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## CASE REPORTS



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## ORIGINAL ARTICLES



## CASE REPORTS

ORIGINAL ARTICLE

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# Evaluation of the Antidepressant Effect of Propolis in Chronic Unpredictable Mild Stress-Induced Depression Model in Rats

## Sıçanlarda Kronik Öngörülemeyen Hafif Stres Kaynaklı Depresyon Modelinde Propolisin Antidepresan Etkisinin Değerlendirilmesi

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### ABSTRACT

**Aim:** In this study, the antidepressant effect of propolis was investigated in a model of chronic unpredictable depression in rats.

**Materials and Methods:** Wistar-Albino male rats were used in the study and were divided into four groups as propolis, stress, stress + propolis, and control groups. Eight animals were assigned to each group. The experimental protocol was applied to the stress groups for 60 days, and the animals were exposed to different stressors. Propolis extract (100 mg/kg) was administered orally to propolis and stress + propolis groups throughout the experimental protocol. As a result of depression modeling, the Forced Swimming Test, Sucrose Preference Test, and Elevated Plus Maze Test were applied for behavioral evaluation. Twenty-four hour urine samples were collected for quantitative analysis of serotonin 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxy indole acetic acid (5-HIAA) in urine by liquid chromatography-tandem mass spectrometry method. The animals were sacrificed as a result of the experiment process.

**Results:** It was seen that there was a statistical difference for behavioral tests between the groups ($p<0.05$). The administration of propolis to rats under stress has been shown to alter sugar consumption in rats ($p<0.05$). For Forced Swimming Test, there was a statistical difference between the stress group and the other groups. For 5-HT and 5-HIAA levels, there was no significant difference between the groups ($p>0.05$).

**Conclusion:** The findings have shown that propolis extract may help to prevent depression, thanks to its antidepressant-like effects.

**Keywords:** Serotonin, depression, propolis, chronic unpredictable stