemoterapi.

## INTRODUCTION

Doxorubicin (DOX), also known as adriomycin, is an efficient chemotherapeutic drug belonging to the group of antracycline antibiotics. It has a very broad spectrum and is extensively used in the therapy of numerous cancers such as leukemias, lymphomas and various solid tumors (1). However, the clinical use of DOX is restricted due to its significant toxic side effects in some non-target organs including the liver, testis and heart (2-5). It has been known that the liver is one of the most vulnerable organs to DOX damage and liver damage is a common DOX-related side effect observed in the treatment of other types of cancer using this drug (3). Even though the mechanism of DOX-mediated liver injury is not known exactly, results from previous studies suggest that it induces the formation of reactive oxygen species (ROS), nucleic acids strand breakage and apoptosis (3, 4).

Liver is the principle organ responsible for the detoxification of chemicals, excretion of wastes in bile and various critical roles such as the synthesis, storage and redistribution of carbohydrates, lipids and proteins (6). Like other drugs, DOX is metabolized in the liver and then excreted in bile. If liver function is impaired, the elimination of DOX slows down and it begins to accumulate in the body and causes adverse effects on other tissues. Thus, understanding the mechanisms of DOX-induced toxicity in the liver is very important to eliminate these toxic effects, to increase efficiency of this drug and to reduce the damages to other tissues.

Propolis (PRPLS), a bee product formed by honey bees to protect the hive from intruders, contains hundreds of compounds with powerful antioxidant properties including phenols, flavonoids, terpenoids and vitamins. Among these compounds polyphenols and flavanoids have been suggested to be responsible for the biological activities of it (7). On the other hand, these chemical compounds vary according to flora, geographical origin, honeybee subspecies, collection season and PRPLS extraction method (8). In previous studies, it has been shown that PRPLS has antioxidant, antibacterial, antiviral, antifungal, anti-inflammatory and immunomodulatory effects on biological systems (9). Based on these studies, it can be considered that the use of PRPLS together with chemotherapy may be effective in reducing or stopping the toxicity of DOX on healthy tissues. Previously, the protective effects of polyphenolic extracts from Algerian PRPLS, aqueous extract of Egyptian PRPLS and ethanolic extract of Indian PRPLS on different tissues against the toxical impacts of DOX have been demonstrated (2, 4, 5, 10). However, in the literature, there is no study regarding the protective effect of Turkish PRPLS (Yığılca) against the toxicity of DOX on liver tissue.

The proper functioning of a tissue is related to its structure, while the structure and function of a tissue depend on the physicochemical properties and compositions of the biomolecules in it. Thus, compositional and structural changes in a tissue can be indicators of various metabolic disorders. Fourier Transform Infrared (FTIR) spectroscopy is a high-tech product that enables the examination of structural and compositional changes in tissues. It measures the vibrations of molecules at different wavelengths, enables the visualization and characterization of different vibrational groups and therefore provides valuable information about biological systems. Using FTIR spectroscopy, molecular alterations in cellular constituents such as carbohydrates, proteins, nucleic acids and lipids can be determined at the functional group level. In FTIR spectrometers by utilizing the Attenuated Total Reflectance (ATR) unit, it is possible to determine such alterations more quickly and accurately by minimizing sample preparation or by examining samples directly, regardless of sample thickness. Detection of alterations in the band area ratios, wavenumbers and bandwidths of ATR-FTIR bands gives information about the amounts of biomolecules, their interactions with each other, the level of lipid peroxidation and secondary structures of proteins in a tissue (11-13).

Although it has been shown that DOX has many toxic impacts on the liver tissue, the studies investigating the toxic effects of this drug on the liver are generally at histological or biochemical level (3, 4, 14) and do not provide enough information about the structural, compositional and functional changes that may have caused the pathology in the liver tissue. The purpose of the current study is to reveal the damages caused by DOX in liver tissue at molecular level and to evaluate the protective effect of Turkish PRPLS against the damaging effect of DOX on this tissue by using ATR-FTIR spectroscopy.

## MATERIAL AND METHODS

**Propolis:** PRPLS was obtained from the Düzce University Beekeeping Research and Application Centre, Düzce, Turkey. Since ethanol is a good solvent for PRPLS and not toxic for organisms, ethanolic extraction of PRPLS was preferred (5, 10). Ethanolic extracts of PRPLS (completing the volume of 20 g of PRPLS to 100 ml with 96% ethanol) were prepared, left for 5 days in the dark under moderate shaking and filtered utilizing filter paper. The filtrate was evaporated by utilizing a vacuum evaporator. Then the residual was dissolved in 70% ethanol and stored at +4 oC until use.

**Animal Experiments:** Animal experimental design of the current study was approved by the Düzce University Experimental Animals Ethics Committee (2019/1/9). Male Sprague-Dawley rats

(10-12 weeks old, 250-300 g) were housed in a 12-h light:12-h dark photo period with standard rat diet and water ad libitum at room temperature (22 ± 2 °C). Animals were administered 200 mg/kg PRPLS ethanolic extract by oral gavage daily for 20 days (2) and DOX (Adrimisin, Saba Pharma, İstanbul) intraperitoneally (i.p.) in six divided doses (2.5 mg/kg) with a total cumulative dose of 15 mg/kg (15). The rats were separated into 4 groups: 1. Control group (n=7): Animals were given the solvent of PRPLS (ethanol) for 20 days by oral gavage and the solvent of DOX i.p. (saline) on days 10, 12, 14, 16, 18 and 20. 2. DOX group (n=7): Animals were given the solvent of PRPLS for 20 days and DOX (2.5 mg/kg; i.p.) on days 10, 12, 14, 16, 18 and 20. 3. PRPLS group (n=6): Animals were given PRPLS daily for 20 days and the solvent of DOX on days 10, 12, 14, 16, 18 and 20. 4. PRPLS + DOX group (n=7): Animals were given PRPLS daily for 20 days and DOX (2.5 mg/kg; i.p.) on days 10, 12, 14, 16, 18 and 20. 24 hrs after the last administrations, the rats were euthanized; the liver tissues were removed and stored at -80 °C until use.

**Spectroscopic Studies:** The spectra of liver tissues were obtained with a Spectrum Two FTIR spectrometer (Perkin-Elmer Ltd., UK) attached with an ATR accessory. 0.5 X 0.5 X 0.1 cm sized samples cut from three different parts of the rat liver were put on the diamond/zinc-selenite crystal of the ATR and treated with nitrogen gas (N2) for five minutes to get rid of the water in the environment. The tissue was compressed by applying 100 force gauge pressure to ensure a smooth surface contact. The spectra were collected with a scanning number of 64 at a resolution of 4 $cm^{-1}$ in the 4000-400 $cm^{-1}$ wavenumber range at room temperature. In order to improve the validity of the results, 3 replicates were scanned from the neighboring portions of these chosen parts of each sample and the averages of these spectra were taken and analyzes and statistical tests were performed on the average spectra (11).

Spectral analyses were performed using Perkin Elmer Spectrum 100 and OPUSNT (Bruker Optics, Reinstetten, Germany) softwares. Raw mean spectra were used for detailed analysis. Bandwidth and wavenumber values were calculated from 75% of height. For visual representation, the averaged spectra were baseline corrected and normalized. The second derivative vector normalization method was applied to the 1700-1600 cm-1 region, which consists of unresolved bands, to determine the protein secondary structure changes (11).

**Statistical Analysis:** The statistical analyses were conducted using GraphPad Prism (Version 9.3.1) software. Data were subjected to the Shapiro-Wilk test to characterize their normality. Since the data did not show a normal distribution, the ATR-FTIR results for each group were statistically analyzed by employing non-parametric Kruskal-Wallis test followed by Dunn's multiple comparisons test. The values are expressed as "mean ± standard deviation". The calculated p values less than 0.05 were considered as statistically significant.

## RESULTS

Figures 1A-C depict the average ATR-FTIR spectra of rat livers of experimental groups in the wavenumber ranges of 3700-3025 $cm^{-1}$, 3025-2800 $cm^{-1}$ and 1800-950 $cm^{-1}$. In these figures, the essential bands are numbered and assignment of each band according to the literature is given in Table 1.

**Table 1.** Major bands in the ATR-FTIR spectrum of the rat liver and their assignments (11, 12).

| Band No | Wavenumber ($cm^{-1}$) | Band Assignment |
|---|---|---|
| **1** | **3313** | **Amide A:** Proteins (N-H stretching), O-H stretching of polysaccharides, water |
| **2** | **3012** | **Olefinic HC=CH stretching:** Unsaturated lipids |
| **3** | **2965** | **$CH_3$ antisymmetric stretching:** Lipids and protein side chains |
| **4** | **2929** | **$CH_2$ antisymmetric stretching:** Mainly lipids |
| **5** | **2874** | **$CH_3$ symmetric stretching:** Mainly proteins |
| **6** | **2856** | **$CH_2$ symmetric stretching:** Mainly lipids |
| **7** | **1743** | **Carbonyl (C=O) ester stretching:** Triglycerides, cholesterol esters and phospholipids |
| **8** | **1640** | **Amide I:** Proteins (80 % C=O stretching) |
| **9** | **1547** | **Amide II:** Proteins (40% C-N stretching, 60% N-H bending) |
| **10** | **1456** | **$CH_2$ bending:** Mainly lipids |
| **11** | **1400** | **$COO^-$ symmetric stretching:** Lipids and proteins |
| **12** | **1308** | **Amide III:** Proteins |
| **13** | **1238** | **$PO_2^-$ antisymmetric stretching:** Phospholipids and nucleic acids |
| **14** | **1153** | **CO-O-C antisymmetric stretching:** Phospholipids, cholesteryl esters and nucleic acids |
| **15** | **1120** | **Ribose ring vibrations:** RNA |
| **16** | **1082** | **$PO_2^-$ symmetric stretching:** Phospholipids and nucleic acids<br>**C–O stretching:** Polysaccharides, glycolipids |
| **17** | **1033** | **C–O stretching:** Glycogen |
| **18** | **1025** | **DNA vibrations** |
| **19** | **972** | **C-$N^+$-C stretching:** Nucleic acids, ribose-phosphate main chain vibrations of RNA |

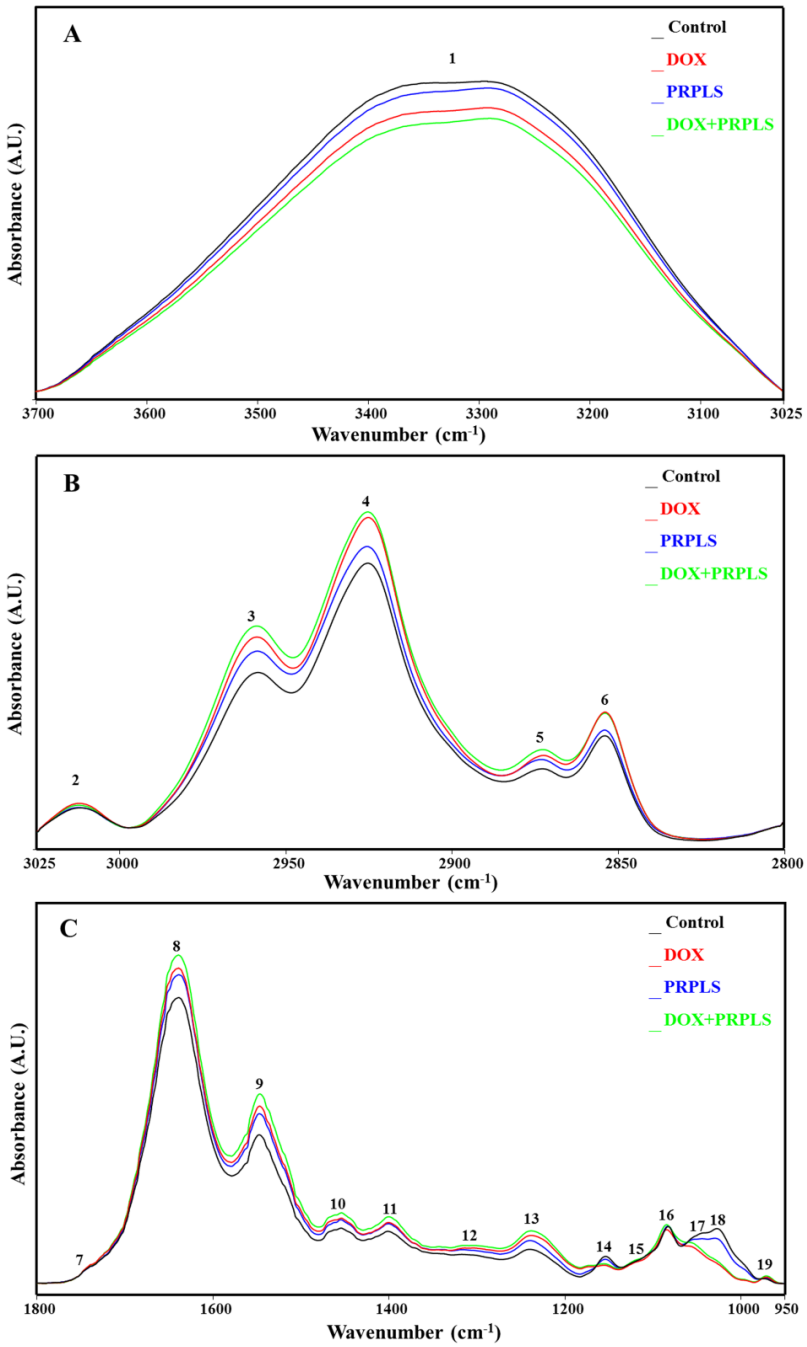


**Figure 1.** Average, baseline corrected and normalized ATR-FTIR spectra of control, DOX, PRPLS and DOX + PRPLS treated rat liver tissues in the A) 3700-3025, B) 3025-2800 and C) 1800-950 $cm^{-1}$ wavenumber regions. Spectra were normalized with respect to the $CH_2$ antisymmetric stretching band (A) and to the Amide A band (B and C).

In the current study, the area ratios of some specific infrared bands, which provide information about the amounts of the molecules, were analyzed to eliminate errors that may result from differences in experimental conditions (11, 13). The comparisons of area ratios of infrared bands between the control and treated groups are given in Table 2. The area ratios utilized in our study, the functional groups utilized to obtain these ratios and the functions of the calculated ratios are presented as Supplementary Material (Supplementary Table 1).

Changes in the amounts of saturated lipids were examined by calculating the band area ratio of $CH_2$ sym./$CH_2$ sym. + antisym. (13). As seen from Table 2, this ratio increased significantly in the DOX and DOX + PRPLS administered groups compared to control group. The olefinic HC=CH/lipid ratio, which was used to detect the alterations in the amount of unsaturated lipids, decreased significantly in the DOX and DOX + PRPLS treated groups in comparison with control group (Table 2).

**Table 2.** Alterations in the band area ratios of a subset of ATR-FTIR bands obtained from the liver spectra of control, DOX, PRPLS and DOX + PRPLS treated rats.

| Band Area Ratio | Control | DOX | PRPLS | DOX + PRPLS |
|---|---|---|---|---|
| $CH_2$ sym./$CH_2$ sym + antisym. | 1.306±0.022 | 1.366±0.025* | 1.325±0.044 | 1.377±0.046* |
| Olefinic HC=CH/ Lipid | 0.042±0.004 | 0.034±0,004* | 0.040±0.004 | 0.032±0.004* |
| Amide I/Amide I + II | 7.024±0.457 | 8.107±0.440* | 7.485±0.746 | 8.250±0.798*+ |
| RNA/Protein | 0.055±0.008 | 0.043±0.007* | 0.047±0.006 | 0.043±0.003* |
| RNA/Lipid | 0.669±0.067 | 0.509±0.039** | 0.612±0.077 | 0.532±0,059* |
| DNA/Protein | 0.152±0.016 | 0.046±0.009*** | 0.122±0.02# | 0.049±0.005** |
| DNA/Lipid | 1.715±0.145 | 0.542±0.066*** | 1.459±0.238# | 0.630±0.087** |
| RNA/DNA | 0.375±0.024 | 1.235±0.088** | 0.447±0.075 | 1.063±0.100** |
| C-O/Protein | 0.315±0.035 | 0.122±0.021** | 0.267±0.051# | 0.126±0.018** |
| C-O/Lipid | 3.823±0.419 | 1.449±0.252*** | 3.422±0.209# | 1.556±0.174** |
| C-O/$PO_2^-$ | 1.409±0.170 | 0.657±0.090** | 1.262±0.159# | 0.705±0.080** |
| $CH_2$ antisym./$CH_3$ antisym. | 2.512±0.170 | 2.251±0.097* | 2.407±0.278 | 2.258±0.202* |
| C=O/Lipid | 1.154±0.055 | 0.950±0.079** | 1.078±0.085 | 0.941±0.068** |
| $CH_3$ antisym./Lipid | 0.223±0.015 | 0.269±0.009** | 0.237±0.015 | 0.278±0.020** |
| Amide I/Amide II | 2.361±0.086 | 2.173±0.078* | 2.239±0.212 | 2.118±0.161* |
| Lipid/Protein | 0.082±0.002 | 0.085±0.001* | 0.078±0.007 | 0.084±0.002* |

Degree of significance was denoted as $^{*,\#,+}p<0.05$, $^{**,\#\#,++}p<0.01$, ***,$^{\#\#\#,+++}$ p<0.001. (*): control vs. DOX, PRPLS and DOX + PRPLS; (#): DOX vs. PRPLS and DOX + PRPLS; (+): PRPLS vs. DOX + PRPLS

Alterations in the protein concentrations were evaluated by calculating Amide I/Amide I + II ratio (13). As can be seen from Table 2, this ratio increased significantly in the DOX and DOX + PRPLS treated groups compared to control group. In addition, the DOX + PRPLS treated group presented significantly higher level of this ratio in comparison with those observed in the PRPLS group. The band at 1120 $cm^{-1}$ arises from ribose ring vibrations in the RNA and gives information about the RNA molecules in the system. Using this band, RNA/protein and RNA/lipid ratios were calculated to attain information about the amount of RNA in the tissue (12). To obtain information about the amount of DNA, DNA/protein and DNA/lipid ratios were calculated using the 1025 $cm^{-1}$ band, which is a special DNA band (12). All these ratios decreased significantly in the DOX and DOX + PRPLS administered groups compared to control group (Table 2). The changes in the RNA/DNA ratio were utilized to obtain information about the transcription status of the cells (12). As can be seen from Table 2, this ratio increased significantly in the DOX and DOX + PRPLS treated groups compared to control groups. As also seen from Table 2, DNA/protein and DNA/lipid ratios were found to be significantly higher in the PRPLS treated group when compared to DOX administered group.

Since the C-O stretching band, which appears at 1033 $cm^{-1}$, gives information about glycogen, C-O/protein and C-O/lipid ratios were calculated to analyze the alterations in the concentration of glycogen in the system (11). As seen from Table 2, these ratios significantly decreased in the DOX and DOX + PRPLS treated groups in comparison with control group. To attain information about the changes in the metabolic activity of the tissue, C-O/$PO_2^-$ (glycogen/phosphate) ratio was used (12) and it has been observed that this ratio decreased significantly in the DOX and DOX + PRPLS treated groups compared to control group (Table 2). C-O/protein, C-O/lipid and C-O/$PO_2^-$ ratios were also found to be significantly higher in the PRPLS treated group when compared to DOX administered group.

$CH_2$ antisym./$CH_3$ antisym. ratio was utilized to detect the changes in the chain length of phospholipids, while the C=O/lipid and $CH_3$ antisym./lipid ratios were utilized to detect the changes in the carbonyl status of and methyl concentration in the tissue, respectively (11, 16). As seen from Table 2, the $CH_2$ antisym./$CH_3$ antisym. and C=O/lipid ratios decreased and the $CH_3$ antisym./lipid ratio increased significantly in the DOX and DOX + PRPLS treated groups in comparison with control group. To obtain information about the structural alterations in the proteins, the ratio of Amide I/Amide II was calculated (11) and it has been seen that this ratio decreased significantly in the DOX and DOX + PRPLS treated groups compared to control group. In addition, to compare the relative alterations in the amounts of lipids and proteins, the lipid/protein ratio was calculated (16). This ratio increased significantly in the DOX and DOX + PRPLS treated groups in comparison with control group (Table 2).

The alterations in the wavenumbers of the $CH_2$ antisymmetric and symmetric stretching bands and bandwidth of the $CH_2$ antisymmetric stretching band were analyzed to examine the order and fluidity of the biological membrane (16). As seen from Table 3, the wavenumber values of the $CH_2$ antisymmetric and symmetric stretching bands shifted to higher values in the DOX and DOX + PRPLS treated groups and the bandwidth of the $CH_2$ antisymmetric stretching band increased significantly in the same groups compared to control group.

**Table 3.** Alterations in the wavenumber and bandwidth values of a subset of ATR-FTIR bands obtained from the liver spectra of control, DOX, PRPLS and DOX + PRPLS treated rats.

| Functional Group | Control | DOX | PRPLS | DOX+PRPLS |
|---|---|---|---|---|
| **Peak position (Wavenumber)** | | | | |
| $CH_2$ antisym. str. | 2928.031±0.628 | 2928.996±0.733* | 2928.361±0.911 | 2928.646±0.342* |
| $CH_2$ sym. str. | 2855.149±0.390 | 2855.75±0.411* | 2855.421±0.342 | 2855.739±0.366* |
| Amide I | 1640.674±0.324 | 1639.791±0.871* | 1640.183±0.674 | 1639.964±0.201** |
| **Bandwidth** | | | | |
| $CH_2$ antisym. str. | 9.609±0.156 | 9.903±0.103* | 9.778±0.716 | 9.941±0.316* |
| Amide I | 34.556±0.221 | 34.116±0.291* | 34.307±0.385 | 33.866±0.558* |

Degree of significance was denoted as $^{*,\#,+}p<0.05$, $^{**,\#\#,++}p<0.01$, ***,$^{\#\#\#,+++}$ $p<0.001$. (*): control vs. DOX, PRPLS and DOX + PRPLS; (#): DOX vs. PRPLS and DOX + PRPLS; (+): PRPLS vs. DOX + PRPLS.

In order to attain information about the conformational alterations in proteins, the wavenumber and bandwidth of the Amide I band were analyzed (11). As seen from Table 3, the wavenumber of this band shifted to lower values and its bandwidth decreased in the DOX and DOX + PRPLS groups in comparison with control group. To evaluate the changes in the secondary structures of proteins, vector normalized second derivative ATR-FTIR spectra were analyzed in the Amide I band region (11). As seen from Table 4, the intensities of the turn and random coil structures increased significantly and the intensities of the alpha-helix and beta-sheet structures decreased significantly in the DOX and DOX + PRPLS treated groups. As also seen from Table 4, the intensity of alpha-helix structure decreased and the intensity of random coil structure increased significantly in the DOX + PRPLS treated group compared to the PRPLS treated group.

**Table 4.** Alterations in the intensity values of the principle protein secondary structures from the Amide I band region of vector normalized second derivative liver spectra of control, DOX, PRPLS and DOX + PRPLS treated rats.

| Functional Group | Control | DOX | PRPLS | DOX+PRPLS |
|---|---|---|---|---|
| Turns (1684 $cm^{-1}$) | 0.130 ± 0.005 | 0.137 ± 0.005* | 0.132 ± 0.007 | 0.139 ± 0.008* |
| Alpha-helix (1651 $cm^{-1}$) | 0.298 ± 0.007 | 0.283 ± 0.008* | 0.309 ± 0.009 | 0.283 ± 0.011*+ |
| Random coil (1640 $cm^{-1}$) | 0.111 ± 0.007 | 0.127 ± 0.009* | 0.109 ± 0.010 | 0.129 ± 0.009*+ |
| Beta-sheet (1633 $cm^{-1}$) | 0.111 ± 0.014 | 0.075 ± 0.009** | 0.097 ± 0.019 | 0.077 ± 0.009** |

Degree of significance was denoted as $^{*,\#,+}p<0.05$, $^{**,\#\#,++}p<0.01$, ***,$^{\#\#\#,+++}$ $p<0.001$. (*): control vs. DOX, PRPLS and DOX + PRPLS; (#): DOX vs. PRPLS and DOX + PRPLS; (+): PRPLS vs. DOX + PRPLS.

## DISCUSSION

Alterations in the structure and composition of lipids, which are the main components of cell membranes and important storage molecules in liver cells, may cause important dysfunctions in this tissue. In this study, the increase detected in the ratio of $CH_2$ sym./$CH_2$ sym.+antisym. after DOX administration showed that DOX induced an increase in the concentration of saturated lipids in rat liver (13). This result suggested that DOX administration caused alterations in lipid metabolism, resulting in lipid accumulation in the liver. It has been reported that DOX increased the production of ceramides, dihydro-ceramides, sphingosine and dihydrosphingosine, which is an indicator of a disorder in the sphingolipid metabolism in the liver (17). In addition, it has been reported that DOX caused liver steatosis (fatty liver) in different experimental animals (1, 18). It has been known that liver steatosis also causes a decrease in glycogen concentration (19). In a previous study, an increase in the amount of lipid together with a decrease in the amount of glycogen during liver steatosis has been demonstrated via FTIR spectroscopy (20). Indeed, when the C-O/protein and C-O/lipid ratios were analyzed to determine the alterations in glycogen amount in the liver tissue, a decrease upon DOX administration was observed. This result indicated that DOX led to a decrease in glycogen amount in rat liver tissue (11). In consistence with our result, it has been reported that DOX reduced insulin-induced glucose uptake and glycogen synthesis in liver cells and glycogen stores in the heart tissue of rats (17, 21). Thus, the decrease observed in the amount of glycogen might have arisen from the significant changes induced by DOX in glycogen and lipid metabolism in the tissue. Changes in the amount of lipid and glycogen, which are the main energy storage materials of the liver, affect the energy metabolism of the system. Since excessive amounts of carbohydrates and proteins are converted to lipids and stored in the liver, our results indicated that the energy metabolism of the liver was significantly affected by DOX. The decrease observed in the glycogen/phosphate (C-O/$PO_2^-$) ratio after DOX administration, also confirmed that DOX induced significant changes in the metabolic activity of the liver tissue (12).

The double bonds in unsaturated fatty acids are extremely vulnerable to lipid peroxidation, which is a molecular injury mechanism initiated by ROS and an indicator of high oxidative stress in

tissues. If unsaturated fatty acids in lipids are attacked by ROS, a lipid peroxidation chain reaction starts and these harmful reactions lead to the breakdown of lipids and ultimately a decrease in unsaturated fatty acid concentration, namely olefinic bonds (22). For this reason, in biological FTIR spectroscopy studies the olefinic HC=CH/lipid ratio (unsaturated/saturated ratio) is utilized as lipid peroxidation index (11). In this study, the decrease noticed in the olefinic HC=CH/lipid ratio indicated that DOX caused a decrease in the concentration of unsaturated lipids in liver tissue. It has been known that semiquinone electron fragments formed in the liver after DOX administration produce ROS by reducing oxygen and these oxidative free radicals participate in the DOX-induced lipid peroxidation (14). Consistent with the literature, our findings also showed that DOX caused an increase in the level of lipid peroxidation possibly by inducing oxidative stress in liver tissue. In a previous FTIR spectroscopy study related to lipid peroxidation, it has been found that there is also a decrease in the $CH_2$ and carbonyl (C=O) groups and an increase in the $CH_3$ groups in the tissue of interest (11). When these parameters were examined in this study, it has been observed that $CH_2$ antisym./$CH_3$ antisym. and C=O/lipid ratios decreased and $CH_3$ antisym./lipid ratio increased significantly after DOX administration. These changes showed that lipids were broken down by ROS into smaller fragments, which have less $CH_2$ and C=O groups and more $CH_3$ groups, and could be attributed to lipid peroxidation in the DOX-treated tissue. These findings demonstrated that the action mechanism of DOX is associated with its potential for induction of oxidative stress and lipid peroxidation. In earlier studies, it has been indicated that DOX caused lipid peroxidation in the liver and other tissues. For example, Afsar et al. (23) have shown that hepatotoxins, which were formed as a result of the generation of ROS after DOX administration in the liver, caused a disruption in lipid profile and an increase in lipid peroxidation by reacting with polyunsaturated fatty acids. Ozdoğan et al. (24) have shown that elevated ROS production after DOX administration was directly related to the damage occurring in cardiac myocyte membranes via lipid peroxidation. In addition, it has been shown that DOX led to alterations in the level of endogenous antioxidant enzyme activities and formation of lipid peroxidation products (malondialdehyde) by causing oxidative stress in various tissues (4, 18). Thus, the decreases observed in the olefinic HC=CH/lipid, $CH_2$ antisym./$CH_3$ antisym. and C=O/lipid ratios and the increase in the $CH_3$ antisym./lipid ratio indicated that DOX caused lipid peroxidation in rat liver tissue.

In this study, the increase observed in the ratio of Amide I/Amide I+II showed that there was an increase in the concentration of proteins in the liver after DOX administration (13). This increment in the amount of proteins might be due to an increase in protein synthesis in this tissue. In previous studies, it has been shown that liver enzymes, such as alanine transaminase and aspartate transaminase increased after DOX administration (14). In addition, it has been reported that the amount of cysteine, an amino acid in thiol structure, increased in the liver after DOX administration (25). Therefore, this increase in the amount of protein might be due to the increase in the concentration of some enzymes and some amino acids after DOX administration in the liver.

The decreases detected in the wavenumber and bandwidth values of the Amide I band after DOX administration indicated a change in protein conformation (13). This finding was also supported by the decrease observed in the Amide I/Amide II ratio after DOX administration. It has been known that any pathological condition in the liver tissue causes a decrease in the Amide I/Amide II ratio and thus various alterations in the protein structure (11). The findings of the vector normalized second derivative spectra analyses revealed more detailed information about the changes in the secondary structure of proteins. The increase observed in the random coil structure showed that DOX caused protein denaturation in the liver cells (11). The protein denaturation observed in the liver might have resulted from the increase in the amount of ROS induced by DOX. The appropriate functioning of proteins is dependent on their three-dimensional structure and errors that may occur during protein folding can lead to differences in protein secondary structure and thus functional damage. The increase in DOX-induced ROS formation might have caused changes in the protein secondary structure by changing the redox potential of the cell (26). Our results are consistent with previous studies that have reported that ROS formed in the tissue after DOX administration caused deterioration in protein structure. Previously, Oz et al. (21), have shown that myofibril proteins in heart cells had more disordered structures after DOX administration. Yagmurca et al. (3) have shown that DOX caused protein oxidation together with lipid peroxidation in rat liver tissue. Oxidation of proteins (e.g. enzymes) causes significant alterations in their structure and function that are important for cells.

The alterations in proteins observed in DOX-treated groups might have also been the result of changes in the levels of gene expression or protein synthesis. In this study, decreases observed in the RNA/lipid, RNA/protein, DNA/lipid and DNA/protein ratios showed that the concentrations of nucleic acids in liver tissue decreased after DOX administration. Since DOX is a moderately lipophilic drug, it has a high binding capacity to the cellular and nuclear membranes and nucleic acids. Therefore, it may accumulate in the nuclei of liver

cells and cause DNA damage. It has been known that one of the mechanisms of action of DOX is to block DNA and RNA synthesis by locating between adjacent base pairs (27). DOX contains an amino sugar and an anthracycline ring. The aglycone part of the drug enters between DNA and RNA, binds ionically to these molecules to stabilize the intercalation of the ionic sugar structure, and as a result, DNA and RNA are deformed during synthesis (4). Thus, the decrease in the amount of nucleic acids in the tissue observed by analysing the DNA and RNA bands might have resulted from the inhibition of the synthesis of these molecules by DOX. In addition, it has been known that ROS formed in the liver after DOX administration oxidizes DNA and RNA and causes deterioration in their structures (27). The decrease noticed in the RNA/lipid ratio also implied some alterations in the proliferation state or growth capacity of cells after DOX administration (12). Regarding the nucleic acids, the RNA/DNA ratio was also calculated and an increase in this ratio was observed after DOX administration. This increase showed that the transcription rate of some genes was higher in DOX-treated groups than in the control group (12). It has been known that the transcription level of the genes responsible for the synthesis of proteins; that is the amount of mRNA; increases after DOX administration. For example, in previous studies it has been reported that hepatic injury induced by DOX begins with the expression of genes responsible for drug delivery, cell cycle progression, oxidative stress response, mitochondrial disorders, apoptosis, DNA damage and DNA repair (28).

In the current study, to determine the effect of DOX on the relative amounts of lipids and proteins in the system, the lipid/protein ratio was evaluated. This ratio also enables the analysis of changes in lipid and protein asymmetry, which are important indicators for cell functions. In addition, a change in this ratio indicates a modification in the structure, arrangement and fluidity of the membrane (11). A significant increase in the lipid/protein ratio was detected after DOX administration in the liver tissue. In our study, since an increase in both lipid and protein amount after DOX administration has been detected, the increase in this ratio indicated that the increase in lipid amount was higher. This result showed that DOX affected lipid metabolism more than protein metabolism in the liver (11, 12). This increase also showed that DOX caused alterations in lipid and protein asymmetry in the liver cell membranes and supported the result that it caused alterations in lipid and protein metabolism. Since the alterations in lipid and protein asymmetry significantly affect membrane function, they may cause significant changes in the concentrations of ions inside and outside the cells.

Changes in the parameters associated with the order and fluidity of the phospholipids, the main components of the cellular membrane, affect the normal functioning of membranes. The shifts towards higher values detected in the wavenumbers of the $CH_2$ antisymmetric and symmetric stretching bands after DOX administration indicated a decrease in the order and an increase in the acyl chain flexibility of membrane lipids (11, 16). A significant increase observed in the bandwidth of the $CH_2$ antisymmetric stretching band indicated that DOX caused an increase in the fluidity of membrane. These alterations might be due to the differences in the ratios of different lipid species and the lipid/protein ratio as a result of the oxidative stress induced by DOX. In addition, increased lipid peroxidation due to DOX administration might have caused some changes in membrane functions, loss of membrane structure integrity and inactivation of many membrane-bound protein receptors and enzymes (5, 10). In a previous study using electron paramagnetic spectroscopy, it has been shown that oxidative damage in the liver caused a decrease in membrane order (29). Biological membranes control a number of important functions such as signal transmission, material transfer and the activity of membrane-bound enzymes. In addition, the changes in membrane order and dynamics, secondary structures of proteins in membranes and molecular content of membranes cause many diseases by damaging the integrity and function of ion channels (11). Therefore, the order and fluidity of membrane should be at the optimum level in cells and this is very important for the degree of permeability to DOX. In consistence with previous studies, our results indicated that DOX disrupted the normal function of the cell membrane by changing its order and fluidity. These harmful effects on cellular membranes might be one of the toxic action mechanisms of DOX in biological tissues.

In order to obtain information about the effects of PRPLS on the molecules in the liver tissue, a group of animals was given PRPLS only. Our results indicated that PRPLS did not induce any significant change on the composition and structure of biomolecules in the liver when given alone to animals compared to the control group. This finding showed that PRPLS is not toxic to the body and it can be used for various purposes in medicine.

The results of the group that was given PRPLS before DOX administration showed that there were significant changes in the analyzed FTIR parameters compared to the control group and all these changes were in parallel with those observed in the DOX-treated group. This finding demonstrated that PRPLS was inadequate to prevent the harmful effects of DOX on molecules in liver tissue. It has been known that DOX is predominantly metabolized in the liver and the liver is one of the organs where DOX is mostly accumulated (28). For this reason, DOX caused

significant harmful effects on the molecules in the liver at the dose and time administered in this study. In the literature, there are a few studies showing the protective effects of PRPLS collected from different geographical regions of the world against DOX toxicity in various organs by using different methods (4, 5, 10). However, in those studies, generally a single dose of acute DOX was administered to the animals and the PRPLS doses and application times used were different from the current study's. Therefore, the reason why PRPLS could not show any protective effect against the toxicity of DOX might be that DOX at the dose and application times used in this study caused major damage on the molecules in the liver tissue and PRPLS at the dose and application times used in the current study was not sufficient to eliminate these significant toxic effects of DOX. A higher dose and longer application time of PRPLS may be required to protect the liver tissue from damaging effect of DOX. Since we believe that non-significant results are also an important building block for scientific studies, we think that our finding about the lack of protective effect of PRPLS at the dose and application times used in this study will shed light on future studies on this important bee product.

## CONCLUSION

The findings of the current study showed that DOX caused significant deleterious effects on the composition, structure and function of the biomolecules in rat liver. DOX administration induced a significant decrease in the amount of glycogen and nucleic acids and a significant increase in the amount of lipid and protein in the liver. The decrease in the olefinic HC=CH/lipid ratio observed after DOX administration indicated an increase in oxidative stress and as a result lipid peroxidation occured in the liver. In addition, the decreases observed in the $CH_2$ antisym./$CH_3$ antisym. and C=O/lipid ratios and the increase in the $CH_3$ antisym./lipid ratios indicated that lipids were fragmented and the amount of short-chained lipids increased as a result of lipid peroxidation in the liver tissue after DOX administration. The increase observed in the amount of random coil structures showed that protein denaturation occured in the tissue due to DOX. The decrease in the C-O/$PO_2^-$ ratio and the increase in the lipid/protein ratio indicated that DOX caused significant alterations in carbohydrate, lipid and protein metabolism in the liver tissue. In addition, DOX administration induced a decrease in the order of the phospholipid chains of the liver cell membranes and an increase in membrane fluidity. It has been known that one of the mechanisms of the toxic effect of DOX in the cell is to attach to the cell membrane and damage cellular functions. Therefore, this finding regarding the cell membrane is very important as it has revealed another toxic action mechanism of DOX for the first time – DOX damages the cell by changing the membrane order and fluidity.

This is the first report revealing the structural and related functional effects of DOX on the liver tissue at the molecular level by utilizing ATR-FTIR spectroscopy. Understanding the toxic action mechanisms of DOX in the liver is very important to increase the efficacy of this drug in malignant cells and reduce its toxic effects on healthy tissues. The fact that PRPLS did not cause any changes in the liver tissue when given alone could be used in the process of integrating this precious bee product into medicine, especially pharmacology. In addition, our study has shown that ATR-FTIR spectroscopy is a practical technique for examining the effects of a chemotherapeutic agent in a biological tissue.

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**Supplementary Table S1.** Band area ratios, the respective functional groups used to calculate those ratios and their assignments.

| Band Area Ratio | Functional Groups Utilized | Function |
|---|---|---|
| $CH_2$ sym./$CH_2$ sym + antisym. | $CH_2$ symmetric stretching (# 6)/$CH_2$ symmetric stretching (# 6) + $CH_2$ antisymmetric stretching (# 4) | Saturated lipid concentration of the system |
| Olefinic CH=CH /Lipid | Olefinic=CH stretching (# 2)/$CH_3$ antisymmetric stretching (# 3) + $CH_2$ antisymmetric stretching (# 4) + $CH_2$ symmetric stretching (# 6) | Unsaturation level of the system (lipid peroxidation) |
| Amid I/Amid I + II | Amide I (# 8)/Amide I (# 8) + Amide II (# 9) | Protein concentration of the system |
| RNA/Protein | Ribose ring vibrations (# 15)/Amide II (# 9) | RNA concentration of the system |
| RNA/Lipid | Ribose ring vibrations (# 15)/ $CH_3$ antisymmetric stretching (# 3) + $CH_2$ antisymmetric stretching (# 4) + $CH_2$ symmetric stretching (# 6) | |
| DNA/Protein | DNA vibrations (# 18)/Amide II (# 9) | DNA concentration of the system |
| DNA/ Lipid | DNA vibrations (# 18)/ $CH_3$ antisymmetric stretching (# 3) + $CH_2$ antisymmetric stretching (# 4) + $CH_2$ symmetric stretching (# 6) | |
| RNA/DNA | DNA vibrations (# 18)/ Ribose ring vibrations (# 15) | Transcription status of the system |
| C-O/Protein | C-O stretching (# 17)/Amide II (# 9) | Glycogen concentration of the system |
| C-O/Lipid | C-O stretching (# 17)/$CH_3$ antisymmetric stretching (# 3) + $CH_2$ antisymmetric stretching (# 4) + $CH_2$ symmetric stretching (# 6) | |
| C-O/$PO_2^-$ sym. | C-O stretching (# 17)/ $PO_2^-$ symmetric stretching (# 16) | Metabolic activity of the system |
| $CH_2$ antisym./$CH_3$ antisym. | $CH_2$ antisymmetric stretching (# 4)/ $CH_3$ antisymmetric stretching (# 3) | Chain length of the lipids |
| C=O/Lipid | Carbonyl ester stretching (# 7)/ $CH_3$ antisymmetric stretching (# 3) + $CH_2$ antisymmetric stretching (# 4) + $CH_2$ symmetric stretching (# 6) | Carbonyl status of the system |
| $CH_3$ antisym./Lipid | $CH_3$ antisymmetric stretching (# 3) / $CH_3$ antisymmetric stretching (# 3) + $CH_2$ antisymmetric stretching (# 4) + $CH_2$ symmetric stretching (# 6) | Methyl concentration of the system |
| Amid I/Amid II | Amide I (# 8)/ Amide II (# 9) | Changes in protein structure and conformation |
| Lipid/Protein | $CH_3$ antisymmetric stretching (# 3) + $CH_2$ antisymmetric stretching (# 4) + $CH_2$ symmetric stretching (# 6)/ Amide II (# 9) | Comparison of the relative changes in the concentrations of the lipids and proteins |

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RESEARCH ARTICLE

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# Prolonged Capillary Refill Time Indicates Early Nailfold Capillaroscopy in Systemic Sclerosis

**ABSTRACT**

**Objective:** Systemic sclerosis (SSc) is a progressive connective tissue disorder that features vascular injury and persistent fibrosis with an autoimmune background. The hypoxic state at the capillary caused by SSc can be assessed with several methods. This study thus investigated the capillary refill time (CRT) effectivity in evaluating peripheral circulation in SSc patients.

**Methods:** This prospective, case-control study was conducted with SSc patients and gender and age-matched healthy controls. The CRT measurements were performed by a rheumatologist unaware of patients' records with a smartphone camera in the optimized test ambiance. A video processing software was then applied for the captured videos.

**Results:** 61 patients with SSc and 60 controls participated in this study. According to disease involvement, the patients were then divided into diffuse cutaneous SSc (dSSc) and limited cutaneous SSc. Mainly, CRT was prolonged in the patient group than in the control group. CRT was also prolonged in patients with pathological capillaroscopy patterns in the nail fold capillaroscopy (NFC), below 50 years old, or whose disease duration was over three years. Per disease involvement, patients in the dSSc group with pathologic NFC had prolonged CRT results and higher pulmonary artery pressure levels. The use of CRT for NFC positivity in patients with SSc was practicable (AUC: 0.717;95% CI 0.714-0.942; 83.95% accuracy; 67.9% sensitivity, 100% specificity, 100% positive predictive value, 21.7% negative predictive value, P=0.015).

**Conclusions:** CRT is markedly prolonged in patients with SSc. Evaluating CRT with the NFC positivity may provide pulmonary progression predictable, notably in dSSc patients.

**Keywords:** Capillaroscopy, Capillary Refill Time, Pulmonary Hypertension, Systemic Sclerosis.

# Uzamış Kapiller Dolum Süresi, Sistemik Sklerozlu Hastalarda Tırnak Yatağı Kapillaroskopisinin Erken Yapılmasını Vurgular

**ÖZET**

**Amaç:** Sistemik skleroz (SSk), otoimmün bir arka planda vasküler hasar ve kalıcı fibrozisi içeren ilerleyici bir bağ dokusu hastalığıdır. SSk'nin kapillerdeki sebep olduğu hipoksik durum çeşitli yöntemlerle değerlendirilebilir. Bu çalışma bu nedenle, SSk hastalarında periferik dolaşımı değerlendirmede kapiller dolum zamanının (KDZ) etkinliğini değerlendirmiştir.

**Gereç ve Yöntem:** Bu prospektif, vaka-kontrol çalışması, SSk hastaları ile cinsiyet ve yaşça eşleştirilmiş sağlıklı kontrollerle yürütülmüştür. KDZ ölçümleri, hasta kayıtlarından bilgisi olmayan bir romatolog tarafından bir akıllı telefon kamerası ile uygun hale getirilmiş bir test ortamında yapıldı. Kayıt edilen videolar için bir video yazılımı uygulandı.

**Bulgular:** 61 SSk hastası ve 60 kontrol bu çalışmaya katıldı. Hastalık tutulumuna göre hastalar diffüz kutanöz SSk (dkSSk) ve limitli kutanöz SSk olarak ikiye ayrıldı. Esasen, KDZ hasta grubunda kontrol grubuna göre uzamıştı (p = 0.003). KDZ, tırnak yatağı kapilleroskopisi (TYK) patolojik paternde olan 50 yaş altındaki ya da hastalık süresi 3 yıldan fazla olan hastalarda uzamıştı. Hastalık tutulumuyla beraber, TYK'si patolojik olan dkSSk grubundaki hastalarda KDZ sonuçları uzamıştı ve pulmoner arter basıncı seviyeleri daha yüksekti. SSk'lı hastalarda TYK pozitifliği için KDZ kullanımı uygulanabilirdi (AUC: 0.717;95% CI 0.714-0.942; 83.95% doğruluk; 67.9% duyarlılık, 100% özgüllük, 100% pozitif prediktif değer, 21.7% negatif prediktif değer, P=0.015).

**Sonuç:** SSk'lı hastalarda KDZ belirgin şekilde uzamaktadır. KDZ'nin TYK pozitifliği ile değerlendirilmesi, özellikle dkSSk hastalarında pulmoner progresyonun öngörülebilir olmasını sağlayabilir.

**Anahtar Kelimeler:** Kapilleroskopi, Kapiller Dolum Zamanı, Pulmoner Hipertansiyon, Sistemik Skleroz.

## INTRODUCTION

Systemic Sclerosis (SSc) is an autoimmune disease in which excessive connective tissue increase influences the entire human body (1). Intense fibroblastic activity and excessive collagen production were identified in the disease (2). SSc manifests a heterogeneous organ involvement and severity. The skin is primarily affected; cardiac, pulmonary, gastrointestinal (GI) tract, renal, digital, and musculoskeletal involvements may also be detected (3). The first disruption in the skin is edema, followed by thickening and hardening due to excessive collagen production (2, 4). Raynaud's phenomenon (RP), which is reversible vasospasm due to functional changes in the digital arteries of the extremities, can be the first clinical sign (1, 5). Pallor, paresthesia, pain, augmented edema appearing in the fingers in the form of attacks, and the progression of angiopathies secondary to collagen deposition led to ischemic ulcers and atrophies in the fingers (1, 6). The formation of digital ulcers indicates a worse course in disease progressions, such as cardiovascular worsening and decreased survival (7, 8). Like cardiologic involvement, musculoskeletal, renal, and pulmonary complications are also prognostic (3).

Evaluating digital involvements among cutaneous manifestations is crucial in SSc patients. In physical examination, possible clues such as swollen puffy fingers, non-pitting edema of the hands, skin thickening, perioral skin tightening, loss of fingertip tissue, tendon friction rubs, calcinosis cutis, cutaneous hyperpigmentation and telangiectasias, abnormal nail fold capillaroscopy (NFC) can be detected (9, 10). NFC is an uncomplicated, invivo technique that can predict the SSc prognosis and mediate the effects of autoantibodies (11, 12). In addition, NFC, where the fourth or fifth finger is usually selected in practice, is mainly used to distinguish the RP origin (13). Evaluating the architecture of the skin capillaries can point out the morphology, density, size, microhemorrhages, avascularity, and neoangiogenesis (14). Therfore, a pathological NFC consisted of dilated and giant capillaries, hemorrhages, disorganized vascular arrays, ramified/bushy capillaries, and capillary losses (15).

Capillary refilling time (CRT), which is ancient but functional use, is a physical examination method that can evaluate skin arterioles at the bedside. CRT effectively assesses inconvenient circumstances of the peripheral vascular bed's fullness (16). In CRT, mainly, the filling rate of the arteriole and capillary system is observed. A time of > 2 s is considered one means of proving circulatory disorder (17). However, some situations reduce the reliability of CRT, such as age, ambient light, ambient temperature, observer reliability, and applied pressure; therefore, the examination takes effort, attention, and time (16, 18).

There is no conventional method other than NFC to evaluate the digital involvement at the micro-level in patients with SSc. A test such as CRT, which can predict peripheral tissue perfusion, has not yet been applied in patients with SSc. The evaluations and results of our study thus contribute to NFC findings and determine CRT's role in the prognosis of SSc.

## MATERIAL AND METHODS

Our prospective case-control study was conducted with SSc patients diagnosed according to 2013 ACR/EULAR Classification Criteria for Scleroderma (19) and an age-matched control group. The local ethics committee approved the study method (2020/96), and the participants signed informed consent forms were collected prior to the study. The patients' demographic features and laboratory parameters were noted simultaneously. Those over 18 years of age who had at least a hemoglobin (Hb) level of 10 gr/dL did not take drugs or food that can cause vascular response (decongestant, caffeine, or tea intake) within the preceding 24 h were included in the study. At the same time, those with nail bed impropriety (cosmetic application, manicure, onychomycosis) and smoking were excluded.

The patients were categorized into two groups based on the SSc disease severity (20). Accordingly, patients with a clinical evaluation (objectively documented RP plus anyone: SSc-type nail fold capillary pattern/SSc selective autoantibodies or a subjective RP plus both: SSc-type nail fold capillary pattern and SSc selective antibodies) were rated as limited cutaneous (lSSc); and those with criteria for lSSc plus proximal cutaneous changes were rated as diffuse cutaneous (dSSc) involvement. Likewise, the control group consisted of age-matched healthy individuals with no comorbidities.

NFC assessments were performed after patients were kept at room temperature in a sitting position for at least 15 minutes. Mainly the 4th and 5th, though all fingers were evaluated. After the immersion oil (Mr Salt-E, Chrystal distribution, CA, USA) was applied, the procedure was performed with a capillaroscope (AM4113T-R4-70x magnification, Dino-Lite digital microscope, Class B, Taiwan). Findings of NFC in 20x magnification were accepted as the presence of neoangiogenesis, capillary elongation/tortuosity (>4 times the average width), reduced capillary density (<7 capillaries/mm), avascular area (the absence of two or more consecutive loops), hemorrhage (positivity), giant loops (> 50 µm) and abnormal blood flow (21).

The CRT measurements were made with materials (a smartphone, freeware software, physical

programming platform) found easily in ordinary outpatient clinic conditions. The patients who obtained the CRT course were unaware of the laboratory results and NFC findings. Firstly, the body temperatures were taken from all participants to obtain proper conditions for the CRT measurements, and results were considered normal between 36.5 - 38 Celcius (22). Next, the patient and control group were matched by age not to cause an age deflection effect. Then, all measurements were made between 9 - 10 am to keep the optimum light level; furthermore, the measurement area's light exposure was set to a brightness level of 300-500 lux via a photometric application (Lux-Meter) already installed the smartphone. Finally, we designed a 3D measurement platform and a manufacturing procedure with Ultimaker Cura to standardize each evaluation. The middle finger was preferred for CRT measurement, as it has the most extended terminal phalanx between the fingers and thus has more blood supply. In addition, the measurements were performed by only one rheumatologist to avoid measurement technique variations.

The images were recorded twice with a regular smartphone placed on a focused tray on the platform to observe the finger placement area. The chosen finger to be measured was placed in the appropriate position. Adequate pressure was applied to the nail root with a transparent, flat plastic tool until whitening was achieved, then the plastic tool was immediately pulled from the smartphone's camera frame after 5 s. All stages were recorded in mp4 video format via the same smartphone placed on the platform. The images were evaluated by using the color threshold filter of the VLC media player software (ver. 3.0.5) to determine the observed blood flow in the images. The time between the blood flow disappeared and the nail color completely re-regulated was recorded on the screen in ms. The average result of the two measurements for each finger was recorded as the current CRT value. All the equipment used for CRT measurement is presented in Figure 1.

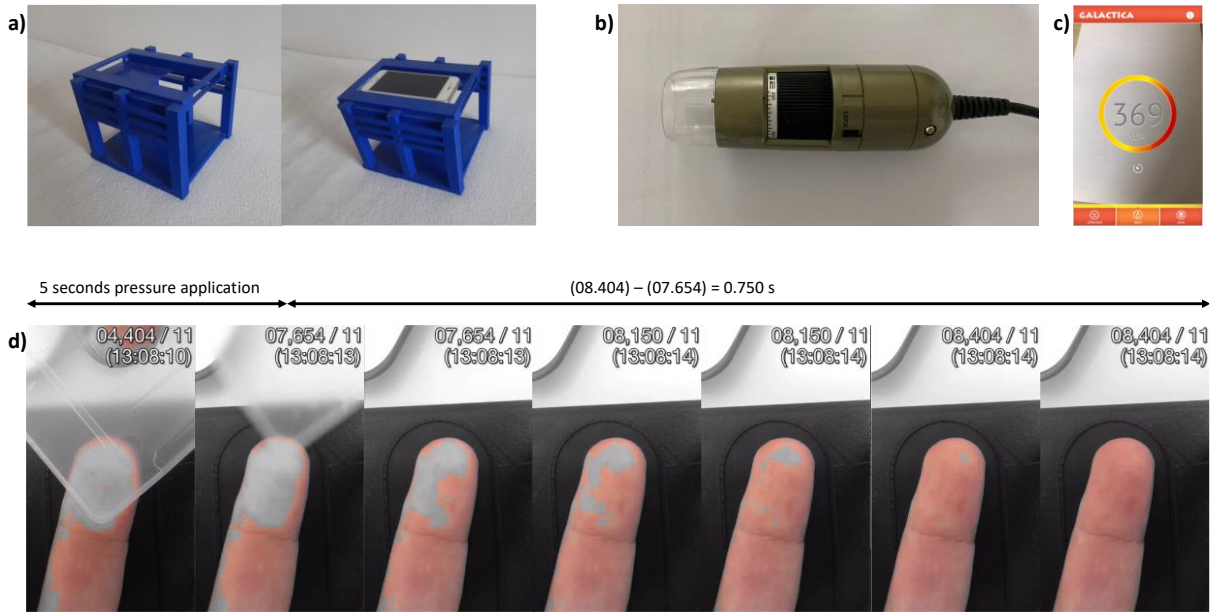



**Figure 1.** The pieces of equipment used for CRT measurement, a) A smartphone placed on a 3D designed measurement platform; b) Digital microscope (Dino-Lite); c) Photometric application (Lux-Meter); d) Sequential snapshots from the evaluated video with VLC player in color threshold filter mode.

**Statistical Analysis:** We used SPSS ver. 22 (SPSS Inc., Chicago, IL, USA) for the statistical analyses. The Pearson correlation was used to correlate normally distributed data, whereas, in non-normal data, Spearman correlation was used. The Chi-square test was preferred in categorical data evaluation. We also used independent t-tests to compare the CRT results between the patients with SSc and the control group. In continuous data pinpointing comparisons with CRT and in comparisons according to disease severity, an independent t-test was used in normal distributions and the Mann–Whitney U-test in non-normal distributions. The area under the receiver-operating characteristics (ROC) curve (AUC) and 95% confidence intervals (CI) were used to assess the ability of each CRT of the patient and control groups to the NFC distinction. In all calculations, a p-value of 0.05 was accepted as significant.

**RESULTS**

This study included a total of 61 out-patients (mean age 48.78±12.56 years) and 60 controls (mean age 49.33 ± 12.75 years). The longest measurement among all CRTs was 5,736 s. Again, 33.1% of all measured CRTs were prolonged. Meanwhile, 95% of those were in the patient group. None of the participants were smoking. Two cases in the lSSc and one in the dSSc group had a level of

Hb below 10 gr/dL; they were excluded from the study. The remained demographic, laboratory, and characteristic features of the patients with SSc subgroups are detailed in Table 1. The control group was also matched by gender to the patient group (56 female, four male). Only 3.3% of the CRTs in the control group were measured as prolonged. The mean CRT results in the control group were 1.315±0.33. No laboratory procedure was performed in the control group, except for fingertip oxygen saturation measurement. The mean oxygen saturation level in the control group was above 96%.

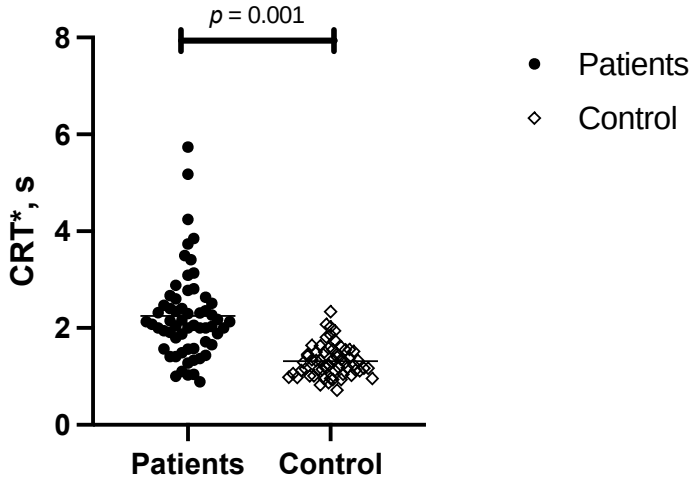
**Table 1.** Demographic, characteristics features and laboratory results of the SSc patients.

| | All patiens (n = 61) | lSSc (n = 24) | dSSc (n = 37) | *P* value |
|---|---|---|---|---|
| **Demographic & Clinics** | | | | |
| Gender, F/M (n) | 57/4 | 23/1 | 34/3 | 0.484 |
| Age, (year) | 48.78±12.56 | 48.37±8.70 | 49.05±14.64 | 0.768 |
| SSc (year) | 4.23±2.38 | 4.1±2.42 | 4.32±2.39 | 0.738 |
| Dispnea, n (%) | 17 (27.9) | 1 (4.2) | 16 (43.2) | **0.001** |
| Involvement | | | | |
| Joint, n (%) | 13 (21.3) | 2 (8.3) | 11 (29.7) | **0.046** |
| Cutaneus, n (%) | 53 (86.9) | 21 (87.5) | 32 (86.5) | 0.909 |
| Extra-cutaneus, n (%) | 38 (62.3) | 13 (54.2) | 25 (67.6) | 0.291 |
| Renal, n (%) | 1 (1.6) | 1 (4.2) | 0 (0) | 0.393 |
| GI, n (%) | 38 (62.3) | 13 (54.2) | 25 (67.6) | 0.291 |
| Digital ulcer, n (%) | 3 (4.9) | 0 (0) | 3 (8.1) | 0.272 |
| **Laboratory results** | | | | |
| WBC, $x10^9$/L | 7.1 (5.75-8) | 7.1 (6.02-8) | 7.2 (5.6-7.95) | 0.819 |
| ANC, $x10^9$/L | 4.3 (3.1-4.9) | 4.3 (3.32-5.12) | 4.1 (2.95-4.9) | 0.555 |
| ALC, $x10^9$/L | 1.85 (1.55-2.5) | 1.87 (1.62-2.5) | 1.8 (1.5-2.5) | 0.762 |
| Plt count, $x10^9$/L | 288 (243-338) | 289 (256-346) | 284 (242-334) | 0.631 |
| Hb, gr/dL | 13.02±1.28 | 12.67±1.25 | 13.25±1.28 | 0.077 |
| Esr, mm/h | 16 (5-28) | 16.5 (6.75-26.5) | 16 (4.14-31.5) | 0.492 |
| CRP, mg/L | 4.48 (2.04-10.4) | 3.96 (1.83-13.5) | 4.83 (2.5-9.75) | 0.900 |
| (+) ANA, n (%) | 57 (93.4) | 21 (87.5) | 36 (97.3) | 0.290 |
| (+) anti-Scl-70, n (%) | 18 (29.5) | 0 (0) | 18 (48.6) | **0.001** |
| (+) anticentromere, n (%) | 21 (34.4) | 12 (50) | 9 (24.4) | **0.039** |
| (+) RF, n (%) | 3 (4.9) | 3 (12.5) | 0 (0) | 0.056 |
| (+) NFC, n (%) | 56 (91.8) | 22 (91.7) | 34 (91.9) | 0.660 |
| (+) RP, n (%) | 58 (95.1) | 22 (91.7) | 36 (97.3) | 0.556 |
| mPAP, mmHg | 27 (25-30) | 27 (25-29) | 28 (24-30.5) | 0.667 |
| Abnormal Thoraks CT,n(%) | 23 (37.7) | 0 (0) | 23 (62.2) | **0.001** |
| NSIP, n (%) | 21 (34.4) | 2 (8.3) | 19(51.4) | **0.001** |
| UIP, n (%) | 3 (4.9) | 0 (0) | 3 (8.1) | 0.272 |
| CRT, ms | 2.248±0.927 | 2.347±1.019 | 2.184±0.871 | 0.585 |
| **Treatment** | | | | |
| HCQ, n (%) | 60 (98.3) | 23 (95) | 37 (100) | 0.210 |
| ISx Treatment, n (%) | 32 (44.3) | 10 (41.6) | 22 (59.5) | **0.004** |
| AZA, n (%) | 12 (19.6) | 7 (29.1) | 5 (13.5) | 0.133 |
| CP, n (%) | 6 (9.8) | 1 (4.1) | 5 (13.5) | 0.231 |
| RIX, n (%) | 4 (6.5) | 2 (8.3) | 2 (5.4) | 0.651 |
| MMF, n (%) | 3 (4.9) | 0 (0) | 3 (8.1) | 0.152 |

*P* values are the comparison of lSSc and dSSc groups. (Chi-square test or Mann-Whitney U test); The mean data are given as mean ± standard deviation. The median data are presented as median and 25-75% quartiles. Data are the number, n (%), or n/N (%); **ALC**, Absolute lymphocyte count; **ANA**, Antinuclear antibody; **ANC**, Absolute neutrophil count; **anti-Scl-70**, Anti-topoisomerase I; **AZA**, Azathioprine; **CENB** , Centromeric protein B; **CP**, Cyclophosphamide; **CRP**, C-reactive protein; **CRT**, Capillary refill time, **CT**, Computed tomography; **dSSc**, Diffuse cutaneous systemic sclerosis; **ESR**, Erithrocyte sedimentation rate; **GI**, Gastrointestinal; **Hb**, Hemoglobin; **HCQ**, Hydroxychloroquine; **lSSc**, Limited cutaneous systemic sclerosis; **ISx**, Featured immunosuppressive treatments; **MMF,** Mycophenolate mofetil; **mPAP**, Mean pulmonary artery pressure; **NFC**, Nailfold capillaroscopy; **NSIP**, Non-specific interstitial pneumonia; **Plt**, Platelet; **RF**, Rheumatoid factor; **RIX**, Rituximab; **RP**, Raynaud's phenomenon; **UIP**, Usual interstitial pneumonia; **WBC**, White blood cell.

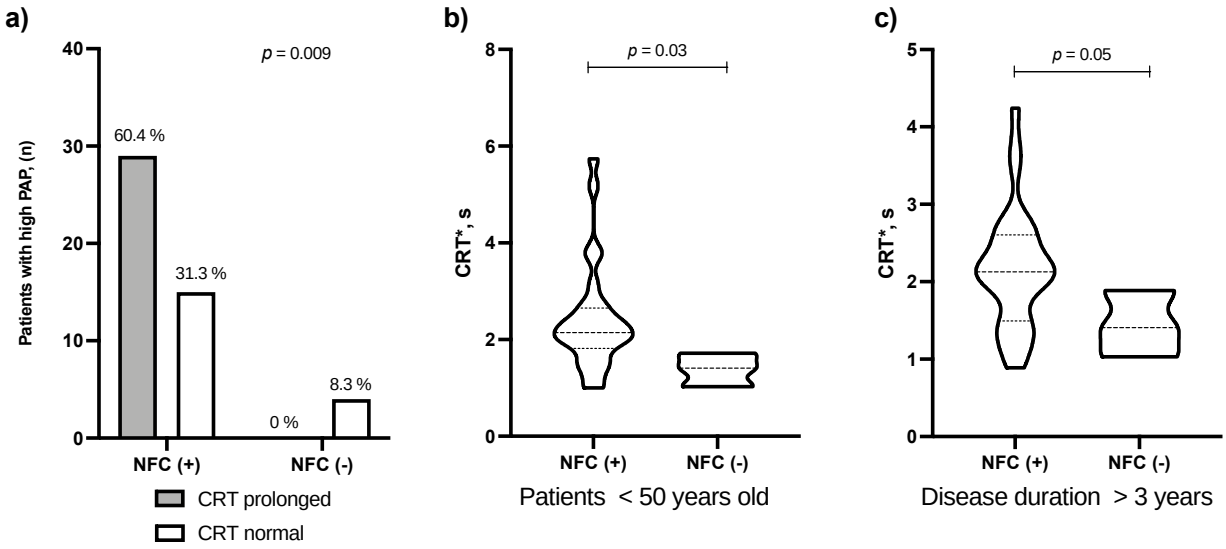
There was significant increase in CRT measurements in the patient group compared to the control group ($p = 0.001$, $F = 18.894$, $\eta = 0.353$) (Figure 2). Unlike, age and gender comparison did not reveal statistical significance between both groups. Among the correlated results, besides CRT and NFC positivity, which was statistically significant ($p = 0.003$), CRT also showed statistical

significance with RP (p = 0.022) and receiving immunosuppressive treatment (p = 0.042). Other remarkable findings were graphically shown in Figure 3. Accordingly, all patients with positive NFC and prolonged CRT had higher pulmonary artery pressure (PAP) levels (>24 mmHg) (Figure 3a) (23). Moreover, a Mann-Whitney U test revealed a difference in CRT in those under 50 years of age who had positive NFC (p = 0.030, η= 0.152) (Figure 3b). There is also evidence of CRT prolongation in all patients with more than three years of disease and positive NFC (p = 0.005, η= 0.125) (Figure 3c).




***CRT,** Capillary refill time

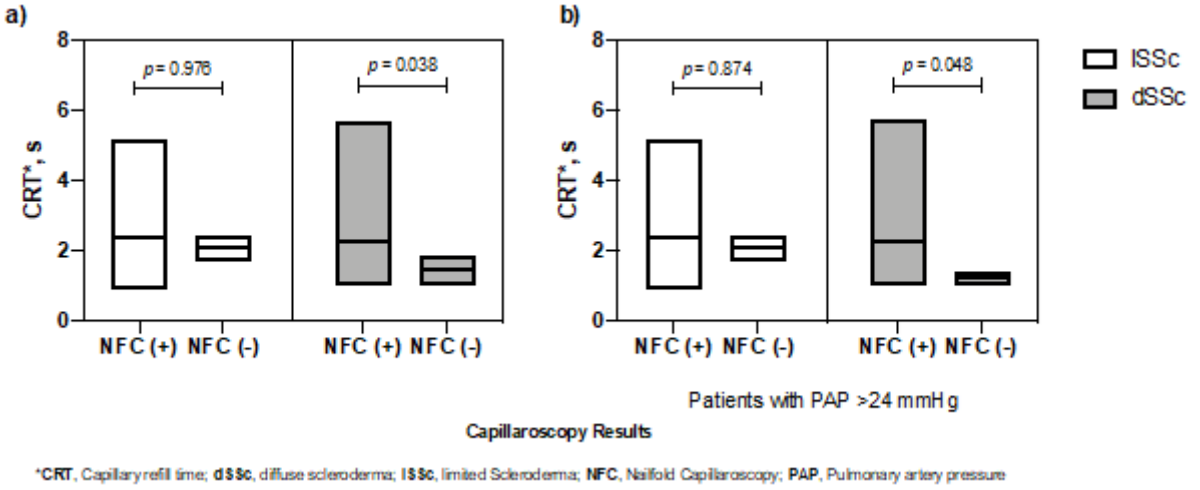
**Figure 2.** CRT distribution of all participants.




*** CRT**, Capillary refill time; **NFC**, Nail fold capillaroscopy; **PAP**, Pulmonary artery pressure

**Figure 3 a**) Distributions of categorized CRT results and the nailfold capillaroscopy (NFC) positivity in SSc patients with high pulmonary artery pressure levels, **b**) CRT variations in SSc patients below 50 years of age with positive NFC, **c**) CRT changes in the NFC positive SSc patients with a disease duration of more than three years.

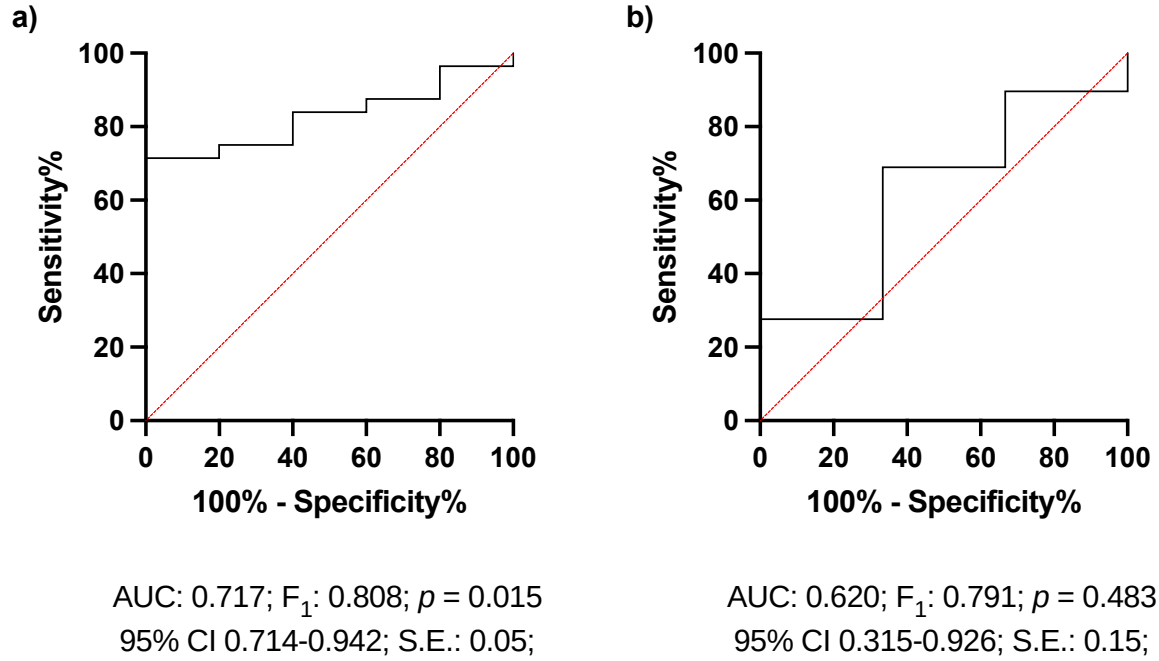
In subgroup analysis, CRT measurements did not reveal a statistical significance between the dSSc and lSSc groups (p = 0.585). Similarly, the lack of statistical significance persisted between CRT and capillaroscopy, computed tomography scans of thorax evaluations. However, CRT was significantly prolonged in the dSSc group with positive NFC testing (p = 0.038) (Figure 4a). In addition, those with a positive NFC in the dSSc group had longer CRTs at high PAP levels (p = 0.048) (Figure 4b).




*CRT, Capillary refill time; dSSc, diffuse scleroderma; lSSc, limited Scleroderma; NFC, Nailfold Capillaroscopy; PAP, Pulmonary artery pressure

**Figure 4**. **a**) CRT comparisons in the dSSc and lSSc patients with nail fold capillaroscopy assessments, **b**) CRT diversions in dSSc and lSSc patients with pulmonary hypertension.

The evaluations for the discrimination significance of CRT measurement were given in Figure 5. Accordingly, CRT had a fair distinctiveness between NFC positive and negative subgroups with 83.95% accuracy (67.9% sensitivity, 100% specificity, 100% positive predictive value, 21.7% negative predictive value, 0.717 ROC AUC) (Figure 5a). Conversely, NFC had a poor prediction on the RP positivity (65.5% sensitivity, 100% specificity, 100% positive predictive value, 13% negative predictive value, 0.620 ROC AUC, 82.7% accuracy) (Figure 5b).




*AUC, Area under the curve; CI, Confidence interval; $F_1$, $F_1$ score; S.E., Standart Error

**Figure 5.** ROC curve analysis for indicative value of CRT, **a)** In patients with pathological capillaroscopy patterns in the nail fold capillaroscopy, **b)** In patients with positive Raynaud's phenomenon.

Among the correlations; CRT was positively correlated with the NFC positivity (p = 0.02, r = 0.384), RP (p = 0.22, r = 0.292), whereas platelet count was negatively correlated with the NFC positivity (p = 0.033, r = -0.273), RP (p = 0.022, r = -0.293) and skin ulceration (p = 0.014, r = -0.314), CRT was negatively correlated with receiving immunosuppressive therapy (p = 0.043, r = -0.26), and absolute lymphocyte count was positively correlated with GI involvement (p = 0.029, r = -0.28), were remarkable.

## DISCUSSION

In this prospective, case-control study, we evaluated CRT measurements in patients with SSc. We found a significant CRT prolongation compared to the control group. CRT results were significantly prolonged, especially in those with a positive capillaroscopy. There was no difference between both dSSc and lSSc subgroups per disease severity. However, the CRP was also detected prolonged in higher PAP levels, <50 years of age, and >3 years disease duration. Since SSc is a progressive disorder, predicting an involvement before target-organ damage will favor the patient. The NFC is one of the implements prominently used in arterial involvements linked to mortality (12). An early evaluation of microangiopathy in SSc patients demonstrates the importance of capillaroscopy; however, specified optical magnification is required for the evaluation, which is not ubiquitous (24). At this point, CRT is a free method that can be performed at the bedside, does not consume much time, but requires attention. Our study detected significant CRT prolongation in patients with positive capillaroscopy. Whether the CRT was not an objective evaluation tool that would not be wholly replaced with NFC, it may be eligible to make NFC more emphasis in patients with prolonged CRTs.

The hand involvement was common in the SSc progression. There have already been implemented and novel located devices that can evaluate the peripheral circulatory system in this regard (25). Since the NFC only defines the anatomical structures of the nail fold, a dynamic device that also evaluates the functional nail fold state, such as oxygenation, was featured to take the pole position. Thermography, Photoplethysmo-graphy, Photoacoustic and high-frequency ultrasound, and laser-assisted imaging such as Laser Doppler Flowmetry, Laser Doppler Perfusion, Laser speckle contrast analysis were some of the prominent functional measures (26-28). However, the common problem was that the imaging could be technically limited in patients with significant digital contractures. Unfortunately, these multifunctional devices can be found in well-equipped health centers. As a result, functional measurement methods were not as objective as capillaroscopy, which is not affected by other physical dilemmas but requires significant experience; instead of considering them as an alternative to capillaroscopy, it should be a goal to prioritize the NFC when these tests are unfavorable.

Once genetic and environmental factors lead to endothelial damage in SSc patients, the apoptosis in endothelial cells would become inevitable via the anti-endothelial antibodies produced by B lymphocytes against the inflammation site (6). Secondary to impaired vasculogenesis, proliferative or destructive but obliterative vasculopathy mainly caused tissue hypoxia. Therefore, determining tissue hypoxia in the early stage of the disease was substantial. An examination approach such as CRT, which has proven itself in various cases, may also be used for a hypoxic setting (29-31). Without absolute objectivity, the CRT evaluates peripheral circulation in a fundamental approach in 2 s. Nevertheless, CRT was measured to be prolonged more per decade increase due to decreased vascular compliance (32).

Similarly, as age advances, the destruction of endothelial cells increases, and their regeneration ability weakens (33). Our study found that CRT results were significantly prolonged in patients below 50 years of age or whose disease duration was over three years. Taken together, this demonstrates that advancing age harms the capillaries similar to SSc. In addition, it may be possible to comment as follows; the CRT prolongation might be practical below 50 years old in SSc patients with positive NFC; however, the time gap in the NFC elongation was closed with the negative effect of increasing age on capillaries beyond the 50s.

A typical CRT can estimate adequate superior vena cava oxygen saturation. A long-term study formalized this issue; a CRT of $\leq$ 2 s was associated with the superior vena cava oxygen saturation of 70% (34). SSc patients with cardiac involvement or pulmonary artery hypertension secondary to pulmonary involvement worsened the central venous pressure levels were clinically presented with dyspnea. Based on this pathogenesis, our study also evaluated the potential relationship between PAP levels and CRT positivity. As a result, we noticed that CRT prolongation was statistically significant in dSSc patients with a PAP level of >20 mmHg compared to the lSSc subgroup. Surprisingly, these patients with pulmonary hypertension also had NFC positivity. These results may partly explain that dyspnea leading to local hypoxemia can trigger RF, which induces hypoxemia at peripheral tissues. While there was no statistical difference in CRT measurements between subgroups, CRT may predict PHT in patients with SSc, based on the difference when PAP level is >20 mmHg in the dSSc subgroup.

Unfortunately, this study could not encompass the entire cutaneous SSc involvements by CRT alone. However, CRT will already be prolonged in both groups since RP and nail fold involvement are diagnostic criteria in both dSSc and ISSc groups. If CRT measurements had been measured with a more sensitive and technologically dynamic method, the errors during application would have been minimized. However, the study was methodized via an ordinary smartphone to create awareness in medical practice without sophisticated devices. Nevertheless, we declared that CRT could not be equivalent to capillaroscopy. Another issue is that the platform used for focusing

was a 3D-printed instrument that could not be easily and rapidly manufactured. However, as smartphones can auto-focusing, using the platform is not indispensable.

In conclusion, CRT has been found relatively prolonged in patients with SSc. Additionally, detecting CRT prolongation was highly prevalent in SSc patients with positive capillaroscopy. To the best of our knowledge, no data was found on the association between SSc and CRT. Our study findings propose that appropriate time should be allocated for CRT measurement in SSc patients to manage the proper approach for early capillaroscopy, even though there was no physical clue for the nail fold involvement. Further studies may investigate whether capillary refill time can be an alternative to predict underlying connective tissue disease in patients with Raynaud's phenomenon in centers where capillaroscopy is unavailable.

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RESEARCH ARTICLE

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# The Association between Migraine and Sleep Quality

**ABSTRACT**

**Objective:** Migraine and sleep disorders are common health problems in the community and cause loss of labor. There are studies showing that there is a relationship between migraine and sleep quality and these two conditions worsen each other. The aim of this study was to determine the relationship between migraine and sleep quality.

**Methods:** This is a case-control type cross-sectional study consisting of a total of 454 participants, included migraine patients, patients with non-migraine headaches and patients without headache complaint admitted to Family Medicine outpatient clinics between October 2017 and March 2018. Sociodemographic data form, Identity Migraine test, International Headache Society diagnostic criteria questionnaire, Pittsburgh sleep quality scale (PSQI) and Epworth day sleepiness scale (Epw) were applied to the participants by face to face interviews.

**Results:** The total PSQI score was 6.5 ± 3.1, and significantly different between the groups. Patients with diagnosis of migraine had a higher PSQI score and poor sleep quality rate than the control groups. There was no correlation between the frequency of migraine attacks and PSQI scores. Extreme sleepiness in day time for the migraine group (30.7%) was higher than the control groups and there was no correlation between the frequency of migraine attacks and Epw scores.

**Conclusions:** Poor sleep quality and daytime sleepiness rates in migraine patients were higher than those with non-migraine headache patients and patients without headache complaints. This may be due to the fact that migraine is a specific problem affecting sleep or the frequency and severity of headache in the migraine patients are greater than that of the non-migraine headache patients and headache-free participants.

**Keywords:** Headache, Migraine, Sleep Quality, Daytime Sleepiness, Epworth Sleepiness scale, PSQI.

# Migren ve Uyku Kalitesi Arasındaki İlişki

**ÖZET**

**Amaç:** Migren ve uyku bozuklukları toplumda sık görülen ve iş gücü kaybına neden olan sağlık sorunlarıdır. Migren ile uyku kalitesi arasında bir ilişki olduğunu ve bu iki durumun birbirini kötüleştirdiğini gösteren çalışmalar mevcuttur. Bu çalışmanın amacı migren ile uyku kalitesi arasındaki ilişkiyi belirlemektir.

**Gereç ve Yöntem:** Vaka kontrol tipindeki kesitsel çalışmamız Ekim 2017-Mart 2018 tarihleri arasında Aile Hekimliği polikliniklerine başvuran migren tanılı hastalar, migren dışı baş ağrısı olan hastalar ve baş ağrısı şikayeti olmayan hastalar olmak üzere toplam 454 katılımcıyla yürütüldü. Sosyodemografik Veri formu, Kimlik Migren testi, Uluslararası Baş Ağrısı Derneği tanı kriterleri anketi, Pittsburgh uyku kalitesi ölçeği (PSQI) ve Epworth gündüz uykululuk ölçeği (Epw) katılımcılara yüz yüze görüşme yoluyla uygulandı.

**Bulgular:** Toplam PSQI skoru 6.5 ± 3.1 idi ve gruplar arasında istatistiksel olarak anlamlı fark vardı. Migren tanılı hastaların, kontrol gruplarına göre daha yüksek PUKİ puanı ve kötü uyku kalitesi oranı vardı. Migren ataklarının sıklığı ile PUKİ puanları arasında istatistiksel olarak anlamlı bir ilişki yoktu. Migren grubunda gündüz aşırı uyku hali (%30.7) kontrol gruplarına göre daha yüksekti ve migren ataklarının sıklığı ile Epw skorları arasında anlamlı ilişki saptanmadı.

**Sonuç:** Migren hastalarında kötü uyku kalitesi ve gündüz uykulu olma oranları, migren olmayan baş ağrısı hastalarına ve baş ağrısı şikayeti olmayanlara göre daha yüksekti. Bunun nedeninin migrenin uykuyu etkileyen spesifik bir problem olması veya migren hastalarında baş ağrısının sıklığının ve şiddetinin, migren olmayan baş ağrısı hastalarına ve baş ağrısı olmayanlara göre daha fazla olduğu düşünülebilir.

**Anahtar Kelimeler:** Baş Ağrısı, Migren, Uyku Kalitesi, Gündüz Uykululuğu, Epworth Uykululuk Ölçeği, PSQI.

## INTRODUCTION

Migraine, which is characterized by periodic, usually unilateral pain, is a headache syndrome that can start in childhood, adolescence or early adulthood, and shows varying frequency and familial characteristics in later years (1). Migraine ranked second among the diseases that caused the most functional loss in 2016 due to its effects that can limit the person (2). It is a disease characterized by throbbing headaches, as well as some neurological, gastrointestinal and autonomic symptoms (2). According to the frequency of attacks, it is classified as light migraine (1-4 attacks per month), moderate migraine (5-8 attacks per month), severe migraine (9-14 attacks per month), and chronic migraine (> 14 attacks per month) (3).

Etiopathogenesis of migraine is defined as a neurovascular reaction caused by specific external stimuli or frequent changes in the central nervous system (4). Stress, mental tension, insomnia, hunger, fatigue and noise are known as the most common triggering factors of migraine (5). The diagnosis is based on the character of the headache and the accompanying symptoms (6). Four stages of migraine have been identified and these consist of prodromal period, aura, headache, and recovery (7).

The worldwide prevalence of migraine have been reported to be 5%, while in Turkey it ranges between 5% -20% and the average has been reported as 16.4% (8, 9). The prevalence was reported to be 15% in Çanakkale, the city where the study was conducted (10).

Sleep, a natural process that provides energy conservation, development and repair of the nervous system in all mammals, associates with many components of a biological structure, especially the nervous system controlling intracellular mechanism stimulation, automatic functions, behavior, and cognitive functions (11). Poor sleep quailty is considered among the factors that trigger migraine (5).

In the biological rhythm and its formation, the sleep-wake cycle influenced by the circadian rhythm that's formed with the repetition of 24-hour stages is decisive. The circadian rhythm is regulated by the suprachiasmatic nucleus in the anterior hypothalamus (12). 7.5-8 hours of sleep is sufficient for adults (13). Sleep deprivation or interruption may cause fatigue, drowsiness, headache, anxiety, impaired concentration, confusion, perception disorder, learning disability, developmental delay, health problems and an increase in accidents (14). The prevalence of sleep disorders in the population ranges from 10% to 30% (15).

There is a complex relationship between headaches and sleep disorders. It cannot be clearly elucidated whether sleep disorders cause headache or headache causes sleep disorders (16). Some studies show that poor sleep quality triggers migraine or intensifies migraine attacks. Sleep disorders are often reported as migraine triggers, but further studies are needed to better understand the relationship between these entities (17).

The aim of this study was to investigate the sleep quality of patients admitted to a local tertiary hospital, as well as to compare and determine the effect of the sleep quality of patients diagnosed with migraine and those with non-migraine headache and headache-free.

## MATERIAL AND METHODS

**Population and Sample:** The population of the study, which is an epidemiological study in case-control type, consists of individuals between the ages of 18-65 who admitted to Çanakkale Onsekiz Mart University Research and Practice Hospital Family Medicine outpatient clinics between October 1, 2017 and March 30, 2018.

The study was carried out in the case group of migraine patients and two control groups: with non-migraine headaches and without headache (no primary or secondary headaches and less than 6 headaches per year).

1564 people were questioned for headaches to determine the participants.1077 individuals did not agree to participate in the study. 487 individuals who agreed to participate in the study and gave written consent, were included in the study and interviews were conducted through face to face. 33 participants were excluded due to missing data. The study was completed with a total of 454 participants, 150 in the migraine group, 155 in the non-migraine group and 149 in the headache-free group. Migraine group was divided into 4 subgroups as mild, moderate, severe and chronic migraine.

Patients aged 18 and 65, who agreed to participate in the study and did not meet the exclusion criteria, were included in the study. Patients, who did not agree to participate in the study or those with pregnancy/breastfeeding, a history of analgesic abuse (more than 15 analgesic use in the last month), craniospinal surgery, neck trauma, craniocervical vascular/non-vascular disease, and a diagnosis of a disease that may impair the perception of reality, substance abuse or withdrawal, active infection, homeostasis disorders, psychiatric disorders, and cranial neuralgias that could cause secondary headaches, were excluded from the study.

Ethics approval was obtained from the Committee of ÇOMU Clinical Research Ethics on 07/06/2017 (E.61936) and carried out according to the requirements of the Declaration of Helsinki.

**Data Collection Tools**

**Sociodemographic Data Form:** This form consists of questions about date of birth, gender, educational status, marital status, occupation, work, smoking, exposure to cigarette smoke, alcohol use, and daily coffee consumption. The form was

prepared by the researchers and applied to each participant.

**Identity (ID) Migraine Test:** ID Migraine Test was developed in 2003 by Lipton in the US to facilitate the diagnosis of migraine patients, especially in primary health care units (18). The sensitivity, specificity and positive predictive value of the ID Migraine Test were found to be as 81%, 75%, and 93%, respectively. Turkish valid and reliable version of this test was used in our study (19). 2 questions were asked before the screening of patients with headache in the last 3 months. These questions were: "Do your headaches affect your work, school or entertainment life?" and "Did you think you should talk to your doctor for your headache?". ID Migraine Test is applied to those who answer yes to any of these questions. The test contains questions related to at least one daily restraint because of nausea, photosensitivity and pain (19). Those who answer 2 or more "yes" to the questions in this test are considered positive.

**International Headache Society (IHS) Diagnostic Criteria:** In our study, the diagnostic criteria determined by IHS were used to confirm or to exclude the migraine diagnosis of the patients. Four questions were added to the questionnaire by the researchers, including the frequency of migraine attacks, disease duration, history of drug use, and visual pain scale.

**Pittsburgh Subjective Sleep Quality Scale (PSQI):** PSQI is a self-report scale that assesses sleep quality over a one-month period. PSQI was developed by Buysse and his colleagues in 1989 and has been shown to have adequate internal consistency, test-retest reliability, and validity (20). The validity and reliability of the index in Turkey had been determined by Ağargün, Kara, and Anlar and it was stated to be suitable for Turkish society. Cronbach alpha internal consistency coefficient was found to be 0.80. In the evaluation of PSQI, 18 items participate in the scoring. PSQI has 7 components, including subjective sleep quality, sleep delay, sleep time, habitual sleep activity, sleep disturbance, sleep medication use, and daytime dysfunction. The sum of the scores of these components constitute the total PSQI score. The total score has a value ranging between 0-21. If the total PSQI score is ≤5, it indicates good sleep quality, and >5 indicates poor sleep quality (21).

**Epworth Sleepiness Scale:** It is an 8-item scale which questions the general level of daytime sleepiness of the individual. Epworth Sleepiness Scale aims to evaluate the chance of falling asleep or drowsiness in daily life (22). Each item is scored between 0-3 points. The total score has a value between 0-24. Participants with an Epworth score of >9 are considered to be those with a tendency to daytime sleepiness (22).

**Statistical Analysis:** The frequency and distribution status of the variables were examined, and the adaptations of the continuous variables to the normal distribution were checked. According to the characteristics of the groups, the chi-square, the differences in the means of independent samples, variance analysis tests, correlation tests, and the participants' Pittsburgh and Epworth scores were compared. Bonferroni corrections were made. The test constants and absolute p values were given for each analysis. $P<0.05$ was accepted as the statistical significance limit for all analyses.

## RESULTS

The study was completed with a total of 454 participants. In terms of gender, 301 (66.3%) of the participants were women. The mean age was 38.1 ± 11.8 years [19-65] and 292 (64.3%) were married. The sociodemographic characteristics of the participants according to the study groups are given in Table 1 and the habit characteristics are given in Table 2.

**Table 1.** Sociodemographic characteristics according to study groups

| Study Groups | | Migraine group (n=150) | Non-migraine control group (n=155) | Headache-Free control group (n=149) | Total (n=454) |
|---|---|---|---|---|---|
| Age | | 40.4±11.8 | 35.6±10.9 | 38.6±12.2 | 38.1±11.8 |
| Gender | Female | 121 (%80.7) | 106 (%68.4) | 74 (%49.7) | 301 (%66.3) |
| | Male | 29 (%19.3) | 49 (%31.6) | 75 (%50.3) | 153 (%33.7) |
| Education period (years) | | 11.8±4.2 | 13.0±4.3 | 12.4±4.2 | 12.4±4.3 |
| Marital Status | Married | 106 (%70.7) | 88 (%56.8) | 98 (%65.8) | 292 (%64.3) |
| | Single | 33 (%22.0) | 58 (%37.4) | 41 (%27.5) | 132 (%29.1) |
| | Widow | 11 (%7.3) | 9 (%5.8) | 10 (%6.7) | 30 (%6.6) |

**Table 2.** The habitual characteristics of the participants according to the study groups

| Study Groups | | Migraine group (n=150) | Other headache control group (n=155) | Headache-Free control group (n=149) | Total(n=454) |
|---|---|---|---|---|---|
| Smoking | Never Smoked | 75 (%50.0) | 76 (%49.0) | 79 (%53.0) | 230 (%50.7) |
| | Quit Smoking | 35 (%23.3) | 26 (%16.8) | 23 (%15.4) | 84 (%18.5) |
| | Smoking | 40 (%26.7) | 53 (%34.2) | 47 (%31.5) | 140 (%30.8) |
| Exposure to cigarette smoke | | 57 (%38.0) | 53 (%34.2) | 48 (%32.2) | 158 (%34.8) |
| | No alcohol | 95 (%63.3) | 93 (%60.0) | 83 (%55.7) | 271 (%59.7) |
| | Socially | 24 (%16.0) | 26 (%16.8) | 23 (%15.4) | 73 (%16.1) |
| | 1-3 times a month | 23 (%15.3) | 27 (%17.4) | 28 (%18.8) | 78 (%17.2) |
| | 1-5 times a | 7 (%4.7) | 8 (%5.2) | 11 (%7.4) | 26 (%5.7) |
| | Almost every day | 1 (%0.7) | 1 (%0.6) | 4 (%2.7) | 6 (%1.3) |
| Weekly average alcohol consumption | | 0.4±1.1 | 0.5±1.4 | 0.9±3.8 | 0.6±2.4 |
| Coffee Use | | 1.6±1.4 | 1.5±1.4 | 1.1±1.1 | 1.4±1.3 |

**Sleep Quality:** PSQI responses of all participants were evaluated. Of the total 454 participants, 121 (26.7%) had good sleep quality (PSQI ≤5) and 333 (73.3%) had poor sleep quality (PSQI> 5). The mean PSQI score was 6.5 ± 3.1 [0.0-18.0]. Table 3 shows the average scores of PSQI total and its components according to the study groups of the participants.

**Table 3.** PSQI component and total scores in study groups

| PSQI component | Migraine group | Other headache control group | Headache-Free control group | Total |
|---|---|---|---|---|
| Subjective sleep quality (component 1) | 1.5±0.8 | 1.2±0.7 | 1.0±0.7 | 1.2±0.7 |
| Sleep latency (component 2) | 1.4±0.8 | 1.2±0.8 | 0.9±0.8 | 1.2±0.8 |
| Sleep time (component 3) | 1.9±1.1 | 1.4±1.1 | 1.1±1.1 | 1.5±1.2 |
| Conventional sleep activity (component 4) | 0.1±0.4 | 0.0±0.3 | 0.0±0.2 | 0.0±0.3 |
| Sleep Disorder (component 5) | 1.8±0.7 | 1.3±0.6 | 1.0±0.5 | 1.4±0.7 |
| Use of sleeping pills (component 6) | 0.3±0.8 | 0.2±0.8 | 0.1±0.5 | 0.2±0.7 |
| Daytime sleep dysfunction (component 7) | 1.2±1.0 | 1.0±0.8 | 0.8±0.8 | 1.0±0.9 |
| Total PSQI score | 8.1±3.1 | 6.3±2.8 | 5.2±2.7 | 6.5±3.1 |

There was a statistically significant difference between the groups in terms of total PSQI scores ($X^2$=66,559; <0.001). In post hoc analysis, PSQI scores showed statistically difference between the migraine study group and the non-migraine headache control group (p <0.001), between the migraine study group and the headache-free control group (p <0.001) and the non-migraine control group and headache-free control group (p <0.001).

18 (12.0%) of the 150 participants in the migraine study group, 37 (23.9%) of the 155 participants in the non-migraine headache control group, and 66 (44.3%) of the 149 participants in the headache-free control group had good sleep quality. The results are given in Table 4.

**Table 4.** Sleep quality in study groups

| | Migraine group (n=150) | Other headache control group | Headache-Free control group | Total (n=454) |
|---|---|---|---|---|
| Good sleep quality | 18 (%12.0) | 37 (%23.9) | 66 (%44.3) | 121 (%26.7) |
| Poor sleep quality (PSQI >5) | 132 (%88.0) | 118 (%76.1) | 83 (%55.7) | 333 (%73.3) |
| Total | 150 (%100.0) | 155 (%100.0) | 149(%100.0) | 454 (%100.0) |

Participants' rates of good and poor sleep quality were statistically different among study groups ($X^2$=40,812, p <0,001). When the groups were compared with the binary combinations; there were statistically significant differences between the migraine group and the non-migraine headache group ($X^2$=7.268, p=0.007), between the migraine group and the headache-free group ($X^2$=38.593, p <0.001) and between the non-migraine headache group and the headache-free group ($X^2$=14,147, p <0,001).

The mean attack frequency in migraine group was not statistically different in patients with poor sleep quality (5.4 ± 5.1 days/month) compared

to those with good sleep quality (4.7 ± 3.7 days/month) (U=1090.0; p=0.566). The mean pain severity in the migraine group was also not statistically different between the patients with good sleep quality (6.8 ± 0.7) and those with poor sleep quality (7.2 ± 0.9) (U=890.5; p=0.064).

**Daytime Excessive Sleepiness (DES):** The mean Epworth score of all participants was 6.4 ± 3.7 [0.0-19.0]. Of the 454 participants, 97 (21.4%) had excessive daytime sleepiness. The distribution of the study groups in terms of Epworth scores is given in Table 5.

**Table 5.** Daytime Extreme Sleepiness status in study groups

| | Migraine group | Other headache | Headache-Free | Total |
|---|---|---|---|---|
| Epworth score mean ± SS | 7.2±4.2 | 6.1±3.6 | 6.0±3.2 | 6.4±3.7 |
| No DES (Epworth score ≤9) | 104 (%69.3) | 127 (%81.9) | 126 (%84.6) | 357 (%78.6) |
| DES (Epworth score >10) | 46 (%30.7) | 28 (%18.1) | 23 (%15.4) | 97 (%21.4) |

Epworth scores were significantly different between the study groups ($X^2$=6,822, p=0,033). According to the post hoc analysis, the differences between migraine group and control groups were statistically significant, while the difference between the control groups was not significant (p=0.025; p=0.022; p=0.997, respectively).

The rates of patients with DES were significantly different between study groups ($X^2$= 11.847, p=0.003). When the groups were compared with the double combinations, the rates of participants who had DES were significantly higher in the migraine group (30.7%) than in the non-migraine headache group (18.1%) ($X^2$=6.588; p=0.010) and headache-free group (15.4%) ($X^2$=9.768 p=0.002). There was no statistically significant difference between the non-migraine headache group and the headache free group ($X^2$= 0.376; p=0.540).

The mean frequency of attacks in migraine group was not significantly different between those with daytime sleepiness (5.7 ± 5.5 days/month) and those without daytime sleepiness (5.1 ± 4.7 days/month) (U=2124.5; p=0.269). The mean pain severity in the migraine group did not show statistically difference in patients with daytime sleepiness (7.3 ± 0.8) and those without DES (7.0 ± 0.9) (U=2091.5; p=0.187).

**The Relationship between Migraine Attack Frequency and Sleep Quality and Daytime Extreme Sleepiness:** PSQI and Epworth scores in migraine subgroups are given in Table 6.

**Table 6.** Migraine attack frequency and PSQI and Epworth relationship

| Migraine groups | PSQI score | Epworth score |
|---|---|---|
| Mild Migraine | 8.1±3.3 | 7.2±4.3 |
| Moderate Migraine | 7.9±3.1 | 7.5±4.1 |
| Heavy Migraine | 8.3±2.9 | 7.5±5.0 |
| Chronic Migraine | 9.0±2.8 | 6.5±4.1 |

The rates of patients with good or poor sleep quality were not significantly different in the migraine subgroups ($X^2$=1.097, p=0.778). No correlation was found between PSQI score and migraine attack frequency (r=0.149, p=0.069). There was no correlation between migraine subgroups and PSQI scores (r=0.024, p=0.370).

In migraine subgroups, the rates of those with or without DES were not statistically different ($X^2$= 0.452, p=0.929). No significant correlation was found between the frequency of migraine attacks and the Epworth score (r=0.035, p=0.674). There was no correlation between the subgroups of migraine and Epworth scores (r=-0,020, p=0,812).

Epworth Scale scores in the migraine group were significantly correlated with Pittsburgh Scale scores (r=0.259, p <0.001). The rate of daytime sleepiness (25.2%) in patients with poor sleep quality was significantly higher than those with good sleep quality (10.7%). ($X^2$= 11,078, p=0.001).

## DISCUSSION

Neurological diseases play a major role in the loss of labor worldwide, and migraine is the third leading cause among the diseases that result in loss of labor in the last two decades (23). Migraine is a type of periodic headache with variable severity, frequency and localization. It is accompanied by anorexia, nausea, vomiting and sensitivity to light and sound (24). Its high prevalence has a significant impact on socioeconomic burden and quality of life. So that, it is important to diagnose migraine and reduce the frequency of migraine attacks.

In our study, we investigated the relationship between sleep quality and daytime sleepiness with migraine and non-migraine conditions. Insufficient sleep quality in migraine patients was higher than those with non-migraine headaches and headache-free patients. Poor sleep quality and excessive daytime sleepiness were more common in migraine patients, but no association has been found with the frequency and severity of the attacks. In this context, poor sleep quality and daytime sleepiness can be considered as comorbid conditions caused by migraine. As a result of our study, it was determined that migraine was a disease affecting sleep quality.

The mean number of migraine attacks in our study was observed to be between 2-6 attacks per month in most patients. The proportion of patients

with a high incidence of migraine attacks was higher than other studies reported in the literature (25, 26).

In our study groups, according to the PSQI score, the proportion of participants with poor sleep quality was significantly different in all groups. Recent studies have shown that sleep disorders are more common among migraine patients and that the frequency of attacks is higher in migraine patients with sleep disorders (27). More extensive studies, especially in the last 20 years, have shown that episodic migraine has become chronic in patients with sleep disorders (28). In a study conducted by Kelman and his colleagues to investigate the relationship between sleep and headache, 65% of the patients reported that headache increased with lack of sleep. In the same study, it was observed that sleep was effective in alleviate the headache (29). In our study, the group with the worst sleep quality was the migraine group, while the group with best sleep quality was the headache-free control group. In a case-control type study of melatonin hormone levels in sleep disorders of migraine patients, PSQI score was found to be 7.3 in the control group and 8.6 in the migraine group. As a result of the study, difficulty in falling asleep and poor sleep quality was more common in migraine patients than those in the control group (30). The mean PSQI total score and component scores in this study were similar to our study.

In a study of migraine and insomnia performed by Jiyoung K. and his colleagues with 2695 participants, it was found that migraine had a negative impact on sleep quality and associated with insomnia. The study was carried out in three groups including migraine patients, patients with non-migraine headache and participants without headache. Insomnia prevalence in the migraine group was found to be significantly higher than the control groups. The frequency of attacks in migraine patients with insomnia was 5.7 days/month, while the frequency of attacks in migraine patients without insomnia was determined to be 3.2 days/month and the difference between them was not statistically significant (15). In our study, similar to the study conducted by Jiyoung and his colleagues, there was no significant relationship between sleep quality and attack frequency.

Daytime excessive sleepiness is also more common among migraineurs, like poor sleep quality (31). In a study conducted with 60 female migraine patients who admitted to the neurology outpatient clinics, the mean age of the participants was lower, the mean number of monthly attacks were higher, and the daytime sleepiness ratio (32%) was similar compared to our study. As a result of the study, they argued that as the frequency of migraine attack increases, daytime sleepiness may increase due to insomnia and that migraine attacks may increase as daytime sleepiness increases and daytime sleepiness may be a symptom of migraine (32). However, in our study, in terms of daytime sleepiness status and Epworth scores, no statistically significant difference was found between migraine groups. In other words, there was no association between the frequency of migraine attacks and daytime sleepiness. Daytime sleepiness rate in patients with migraine was higher than those in the control groups. Based on these results, it can be said that migraine causes daytime sleepiness apart from other headaches. In his study, Smitherman found spontaneous recovery of headache in some migraine patients when sleep patterns were improved (33). In a pilot study, Law and his colleagues performed cognitive behavioral therapy to adolescents with migraine and insomnia and achieved successful results (34). Yang and his colleagues have improved the course of migraine with sleep behavioral therapies in their study (35).

As it is understood from these studies and our study, migraine is a disease which disturbs sleep quality and causes daytime sleepiness, in contrary to other headache types. This result supports the hypothesis of the study. The sleep quality of migraineurs is worse than patients with non-migraine headaches and headache-free participants. This may be due to the higher number of attacks in migraine patients and/or the severity of the attacks.

In the literature, there are similar studies regarding the subject, but our work is complementary in some respects. In our study, the risk of the results being affected by the participant distributions was reduced by keeping the participant numbers close between the groups. The use of validated scales have strengthened our work and reduced the subjective approach. In determining the method of our study, two separate control groups were generated, so that the relationship between migraine and sleep was examined on stronger ground, and migraine subgroups were formed to determine the degree of sleep and migraine relationship.

**Limitations and Future Research:** Since the data collection phase of our study was limited to 6 months, our study was conducted with a small number of participants. Since participants were selected from a specific region and surveys were used as data sources, different results could be obtained if the study was conducted in other communities or societies. This study was conducted in a tertiary hospital so results should be generalised cautiously at the population level.

## CONCLUSION

The sociodemographic characteristics of the study groups were consistent with similar studies. Poor sleep quality and excessive daytime sleepiness in migraine patients were higher than those with non-migraine headache and headache-free participants. This may be due to the fact that migraine is a specific problem affecting sleep or

headache frequency and severity is greater than that of other chronic headache types. The effect of sleep quality or daytime excessive sleepiness on the frequency and severity of attacks among migraine patients were not statistically significant. This situation suggests that in sleep-migraine relationship, migraine disrupts sleep quality rather than poor sleep quality worsens migraine attacks.

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RESEARCH ARTICLE

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# May Argyrophilic Nucleolar Organizer Regions be the New Marker of a Hypoxic Response in Non ST Elevation Myocardial Infarction?

**ABSTRACT**

**Objective:** Non-ST elevation myocardial infarction (NSTEMI) is a type of acute coronary syndrome and its' incidence is similarly high to ST-elevation myocardial infarction. Nucleolar organizing regions (NORs) are located of the secondary structure of acrocentric chromosome and composed of proteins and ribosomal DNA (rDNA) some of which are argyrophilic. We aimed to investigate the change of AgNOR proteins, which increase in hypoxia, in patients with NSTEMI.

**Methods:** A total of 125 participants, 63 patients with NSTEMI and 62 volunteers without any acute coronary syndrome were included in the study. Echocardiography was performed and both mean AgNOR Number and total AgNOR area/total nuclear area (TAA/TNA) were detected for each individuals.

**Results:** The mean AgNOR number and TAA/TNA ratio were significantly higher in the NSTEMI group than control ($p<0.001$). Also, statistically significant relations between TAA/TNA and all of creatinine, hemoglobin, WBC(µl/ml), monocyte, neutrophil, neutrophil / lymphocyte ratio, monocyte / lymphocyte ratio were detected ($p<0.05$ for all). Also, statistically significant relations between mean AgNOR number and all of fasting blood sugar, HDL, WBC(µl/ml), monocyte, neutrophil, EF were detected ($p<0.001$).

**Conclusions:** Both AgNOR protein amounts may be used as a marker for NSTEMI. It may also contribute to the prediction of the outcomes by providing some prognostic information in NSTEMI.

**Keywords:** AgNOR, Hypoxia, NOR, NSTEMI.

# Arjirofilik Nükleolar Organize Edici Bölgeler Non ST Elevasyonlu Miyokard İnfarktüsünde Hipoksik Yanıtın Yeni bir Belirteci Olabilir mi?

**ÖZET**

**Amaç:** Non-ST elevasyonlu miyokard enfarktüsü (NSTEMI), bir akut koroner sendrom türüdür ve görülme sıklığı ST elevasyonlu miyokard enfarktüsüne benzer şekilde yüksektir. Nükleolar organize edici bölgeler (NORs), akrosentrik kromozomun ikincil yapısında yer alır ve bazıları arjirofilik olan proteinlerden ve ribozomal DNA'dan (rDNA) oluşur. NSTEMI hastalarında, hipokside artan AgNOR proteinlerinin değişimini araştırmayı amaçladık.

**Gereç ve Yöntem:** Toplam 125 katılımcı, 63 NSTEMI hastası ve herhangi bir akut koroner sendrom tanısı olmayan 62 gönüllü çalışmaya dahil edildi. Bütün hastalara ekokardiyografi yapıldı ve her birey için hem ortalama AgNOR sayısı hem de toplam AgNOR alanı/toplam nükleer alan (TAA/TNA) tespiti yapıldı.

**Bulgular:** Ortalama AgNOR sayısı ve TAA/TNA oranı NSTEMI grubunda kontrole göre anlamlı derecede yüksekti ($p<0.001$). TAA/TNA ile, kreatinin, hemoglobin, WBC(µl/ml), monosit, nötrofil, nötrofil/lenfosit oranı, monosit/lenfosit oranı arasında istatistiksel olarak anlamlı ilişkiler tespit edildi (tümü için $p<0.05$). Ayrıca ortalama AgNOR sayısı ile, açlık kan şekeri, HDL, WBC(µl/ml), monosit, nötrofil ve EF arasında istatistiksel olarak anlamlı ilişkiler tespit edildi ($p<0.001$).

**Sonuç:** Her iki AgNOR protein miktarı, NSTEMI için bir markır olarak kullanılabilir. NSTEMI'de bazı prognostik bilgiler sağlayarak sonuçların tahmin edilmesine de katkıda bulunabilir.

**Anahtar Kelimeler:** AgNOR, Hipoksi, NOR, NSTEMI.

## INTRODUCTION

Non-ST elevation myocardial infarction (NSTEMI) is a type of acute coronary syndrome characterized by elevated cardiac biomarkers without persistent ST segment elevation on electrocardiography (1). In line with the increasing incidence of diabetes and the elderly population, the incidence of NSTEMI is similarly high to ST-elevation myocardial infarction (STEMI) (2). Despite many studies to provide primary or secondary prevention, mortality and morbidity rates are not yet at the desired level due to the multitude of gray zones in the pathophysiology of the NSTEMI (3). Many factors that accelerate the development of the disease, especially major risk factors such as diabetes, hyperlipidemia, hypertension, smoking, and genetic predisposition, play an important role in the process starting with endothelial dysfunction and leading to plaque rupture and acute coronary thrombosis (4). It is possible that immune system cells, especially neutrophils, lymphocytes and monocytes, contribute to the pathophysiology with a protective mechanism in the first hours when acute coronary thrombosis begins to occur and inflammation is most active.

Nucleolar organizing regions (NORs) are the locations of ribosomal genes composed of proteins and ribosomal DNA (rDNA) some of which are argyrophilic. After silver staining is applied, NORs can be visualized as localized black spots, particularly with the nucleolar space and are known as "AgNORs". There are studies in the literature emphasizing the importance of AgNOR proteins with various cells such as myocyte (5,6), muscle cells (7), lung cells (8), buccal epithelial cells (9), etc. As a result of these studies, it was determined that the hypoxic state after CO exposure increased the amount of AgNOR protein and that increased AgNOR proteins may have some secondary protective effects against hypoxia (5,6). In NSTEMI, myocardial perfusion is impaired secondary to acute coronary thrombosis, and subsequent ischemia/hypoxia occurs in myocytes. There are no studies in the literature on the evaluation of AgNOR proteins in patients with NSTEMI.

In present study, we aimed to investigate the change in AgNOR proteins in patients with NSTEMI in whom hypoxia is observed significantly. We also aimed to compare AgNOR proteins of NSTEMI subjects to those in control group.

## MATERIAL AND METHODS

**Study Design:** A total of 125 participants, 63 patients who underwent percutaneous coronary intervention with the diagnosis of NSTEMI whom presented to outpatient cardiology clinic of our institution, and 62 volunteers without the diagnosis of any acute coronary syndrome, were included in the study. The study protocol was certified by the local Ethics Committee (ethical approval code:2021/61). Written informed consent was acquired from each participant. Congenital heart disease, severe heart valve disease, atrial fibrillation, hyperthyroidism, hypothyroidism, infection or malignancy were considered as exclusion criteria. The diagnosis of NSTEMI was made according to the 2020 ESC Guidelines for the treatment of acute coronary syndromes in patients without persistent ST-segment elevation (1). Only the responsible lesion was intervened in patients with a diagnosis of NSTEMI who underwent percutaneous coronary intervention. Coronary artery stenosis was determined if the plaques cause 50% or more obstruction in coronary lumen, while hemodynamically insignificant stenosis is determined if the lesions were caused less than 50% stenosis. Blood samples of the patients with a diagnosis of NSTEMI included in the study were obtained within the first 6 hours after the onset of chest pain. Hypertension was defined as the use of antihypertensive drugs or blood pressure ≥140/90 mmHg. Diabetes mellitus was describe as use of antidiabetic therapy or fasting plasma glucose levels > 6.94 mmol/L (>125 mg/dL). Hyperlipidemia was determined as serum total cholesterol ≥5.2 mmol/L, low-density lipoprotein ≥2.6 mmol/L, triglyceride ≥1.7 mmol/L, or using of cholesterol-lowering medication (10). Demographic characteristics and laboratory findings (white blood cells, neutrophil, lymphocyte and monocyte counts, creatinine, total cholesterol, low density lipoprotein (LDL), triglyceride, high density lipoprotein (HDL), hemoglobin levels) of the participants were recorded.

**Electrocardiography and Echocardiography:** All participants' 12-lead resting ECGs were recorded with the NIHON KOHDEN Cardiofax ECG 1250K model. (filter range, 0.05-150 Hz; AC filter, 60 Hz, 10 mm / mv, 25 mm / sec). Siemens acuson SC 2000 model device was used for echocardiography. Ejection fraction, segmental wall motion abnormality, cardiac anatomy and valve functions were evaluated with standard projections according to the guidelines of the American Society of Echocardiography (11).

**Coronary Angiography:** Selective right and left coronary angiography and PCI procedures were performed on the patients in the NSTEMI group with the General Electric INNOVA 2100 IQ

model device using the standard Judkin technique. Coronary arteries were visualized at right and left oblique positions with cranial and caudal angles.

**AgNOR Staining:** Blood samples from both NSTEMI and control groups were distributed on clean slides. After the slides were air-dried for 15 minutes, they were fixed with absolute methanol for 5 minutes at room temperature. Subsequently, silver staining was performed for each slide with the Benn and Perle protocol (12) and slight modification of Lindner (13). The solution prepared by mixing one volume of 1% aqueous formic acid and 2% gelatin with two volumes of 50% silver nitrate was dropped onto the slides and incubated at 37 $^0$C for 15 minutes in the dark. After incubation process, the slides washed with bi-distilled water were made ready for examination.

**Image Analysis of Mean AgNOR Number and Total AgNOR Area/Total Nuclear Area (TAA/TNA) Ratio:** First, silver-stained lymphocyte cells from each individual were photographed with a digital camera system (Digital Sight DS-Fi1c; Nikon) attached to a light microscope (Eclipse 80i; Nikon, Tokyo, Japan). Then, using the "free-hand selection" tool for each core, necessary measurements were made using ImageJ version 1.47t image processing software (14) to determine both the TAA/TNA ratio and the average AgNOR number for each core.

**Statistical Analysis:** The study data were analyzed using the Statistical Package for Social Sciences (IBM Corp., Armonk, NY, USA) 23.0. The data distribution was examined with the Kolmogorov-Smirnov test. Due to the non-normal distribution of the data ($P < 0.05$), non-parametric tests were preferred for statistical analysis. For pairwise comparison of the groups, first descriptive statistics and then Mann-Whitney U tests were used. In addition, polynomial regression test was applied. The $p < 0.05$ was considered statistically significant.

## RESULTS

Totally 125 persons (63 with NSTEMI and 62 with control) were included in the current study. The frequency of male and female gender was 68.3%/31.7% and 64.5%/35.5% for the patient and control groups, respectively. There was no significant difference between the two groups in terms of cardiovascular major risk factors except diabetes mellitus. Among the laboratory findings of groups, creatinine (mg/dL), WBC (White Blood Cell) (µl/ml), monocyte, neutrophil, neutrophil/lymphocyte ratio, Monocyte/Lymphocyte ratio were significantly higher in NSTEMI patients than control group ($p<0.05$ for all). On the contrary, HDL (mg/dL) level was significantly higher in the control group ($p=0.001$). The troponin levels of the patients in the NSTEMI group were 5.658 (0.128-21.3) ng/ml. When echocardiographic parameters were compared, ejection fraction was higher in the control group ($p<0.001$), while the interventricular septal thickness and rate of tricuspid regurgitation were significantly higher in the NSTEMI group ($p<0.05$ for all) (Table 1). The ratio of the culprit lesion in NSTEMI patients were 28(44.4%), 17(27%) and 18 (28.6%) for LAD, CX and RCA, respectively.

Also the mean AgNOR number ((1,3 (1-2,9) vs 1 (1-1,4)) and TAA/TNA ratio (0.2 (0,11-0,45) vs 0,05 (0,03-0,1)) were significantly higher in the NSTEMI group than control ($p=0.000$).

When the TAA/TNA ratio to be considered, statistically significant relations between TAA/TNA and all of creatinine, hemoglobin, WBC(µl/ml), monocyte, neutrophil, neutrophil / lymphocyte ratio, monocyte / lymphocyte ratio were detected (Figure1 and Table 2).

Furthermore, when the mean AgNOR number to be considered, statistically significant relations between mean AgNOR number and all of fasting blood sugar, HDL, WBC(µl/ml), monocyte, neutrophil, EF were detected (Figure 2 and Table 3). Silver stained NOR in the lymphocytes of NSTEMI (a, b, c, d), and control (e,f,g,h) groups (X100 magnification) were given in the Figure 3.

## DISCUSSION

Striking findings of present study showed that mean AgNOR number and TAA/TNA ratio were significantly higher in the NSTEMI group than in the control group.

In previous studies, it was determined that the hypoxic state due to CO exposure caused an increase in AgNOR proteins (15-18). However, it was reported that the TAA/TNA ratio could be used as an indicator to determine the level of CO exposure resulting in hypoxia (16). It has also been reported that the TAA/TNA ratio can be used as a biomarker instead of histopathological evaluation scores in determining the degree of myocardial damage in rats (5). In addition, it has been shown that some information can be obtained in the detection of cardiomyopathy (CM) levels through AgNOR proteins and that these proteins can be used as an alternative to carboxyhemoglobin (CoHb) to detect CO poisoning levels (6).

Considering the role of lymphocytes in inflammation in the first hours of acute coronary thrombosis and myocardial ischemia/hypoxia in patients with NSTEMI, it is expected that there will be some molecular changes controlling inflammation at the cell nucleus level. In our study, the fact that both the TAA/TNA ratio and mean AgNOR number were higher in patients with NSTEMI compared to the control group can be evaluated in the context of these molecular changes.

**Table 1.** Clinical, laboratory and echocardiographic findings of both groups

| | **NSTEMI (n=63) Mean±SD (min-max)** | **Control (n=62) Mean±SD (min-max) (median)** | $\chi^2$ | **p** |
|---|---|---|---|---|
| **Sex (M/F) (%)** | 43(68.3%)/20(31.7%) | 40(64.5%)/22(35.5%) | 0.196 | 0.658 |
| **Diabetes mellitus (Yes/No) (%)** | 24(38.1%)/39(61.9%) | 13(21%)/49(79%) | 4.399 | 0.036 |
| **Hypertension (Yes/No) (%)** | 40(63.5%)/23(36.5%) | 35(56.5%)/27(43.5%) | 0.645 | 0.422 |
| **Hyperlipidemia (Yes/No) (%)** | 35(55.6%)/28(44.4%) | 36(58.1%)/26(41.9%) | 0.080 | 0.777 |
| **FHCD (Yes/No) (%)** | 20(31.7%)/43(68.3%) | 15(24.2%)/47(75.8%) | 0.884 | 0.347 |
| **Smoking(Yes/No) (%)** | 36(57.1%)/27(42.9 %) | 18(29%)/44(71%) | 10.063 | 0.002 |
| | | | **Z** | **p** |
| **Age (years)** | 58.03±10.07(41-74) (59) | 56.69±11.17(34-75) (57.5) | -0.618 | 0.537 |
| **BMI in Diagnosis (kg/m2)** | 27.19±1.76(22.46-32.72) (27.04) | 27.61±3.56(20.86-37.89) (27.26) | -0.452 | 0.651 |
| **Systolic Blood pressure (mmHg)** | 134.65±9.981(120-150) (135) | 133.95±12.647(100-160) (135) | -0.176 | 0.860 |
| **Diastolic Blood pressure (mmHg)** | 86.47±6.03(60-100) (90) | 86.61±7.933(60-100) (90) | -0.471 | 0.638 |
| **Fasting Blood Sugar** | 108.32±31.52(70-180) (90) | 104.07±22.99(77-200) (99.5) | -0.736 | 0.462 |
| **Creatinin (mg/dL)** | 0.91±0.20(0.48-1.50) (0.86) | 0.83±0.16(0.53-1.27) (0.82) | -2.050 | 0.040 |
| **LDL (mg/dL)** | 118.08±38.44(35-210) (120) | 122.18±34.89(50-201) (123.5) | -0.790 | 0.429 |
| **HDL (mg/dL)** | 41.90±12.18(23-97) (39) | 48.31±12.28(25-82) (45) | -3.254 | 0.001 |
| **Triglyceride(mg/dL)** | 161.32±88.55(60-509) (144) | 153.47±67.08(67-349) (134.5) | -0.040 | 0.968 |
| **Total Cholesterol (mg/dL)** | 190.60±44.18(100-325) (185) | 200.95±41.93(120-305) (202.5) | -1.568 | 0.117 |
| **Hemoglobin (g/dL)** | 13.64±1.48(10-17) (13.5) | 14.08±1.35(11.3-17.2)(13.95) | -1.596 | 0.111 |
| **WBC ( µl/ml)** | 11766.67±3836.16(5400-29300) (11600) | 6737.1±1569.19(4300-10600)(6300) | -8.082 | <0.001 |
| **Platelet ($X10^3$)** | 269.52±79.23(136-565) (260) | 245.13±63.65(149-481) (229.5) | -1.874 | 0.061 |
| **Lymphocyte ($X10^3$)** | 2.25±1.09(0.32-6.20) (2.3) | 2.02±0.52(0.65-3.10) (2.015) | -1.190 | 0.234 |
| **Monocyte ($X10^3$)** | 0.76±0.35(0.06-1.7) (0.7) | 0.51±0.18 (0.21-1.46) (0.5) | -4.567 | <0.001 |
| **Neutrophil ($X10^3$)** | 8.54±3.9(2.9-26.50) (7.6) | 4.03±1.35(2.09-8.16) (3.66) | -8.061 | <0.001 |
| **Neutrophil/Lymphocyte** | 5.56±5.65(1-29.34) (3.36) | 2.17±1.1(0.88-7.11) (1.88) | -5.516 | <0.001 |
| **Monocyte/Lymphocyte** | 0.44±0.43(0.04-2.80) (0.34) | 0.27±0.16(0.13-1.12) (0.026) | -3.163 | 0.002 |
| **Mean AgNOR Number** | 1.53±0.52(1-2.90) (1.3) | 1.08±0.12(1-1.4) (1) | -6.699 | <0.001 |
| **TAA/TNA** | 0.21±0.06(0.11-0.45) (0.2) | 0.05±0.02(0.03-0.1) (0.05) | -9.645 | <0.001 |
| | **Echocardiographical Findings** | | | |
| | **STEMI Mean±SD (min-max)** | **Control Mean±SD (min-max)** | **Z** | **p** |
| **EF (n %)** | 50.65±8.18(30-61) (50) | 63.87±2.78(50-65) (65) | -9.326 | <0.001 |
| **IVST (cm)** | 1.18±0.13(0.9-1.7) (1.2) | 1.07±0.14(0.8-1.5) (1) | -4.278 | <0.001 |
| | **STEMI n(%)** | **Control n(%)** | $\chi^2$ | **p** |
| **MR (Yes/No) (%)** | 40(63.5%)/23(36.5%) | 30(48.4%)/32(51.6%) | 2.893 | 0.089 |
| **AR** | 12(19%)/51(81%) | 10(16.1%)/52(83.9%) | 0.184 | 0.668 |
| **PR** | 10(15.9%)/53(84.1%) | 3(4.8%)/59(95.2%) | 4.083 | 0.043 |
| **TR** | 31(49.2%)/32(50.8%) | 22(35.5%)/40(64.5%) | 2.409 | 0.121 |

**FHCD**: Family History of Cardiovascular Disease, **BMI:** Body mass index, **HDL:** High-density lipoprotein, **LDL:** Low-density lipoprotein, **EF:** Ejection Fraction **MR:** Mitral Regurgitation, **TR:** Tricuspid Regurgitation **AR:** Aortic Regurgitation **PR:** Pulmonary Regurgitation, **LAD** :Left Anterior Descending Artery, **LCX:** Left Circumflex Artery **RCA**: Right Coronary Artery, **Min-Max:** Minimum-Maximum**, SD:** Standard deviation, ***=**Statistically significant, **IVST**: interventricular septum thickness, **WBC:**White Blood Cell

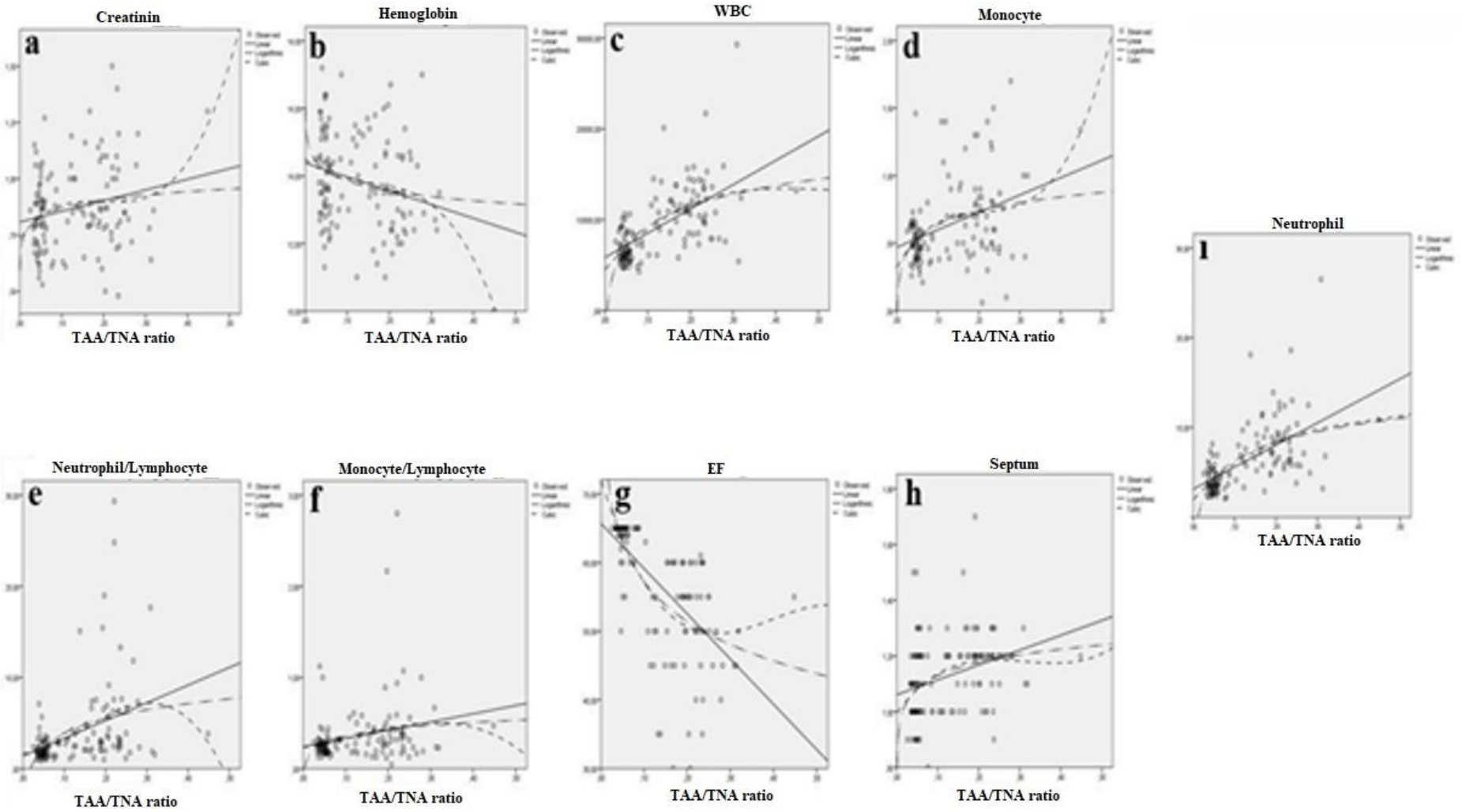


**Figure 1.** The relation between creatinine and TAA/TNA ratio (a), hemoglobin and TAA/TNA ratio (b), WBC and TAA/TNA ratio (c), monocyte and TAA/TNA ratio (d), Neutrophil / Lymphocyte ratio and TAA/TNA ratio (e), monocyte / Lymphocyte ratio and TAA/TNA ratio (f), EF and TAA/TNA ratio (g), Septum and TAA/TNA ratio (h), neutrophil and TAA/TNA ratio (ı) for both groups.

**Table 2.** Model Summary and Parameter Estimates for TAA/TNA and all of creatinin, hemoglobin, WBC, monocyte, neutrophil, Neutrophil / Lymphocyte, Monocyte / Lymphocyte, EF, IVST of both groups

| | | **Model Summery** | | | | | **Parameter Estimates** | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | ***Equation*** | ***$R^2$*** | ***F*** | ***df1*** | ***df2*** | ***sig*** | **Constant** | ***b1*** | ***b2*** | ***b3*** |
| **Creatinin and TAA/TNA** | Linear | 0.049 | 6.297 | 1 | 123 | 0.013 | 0.808 | 0.475 | | |
| | Log | 0.043 | 5.577 | 1 | 123 | 0.020 | 0.991 | 0.053 | | |
| | Cubic | 0.067 | 2.887 | 3 | 121 | 0.038 | 0.733 | 2.612 | -13.487 | 22.710 |
| **Hemoglobin (g/dL) and TAA/TNA** | Linear | 0.064 | 8.428 | 1 | 123 | 0.004 | 14.385 | -4.098 | | |
| | Log | 0.049 | 6.306 | 1 | 123 | 0.013 | 12.882 | -0.422 | | |
| | Cubic | 0.091 | 4.031 | 3 | 121 | 0.009 | 14.499 | -10.038 | 54.043 | -118.653 |
| **WBC(µl/ml) and TAA/TNA** | Linear | 0.370 | 72.208 | 1 | 123 | <0.001 | 5833.129 | 26694.334 | | |
| | Log | 0.382 | 76.079 | 1 | 123 | <0.001 | 16668.897 | 3204.454 | | |
| | Cubic | 0.392 | 26.037 | 3 | 121 | <0.001 | 4478.610 | 54671.794 | -108456.9 | 68986.829 |
| **Monocyte and TAA/TNA** | Linear | 0.137 | 19.546 | 1 | 123 | <0.001 | 0.470 | 1.287 | | |
| | Log | 0.130 | 18.397 | 1 | 123 | <0.001 | 0.977 | 0.148 | | |
| | Cubic | 0.155 | 7.405 | 3 | 121 | <0.001 | 0.320 | 5.387 | -24.579 | 39.227 |
| **Neutrophil and TAA/TNA** | Linear | 0.343 | 64.348 | 1 | 123 | <0.001 | 3.139 | 24.554 | | |
| | Log | 0.349 | 65.813 | 1 | 123 | <0.001 | 13.045 | 2.921 | | |
| | Cubic | 0.359 | 22.552 | 3 | 121 | <0.001 | 1.978 | 49.205 | -101.313 | 80.073 |
| **Neutrophil / Lymphocyte and TAA/TNA** | Linear | 0.151 | 21.895 | 1 | 123 | <0.001 | 1.374 | 19.458 | | |
| | Log | 0.156 | 22.729 | 1 | 123 | <0.001 | 9.270 | 2.335 | | |
| | Cubic | 0.177 | 8.671 | 3 | 121 | <0.001 | 1.690 | 2.191 | 160.047 | -354.778 |
| **Monocyte / Lymphocyte and TAA/TNA** | Linear | 0.056 | 7.279 | 1 | 123 | 0.008 | 0.240 | 0.905 | | |
| | Log | 0.056 | 7.357 | 1 | 123 | 0.008 | 0.604 | 0.107 | | |
| | Cubic | 0.062 | 2.685 | 3 | 121 | 0.050 | 0.235 | 0.679 | 3.761 | -10.236 |
| **EF and TAA/TNA** | Linear | 0.414 | 86.725 | 1 | 123 | <0.001 | 65.687 | -65.816 | | |
| | Log | 0.470 | 109.203 | 1 | 123 | <0.001 | 38.073 | -8.290 | | |
| | Cubic | 0.484 | 37.772 | 3 | 121 | <0.001 | 71.984 | -200.729 | 565.007 | -473.520 |
| **IVST and TAA/TNA** | Linear | 0.107 | 14.686 | 1 | 123 | <0.001 | 1.061 | 0.533 | | |
| | Log | 0.124 | 17.351 | 1 | 123 | <0.001 | 1.286 | 0.068 | | |
| | Cubic | 0.127 | 5.891 | 3 | 121 | 0.001 | 0.995 | 2.026 | -6.893 | 7.386 |

**IVST:** Interventricular septum thickness, **EF:** Ejection Fraction, **WBC:**White Blood Cell

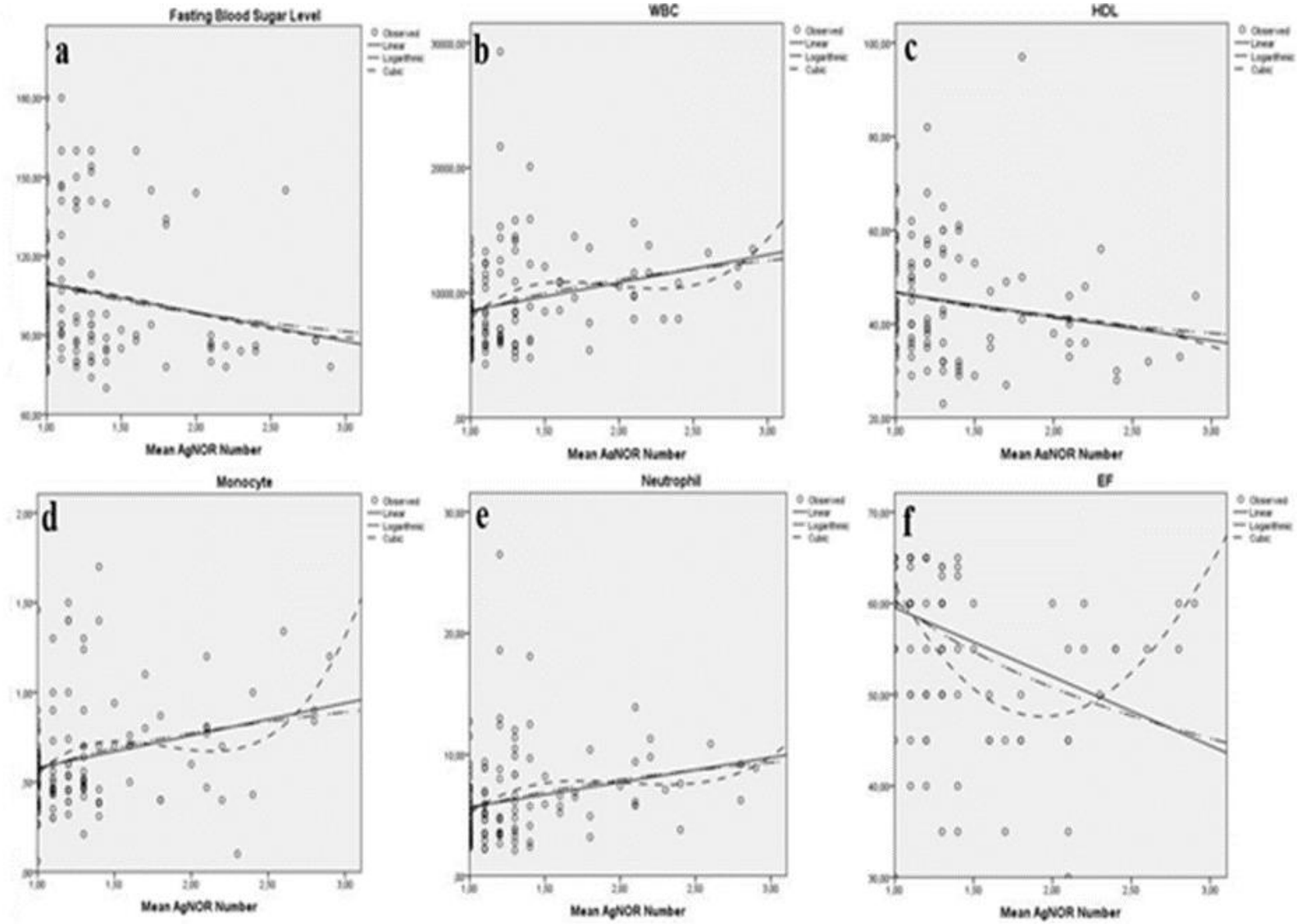


**Figure 2.** The relation between fasting blood sugar and mean AgNOR number (a), WBC and mean AgNOR number (b), HDL and mean AgNOR number (c), monocyte and mean AgNOR number (d), neutrophil and mean AgNOR number (e) EF and mean AgNOR number (f) for both groups.

**Table 3.** Model Summary and Parameter Estimates for Mean AgNOR number and all of fasting blood sugar, HDL, WBC, monocyte, neutrophil, EF of both groups

| | | **Model Summery** | | | | | **Parameter Estimates** | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | ***Equation*** | ***$R^2$*** | ***F*** | ***df1*** | ***df2*** | ***sig*** | **Constant** | ***b1*** | ***b2*** | ***b3*** |
| **Fasting Blood Sugar and Mean AgNOR** | Linear | 0.030 | 3.853 | 1 | 123 | 0.052 | 120.502 | -10.928 | | |
| | Log | 0.029 | 3.688 | 1 | 123 | 0.057 | 110.025 | -16.950 | | |
| | Cubic | 0.031 | 1.275 | 3 | 121 | 0.286 | 102.664 | 21.572 | -18.220 | 3.175 |
| **HDL and Mean AgNOR** | Linear | 0.032 | 4.005 | 1 | 123 | 0.048 | 51.730 | -5.084 | | |
| | Log | 0.031 | 3.995 | 1 | 123 | 0.048 | 46.892 | -8.045 | | |
| | Cubic | 0.032 | 1.328 | 3 | 121 | 0.268 | 61.187 | -22.707 | 10.162 | -1.824 |
| **WBC(µl/ml) and Mean AgNOR** | Linear | 0.064 | 8.445 | 1 | 123 | 0.004 | 6359.450 | 2226.720 | | |
| | Log | 0.074 | 9.825 | 1 | 123 | 0.002 | 8419.444 | 3785.483 | | |
| | Cubic | 0.098 | 4.374 | 3 | 121 | 0.006 | -20093.4 | 49688.181 | -26078.23 | 4445.557 |
| **Monocyte and Mean AgNOR** | Linear | 0.067 | 8.771 | 1 | 123 | 0.004 | 0.401 | 0.179 | | |
| | Log | 0.070 | 9.209 | 1 | 123 | 0.003 | 0.570 | 0.291 | | |
| | Cubic | 0.104 | 4.674 | 3 | 121 | 0.004 | -1.962 | 4.642 | -2.615 | 0.477 |
| **Neutrophil and Mean AgNOR** | Linear | 0.059 | 7.718 | 1 | 123 | 0.006 | 3.637 | 2.038 | | |
| | Log | 0.069 | 9.057 | 1 | 123 | 0.003 | 5.518 | 3.479 | | |
| | Cubic | 0.085 | 3.763 | 3 | 121 | 0.013 | -16.638 | 37.671 | -19.036 | 3.141 |
| **EF and Mean AgNOR** | Linear | 0.136 | 19.387 | 1 | 123 | <0.001 | 67.095 | -7.559 | | |
| | Log | 0.179 | 26.866 | 1 | 123 | <0.001 | 60.303 | -13.742 | | |
| | Cubic | 0.298 | 17.147 | 3 | 121 | <0.001 | 113.230 | -71.435 | 21.264 | -0.968 |

**IVST**: Interventricular septum thickness, **EF:** Ejection Fraction , **WBC:**White Blood Cell

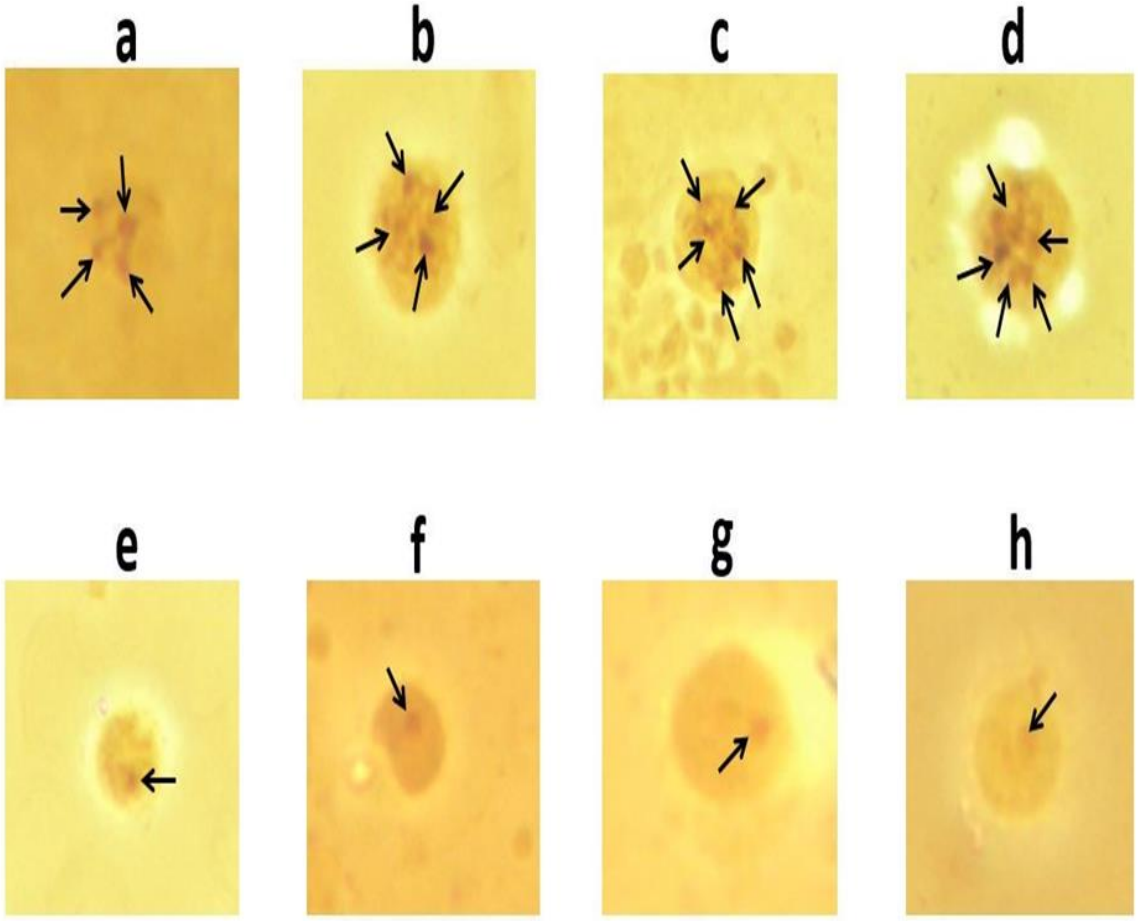


**Figure 3.** Silver stained NOR in the lymphocytes of NSTEMI (a, b, c, d), and control (e,f,g,h) groups (X100 magnification)

In addition, we found that statistically significant relationship between TAA/TNA and all of creatinine, hemoglobin, WBC (µl/ml), monocytes, neutrophils, neutrophil/lymphocyte ratio, monocyte/lymphocyte ratio and statistically significant relationship between AgNOR count and all of fasting blood glucose, HDL, WBC (µl/ml), monocytes, neutrophils, and EF indicate that the effects of AgNOR proteins cannot be ignored in the acute coronary syndrome process. In other words, the significant relationship between AgNOR proteins and cardiovascular risk factors such as high blood sugar, low HDL, anemia, and creatinine and the significant relationship between AgNOR proteins and inflammatory cells such as monocytes and neutrophils, which have very important functions in acute coronary syndrome (3,17,18) seem remarkable.

Until now, many studies have been conducted in the literature regarding the prognostic value of parameters such as WBC count, monocyte count, neutrophil count, neutrophil/lymphocyte ratio, Monocyte/Lymphocyte ratio in patients with acute coronary syndrome (18-22). In our study, we observed that these parameters were parallel to the literature data in patients with NSTEMI and we also witnessed a statistically significant relationship with AgNOR proteins.

Relatively small study cohort and single center nature of the study design are limitaions of present work. Yet, this is the first study in literature reported increased AgNOR in NSTEMI subjects.

**Conclusion**

Both AgNOR protein amounts may be used as a marker for NSTEMI. It may also contribute to the prediction of the outcomes by providing some prognostic information in NSTEMI.

**Conflict of Interest**: The authors declare that there is no conflict of interest

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**Author Contributions:** IHD and RE: Coordination of the study, Manuscript writing, database management, echocardiography data analysis, IHD and RE: Data collection, statistical analysis, IHD and RE: Contribution to the concept, design and critical revision of article.

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RESEARCH ARTICLE

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# Rare Side Effects after Inactiveted Sars-Cov-2 Vaccine (Coronavac)

**ABSTRACT**

**Objective:** To evaluate the frequency, type, onset time, and intensity of the side effects after the Coronavac vaccination in healthcare workers (HCW) and elderly people and also to determine whether COVID-19 disease occurs after vaccination in HCW.

**Methods:** HCW and elderly people who were vaccinated in Gaziantep Medical Park were the cohorts of the study. A questionnaire was applied to HCW, and the data of elderly people were obtained from medical records. The questionnaire had demographic data, medical history also included COVID-19-related and side effects of the Coronavac vaccine.

**Results:** Four hundred twenty-seven questionnaires were analyzed. The most common adverse reaction was pain at the injection site. The incidences of pain after the first and second injections were 23.8% (102/427) and 12.8% (52/405), respectively. And most common systemic side effect was fatigue with an incidence of 18.2% and 10.3% after each dose, respectively. Side effects that kept HCW from going to work after vaccination were not observed. After the two doses of vaccination, only 7 HCW had mild COVID-19 infection. One had a COVID-19 infection after 7 days of the first dose. 354 medical records of elderly people were evaluated. Only one of them reported urticaria after the first dose.

**Conclusions:** Our study found that Coronavac is a well-tolerated and effective vaccine.

**Keywords:** Adverse Reaction, Coronavac, COVID-19 Vaccination, Inactivated Vaccine, Efficacy, Healthcare Workers Vaccination, Safety, Side Effects.

# İnaktive Sars-Cov-2 (Coronavac) Aşısı Sonrası Nadir Görülen Yan Etkiler

**ÖZET**

**Amaç:** Sağlık çalışanlarında (SÇ) ve yaşlılarda Coronavac aşısı sonrası görülen yan etkilerin sıklığını, türünü, başlangıç süresini ve yoğunluğunu değerlendirmek ve ayrıca sağlık çalışanlarında aşılama sonrası COVID-19 hastalığının gelişip gelişmediğini belirlemektir.

**Gereç ve Yöntem:** Çalışmanın kohortunu Gaziantep Medikal Park hastanesinde aşılanan sağlık çalışanları ve yaşlı hastalar oluşturmuştur. Sağlık çalışanlarına anket uygulandı ve yaşlı hastaların verileri tıbbi kayıtlardan elde edildi. Anket demografik veriler, COVID-19 dahil tıbbi geçmiş ve Coronavac aşısının yan etkilerini içermekteydi.

**Bulgular:** Dörtyüz yirmi yedi anket analiz edildi. En yaygın görülen yan etki enjeksiyon bölgesinde ağrı idi. Birinci ve ikinci enjeksiyonlardan sonra ağrı insidansı sırasıyla %23.8 (102/427) ve %12.8 (52/405) idi. En yaygın sistemik yan etki, her dozdan sonra sırasıyla %18.2 ve %10.3'lük bir insidansla, yorgunluktu. Aşılamadan sonra sağlık çalışanlarının işe gitmesine engel olabilecek yan etkiler gözlenmedi. İki doz aşılamadan sonra sadece 7 sağlık çalışanı hafif COVID-19 enfeksiyonu geçirdi. İlk dozdan 7 gün sonra bir kişi COVID-19 enfeksiyonu geçirdi. Yaşlılara ait 354 adet tıbbi kayıt incelendi. Bunlardan sadece bir tanesinde ilk dozdan sonra ürtiker bildirildiği tespit edildi.

**Sonuç:** Bizim çalışmamız, Coronavac aşısının iyi tolere edilen ve etkili bir aşı olduğunu tespit etmiştir.

**Anahtar Kelimeler:** İstenmeyen Olaylar, Coronavac, COVID-19 Aşısı, İnaktive Aşı, Etkinlik, Sağlık Çalışanlarının Aşılanması, Güvenlik, Yan Etkiler.

## INTRODUCTION

There have been 176.693.988 confirmed cases of COVID-19 (Coronavirus disease 2019), including 3.830.304 deaths, reported to WHO (The World Health Organization) as of 17 June 2021, worldwide (1). The COVID-19 virus spreads primarily through droplets of saliva or discharge from infected people and affects different people in various ways. Clinical characters of COVID-19 range from asymptomatic to acute respiratory distress syndrome, multiple organ dysfunction, and death (2).

COVID-19 has not a specific treatment yet. The best way to control this COVID-19 pandemic is vaccination. Now, there are many types of vaccines (3). In Turkey, on January 13, 2021, the vaccination program started with Coronavac which is an inactivated SARS-CoV-2 vaccine developed by the Chinese biotechnology company Sinovac against coronavirus. Firstly Coronavac vaccine was applied to healthcare workers, disabled, and elderly people. On 01 June 2021, WHO gave emergency use permission to the CoronaVac vaccine (3).

To control this COVID-19 pandemic, we have to give efficient and harmless vaccines to people. Most people and some healthcare workers have questions about the safety and effectiveness of the COVID-19 vaccines, and they want to have more evidence about vaccines before vaccination. In the literature, there are few studies about COVID-19 vaccines' safety and efficacy. In this study, we want to analyze Coronavac side effects and effectiveness in real-life conditions.

## MATERIAL AND METHODS

**Study Design and Population:** We applied a questionnaire to healthcare workers and staff who were volunteered to take a part in the study and worked in Gaziantep Medical Park Hospital in Turkey. Ethics committee approval was taken from the Ministry of Health of Turkey and Hatay Mustafa Kemal University ethics committee for this study.

We designed a self-administered questionnaire. The questionnaire contains basic demographic information, such as age, sex, occupation, health status, COVID-19 anamnesis, date of COVID-19 vaccination, and adverse reactions of Coronavac. The part of adverse reactions of the questionnaire includes local reactions as edema, redness, pain, itch, bruising, movement restriction, and systemic reactions as confusion, faint, fever, chills, fatigue, joint pain, muscle pain, skin rash, appetite, headache, dizziness, nausea, vomiting, abdominal pain, cough, shortness of breath, diarrhea, chest pain, palpitation, throat ache, somnolence, insomnia. We classified these adverse reactions as mild, moderate, and severe.

We obtained the data of elderly individuals who were applied the Coronavac vaccine in our hospital, from their medical records. The data include their age, co-morbidities, and side effects of the vaccine.

Descriptive statistics were used, and the results were expressed as mean (SD) and percentage.

## RESULTS

We applied 450 questionnaires to HCW, but 427 questionnaires were appropriate to analyze. 405 HCW got two doses of vaccine. 22 HCW did not receive the second dose of the vaccine. 11 of them reported why they did not get the second dose. One had another health problem, three of them were waiting for the interval which was four weeks after the first dosage, three of them forgot to vaccinate, one had severe fatigue, so did not want the second dose, one thought that the vaccine was not effective.

Demographic and clinical characteristics of HCW are given in Table 1. 101 HCWs (23.65%) had COVID-19 infection before vaccination, 8 (1.87%) had COVID-19 infection after vaccination. Three HCWs became infected with COVID-19 after 40 days, two after 10 days, one after 50 days, and one after 63 days from the second dose of vaccine. One HCW had a COVID-19 infection seven days after the first dose of the vaccine.

**Table 1.** Demographic and clinical characteristics of healthcare workers

| Characteristics (n=427) | findings |
|---|---|
| Age, mean (SD), y | 30.47 (9.29) |
| Female, No. (%) | 277 (64.87) |
| Co-morbidities | |
| diabetes mellitus | 6 (1.40) |
| anemia | 1 (0.23) |
| asthma | 3 (0.70) |
| hypertension | 5 (1.17) |
| chronic urticaria | 1 (0.23) |
| multiple sclerosis | 1 (0.23) |
| Fabry's disease | 1 (0.23) |
| allergic rhinitis | 3 (0.70) |
| hyperlipidemia | 1 (0.23) |
| anxiety disorder | 1 (0.23) |
| COVID-19 infection | |
| Before vaccination | 101 (23.65) |
| After vaccination | 8 (1.87) |

Local and systemic side effects of the vaccine are given in Table 2. The most common side effect as a local reaction was mild pain with 12.1% after the first dose, and 6.7% after the second dose. There was mild fatigue in 8.0% after the first dose and 5.9% after the second dose of vaccine as a systemic reaction.

**Table 2.** Local and systemic side effects of vaccine

| | After 1 vaccination (n=427) | | | After 2 vaccination (n=405) | | |
|---|---|---|---|---|---|---|
| | Mild | moderate | severe | Mild | moderate | severe |
| Local reactions, No. (%) | | | | | | |
| Edema | 18 (4.2) | 4 (0.9) | 1 (0.2) | 12 (3.0) | 0 (0) | 0 (0) |
| Redness | 16 (3.7) | 7 (1.6) | 0 (0) | 10 (2.5) | 3 (0.7) | 0 (0) |
| Pain | 52 (12.1) | 34 (8.0) | 16 (3.7) | 27 (6.7) | 19 (4.7) | 6 (1.5) |
| Itch | 17 (4.0) | 7 (1.6) | 1 (0.2) | 10 (2.5) | 2 (0.5) | 1 (0.2) |
| Bruising | 10 (2.3) | 5 (1.2) | 0 (0) | 7 (1.7) | 1 (0.2) | 0 (0) |
| Movement restriction | 15 (3.5) | 12 (2.8) | 7 (1.6) | 11 (2.7) | 5 (1.2) | 0 (0) |
| Systemic reactions, No. (%) | | | | | | |
| Fatigue | 34 (8.0) | 26 (6.1) | 18 (4.2) | 24 (5.9) | 12 (3.0) | 6 (1.5) |
| Joint pain | 17 (4.0) | 27 (6.3) | 11 (2.6) | 15 (3.7) | 8 (2.0) | 3 (0.7) |
| Muscle pain | 19 (4.4) | 26 (6.1) | 10 (2.4) | 13 (3.2) | 9 (2.2) | 5 (1.2) |
| Skin rash | 10 (2.3) | 1 (0.2) | 0 (0) | 7 (1.7) | 0 (0) | 0 (0) |
| Appetite | 6 (1.4) | 7 (1.6) | 4 (0.9) | 7 (1.7) | 1 (0.2) | 0 (0) |
| Headache | 20 (4.7) | 15 (3.5) | 18(4.2) | 12 (3.0) | 10 (2.5) | 6 (1.5) |
| Dizziness | 14(3.3) | 9(2.1) | 6 (1.4) | 11 (2.7) | 4 (0.1) | 2 (0.5) |
| Nausea | 11(2.6) | 9(2.1) | 4 (0.9) | 7 (1.7) | 2 (0.5) | 0 (0) |
| Vomit | 10 (2.3) | 1(0.2) | 2 (0.5) | 7 (1.7) | 1 (0.2) | 0 (0) |
| Abdominal pain | 9 (2.1) | 3 (0.7) | 1 (0.2) | 7 (1.7) | 0 (0) | 0 (0) |
| Cough | 9 (2.1) | 2 (0.5) | 2 (0.5) | 6 (1.5) | 2 (0.5) | 2 (0.5) |
| Shortness of breath | 9 (2.1) | 1 (0.2) | 2 (0.5) | 6 (1.5) | 1 (0.2) | 0 (0) |
| Diarrhea | 9 (2.1) | 1 (0.2) | 0 (0.0) | 6 (1.5) | 0 (0) | 0 (0) |
| Chest pain | 9 (2.1) | 1 (0.2) | 3 (0.7) | 6(1.5) | 2 (0.5) | 2 (0.5) |
| Palpitation | 11(2.6) | 3 (0.7) | 4 (0.9) | 7 (1.7) | 2 (0.5) | 2 (0.5) |
| Throat ache | 9 (2.1) | 2 (0.5) | 1 (0.2) | 7 (1.7) | 0 (0) | 1 (0.2) |
| Somnolence | 16 (3.7) | 11 (2.6) | 8 (1.8) | 10 (2.5) | 9 (22) | 4 (0.1) |
| Insomnia | 11(2.6) | 3 (0.7) | 2 (0.5) | 7 (1.7) | 1 (0.2) | 0 (0) |

As local reactions, there were mild, moderate, and severe pain in 12.1%, 8.0%, and 3.7% of patients, after the first vaccine and 6.7%, 4.7%, 1.5%, after the second vaccine, respectively. As systemic reactions, there were mild, moderate, and severe fatigue in 8%, 6.1%, and 4.2% of patients after the first vaccine and in 5.9%, 3%, 1.5% of patients after the second vaccine, respectively.

As systemic reactions, confusion, faint, fever, and chills were analyzed as 0.9%, 0%, 3.7%, and 1.6% after the first dose and as 0.5%, 0.5%, 2.0%, and 0.7% after the second dose of vaccination, respectively. There were no significant problems in their follow-up.

354 people older than 64 years who were vaccinated with Coronavac vaccine in our hospital, were included in this study. The mean (SD) age of these people was 71.3 (5.9) years, and most of them were women (n: 207 [58.5%]). There was hypertension in 119 patients (33.6%), diabetes mellitus in 88 (24.8%), cancer in 28 (7.9%), chronic obstructive pulmonary disease (COPD) in 7 (2%), heart disease in 9 (2.5%), asthma in 22 (6.21%), stroke, liver disease and rheumatoid arthritis in 2 (0.6%) parkinson's disease and epilepsy in 3 (0.84%), kidney disease in 5 (1.41%) as comorbidities.

## DISCUSSION

Until today, no specific therapy for COVID-19 has been found. So an effective, side effect free, and easy to apply vaccine is the most necessary agent to control the COVID-19 pandemic. According to the WHO, as of June 18, 2021, 287 vaccine candidates were under clinical evaluation for COVID-19, and 102 candidate vaccines were in the clinical phase and 185 were in the preclinical phase (4). In Turkey, on January 13, 2021, the vaccination program started with Coronavac which is an inactivated SARS-CoV-2 vaccine developed by the Chinese biotechnology company Sinovac and got Emergency Use Authorization from the Turkish Medicines and Medical Devices Agency. CoronaVac (Sinovac Life Sciences, Beijing, China) is an inactivated virus vaccine which was one of the earliest to add the COVID-19 vaccine trials series in April 2020 (5). Based on its efficacy and safety results of phase I/2 trials, the vaccine was approved for emergency use in several countries (6).

In our country, the vaccination program has started as of January. However, many people and also doctors had questions about the efficacy and side effects of the vaccine (7). The only way to remove the question marks is to increase knowledge of real-life data and literature. We aimed to contribute to the literature by determining our own experience with this study.

Vaccination had started among healthcare workers in our country in February. We carried out this study at an average of 4 months after vaccination and only 8 people were infected in the hospital after vaccination. One of them was one week after the first vaccination. It was not clear whether this case was a pre-vaccine infection or a post-vaccine infection. These cases with COVID-19 infection after vaccination were followed up and treated in the Infectious Diseases and Clinical Microbiology department of our hospital. They had the disease with mild symptoms. Hospitalization of these patients was not required and COVID-related lung infection was not observed in any of the

patients. Compared to pre-vaccination, this number was very low. Meanwhile, we showed the efficacy. Inactivated SARS-CoV-2 vaccines had a low incidence of adverse reactions compared to other candidate vaccines and this was shown in many studies (8-10). Zhang M-X et al. showed in their study that the inactivated Coronavac vaccine has an acceptable safety profile among healthcare workers because of the low incidence of self-reported adverse reactions. And they documented that the most common adverse reaction was local pain at the injection site, with an incidence of 9.6% after the first dose and 10.7% after the second dose, accounting for 61.8% and 73.0% of adverse reactions, respectively (11). In a study from our country, it was demonstrated that the most common systemic side effect experienced after the Coronavac was fatigue, and the most common local side effect was pain (12). According to the evaluation report of the Food and Health Bureau (FHB) of Hong Kong SAR on Coronavac, the "very common" adverse reactions (≥10%) were injection site pain, headache, and fatigue (13). The results of our study are similar to all these studies. In our study, the most common adverse reaction was local pain in HCW. The systemic side effects that followed were fatigue and joint pain.

One of the significant aspects of our study is to show that Coronavac can be used safely over the age of 64. Urticarial plaques were found in the body of only one patient among those vaccinated over 64 years of age. The patient recovered with symptomatic treatment. No side effect was observed after the second dose of the vaccine. Real-life data was the advantage of our study. In addition, this study revealed very clear data about the effectiveness and safety of the vaccine, because HCW applied to the infectious disease's polyclinic of our hospital when COVID infection occurred instead of other clinics.

Our study has few limitations. One limitation is that it was single-centered. The other limitation is that we could not survey people aged 65 and over. So that, we could not investigate the minor side effects which elderly people do not need to report.

In conclusion, we demonstrated that Coronavac is an effective and well-tolerated vaccine also for elderly people.

**Conflicts of interest:** None.

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RESEARCH ARTICLE

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# The Role of Some Parameters in Diagnosis in the Absence of PCR in the Children with COVID-19

**ABSTRACT**

**Objective:** Infectious diseases cause inflammation in the human body and produce numerical and functional changes in peripheral blood cells. Coronavirus disease-19 (COVID-19) is also an infectious disease diagnosed by the Polymerase Chain Reaction (PCR) test. However, PCR testing may not always be available. The aim of this study is to show the effect of numerical and functional changes in blood parameters on the diagnosis of COVID-19 in children.

**Methods:** In this retrospective study, 296 patients and 286 healthy children were included. Nasopharyngeal swabs were collected. The swabs were analyzed by Real-time PCR. Independent-t/Mann-Whitney-U tests were applied; Receiver Operating Characteristic (ROC) curves and logistic regression modelling were evaluated.

**Results:** Gender and age distributions of the groups were similar ($p>0.05$). There were significant differences between the two groups in terms of white blood cell (WBC) ($p<0.001$), neutrophil ($p<0.001$), thrombocyte ($p<0.001$), lymphocyte ($p<0.001$), mean platelet volume (MPV) ($p=0.009$), lactate dehydrogenase (LDH) ($p=0.003$), C-reactive protein (CRP) ($p<0.001$) and aspartate aminotransferase (AST) ($p=0.002$). It was found in ROC curve analyses, while LDH ($p<0.001$) and CRP ($p<0.001$) values were higher in patients, MPV ($p=0.03$), platelet ($p=0.04$), and neutrophil ($p<0.001$) values of them were lower. The best model in logistic regression was the model that included hemoglobin, neutrophil, lymphocyte, thrombocyte, LDH and CRP.

**Conclusions:** Rapid diagnosis of COVID-19 are crucial for public health. PCR, required for definitive diagnosis, may not always be achieved, so easier and cheaper methods are needed. This study supports the diagnosis of COVID-19 in the children in the absence of PCR.

**Keywords:** COVID-19, Children, Laboratory Parameters, Diagnosis.

# COVID-19'lu Çocuklarda PCR Yokluğunda Tanıda Bazı Laboratuvar Parametrelerinin Rolü

**ÖZET**

**Amaç:** Enfeksiyon hastalıkları vücutta inflamasyon oluşturarak periferik kan hücrelerinde sayısal ve fonksiyonel değişikliklere neden olur. Coronavirüs hastalığı-19 (COVID-19), Polimeraz Zincir Reaksiyonu (PCR) testinin ile teşhis edilen bulaşıcı bir hastalıktır. Ancak PCR testi her zaman temin edilemeyebilir. Bu çalışmanın amacı, çocuklarda kan parametrelerindeki sayısal ve fonksiyonel değişikliklerin PCR olmadığı durumda COVID-19 hızlı teşhisine etkisini değerlendirmektir.

**Gereç ve Yöntem:** Çalışmaya 296 COVID-19 hasta ve 286 sağlıklı çocuk dahil edildi. Bilimsel araştırma için gereken izinler alındı. Değişkenlerin normal dağılımına uygunluğuna göre analizlerde bağımsız-t veya Mann-Whitney-U testleri uygulandı. Receiver Operating Characteristic (ROC) eğrileri ve lojistik regresyon modellemesi değerlendirildi.

**Bulgular:** Grupların cinsiyeti ve yaşı benzerdir ($p>0.05$). Beyaz küre (WBC) ($p<0.001$), nötrofil ($p<0.001$), trombosit ($p<0.001$), lenfosit ($p<0.001$), ortalama trombosit hacmi (MPV) ($p=0.009$), laktat dehidrojenaz (LDH) ($p=0.003$), C-reaktif protein (CRP) ($p<0.001$) ve aspartat aminotransferaz (AST) ($p=0.002$) açısından iki grup arasında istatistiksel olarak anlamlı farklılıklar bulunmaktadır. ROC eğrisine bakıldığında, hastalarda LDH ($p<0.001$) ve CRP ($p<0.001$) daha yüksekken MPV ($p=0.03$), trombosit ($p=0.04$) ve nötrofil ($p<0.001$) sonuçları daha düşüktür. Lojistik regresyonda en iyi model hemoglobin, nötrofil, lenfosit, trombosit, LDH ve CRP'yi içeren modeldi.

**Sonuç:** COVID-19'un PCR yokluğunda hızlı teşhisi halk sağlığı için çok önemlidir. Kesin tanı için gerekli olan Gerçek Zamanlı Polimeraz Zincir Reaksiyonu (RT-PCR) her zaman sağlanamayabilir, bu nedenle daha kolay ve daha ucuz yöntemlere ihtiyaç vardır. Sınırlı sayıda katılımcıya rağmen bu çalışma çocuklarda labaratuvar parametreleri ile Covid-19'da hızlı tanıyı desteklemektedir.

**Anahtar Kelimeler:** COVID-19, Çocuk, Laboratuvar Parametreleri, Tanı.

## INTRODUCTION

Coronavirus disease-19 (COVID-19) has been recognized as a pandemic with the announcement of the World Health Organization (WHO) on March 12, 2020 (1). COVID-19 incidence in young children has increased over time due to not wearing a mask and not following social distance rules (2). Children with COVID-19 positive are thought to be carriers with mild symptoms or asymptomatic; they can play an important role in the spread of the disease (3,4). In the literature, it is emphasized even if most of the children with COVID-19 have mild symptoms or asymptomatic, in a part of them that have also a serious disease can cause morbidity or mortality (5).

In many diseases, peripheral blood cells undergo numerical and functional changes as response to inflammation in the body (6). It is still under investigation whether some parameters which can be easily obtained from peripheral blood counts, such as total white blood cell (WBC), neutrophil (polymorph nuclear leukocytes - PMNL), lymphocyte, platelet, mean platelet volume (MPV), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and C-Reactive Protein (CRP), can quickly detect infectious or inflammatory diseases (7-9).

This study hypothesizes that it can be possible the diagnosis of COVID-19 disease with laboratory parameters in the absence of Polymerase Chain Reaction (PCR). Accordingly, this study aims to reveal effect of COVID-19 disease on the blood parameters, to evaluate the blood parameters of children and adolescents, to provide outcomes that can help diagnose quickly, when PCR test cannot be performed.

## MATERIAL AND METHODS

**Design of the Study:** This study was planned as a retrospective descriptive study. All patients under the age of 18 (296 participants) who applied to the hospital in Şanlıurfa between September 1 and November 30, 2020 and were positive for the COVID-19 PCR test were included in the patient population group of the study. The control group consisted of 286 healthy children who applied to the general pediatric clinic for control evaluation between the same dates, without any diagnosis/signs of infection and negative COVID-19 PCR test. The participants in both groups were matched as much as possible in terms of age and gender; otherwords, the age and gender distribution of the participants in both groups was similar (Table 1).

**Table 1.** Patient and control groups' socio-demographic variables

| Socio-demographic characteristics | | Patient | | Control | | P value |
|---|---|---|---|---|---|---|
| | | n | % | n | % | |
| **Sex** | Girl | 141 | 47.6 | 137 | 47.9 | 0.95 |
| | Boy | 155 | 52.4 | 149 | 52.1 | |
| | **Total** | **296** | **100.0** | **286** | **100.0** | |
| **Age** | 0 | 26 | 8.8 | 20 | 7.0 | |
| | 1 | 9 | 3.1 | 9 | 3.1 | |
| | 2 | 14 | 4.7 | 15 | 5.2 | |
| | 3 | 16 | 5.4 | 14 | 4.9 | |
| | 4 | 11 | 3.7 | 10 | 3.5 | |
| | 5 | 11 | 3.7 | 11 | 3.8 | |
| | 6 | 10 | 3.4 | 14 | 4.9 | |
| | 7 | 12 | 4.1 | 11 | 3.8 | |
| | 8 | 18 | 6.1 | 18 | 6.3 | |
| | 9 | 9 | 3.1 | 8 | 2.8 | 0.82 |
| | 10 | 24 | 8.1 | 21 | 7.3 | |
| | 11 | 12 | 4.1 | 12 | 4.2 | |
| | 12 | 15 | 5.1 | 15 | 5.2 | |
| | 13 | 17 | 5.8 | 17 | 5.9 | |
| | 14 | 17 | 5.8 | 17 | 5.9 | |
| | 15 | 18 | 6.1 | 18 | 6.3 | |
| | 16 | 25 | 8.5 | 25 | 8.7 | |
| | 17 | 31 | 10.5 | 31 | 10.8 | |
| | **Total** | **295** | **100.0** | **286** | **100.0** | |

### Sampling

**Inclusion Criteria:** The patients, who admitted to the hospital due to clinical symptoms similar to COVID-19, were younger than 18 years of age and had confirmed COVID-19 infection with RT-PCR, were included in the patient group.

**Exclusion Criteria:** The persons were excluded from the study if they had negative PCR test or had the Multisystem Inflammatory Syndrome in Childhood (MISC), chronic lung disease, diabetes mellitus, congenital heart disease, malignancy, immunodeficiency or recent traumatic history.

**RT-PCR Test and Analyses:** The nasopharyngeal swab samples were collected from the individuals with suspected COVID-19 infection. The samples were analyzed on the Real-time PCR device after nucleic acids were isolated, thus the samples which were positive for targeted DNA fragments were accepted as positive. Before of the treatments, the blood samples were taken from each participant. Complete blood counts were examined with an automatic blood counter (Abbott Celldyn-3500, IL, USA). Also, for some biochemical parameters, such as AST, ALT, LDH, and CRP, 2 cc venous blood samples were taken from each patient and the levels of the biochemical parameters were measured using a spectrophotometric chemical analyzer (Architect-C16000, Abbott Diagnostics, Abbott Park, IL, USA). There was not any sources of funding for analyzes in this research.

**Ethics Declarations:** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The scientific research permit from the Ministry of Health and the ethics committee approval from the Ethics Committee of Harran University were taken (Date: August 31, 2020, /No.: HRU/20.15.27).

**Statistical Analysis:** The data were analyzed using SPSS.v25. The normality of the variables was evaluated using Kruskal-Wallis and Shapiro-Wilk tests, histogram, scatter plot, and Skewness-Kurtosis values. In comparisons between the patient and control groups, the Independent t-test and the Mann-Whitney U test were used. Pearson and Fisher Chi-Square tests were used to compare the categorical data. ROC curve was used and the model was made with logistic regression modelling. P<0.05 was accepted significant.

## RESULTS

There is no significant difference between control and patient groups in terms of gender and age (p>0.05) (Table 1). There is a significant difference between the patient and control groups for the WBC (p<0.001), neutrophil (p<0.001), platelet count (p<0.001), MPV (p=0.009), LDH (p=0.003), lymphocyte count (p<0.001), CRP (p<0.001), and AST (p=0.002) values (Table 2).

**Table 2.** Patient and control groups' some laboratory values

| Variables* | Group | n | Mean† | SD† | SE† | T test† | | p value |
|---|---|---|---|---|---|---|---|---|
| | | | | | | df† | t† | |
| **Hemoglobin** | **Patient** | 296 | 12.7341 | 1.36901 | 0.07957 | 579 | 0.898 | 0.369 |
| | **Control** | 285 | 12.5921 | 2.33505 | 0.13832 | 455.101 | 0.890 | 0.374 |
| **WBC*** | **Patient** | 296 | 6.4219 | 2.48329 | 0.14434 | 580 | -5.746 | **<0.001** |
| | **Control** | 286 | 7.5905 | 2.42068 | 0.14314 | 579.954 | -5.749 | **<0.001** |
| **PMNL*** | **Patient** | 296 | 2.7815 | 1.56230 | 0.09081 | 580 | -6.361 | **<0.001** |
| | **Control** | 286 | 3.6084 | 1.57314 | 0.09302 | 579.010 | -6.360 | **<0.001** |
| **Thrombocytes** | **Patient** | 296 | 277.1115 | 79.02935 | 4.59349 | 579 | -5.230 | **<0.001** |
| | **Control** | 285 | 318.9344 | 111.55767 | 6.60811 | 510.108 | -5.197 | **<0.001** |
| **MPV*** | **Patient** | 273 | 10.0886 | 1.00373 | 0.06075 | 549 | 2.636 | **0.01** |
| | **Control** | 278 | 9.7850 | 1.62248 | 0.09731 | 463.316 | 2.647 | **0.01** |
| **LDH*** | **Patient** | 212 | 276.6651 | 83.01034 | 5.70117 | 251 | 2.958 | **0.003** |
| | **Control** | 41 | 235.0488 | 79.44179 | 12.4067 | 58.185 | 3.048 | **0.003** |

| Variables | | n | MR‡ | SoR‡ | U‡ | Z‡ | p value |
|---|---|---|---|---|---|---|---|
| **Lymphocyte** | **Patient** | 295 | 261.30 | 77084.00 | 33424.000 | -4.269 | **<0.001** |
| | **Control** | 285 | 320.72 | 91406.00 | | | |
| **CRP*** | **Patient** | 290 | 253.69 | 73569.00 | 15606.000 | 5.949 | **<0.001** |
| | **Control** | 162 | 177.83 | 28809.00 | | | |
| **AST*** | **Patient** | 293 | 238.53 | 69888.00 | 18598.000 | 3.153 | **0.002** |
| | **Control** | 155 | 197.99 | 30688.00 | | | |

* WBC: White Blood Cell; PMNL: Polymorphonuclear leukocytes; MPV: Mean Platelet Volume; LDH: Lactate Dehydrogenase; CRP: C-Reactive Protein; AST: Aspartate Aminotransferase; †SD: Std. deviation; SE: Std. error; df: Degree of freedom; t: Independent Sample T – Test
‡ MR: Mean rank; SoR: Sum of ranks; U:Mann Whitney U Test; Z:Z score

In ROC curve analysis, it is showed that LDH (p<0.001) and CRP (p<0.001) values are higher in the patient group while MPV (p=0.03), platelet (p=0.04), and neutrophil (p<0.001) are lower in the patient group. The values that have highest area under the curve are respectively CRP (0.741) and LDH (0.704) (Table 3). The ROC curves of the variables can be seen in Figure.

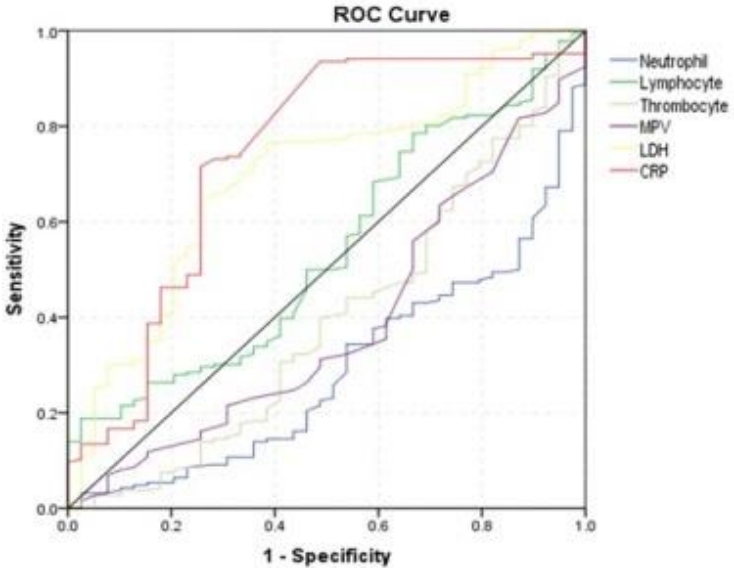


Figure 1. ROC curve for some laboratory parameters

**Table 3.** ROC curve of some laboratory values of patient and control groups

| | Diagnostic scan | | ROC curve | | | |
|---|---|---|---|---|---|---|
| **Variable** | **Cut off** | **Sensitivity** | **Specificity** | **Area** | **95% confidence interval** | **p value** |
| **Neutrophil** | **2.94** | 0.39 | 0.39 | 0.291 | 0.208-0.374 | **<0.001** |
| **Lymphocyte** | **2.17** | 0.51 | 0.51 | 0.544 | 0.449-0.639 | 0.443 |
| **Thrombocyte** | **285500** | 0.44 | 0.46 | 0.393 | 0.291-0.496 | **0.037** |
| **MPV** | **10.05** | 0.37 | 0.39 | 0.390 | 0.293-0.487 | **0.026** |
| **LDH** | **239.5** | 0.69 | 0.69 | 0.704 | 0.614-0.793 | **<0.001** |
| **CRP** | **0.74** | 0.74 | 0.74 | 0.741 | 0.644-0.838 | **<0.001** |

First logistic regression models has be analyzed by including hemoglobin, PMNL, lymphocyte count, and platelet count. At second model, the MPV has be added to the model. In the third model, the regression analysis has included hemoglobin, PMNL, lymphocyte count, and platelet count, LDH and CRP. This last model is the most revealing of the models made (Table 4).

**Table 4.** Logistic regression models

| | | | | | | | **95% CI†** | |
|---|---|---|---|---|---|---|---|---|
| **Variables** | **B†** | **SE †** | **Wald** | **df†** | **p value** | **Exp(B)** | **Lower** | **Upper** |
| **Model 1** | | | | | | | | |
| **Hemoglobin** | 0.035 | 0.046 | 0.565 | 1 | 0.45 | 0.966 | 0.882 | 1.058 |
| **Neutrophil** | -0.307 | 0.062 | 24.811 | 1 | **<0.001** | 1.360 | 1.205 | 1.535 |
| **Lymphocyte** | 0.003 | 0.057 | 0.003 | 1 | 0.96 | 0.997 | 0.891 | 1.115 |
| **Thrombocyte** | -0.004 | 0.001 | 11.759 | 1 | **0.001** | 1.004 | 1.002 | 1.006 |
| **Constant** | 1.627 | 0.695 | 5.475 | 1 | 0.02 | 0.197 | | |
| **Model 2** | | | | | | | | |
| **Hemoglobin** | 0.019 | 0.048 | 0.153 | 1 | 0.70 | 0.981 | 0.893 | 1.079 |
| **Neutrophil** | -0.290 | 0.063 | 21.056 | 1 | **<0.001** | 1.337 | 1.181 | 1.514 |
| **Lymphocyte** | 0.005 | 0.059 | 0.006 | 1 | 0.94 | 0.995 | 0.886 | 1.118 |
| **Thrombocyte** | -0.004 | 0.001 | 10.359 | 1 | **0.001** | 1.004 | 1.001 | 1.006 |
| **MPV** | 0.109 | 0.071 | 2.367 | 1 | 0.12 | 0.897 | 0.780 | 1.030 |
| **Constant** | 0.647 | 1.062 | 0.371 | 1 | 0.54 | 0.524 | | |
| **Model 3** | | | | | | | | |
| **Hemoglobin** | 0.141 | 0.124 | 1.292 | 1 | 0.26 | 0.869 | 0.681 | 1.107 |
| **Neutrophil** | -0.351 | 0.123 | 8.106 | 1 | **0.004** | 1.420 | 1.115 | 1.807 |
| **Lymphocyte** | 0.429 | 0.207 | 4.295 | 1 | **0.04** | 0.651 | 0.434 | 0.977 |
| **Thrombocyte** | -0.005 | 0.003 | 4.174 | 1 | **0.04** | 1.005 | 1.000 | 1.010 |
| **CRP** | 0.555 | 0.223 | 6.175 | 1 | **0.01** | 0.574 | 0.371 | 0.889 |
| **LDH** | 0.006 | 0.003 | 3.904 | 1 | **0.048** | 0.994 | 0.989 | 1.000 |
| **Constant** | -0.675 | 1.938 | 0.122 | 1 | 0.72 | 1.965 | | |

* MPV: Mean Platelet Volume; LDH: Lactate Dehydrogenase; CRP: C-Reactive Protein
†B: beta; SE: Std. Error; df: Degree of freedom; Cl: Confidence interval

## DISCUSSION

COVID-19 disease progresses with mild symptoms or asymptomatic in children (3, 4). Due to the high risk of transmission of the virus, it is important to diagnose the disease early and rapidly. Some blood parameters, which are fast and cheaply obtainable, could be useful in the diagnosis of COVID-19. In this study, the role of widely accessible, fast and cheap blood parameters, such as WBC, PMNL, platelet and lymphocyte counts, MPV, ALT, AST, CRP and LDH, have be evaluated in the absence of PCR in the diagnosis of COVID-19 patients.

The literature show that COVID-19 patients have different laboratory results (10-16). Shen et al. (10) have found that their leukocyte count was normal or low while their lymphocyte count was low. Chen et al. (11) also have found that lymphocyte count was significantly low in the patients with COVID-19; moreover, they found that the WBC, PMNL and platelet count were low, but it was not statistically significant. Liu et al. (12) have showed a significant decrease in lymphocyte count in adult patients with Covid-19; it is inversely proportional to the viral load in the respiratory tract and the severity of the disease. In our study, while it is not detected a significant difference in hemoglobin value between patient and control groups, WBC, neutrophil, thrombocyte, lymphocyte values are in patients less than controls. These findings suggest that although COVID-19 infection is a viral infection, it can cause different outcomes in blood parameters compared to many infections.

A similar situation exists for biochemical parameters, also. In our study, it is seen MPV, LDH, CRP, and AST values are in patients higher than the control group, significant. However, Xia et al. (13) have reported ALT value increased in 25% of the patients and CRP value increased in 45% of the cases. Zhang et al. (14) have determined while the CRP value of 59% of the cases and LDH of 82% of the cases had an increase, there is no significant difference in ALT. Cai et al. (15) have stated CRP of 30% of the cases, ALT of 10% of them, AST of 20% of them, LDH value of 30% of them are increasing. Wang et al. (16) have determined while CRP increased in 9.7% of the cases, it decreased by 3.2% of them. They have reported ALT increased in 22% of the patients and AST increased in 22% of the cases. So, it should be kept in mind that different results may be seen in different patient groups before making a quick and erroneous decision while evaluating the disease.

In this study, While ROC Curve analysis of lymphocyte count did not give significant results, the area under the curve of the MPV was below 0.40, and it was found that CRP (0.74) and LDH (0.70) have highest area under the curve (Table 3; Figure). While Gumus et al. (17) stated that there was a difference in the MPV and lymphocyte count values between the patient and control groups, Seyit et al. (18) showed that the sensitivity of LDH and CRP was 60% and 66%, respectively, and similar to the results of our study, they found that there was no difference between the patient and control groups in the MPV. Due to the different results in the literature, regression modeling was performed to include more than one parameter in this study (Table 4). The model to which LDH and CRP parameters were added was found to be more explanatory compared to the model with basic CBC parameters. In the literature, it is underlined, LDH levels in COVID-19 patients are seen high and these results are associated severe disease, infection-related lung damage, and hospitalization as it plays a role in the energy mechanism in all cells is an important enzyme (19-21). Especially, owing to tissue damage in lung diseases, such as pulmonary fibrosis, LDH levels increase, and these results are accepted that support to viral infection (21). In a study, in which mortality modelling of COVID-19 infection is made, increase of LDH level is evaluated that associates with COVID-19 mortality (22). So, serum LDH may be a biomarker to detect patients have high risk.

Some results are also reported for CRP; it is emphasized that high CRP level may be important in grading the severity of the disease (18). Particularly, in the literature, it is mentioned CRP correlates lung damage and pneumonia in the early stage of the disease (23) and it is higher with mild versus severe disease (24). While a meta-analysis's results, it is seen that levels of white blood cell count elevated in this patients, another meta-analysis's results showed that while lymphocytes were decreased, inflammation markers were increased in COVID-19 infection (23, 24). On account of, in light of all these data, it is considered that in the absence of PCR, the evaluation of some laboratory parameters, such as neutrophil, lymphocyte and thrombocyte, primarily CRP and LDH, may be quite useful in the diagnosis of COVID-19 and early isolation, and early initiation of treatment.

This study has some limitations, also. Although it is tried to match the patient and control groups as much as possible, as in all case-control studies, it has not be possible to match them completely. On the other hand, it is seen that the evaluation of the laboratory findings, as well as the clinical picture, increases the chance of diagnosis.

## CONCLUSION

Consequently, the diagnosis of the disease in the absence of PCR, and treatment and isolation of COVID-19 patients are very important for the protection of both the individual and the public health. This study and other similar studies can provide valuable information that can contribute to diagnosis and intervention in some countries, which cannot perform the PCR or pediatric patients before receiving RT-PCR test results of patients with suspected COVID-19.

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RESEARCH ARTICLE

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# Initial Signs and Symptoms in Suspected Patients Admitted to Triage Outpatient Clinic of Coronavirus Disease 2019 (COVID-19): A Single-Center Experience

**ABSTRACT**

**Objective:** Coronavirus disease (COVID-19) is a worldwide pandemic with a huge burden of illness, high economic costs, and mortality rates. This study sought to compare clinical signs and symptoms among adult COVID-19 patients admitted to triage outpatient clinics.

**Methods:** In this observational retrospective study, clinical symptoms, biochemical parameters, and chest computed tomography (CT) of 1745 suspected patients admitted to COVID-19 triage outpatient clinic between 01 April 2020-01 September 2020 were analyzed.

**Results:** A total of 650 (37.2%) of 1745 patients who were admitted to triage outpatient clinic were diagnosed as COVID-19 by PCR confirmation. Of the participants, 88.1% had at least one symptom, 11.9% were asymptomatic. Almost half of the patients (50.1%) had a history of exposure including contact with COVİD-19 confirmed cases. COVID-19 (+) patients were more diabetic and hypertensive than negative cases. In patients who underwent chest CT imaging, 40.6% (310) had pneumonic infiltrations compatible with COVID-19 pneumonia. Hemoglobin, leukocyte, neutrophil, lymphocyte, and platelet counts were lower, but CRP levels were higher in patients with COVID-19. Multivariate logistic regression analysis revealed that older age (OR=1.020; p=0.018), contact with confirmed COVID-19 patient (OR=1.907, p=0.009), fever (OR=1,588, p=0,001), fatigue (OR=2.075, p=0.009), cough (OR= 2.301, p<0.001) were significantly associated with increased odds of PCR (+) status.

**Conclusions:** Predictive factors associated with PCR (+) test results were older age, history of contact with confirmed COVID-19 patient, high fever, fatigue, cough in our study. Some symptoms could have a significant relationship with PCR positivity, which requires a more careful approach during the first admission to healthcare facilities.

**Keywords:** COVID-19 Virus, Polymerase Chain Reaction, Signs and Symptoms.

# Koronavirüs Hastalığı 2019 (COVID-19) Triyaj Polikliniğine Başvuran Şüpheli Hastalarda Başlangıç Belirti ve Semptomları: Tek Merkez Deneyimi

**ÖZET**

**Amaç:** Koronavirüs hastalığı (COVID-19) Dünya çapında yaygın olarak yüksek ekonomik yük ve ölüm olanlarıyla pandemiye neden olmuştur. Bu çalışma, triyaj polikliniğine başvuran yetişkin COVID-19 hastalarında görülen klinik belirti ve semptomlar arasındaki ilişkiyi araştırmayı amaçlamaktadır.

**Gereç ve Yöntem:** Bu gözlemsel retrospektif çalışmada, 01 Nisan 2020-01 Eylül 2020 tarihleri arasında COVID-19 triyaj polikliniğine başvuran 1745 şüpheli hastanın klinik semptomları, biyokimyasal parametreleri ve akciğer tomografisi (BT) bulguları analiz edilmiştir.

**Bulgular:** Triyaj polikliniğine başvuran toplam 1745 hastanın 650'sine (%37,2) PCR testi ile COVID-19 tanısı konuldu. Katılımcıların %88,1'i en az bir semptoma sahipti, %11,9'u ise asemptomatikti. Hastaların neredeyse yarısında (%50,1) PCR test sonucu pozitif olan COVİD-19 vakalarıyla temas öyküsü vardı. COVID-19 (+) hastalar, negatif vakalara göre daha yüksek oranda diyabet ve hipertansiyon hastasıydı. Akciğer BT görüntülemesi yapılan hastaların %40,6'sında COVID-19 pnömonisi ile uyumlu pnömonik infiltrasyonlar vardı. COVID-19 hastalarında hemoglobin, lökosit, nötrofil, lenfosit ve trombosit sayıları daha düşüktü, ancak CRP seviyeleri daha yüksekti. Çok değişkenli lojistik regresyon analizi sonucuna göre, ileri yaş (OR=1.020; p=0.018), PCR (+) COVID-19 hastasıyla temas (OR=1.907, p=0.009), ateş (OR=1.588, p=0.001), yorgunluk (OR= 2.075, p=0.009), öksürük (OR= 2.301, p<0.001) ile PCR (+) olma arasında pozitif yönde anlamlı ilişki tespit edilmiştir.

**Sonuç:** Çalışmamızda PCR (+) test sonuçları ile ilişkili faktörler ileri yaş, COVID-19 (+) hastayla temas öyküsü, yüksek ateş, yorgunluk, öksürük olarak tespit edilmiştir. COVID-19 şüphesi olan hastalar farklı semptomlarla sağlık kuruluşuna başvursa da, bazı semptomların PCR pozitifliği ile anlamlı ilişkisi vardır, bu da sağlık kuruluşlarına ilk başvuru sırasında daha dikkatli bir yaklaşım gerektirir.

**Anahtar Kelimeler:** COVID-19 Virüsü, Polimeraz Zincir Reaksiyonu, Belirti ve Semptomlar.

## INTRODUCTION

The new coronavirus (2019-nCoV) disease, first identified in Wuhan, China in December 2019, was declared a pandemic disease (COVID-19) by the World Health Organization (WHO) as of March 11, 2020. The WHO defined COVID-19 as a public health problem that required urgent action around the world (1). The first case of COVID-19 in Turkey was reported on March 11, 2020, and the first death due to COVID-19 was reported on March 18, 2020 (2). COVID-19 has become an important public health problem, and recognizing the disease has gained importance in Turkey and all around the world with increased case and death numbers (3).

The range of symptoms of COVID-19 cases varied from asymptomatic to severe respiratory symptoms, extrapulmonary symptoms, and even death. The first step in recognizing the disease was detecting its associated vital signs and symptoms (4). The most frequently reported signs and symptoms at the onset of COVID-19 include fever (77-98%), cough (46-82%), myalgia or fatigue (11-52%), and dyspnea (3-31%). Other less reported symptoms are sore throat, headache, sputum, loss of taste, loss of smell, diarrhea, and cough, etc (5). Although the clinical characteristics of COVID-19 patients have been examined, many features still are not fully revealed (6). The non-specificity of the symptoms detected in the studies and the variability in their incidence are remarkable. Some patients continue to present with atypical symptoms and clinical course (7).

Early diagnosis regarding baseline symptoms may ease to control of the pandemic spread and decrease morbidity and mortality rates. Preventing the dissemination has an essential role particularly in areas that have limited access to real-time reverse-transcription polymerase chain reaction (PCR) analysis (8). Chest computed tomography (CT) is highly diagnostic to demonstrate COVID-19 specific images in suspected patients. But, changes based on severe lung abnormalities caused by COVID-19 infection are distinct on chest CT images nearly 10 days after the first clinical characteristics (9). These restrictions in diagnosing increase the importance of initial symptoms to suspect from COVID-19 cases in outpatient clinics (10).

The present study sought to determine the initial symptoms of patients who applied to the Coronavirus (COVID-19) triage outpatient clinic. Besides, the relationship of the symptoms with laboratory results and imaging methods was examined.

## MATERIAL AND METHODS

**Study Design and Participants:** This retrospective study focused on the initial clinical features of patients with suspected COVID-19 who presented to the Gulhane Training and Research Hospital Coronavirus (COVID-19) triage outpatient clinic. The data of 1745 patients who applied to the triage outpatient clinic between 01.04.2020 and 01.09.2020 were analyzed.

Patient files were scanned through the hospital database system and the necessary data were transferred to the data collection form. Patients who were thought to be suspected of COVID-19 and requested Reverse Transcription Polymerase Chain Reaction (RT-PCR) test regarding the initial evaluation were included in the study. The symptoms, examination findings, laboratory results, and CT results of the patients were obtained from the hospital database system. The relationship between symptoms and laboratory-imaging outcomes was investigated using appropriate statistical tests. Patients who were evaluated in the COVID-19 triage outpatient clinic and clinically likely to be Covid disease were included in the study. Patients under the age of 18 were not included in the study.

**Ethical Approval:** Written approval was obtained from the Health Sciences University Non-Invasive Ethics Committee for conducting the study. The study was started after obtaining administrative permission from Health Sciences University Gulhane Health Research and Application Center Medical Specialty Ethics Committee.

**Statistical Analysis:** Statistical evaluations were performed by running the SPSS (version 22.0; SPSS, INC., Chicago, IL, USA). The normality of distribution of continuous variables was evaluated using the Kolmogorov-Smirnov test, histogram, and Shapiro-Wilk test. Descriptive statistics were expressed as mean ± standard deviation or median (minimum-maximum) for continuous variables and as the number of cases and percentage for categorical variables. Quantitative data were evaluated using a Student t-test or the Mann-Whitney U test, as appropriate. Comparison of categorical variables was performed using the chi-square test. p-value $<0.05$ was considered statistically significant. logistic regression was used to assess the effect of signs and symptoms affecting PCR (+) status for multivariate analysis,

## RESULTS

In total, 1745 patients with the suspected COVID-19 were included in the study. A total of 650 (37.2%) of 1745 patients who were admitted to the triage outpatient clinic were diagnosed as COVID-19 by PCR confirmation. Of the patients, mean age was 42.19±16.17 (17-93, median=40), and 50.8% (887) was male. The median age for COVID-19 (+) patients was 43 (18-92) years, and 38 (17-93) years for COVID-19 (-) cases ($p<0.001$) (Table 1). Of the participants, 88.1% had at least one symptom, 11.9% were asymptomatic. Almost half of the patients (50.1%) had a history of exposure including contact with COVID-19 confirmed cases. Hypertension and diabetes

mellitus were the most common chronic diseases (respectively, 41.1%, 30.4%). COVID-19 (+) patients were more diabetic and hypertensive than negative cases (respectively, 37.0% vs. 25.8%, p=0.006; 47.2% vs. 36.8%, p=0.017). The distribution of the demographic characteristics of the participants who were admitted to the triage outpatient clinic is given in Table 1.

**Table 1.** Comparison of demographic characteristics of the patients who were admitted to triage outpatient clinic (n=1745)

| **Characteristics** | **All suspected patients** | **PCR-confirmed COVID-19 (-)** | **PCR-confirmed COVID-19 (+)** | **p*** |
|---|---|---|---|---|
| | **Median (min-max)** | **Median (min-max)** | **Median (min-max)** | |
| **Age (**years) | 40 (17-93) | 38 (17-93) | 43(18-92) | **<0.001¥** |
| | **n (%)** | **n (%)** | **n (%)** | |
| **Age groups (**years) | | | | **<0.001** |
| <29 | 23.5 (402) | 26.8 (289) | 17.8 (113) | |
| 29-39 | 25.5 (436) | 26.6 (287) | 23.5 (149) | |
| 40-51 | 24.8 (424) | 24.6 (265) | 25.0 (159) | |
| ≥52 | 26.3 (450) | 21.9 (236) | 33.7 (214) | |
| **Gender** | | | | 0.068 |
| Male | 50.8 (887) | 52.5 (575) | 48.0 (312) | |
| Female | 49.2 (858) | 47.5 (520) | 52.0 (338) | |
| **Contact with a COVID-19 patient** | 50.1 (875) | 49.2 (539) | 51.7 (336) | 0.319 |
| **Smoking** | 33.2 (579) | 32.7 (358) | 34.0 (221) | 0.575 |
| **Comorbid diseases** (n=526) | 30.1 (526) | 28.3 (310) | 33.2 (216) | **0.030** |
| DM | 30.4 (160) | 25.8 (80) | 37.0 (80) | **0.006** |
| HT | 41.1 (216) | 36.8 (114) | 47.2 (102) | **0.017** |
| Asthma/COPD | 22.8 (120) | 25.2 (78) | 19.4 (42) | 0.124 |
| CVD | 23.4 (123) | 22.3 (69) | 25.0 (54) | 0.465 |
| Malignancies | 5.5 (29) | 6.1 (19) | 4.6 (10) | 0.459 |

*Chi-square test. ¥ Mann Whitney-U test; *DM, Diabetes mellitus; HT, Hypertension; COPD, Chronic Obstructive Pulmonary Disease; CVD, Cardiovascular disease; PCR, Polymerase Chain Reaction.*

Based on patient's medical history and clinical examination, complaints including acute cough (57.6% vs. 48.7%, p=0.001), myalgia (47.6% vs. 41.5%, p=0.021), and fatigue (28.3% vs. 21.1%, p=0.001) were more common in COVID-19 patients, while sore throat (43.6% vs 34.0%, p=0.000) and diarrhea (22.1% vs. 14.9%, p=0.001) were more common in COVID-19 (-) patients. Fever (≥38 C) was higher (35.3% vs. 20.4%, p<0.000) and $O_2$ saturation was lower (97.59±2.331 vs. 98.07±2.122) in patients with COVID (+). The median number of days between initial symptom and outpatient clinic presentation was longer in diagnosed patients than undiagnosed patients (3 vs. 2 days, p=0.007). Other comparisons of symptoms of the PCR-confirmed COVID-19 (+) and COVID-19 (-) cases were depicted in Table 2.

**Table 2.** Comparison of symptoms of the PCR-confirmed COVID-19 (+) and COVID-19 (-) cases (n=1537)

| **Symptoms (%-n)** | **All suspected patients** | **PCR-confirmed COVID-19 (-)** | **PCR-confirmed COVID-19 (+)** | **p*** |
|---|---|---|---|---|
| | **n (%)** | **n (%)** | **n (%)** | |
| **Headache** | 35.9 (552) | 35.9 (345) | 35.9 (207) | 0.988 |
| **Fatigue** | 23.8 (366) | 21.1 (203) | 28.3 (163) | **0.001** |
| **Chill** | 19.1 (293) | 16.1 (155) | 24.0 (138) | **<0.001** |
| **Sore throat** | 40.0 (615) | 43.6 (419) | 34.0 (196) | **<0.001** |
| **Acute Cough** | 52.0 (799) | 48.7 (468) | 57.6 (331) | **0.001** |
| **Dyspnea** | 27.1 (416) | 27.2 (261) | 26.9 (155) | 0.915 |
| **Myalgia** | 43.8 (673) | 41.5 (399) | 47.6 (274) | **0.021** |
| **Loss of taste** | 15.8 (243) | 13.6 (131) | 19.4 (112) | **0.002** |
| **Loss of smell** | 13.5 (208) | 12.0 (115) | 16.1 (93) | **0.020** |
| **Diarrhea** | 19.4 (298) | 22.1 (212) | 14.9 (86) | **0.001** |
| **Rhinore** | 5.1 (78) | 5.0 (48) | 5.2 (30) | 0.854 |
| **Low-Back pain** | 4.0 (61) | 4.4 (42) | 3.3 (19) | 0.297 |
| **Chest pain** | 4.0 (61) | 4.0 (38) | 4.0 (23) | 0.970 |
| **Nausea** | 7.0 (108) | 7.2 (69) | 6.8 (39) | 0.761 |
| **Vomitting** | 3.5 (54) | 3.7 (36) | 3.1 (18) | 0.522 |
| **Sweating** | 2.0 (30) | 1.6 (15) | 2.6 (15) | 0.152 |
| **Other Symptoms** | 3.9 (60) | 3.7 (36) | 4.2 (24) | 0.680 |
| **Fever (≥38 C)** | 26.0 (363) | 20.4 (179) | 35.3 (184) | **<0.001** |
| | **Mean-SD** | **Mean-SD** | **Mean-SD** | |
| **Saturation $O_2$** | 97.89±2.21 | 98.07±2.12 | 97.59±2.33 | **<0.001**** |
| | **Median (min-max)** | **Median (min-max)** | **Median (min-max)** | |
| **Symptom Duration (day)** | 2.00 (1-30) | 2.00 (1-30) | 3.00 (1-10) | **0.007***** |

*Chi-square test. **Student t-test. ***Mann Whitney-U test

A chest CT was performed in 763 (43.7%) patients. In patients who underwent chest CT imaging, 40.6% (310) had pneumonic infiltrations compatible with COVID-19 pneumonia. COVID-19 (+) patients had higher rates of chest CT (+) images than undiagnosed cases (49.9% vs. 31.6%; p<0.001) (Figure 1).

In cases with Chest CT, 31.6% (122) of the cases with PCR (-) had pneumonia compatible with COVID-19.

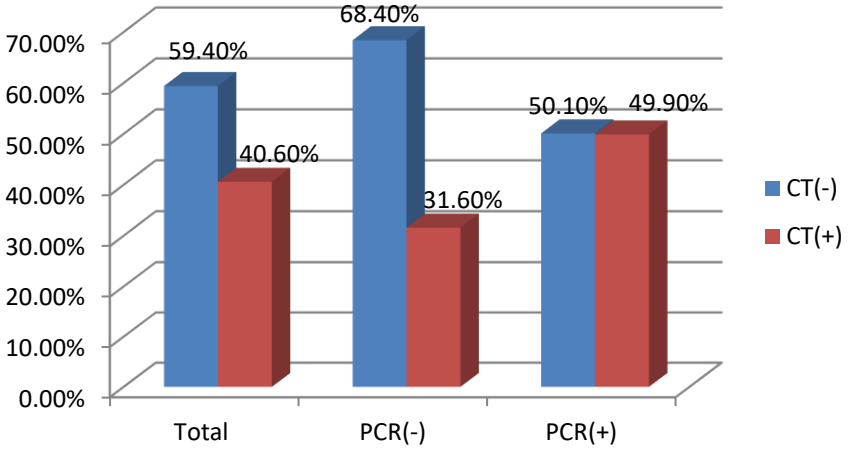


**Figure 1**. Distribution of CT (+) /CT (-) patients based on PCR results

Hemoglobin (13.9 vs. 14.2, p=0.010), leukocyte (5.40 vs. 6.90, p<0.001), neutrophil (3.10 vs. 4.10, p<0,001), lymphocyte (1.40 vs. 1.90, p<0.001), and platelet (202.50 vs. 233.00, p<0.001) counts were lower, but CRP levels were higher (15.00 vs. 9.05, p=0.030) in patients with COVID-19. Other laboratory parameters during admission were similar in the two groups (Table 3).

**Table 3.** Comparison of laboratory findings of the PCR-confirmed COVID-19 (+) and COVID-19 (-) cases (n=1167)

| Parameters | All suspected patients | PCR-confirmed COVID-19 (-) | PCR-confirmed COVID-19 (+) | p* |
|---|---|---|---|---|
| | Median (min-max) | Median (min-max) | Median (min-max) | |
| **WBC** | 6.100(0.600-25.700) | 6.900 (0.6-25.700) | 5.400 (0.8-16.000) | **<0.001** |
| **RBC** | 4.8(1.2-50.2) | 4.9(1.2-50.2) | 4.78 (2.20-12.70) | **0.028** |
| **HB** | 14.1(2.9-24.3) | 14.2 (7.4-22.9) | 13.9(2.9-24.3) | **0.010** |
| **PLT** | 219.00(67.00-1401.40) | 233.00 (72.00-1401.40) | 202.500 (67.00-539.00) | **<0.001** |
| **LYMP** | 1.60(0.10-157.00) | 1.90 (0.20-5.80) | 1.400(0.10-157.00) | **<0.001** |
| **NEUT** | 3.600(0.1-21.6) | 4.100(0.1-21.6) | 3.100(0.4-14.4) | **<0.001** |
| **CRP (n=487)** | 10.2(0.2-342.0) | 9.050(0.2-326.0 | 15.00(0.2-342) | **0.030** |
| **Troponin(n=121)** | 3.4(0.3-1719) | 3.10(0.6-658.0) | 3.45 (0.30-1719) | 0.429 |
| **Ferritin(n=391)** | 78.8(2.3-1500) | 89.0(2.3-593.0) | 75.5(2.60-1500) | 0.230 |
| **D-dimer (n=430)** | 0.39 (0.1-15.7) | 0.39(0.10-14.70) | 0.39(0.10-15.70) | 0.125 |

*: Mann Whitney-U test

Variables with statistical significance based on the univariate analysis were subjected to multivariate logistic regression analysis. Based on a multivariate logistic regression analysis, age (OR=1.020; p=0.018), contact with confirmed COVID-19 patient (OR=1.907, p=0.009), fever at application (OR=1,588, p=0,001), fatigue (OR=2.075, p=0.009), cough (OR= 2.301, p<0.001) remained significantly associated with increased odds of PCR (+) status (Table 4).

**Table 4.** Logistic regression analysis of symptoms and signs affecting PCR (+) status

| Variables | B | p | ORs | 95% C.I.(Lower-Upper) |
|---|---|---|---|---|
| **Age (years)** | 0.020 | **0.018** | 1.020 | 1.003-1.037 |
| **Gender** | -0.215 | 0.343 | 0.807 | 0.517-1.258 |
| **Comorbid diseases** | -0.175 | 0.522 | 0.839 | 0.491-1.435 |
| **Fatigue** | 0.730 | **0.009** | 2.075 | 1.197-3.600 |
| **Chill** | 0.275 | 0.328 | 1.316 | 0.759-2.280 |
| **Sore throat** | -0.305 | 0.187 | 0.737 | 0.469-1.159 |
| **Cough** | 0.833 | **<0.001** | 2.301 | 1.452-3.646 |
| **Myalgia** | 0.345 | 0.137 | 1.412 | 0.896-2.224 |
| **Loss of taste** | 0.806 | 0.088 | 2.239 | 0.886-5.662 |
| **Loss of smell** | -0.483 | 0.354 | 0.617 | 0.222-1.713 |
| **Diarrhea** | -0.243 | 0.399 | 0.784 | 0.445-1.381 |
| **Saturation $O_2$** | -0.021 | 0.741 | 0.979 | 0.864-1.110 |
| **Duration (day)** | 0.002 | 0.948 | 1.002 | 0.932-1.078 |
| **Fever (C)** | 0.463 | **0.001** | 1.588 | 1.197-2.108 |
| **Contact with a COVID-19 patient** | 0.646 | **0.009** | 1.907 | 1.171-3.107 |

## DISCUSSION

More than one-third of PCR results (37.2%) in the study sample were evaluated as COVID-19 (+). Significant variables to predict COVID-19 disease confirmed by PCR test results were advanced age, symptoms of weakness and cough, high fever, and a history of close contact with a COVID-19 patient. Laboratory parameters including WBC, RBC, Hb, Platelet, lymphocyte, and neutrophil counts were significantly lower and CRP values were higher in PCR (+) cases. Based on CT evaluations, COVID 19 (+) chest radiological appearance was significantly higher in PCR (+) patients compared to PCR (-) patients.

**PCR (+) Status:** PCR positivity rates during the admittance to the health care services varied. In a study carried out in Turkey, the positive PCR test ratios were 16.5% during the first admissions (6). Another research from Turkey depicted that 9.8% of all cases were positive regarding RT-PCR test results (11). According to the national weekly report of the COVID-19 database from Turkey on October 16-22, 2021, the number of total tests was 2.497.723, and the total cases were stated as 199.170. The positive ratio of PCR test results performed in that week was 12.54% (12). Another study including 673 patients from Turkey stated that 29.4% of patients admitted to COVID-19 First Evaluation Outpatient Clinic were diagnosed as COVID-19 (13). Research including 116 patients with the suspect from COVID-19 disease who presented to two emergency departments in China for the first time reported 32 patients were confirmed to have COVID-19 by laboratory results. The current study found out 37.2% of all participants were diagnosed with PCR (+) (10). The positivity rate of PCR test results between countries varies according to many factors since the beginning of the pandemic. Among these factors, quarantine practices, vaccination practices and other social protective measures applied by countries in different periods can be counted.

**Age:** Advanced patient age is considered an important risk factor for contracting COVID-19 disease (14,15). In the study conducted in Wuhan, the initial point of the disease, the median age of COVID-19 patients was 65 years, and 38.3% of all patients were over 65 years old (15). Similar results were reported in a retrospective cohort study of approximately 90,000 people (16). In the triage study of Dizman et al. in Turkey, it was determined that the median age of COVID-19 patients was higher than those without COVID-19 (38 vs 35 years) (13). In the current study, the median age of positive cases was significantly higher than negative cases (43 vs. 38 years). In addition, it was determined that the increase in age increased the probability of having PCR positivity. The reason why the median age values in our study are lower than in other studies arises from the data were obtained from patients in the first period of the pandemic and the curfew applied to patients over 65 at that time. It is also considered that PCR positivity increases as a natural consequence of weakening of the immune system, especially in geriatric patients with advanced ages.

**Symptoms:** The symptoms detected during the admission of suspected patients differ in health care facilities. A metaanalysis included 45 studies, cough, fever, headache, dyspnea and diarrhea were the most common complaints (17). Sahin et al. evaluated the first and control admissions of patients who applied to the outpatient clinic with the suspicion of COVID-19 and found that 24.4% of the patients who applied for the first time were asymptomatic. Most clinical common symptoms in patients with PCR (+) test were weakness (73.0%), headache (64.9%), pain (32.2%), cough (56.8%), sore throat (51.4%), and anorexia (45.9%) (6). In another study from Turkey, Mercan et al. stated 15.2% of cases were asymptomatic, whereas 84.8% had at least one symptom. Common symptoms in adults with COVID-19 were cough (26.3%), headache (26.3%), high fever (24.1%), fatigue (21.9%), arthralgia (21.9%), myalgia (19.4%), and malaise (17.5%) (18). In an observational study of 326 COVID patients in Bangladesh, 19.02% were symptom-free. In patients with symptoms, fever was the most common symptom, followed by cough, loss of smell and taste, sore throat, and headache (19). Dizman et al. stated that the proportions of cough, myalgia, loss of smell/taste, and sore throat were more frequent in confirmed COVID-19 patients. The authors declared that the presence of these symptoms may trigger SARS-CoV-2 PCR positivity (13). In a retrospective New York study, the most common symptoms were described as fever, coughing, shortness of breath, muscle pain, and, nausea respectively (20). In a study carried out in Malaysia, though most of the confirmed cases were asymptomatic, those who presented with loss of smell/taste, fever, running nose were significantly associated with positivity due to the multivariate logistic regression analysis (21). Sahin et al. revealed odd's rates of significant complaints about PCR positivity were 2.607 for fever, 2.724 for anorexia, 2.051 for cough, 2.594 for loss of smell, and 2.243 for loss of strength (6).

Based on our patient's medical history, the most common presenting complaints were acute cough (57.6%), myalgia (47.6%), and fatigue (28.3) were common in positive patients, while sore throat (34.0% vs.43.6%) and diarrhea (14.9% vs. 22.1%) were more common in negative patients. Regression analysis demonstrated that fatigue was nearly 2-fold and cough was a 2.3-fold increased likelihood of having PCR (+) status. The fact that the frequency of symptoms observed in PCR (+) patients were observed at different rates in studies may be due to many reasons, especially geographical and ethnic reasons. Another reason for

the determination of different rates may be the difference in sample selection. While the first application was made to triage outpatient clinics in some hospitals, it was made in the emergency department in some hospitals. When similar studies were examined, while the frequency of symptoms was calculated in some studies, the calculation was made on all patients, not considering the asymptomatic patients. In some studies, consistent with our results, evaluations were made on patients with symptoms.

**Fever:** High fever was the most common symptom among the main symptoms of confirmed COVID-19 cases (5,22). The importance of high fever was emphasized in the effective fight against COVID-19 disease among suspected cases (18). In a meta-analysis comprising 38 studies conducted in China, the most common complaint was fever (80.4%) (23). Sun et al. informed that the rate of high fever was observed in 82.1% of their admittants (24), while Tian et al. reported fever in 82.1% (25). Another study demonstrated fever in 55% of outpatients, and 68% of inpatients. In studies conducted in our country, the frequency of high fever in COVID patients varies. (6.2% to 24.1%) (11,18). A study carried out in Malaysia showed that presentation with high fever increased almost 4-fold of having PCR (+) test results (21). In our study, the incidence of fever in patients diagnosed with COVID-19 was significantly higher than in COVID (-) cases (35.3% vs. 20.4%). Patients with a high fever at application was a nearly 1.6-fold increased risk of being PCR (+). It is considered that the most important factor in the detection rates of high fever in studies is the difference in the threshold value of body temperature determined for high fever.

**Contact with a COVID-19 Patient:** One of the most important risks of contracting COVID-19 disease is to be in proximity with patients in the same environment. In almost all countries, the suspected time to contact COVID-19 patients in medical history was accepted as 2 weeks before the onset of symptoms. Zhu et al. stated that history of exposure to COVID-19 patients in the previous 2 weeks was more prevalent in diagnosed patients than negative cases (63% vs. 44%) (23). A study from Turkey reported that contact history was more common in patients with COVID-19 (67.7% vs 52%,) (13). Chow et al. indicated that close exposure history to confirmed SARS-CoV-2 cases was higher in PCR (+) cases than PCR (-) ones (38.1% vs. 11.8%). The odds of being positive were increased by 3.2-fold among those who had close contact with confirmed SARS-CoV-2 cases (5). In our study, it was found that having a history of close contact with a COVID-19 patient increased the probability of PCR (+) nearly 2 times.

**Comorbidities:** The presence of accompanying chronic health issues can affect the outcome of COVID-19 patients. In a meta-analysis, hypertension was the most prevalent comorbid disease with a rate of 21.1% among 1576 patients. Other common comorbidities comprised diabetes mellitus, cardiovascular disease, and respiratory system disease with the prevalence of 9.7%, 8.4%, and 1.5% of the patients, respectively(26). Cheng et al. reported one-third of participants had one or more chronic diseases in laboratory-confirmed adult COVID-19 infection cases. In their study, patients with a severe or critical type of COVID-19 disease were more likely to have accompanying chronic health issues, including DM, hypertension, chronic heart disease, and chronic pulmonary disease, than a mild or moderate type of COVID-19 disease (27). In a study consisting of COVID-19 confirmed cases followed- by primary healthcare services, 25.4% of the patients had a chronic disease including hypertension (16.8%) and diabetes mellitus (7.0%) (18). Previously, one study from Iran showed that diabetes (16.3%) and cardiovascular disorder (21%) were the most frequent comorbidities among the COVID-19 patients. A study from Bangladesh depicted that hypertension (19.2%) and bronchial asthma (17.6%) were the most common co-morbidities in their sample (28), while another study in the same country stated diabetes mellitus, hypertension, and bronchial asthma were most frequently observed in both symptomatic and asymptomatic patients (19). In a study conducted in the first evaluation outpatient clinic in Turkey, 31.5% of cases had comorbid diseases, and the most common chronic conditions were diabetes mellitus and hypertension. However, although comorbid diseases were more prevalent in patients with COVID-19, no statistically significant difference was identified between the positive and negative cases (13). In our study, it was determined that having comorbid diseases increased the risk of PCR positivity approximately 2-fold. Two of the most important of these diseases were DM and HT, as emphasized in other studies. Keeping under the control of concomitant diseases that have been confirmed to have significantly increased COVID-19-related hospital admissions, intensive care rates, and deaths are an indispensable part of being up against the pandemic.

**Chest CT:** Recent studies emphasized the importance of chest CT in the diagnosis of COVID-19 and CT sensitivity has been described as higher than 90% (29,30). Yurt et al. reported that chest CT imaging was administered on 84.8% of the patients admitted to the hospital, and 16.4% showed COVID-19 pneumonia findings. Chest CT (+) rates of PCR (+) patients were significantly lower than PCR (-) cases (23% vs. 15.7%) (11). Dizman et al. stated their local guideline suggested chest CT for the cases with comorbid diseases and/or respiratory symptoms during the evaluation of suspected COVID-19 applicants. By this clinical management, they diagnosed 56 patients whose SARS-CoV-2 PCR was negative, but CT findings

were consistent with COVID-19 among 421 chest CTs (13). In a study conducted in two emergency departments, chest CT scans of suspected patients on their first admission exposed the presence of COVID pneumonia in most of the laboratory diagnosed patients (94%) and 67% of negative cases (10). In our study, CT was performed in 43.7% of the patients. Among those who had a CT scan, 40.6% (310) were evaluated as having COVID-19 radiological appearances. In approximately one-third of the PCR (-) cases, there was an infiltration appearance compatible with COVID-19.

**Laboratory Parameters:** In COVID-19 disease, laboratory parameters, especially complete blood count, are generally compatible with viral infections. It has been stated that it may differ according to the progression and severity of the disease (31). Lymphopenia and high D-dimer levels were reported to be subjected to poor outcomes due to mortality and morbidity (32,33). Dizman et al. stated that their facility was able to analyze the complete blood cell (CBC) in 571 patients and D-dimer in 353 patients. COVID-19 patients compared to negative patients were more likely to have lower lymphocyte and platelet counts. D-dimer values did not differ between the two groups, but ferritin values were higher in COVID-19 (+) cases (13). A China study reported that CBC test results on admission displayed that 22% of positive cases and 5% of negative cases had leukopenia (white blood cell count $<3.5\times10^9$/L), 9% positive cases, and 19% negative cases had neutrophilia (neutrophil count $>6.3\times10^9$/L) and 59% positive cases and 29% negative cases had lymphopenia (lymphocyte count $<1.1\times10^9$/L) (10). In a study from Turkey stated that the mean leukocytes, neutrophils, and platelet counts of COVID patients were lower than those who were not (11). In our study, Hemoglobin (13.9 vs.14.2), leukocyte (5.40 vs. 6.90), neutrophil (3.10 vs. 4.10), lymphocyte (1.40 vs. 1.90), and platelet (202.50 vs. 233.00) counts were lower, but CRP levels were higher (15.00 vs. 9.05) in patients with COVID-19 compared to those of negative cases. Our results were consistent with other studies in the literature. Differences between results may be due to the reference ranges of test kits or the time at which patients are evaluated.

**Study Strengths:** Studies of COVID-19 disease symptoms are generally based on retrospective evaluation of patients' recalled symptom states after hospitalization. Our study, on the other hand, was evaluated by examining the symptoms of the patients who were referred directly to the triage outpatient clinic due to the suspicion of the disease at the time of their admission. Recall bias is minimal. Second, in similar studies, the relationship between symptoms and PCR positivity was evaluated by bivariate analysis. A limited number of studies, including ours, include regression analysis in which ORs are calculated.

**Study Limitations:** Our study has some limitations. Since the study is a single-center study, the participants' COVID-19 symptoms, laboratory parameters, and chest CT results cannot be generalized to all patients. Second, because the study was based on the onset of pandemic data, symptoms, laboratory parameters, and chest CT results may differ, especially in the post-vaccination period.

## CONCLUSION

Early diagnosis of COVID (+) patients and application of adequate isolation or quarantine measures are crucial factors to control the spread of the COVID-19 pandemic. Triage outpatient clinics provide safe healthcare services to suspected patients separating them from other patients admitted to the emergency department.

Risk factors associated with PCR (+) test results were older age, history of contact with confirmed COVID-19 patient, high fever, fatigue, cough in our study. In studies, especially some symptoms and medical history components have a significant relationship with PCR positivity, which requires a more careful approach to these points in terms of COVID-19 disease in the first applications. This situation is important in cases which PCR test could not be performed or in cases with delayed diagnosis.

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RESEARCH ARTICLE

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# The Diagnostic Value of Bladder-Wall Thickness and ARFI Elastography of Bladder Wall amongst Patients with BPH

**ABSTRACT**

**Objective:** The present study identified bladder-wall thickness and, through the use of ARFI elastography, bladder-wall elasticity values amongst patients with benign prostate hyperplasia (BPH), then examined their relationship with the disease diagnosis and progression.

**Methods:** The study included 60 patients with BPH (patient group) and 50 healthy volunteers (control group). All members of the patient and control groups were measured for bladder-wall thickness (BWT) and bladder-wall mean shear-wave velocity (BW mean SWV) values, as well as for uroflowmetry parameters. The patient group was divided into the sub-groups of mild-medium and severe BPH, according to the International Prostate Symptom Score (IPSS). The patient and control groups and their sub-groups were compared amongst themselves.

**Results:** Whilst the BPH group indicated a mean wall thickness of 6.3±2 mm (range: 3-12 mm), the control group yielded a result of 2.8±0.7 mm (range: 2-5 mm), which led to the conclusion that there was a significant difference between these groups ($p<0.01$). The BWmeanSWV value was 1.39±0.5 m/s (range: 0.60-2.65 m/s) for the BPH group and 1.01±0.2 m/s (range: 0.60-1.50 m/s) for the control group, and this also indicated the presence of a significant difference between the groups ($p<0.01$). According to the IPSS, BWT was observed to be significantly higher in the severe sub-group when compared to the mild-medium BPH group [(5.07±1 mm; range: 3-7 mm), (6.8±2 mm; range: 4-12 mm), $p<0.01$).

**Conclusions:** When compared to the control group, patients with BPH showed significantly higher BWT and BWmeanSWV values; these two parameters may provide an additional method in the diagnosis of bladder outlet obstruction secondary to BPH. BWT, increasing in parallel with the severity of BPH, may be utilised in the follow-up for BPH progression.

**Keywords:** Bladder-Wall Thickness, Bladder-Wall Shear-Wave Velocity, ARFI Elastography, Benign Prostate Hyperplasia.

# Benin Prostat Hiperplazili Hastalarda Mesane Duvar Kalınlığı ve Mesane Duvar ARFI Elastografinin Tanı Değeri

**ÖZET**

**Amaç:** Çalışmamızda benign prostat hiperplazili (BPH) hastalarda mesane duvar kalınlığı ve ARFI elastografi yöntemi ile mesane duvarı elastisite değerlerini saptayıp, hastalığın tanısı ve progresyonu ile ilişkisini araştırdık.

**Gereç ve Yöntem:** Çalışmaya 60 BPH'lı hasta (Hasta grubu) ile 50 sağlıklı gönüllü (Kontrol grubu) dahil edildi. Hasta ve kontrol grubunun tamamında mesane duvar kalınlığı (MDK), mesane duvarı MDmeanSWV değerleri ve uroflovmetre parametreleri ölçüldü. Hasta grup; International Prostate Symptom Skoru'na (IPSS) göre hafif-orta ve şiddetli BPH subgruplarına ayrıldı. Hasta–kontrol gurubu ve subgruplar kendi aralarında karşılaştırıldı.

**Bulgular:** BPH'lı grup mesane duvar kalınlığı 6.3±2 mm (3-12 mm) iken kontrol grubunda 2.8±0.7 mm (2-5 mm) olup her iki grup arasında anlamlı fark mevcuttu ($p<0.001$). MDmeanSWV değeri BPH'lı grupta 1.39±0.5 m/s (0.60-2.65 m/s) iken, kontrol grubunda 1.01±0.2 m/s(0.60-1.50) olup her iki grup arasında anlamlı fark mevcuttu($p<0.001$).

IPSS ye göre şiddetli BPH'lı grup, hafif-orta BPH grup ile karşılaştırıldığında MDK, şiddetli subgrupta anlamlı şekilde daha yüksek bulundu [sırasıyla; 5.07±1 (3-7), 6.8±2 (4-12), $p<0.01$].

**Sonuç:** Kontrol grubuna göre BPH'lı hastalarda MDK ile birlikte MDmeanSWV elastografi değerleri belirgin yüksektir, bu iki parametre BPH'ya sekonder gelişen mesane çıkış obstrüksiyon tanısında ilave bir yöntem olabilir. BPH şiddeti ile paralel artış gösteren MDK, BPH progresyonunun takibinde kullanılabilir.

**Anahtar Kelimeler:** Mesane Duvarı Kalınlığı, Mesane Duvarı Shear-Wave Velositesi, ARFI Elastografi, Benign Prostat Hiperplazisi.

## INTRODUCTION

More than 50% of cases of benign prostate hyperplasia (BPH), a disease with an incidence rising in parallel with age, are observed amongst older male patients. 28% of patients of the age of 70 or above exhibit medium-to-severe lower urinary tract symptoms (1-3). The bladder outlet obstruction caused by BPH increases the detrusor pressure and causes the urinary flow rate to decrease during voiding (4). Infravesical obstruction in patients with BPH causes detrusor hypertrophy, which in turn leads to the emergence of irritative urinary symptoms. Bladder outlet obstruction was reported to be non-symptomatic in 52% of patients with BPH (5-6).

According to studies conducted on animals with obstruction of the bladder, there was a significant increase in the bladder-wall thickness due to smooth muscle cell hypertrophy, as well as fibrocystic hyperplasia and collagen accumulation on the bladder wall. These findings were also identified in human patients with bladder outlet obstruction (7-8). Trabecular formation in the bladder and bladder hypertrophy findings can be established through cystoscopy or cystography. However, these techniques do not provide objective or quantitative means for measuring the degree of bladder hypertrophy. Furthermore, the most reliable functional method in the diagnosis of bladder outlet obstruction is the evaluation of urodynamic pressure flow. However, it includes such complications as bleeding and infection and is a time-consuming and costly method (9).

Uroflowmetry is an urodynamic test that enables the non-invasive evaluation of the properties of urinary flow during urination (10). Prostate-induced bladder outlet obstruction causes a lower flow rate, and uroflowmetry provides us with a more objective criterion when compared to the symptoms of the disease in BPH diagnosis (11). The International Prostate Symptom Score (IPSS) is quite useful during the follow-up for disease progression in the identification of treatment modalities by providing a better understanding of BPH-related disease symptoms. IPSS>7 are accepted as important criteria in the diagnosis of BPH (12).

Bladder elasticity emerges secondary to the presence of a higher ratio of connective tissue in the bladder wall than that in the smooth muscle. When compared to the smooth muscle, the bladder becomes more rigid in direct proportion with the increase of the quantity of connective tissue, and can expand to a much lesser degree during filling (13-14-15). Cystometry, considered to be the gold standard in the evaluation of bladder compliance, is an uncomfortable method for patients and poses a risk of infection at the same time. Acoustic radiation force impulse (ARFI) imaging is a new, non-invasive and low-cost method for evaluating tissue elasticity. Integrated into the ultrasound technique, ARFI imaging enables the quantitative and qualitative evaluation of tissue elasticity. The measurement of shear-wave velocity (SWV) in the tissue where the region of interest (ROI) is situated indicates higher SWV and higher rigidity, thereby allowing the identification of the mechanical properties of the tissue (16).

We first identified bladder-wall thickness (BWT) and, through the use of ARFI elastography, differences in bladder elasticity amongst patients with BPH and volunteers, and then we examined the relationship between the severity of BPH and BWT, BWmeanSWV values.

## MATERIAL AND METHODS

**Patients:** The study included 105 patients with BPH that presented at the urology department with lower urinary tract symptoms between August 2014 and May 2015. Fourthy-five of these patients were excluded from the study due to the presence of exclusion criteria such as uncontrolled diabetes mellitus, neurological diseases affecting urinary function, bladder and prostate cancers, history of lower urinary tract surgery, urinary tract infection or urethral stricture. The study included 60 male patients with BPH and 50 healthy volunteer. Age - matched healthy male volunteers, who are free of any systemic disorder and without any urinary tract complaints formed the control group.

The study was approved by the ethics board of Dicle University. Consent forms were collected from the patients and the healthy volunteers.

**Imaging:** Individuals were subjected to B-mode ultrasonography (US) and ARFI elastography imaging through the use of an Acuson S2000 ultrasound system (Siemens Solutions, Mountain View, CA, USA) and a convex probe (4 C1, frequency range: 1–4 MHz). The B-mode US examination was undertaken by a radiologist with 16 years of experience in this field, and the evaluation of the ARFI elastography measurements (Virtual Touch™ Tissue Quantification) was done by the same radiologist (B.A.) with 2 years of experience in this field. The first step was to measure the prostate volume in the BPH and control groups using B-mode US. The prostate volume was calculated using the following formula: $\pi/6 \times$ (transverse diameter $\times$ anteroposterior diameter $\times$ cephalocaudal diameter) (17).

Afterward, BWT was measured on the anterior and right and left lateral bladder walls, and the average of all three measurements was recorded as the wall thickness. The Region of Interest (ROI) was placed onto the bladder wall and the measured SWV value was obtained in metres/second. A total of eight valid SWV measurements were taken for each measurement of the anterior, posterior, right and left bladder wall with full bladder (Figure 1).

**IPSS and Uroflowmetry:** The urinary symptoms of patients were evaluated based on the International Prostate Symptom Score. The total

IPSS score was subcategorised into voiding and storage symptom sub-scores (18). The IPSS requires men to quantitative seven symptoms using a score ranging from 1 to 5 on the basis of their experience in the past 30 days. The IPSS also includes one question that assesses how bothersome their symptoms have been. The answer to this question becomes very important when considering whether to commence treatment for patients with BPH/LUTS. Each severe symptom can be assessed with a maximum score of 5, thus the maximum possible score is 35. A score of zero indicates the absence of any BPH symptoms. A symptom score between 1 and 7 is considered mildly symptomatic, 8-19 is moderately symptomatic, and 20-35 is severely symptomatic. The question about the bothersome nature of the symptoms is scored separately as 0-6 (19). The BPH group was divided into two sub-groups for the identification of disease severity: severe BPH with IPSS≥20 and mild-moderate BPH with IPSS<20.

Uroflowmetry measurements, including the maximum urinary flow rate (Qmax), average urinary flow rate (Qave) and post-voiding residue (PVR), were conducted in the standing position with the use of Bluetooth uroflowmetry (Urodyn+, MMS, Flowmaster, NL). Those with a voided volume of <125 mL were not included. Qmax and PVR measurements were repeated. PVR measurements were undertaken with a bladder scanner (Login C3, Premium, GE, China). Two sub-groups were formed with <10 and ≥10, according to Qmax, and then subjected to comparison (20).

**Statistical Analysis:** SPSS 16.0 (Statistical Package for the Social Sciences version 16.0 for Windows, SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The Kolmogorov-Smirnov test was used for the distribution of data. Data were expressed as mean ± standard deviation. For the evaluation of the continuous variables, we used Student's t-test for parametric data and the Mann-Whitney U test for nonparametric data; the categorical valuables were analyzed with the chi-square test. The relation between the parameters was analyzed with the Spearman and Pearson correlation test. For detecting the effect of parameters on BWmeanSWV values used logistic regression test. In order to predict the severity of BPH, we calculated the areas under the ROC curves for the prostate shear wave speed. A value of $p<0.05$ was accepted as significant.

## RESULTS

Out of the total of 110 patients included in the study, 60 formed the group of BPH patients and 50 formed the control group. The mean age of the control group was 57.1±8 years (range: 50-63 years), whilst the mean age of the BPH group was 60.5±6 years (range: 50-73) years), there was no significant difference between the two groups (p = 0,07). Prostate volume was 66.1±27 ml (range: 21-131 ml) in the BPH group and 19.9±5 ml (range: 10-31 ml) in the control group, indicating a significant difference between the two groups. Whilst the BPH group indicated a mean wall-thickness of 6.3±2 mm (range: 3-12 mm), the control group yielded the result of 2.8±0.7 mm (range: 2-5 mm), which led to the conclusion that there was a significant difference between these groups ($p<0.01$). The BWmeanSWV value was 1.39±0.5 m/s (range: 0.60-2.65 m/s) for the BPH group and 1.01±0.2 m/s (range: 0.60-1.50 m/s) for the control group, and this also indicated the presence of a significant difference between the groups ($p<0.01$) (Figures 2-3).

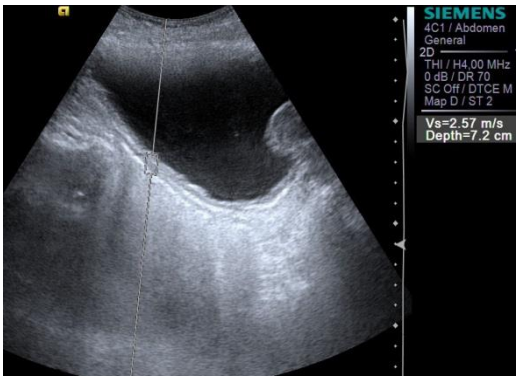


**Figure 1.** Bladder-wall SWV measurement

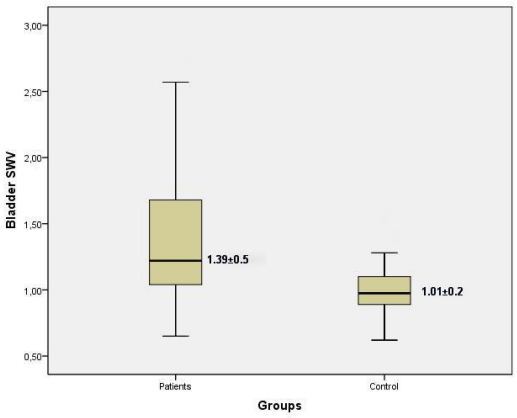


**Figure 2.** Comparison of BWmeanSWV value between patients with BPH and the healthy control group

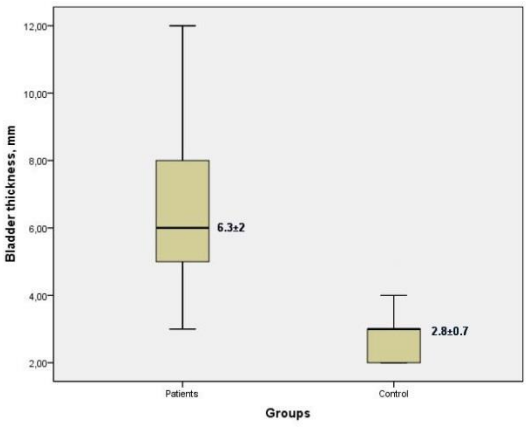


**Figure 3.** Comparison of bladder-wall thickness between patients with BPH and the healthy control group

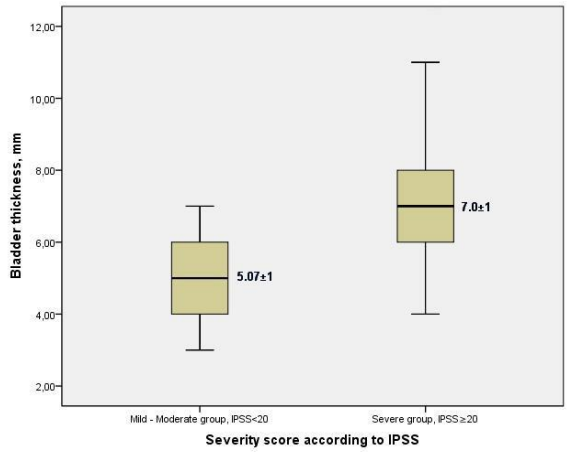


**Figure 4.** Comparison of BW thickness between mild-medium and severe BPH sub-groups according to IPSS

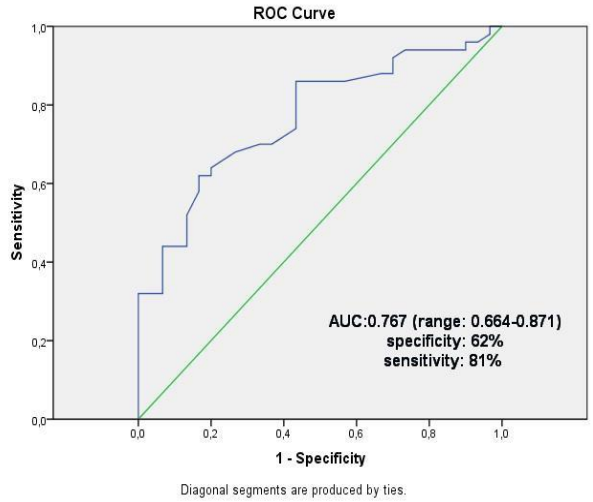


**Figure 5.** Diagnostic performance of BWmeanSWV value in patients with BPH

The correlation analysis undertaken with age, BWT, IPSS score and uroflowmetry parameters through BWmeanSWV identified a significant positive correlation between BWT and IPSS [(r=0.482, p<0.01), (r=247, p=0.04), respectively]. Furthermore, a weak negative correlation was identified in Qmax (r=-0.219, p=0.04) (Table 1).

The logistic regression test gave way to the identification of a significant relation between BWmeanSWV and BWT, with no indication of any other parameter affecting BWmeanSWV (Table 2).

The BPH group was divided into two sub-groups for the identification of disease severity, i.e., severe BPH with IPSS≥20 and mild-moderate BPH with IPSS<20. When the severe BPH group was compared with the mild-moderate BPH group, BWT was found to be significantly higher in the severe sub-group (7.0±1 mm; range: 3-7 mm) (5.07±1 mm; range: 4-12 mm) (p<0.01) (Figure 4). However, BWmeanSWV values were slightly higher in the severe BPH group than in the mild-moderate BPH group, and no statistically significant difference could be identified (1.38±0.4; range: 0.8-2.1) (1.41±0.5; range: 0.6-2.6) (p=0.9).

The comparison between two sub-groups formed with values of <10 and ≥10, based on Qmax, showed a significantly higher BWT in the Qmax<10 sub-group than in the Qmax≥10 sub-group (p<0.01). BWmeanSWV was slightly higher in the Qmax<10 sub-group than in the Qmax≥10 sub-group, with no statistically significant difference identified (Table 3).

The ROC analysis undertaken to assess the diagnostic value of BWmeanSWV for BPH calculated AUC as 0.767 (range: 0.664-0.871), specificity as 62%, and sensitivity as 81% (Figure 5).

**Table 1.** Correlation analysis of BWmeanSWV value in patients with BPH with age, bladder-wall thickness, maximum flow rate and IPSS

| | **r** | **P*** |
|---|---|---|
| Age, year | 0.352 | 0.3 |
| Bladder thickness, mm | 0.482 | < 0.01 |
| Qmax,mL/s | -0.219 | = 0.04 |
| IPSS | 0.247 | 0.04 |

Pearson correlation test * ;IPSS: International Prostate Symptom Score; ; Qmax: Maximum urinary flow rate

**Table 2.** Detecting the effect of age, Prostate volüme, Bladder thickness parameters on the on BWmeanSWV values by logistic regression test.

| Model | | Unstandardized Coefficients | | Standardized Coefficient[a]s | t | Sig.* |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | .675 | .151 | | 4.475 | .000 |
| | Age. year | .001 | .003 | .054 | .405 | .687 |
| | Prostate volüme, mL | -.001 | .002 | -.058 | -.390 | .698 |
| | Bladder thickness, mm | .120 | .033 | .532 | 3.590 | .001 |

a. Dependent Variable: Bladder SWV,* logistic regression test

**Table 3.** Comparison of bladder-wall thickness, BWmeanSWV and uroflowmetry parameters between groups with Qmax scores < 10 and Qmax ≥ 10

| Parameters | | n | Values* | Range | *P*** |
|---|---|---|---|---|---|
| Age,year | Qmax scores < 10 | 30 | 65.9±9 | 47-87 | |
| | Qmax scores ≥ 10 | 20 | 64.4±7 | 52-76 | 0.3 |
| BWmeanSWV,m/s | Qmax scores < 10 | 30 | 1.4±0.5 | 0.65-2.65 | 0.1 |
| | Qmax scores ≥ 10 | 20 | 1.2±0.3 | 0.84-2.15 | |
| Bladder thickness, mm | Qmax scores < 10 | 30 | 7.2±2 | 4-12 | |
| | Qmax scores ≥ 10 | 20 | 5.1±1 | 3-7 | < 0.01 |
| Voiding volüme,mL | Qmax scores < 10 | 30 | 114±71 | 13-275 | |
| | Qmax scores ≥ 10 | 20 | 223±189 | 65-931 | < 0.01 |
| Voiding time,s | Qmax scores < 10 | 30 | 54.8±32 | 12-138 | 0.8 |
| | Qmax scores ≥ 10 | 20 | 56±33 | 13-138 | |
| PVR,mL | Qmax scores < 10 | 30 | 81.4±63 | 0-226 | 0.3 |
| | Qmax scores ≥ 10 | 20 | 65±52 | 0-190 | |

Values are mean±SD *; Mann-Whitney U test**;BPH: Benign prostate hypertrophy; BWmeanSWV: bladder-wall mean shear-wave velocity; IPSS: International Prostate Symptom Score; PVR: post-void residual

## DISCUSSION

BWT was higher in the BPH group than in the control group. Pathological conditions causing bladder outlet obstruction, including BPH, do affect the bladder-wall thickness (21-23). Histologically, smooth muscle cells were reported to lead to the development of hypertrophy, hyperplasia and collagen accumulation between muscle cells. Such muscle hypertrophy weakens the detrusor muscle further (21-24). There are other factors affecting the bladder wall, including age, sex and bladder fullness (8). We minimised the variation in bladder fullness by undertaking the measurements on those with bladder volumes ≥200 ml so that such variations would not affect the BWT measurement. The comparison between mild-medium BPH and severe BPH sub-groups under IPSS showed a significantly higher value for BWT in the severe BPH sub-group. Azab *et al.* identified a positive correlation between BWT and IPSS in patients with BPH (25). Similarly, Parks *et al.* reported a positive correlation between IPSS and BWT (26).

We identified a significantly higher BW mean SWV value in the BPH group in its comparison with the control group. Bladder-wall rigidity escalates along with the increase in connective tissue accumulation brought along by smooth muscle cell hypertrophy induced by bladder outlet obstructions, such as BPH, in the bladder wall. Collagen accumulation appears in the distances between expanded muscle cells caused by the decrease in intermediate cell junctions (13-15,27). The identification of higher bladder-wall elasticity in patients with BPH than in those in the control group may be based upon these histopathological changes. However, we conducted a multi-variance analysis under the notion that age could be an influential factor for bladder rigidity; this established that patient age did not affect the BWmeanSWV value. We determined a positive correlation between BWmeanSWV and IPSS and Qmax in the correlation analysis. Nevertheless, the comparison of BWmeanSWV value between mild-medium BPH and severe BPH groups, calculated according to IPSS, did not provide us with any statistically significant difference between the two groups, even though the BWmean SWV value was slightly higher in the severe BPH group. Studies undertaken with more comprehensive patient series are needed to identify whether BWmeanSWV can be used in the follow-up for disease progression. As a relatively non-invasive method, when compared to the invasive method of cystometry in the evaluation of bladder compliance, US-based ARFI elastography also enables evaluation of the mechanical properties of the bladder wall. Numeric values obtained through ARFI-elastography provide quantitative information that is useful for the evaluation of bladder elasticity.

The present study had certain limitations. As the correlation between the bladder wall elastic module and bladder outlet obstruction could not be clarified, there is a need for further studies as precursors based on the provision of SWV values in the identification of bladder elasticity.

## CONCLUSION

When compared to the control group, patients with BPH show significantly higher BWT and BWmeanSWV values, and these two parameters may provide an additional method in the diagnosis of bladder outlet obstruction secondary to BPH. BWT, increasing in parallel with the severity of BPH, may be utilised in the follow-up of BPH progression.

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**Conflict of Interest:** The authors declare that they have no competing interests.

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CASE REPORT

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# A Rare Infectious Agent: *Elizabethkingia anophelis*; Second Case Reported from Turkey

**ABSTRACT**

*Elizabethkingia anophelis* is a Gram-negative, aerobic, nonmotile bacillus belonging to the *Flavobacteriaceae* family. In recent years, it has emerged as a cause of life-threatening infections, especially in immunocompromised patients. In this study, a 6-month-old baby patient with *E. anophelis* growth in simultaneous tracheal aspirate and urine culture samples sent to investigate the etiology of fever while being followed in the intensive care unit due to the diagnosis of optic glioma is presented. Bacteria identification was performed using the VITEK MS® system, antibiotic susceptibility tests were performed using the VITEK 2 automated system and gradient strip test. Our case is the second *E.anophelis* case reported from Turkey. This case showed that this bacterium would start to appear as a factor in our country. More studies are needed to obtain more detailed information about this bacterium, to determine its transmission routes and resistance mechanisms and to establish appropriate treatment protocols.

**Keywords:** Antibiotic Resistance, *Elizabethkingia anophelis*, Infection, Opportunistic Pathogen.

# Nadir Bir Enfeksiyon Etkeni: *Elizabethkingia anophelis*; Türkiye'den Bildirilen İkinci Olgu

**ÖZET**

*Elizabethkingia anophelis*, *Flavobacteriaceae* familyasına ait Gram negatif, aerobik, hareketsiz basildir. Son yıllarda özellikle bağışıklığı baskılanmış hastalarda yaşamı tehdit eden enfeksiyonların bir nedeni olarak ortaya çıkmıştır. Hastane ortamında kolonize olabilen bakteri, dekontaminasyona dirençlidir. *E. anophelis*, antibiyotiklere karşı oldukça dirençli bir bakteridir. Bu çalışmada, optik gliom tanısı nedeniyle yoğun bakımda takip edilirken, ateş etyolojisini araştırmak için gönderilen eş zamanlı trakeal aspirat ve idrar kültürü örneklerinde *E. anophelis* üremesi olan 6 aylık bebek hasta sunulmuştur. Bakteri identifikasyonu VITEK MS® sistemi, antibiyotik duyarlılık testleri ise VITEK 2 otomatize sistemi ve gradient strip test kullanılarak yapılmıştır. Olgumuz Türkiye'den bildirilen ikinci *E.anophelis* olgusudur. Bu vaka, ülkemizde de bu bakterinin artık etken olarak karşımıza çıkmaya başlayacağını göstermiştir. Bu bakteri hakkında daha detaylı bilgi edinilebilmesi, bulaşma yolları, direnç mekanizmalarının belirlenerek uygun tedavi protokollerinin oluşturulabilmesi için daha fazla çalışmaya ihtiyaç vardır.

**Anahtar Kelimeler:** Antibiyotik Direnci, *Elizabethkingia anophelis*, Enfeksiyon, Fırsatçı Patojen.

## INTRODUCTION

The genus *Elizabethkingia* are aerobic, nonfermentative, non-motile, catalase, oxidase and indole positive Gram negative bacilli that are ubiquitous in the environment including soil, water and other animal reservoirs (1). At the end of the phylogenetic analyzes (16S rRNA gene sequence) of the bacterium known as *Chryseobacterium* genus, which was initially in the *Flavobacteriaceae* family, Kim et al. It has been reported as a new genus by (2).

The first identified strains of the bacterium were *E. meningoseptica* and *E. miricola*, while *E.anophelis*, which was isolated from the midgut of the mosquito, was lastly identified in 2011. *Elizabethkingia* are generally opportunistic nosocomial pathogens and cause meningitis, pneumonia and bacteremia with high mortality rates (3).

After the identification of *E. anophelis* from Anopheles gambiae mosquitoes in Africa, the first case of human infection by this species was reported in 2013, followed by several outbreaks by *E. anophelis* from Singapore, Hong Kong, Taiwan and the United States. (3-6). Today, information about the transmission routes, pathophysiology and antibiotic resistance of *Elizabethkingia* bacteria is still insufficient (5). Bacteria that can colonize in the hospital environment are resistant to decontamination. It is thought that water systems in hospitals act as reservoirs for bacteria that can contaminate and colonize various solutions and devices used in hospitals (4). Although it is known that all genera can be found in soil, water and plants, it has been reported that *E.anophelis* is especially abundant in the midgut of Anopheles gambiae mosquitoes (3,4). Routine phenotypic and biochemical tests often fail to distinguish E. *anophelis* from other bacteria. At the same time, this bacterium is frequently described as *E. meningoseptica* in automated systems (2). However, thanks to newly developed diagnostic methods, there is an increase in the number of cases reported worldwide (2). In infections caused by *E.anophelis*, empirical treatment options are limited due to the lack of multi-drug resistance and drug sensitivity test standards of the bacteria. It is generally seen to be sensitive to fluoroquinolones (7). However, since *E. anophelis* is a newly identified bacterium, information on antibiotic resistance spectra and resistance mechanisms is insufficient (7).

In this study, the clinical features and antibiotic susceptibility of *E.anophelis*, which was isolated for the first time in our center from the tracheal aspirate and urine cultures of a patient hospitalized in the pediatric intensive care unit, will be examined.

## CASE REPORT

A 6-month-old girl, who came to the emergency department with seizure complaints, was intubated and taken to the intensive care unit because her general condition was poor and her breathing was tachypneic. No pathological condition was found in the patient's anamnesis taken from the mother. An intracranial mass (optic glioma?) was detected as a result of radiological imaging, and a brain tumor operation was performed on the 21st day of hospitalization. The patient's general condition was poor in the postoperative period, and his blood culture was taken because his temperature was 38.8 degrees. Fluconazole (3mg/kg) treatment was started in the patient who had *Candida albicans* growth in his blood culture. The tracheal aspirate sample, which was taken simultaneously with the blood culture from the patient and sent to the laboratory, was cultivated on sheep blood agar, eosin methylene blue agar, chocolate agar media. Simultaneously, direct smear was made from the sample and evaluated by gram staining. Gram staining revealed abundant polymorphous leukocytes and gram negative bacilli. There was growth in all plates incubated for 18-24 hours at 35±2°C. (pic 1). Gram negative bacillus growth was observed in gram staining of plaques. Oxidase, indole and catalase tests were positive. Identification of breeding colonies was performed using the VITEK MS® (Bio-Mérieux, Marcy l'Etoile, France) system. Antibiotic susceptibility studies for ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole (TMP/SMX), piperacillin/tazobactam (PRP), ceftazidime, cefepime, imipenem, meropenem, amikacin and gentamicin were performed with the Gradient strip test (Bianalyse, Turkey).

The results of antimicrobial susceptibility tests were evaluated according to other non-*Enterobacterales* bacteria by applying CLSI (Clinical and Laboratory Standards Institute) breakpoints (8). It was observed that the bacterium was resistant to all beta-lactam antibiotics and carbapenems. Only ciprofloxacin and levofloxacin were found to be sensitive. The patient was started on vancomycin (4x80 mg) and piperacillin/tazobactam (4x 80mg/kg) combination therapy. *E.anophelis* growth with the same antibiotic susceptibility profile was detected in the urine culture sent from the patient on the same day. The same bacterial growth was observed in the tracheal aspirate culture sent again on the 10th day of the treatment. The treatment was continued by adding clarithromycin (15mg/kg) suspension. The patient in the pediatric intensive care unit died on the 15th day of treatment.

## DISCUSSION

*E. anophelis*, after being isolated from the midguts of mosquitoes and taxonomically identified in 2011, spread rapidly in many countries and caused fatal opportunistic infections in patients (1). The first reported case of *E.anophelis* in the world is a neonatal meningitis case detected in Africa in

2011. The strain, which was first thought to be *E. meningoseptica*, was found to be a new strain in 16S rRNA analysis (1-3). Later, it was reported to cause infections and epidemics in Singapore, Hong Kong and the United States (5,6,9). The largest outbreak caused by *E.anophelis* was seen in 65 patients and in a hospital in the United States (USA) in immunocompromised patients and caused high mortality rates (30.8%) (6). *E.anophelis* was first reported in our country by Mirza in 2017. Mirza et al. identified 2 of the strains as *E.anophelis* as a result of the 16S rRNA analysis they performed on 5 strains previously identified as *E. meningoseptica* in their center. They isolated the strains from peripheral blood and urine samples of infant patients (8). Our case is the second infection case caused by *E.anophelis* reported from our country.

*E. anophelis* usually causes sepsis and/or meningitis in premature, newborn or adults with underlying disease, especially in immunocompromised, malignancy, chronic renal failure, diabetes mellitus, cirrhosis. In addition, it is seen as a causative agent in pneumonia, catheter-related bloodstream infections, skin and soft tissue infections, urinary tract infections and biliary tract infections (1). Although the majority of infections are hospital-acquired, 89% of the cases in the Wisconsin outbreak were reported to be community-acquired.

It is estimated that mortality rates in infections caused by *E. anophelis* vary between 24-60% (1,6,10). Our case was also a patient with cranial malignancy. In the patient who underwent optic glioma surgery, *E. anophelis* was found to be the causative agent in the tracheal aspirate and urine samples in the postoperative period.

The modes of transmission of *E. anophelis* still remain unclear. Although the bacterium was originally isolated from Anopheles gambiae mosquitoes, there is no evidence to support that human infection is a mosquito-borne disease (9).

In the epidemic seen in the USA, the source of infection was investigated, and the source could not be determined despite the examinations of tap water, food and personal hygiene products (6). In Singapore, it has been suggested that *E. anophelis*, which was detected in the tap water aerators of the hospital, was responsible for the epidemic, and that this bacterium was transmitted to the patients of healthcare workers who infect their hands during hand washing (10). Information on the susceptibility of *E. anophelis* to antimicrobials is quite limited. The fact that bacteria have high resistance rates increases the importance of early diagnosis and appropriate antibiotic treatment (9). Although high resistance rates have been reported in almost all studies on *E. anophelis*, susceptibility to ciprofloxacin, levofloxacin, piperacillin/tazobactam, and piperacillin has been reported in some studies (1,4,6,8). In our case, treatment options became very limited due to the fact that the bacterium was sensitive only to quinolones and the patient was a baby. The patient was started on vancomycin and piperacillin/tazobactam combination therapy. However, the growth of the same bacteria in the tracheal aspirate culture sent on the 10th day of the treatment showed that there was no response to the treatment.

In conclusion; As seen in our study, *E. anophelis* is a bacteria that is highly resistant to antibiotics. It has also been confirmed once again that it is generally sensitive to quinolones. Our case is the second *E.anophelis* case reported from Turkey. This case has shown that this bacterium will begin to appear as a factor in our country as well. More studies are needed to obtain more detailed information about this bacterium, to determine its transmission routes and resistance mechanisms and to establish appropriate treatment protocols.

**Ethics Committee Approval:** Since our study is a case report, ethics committee approval is not required.

**Conflict of Interest:** The authors declared no conflict of interest.

**Author Contributions:** Concept – EÖŞ,ÖA; Supervision-OA; Data collection-EÖŞ-BE-VC; Posted by-ÖA,EÖŞ, MK

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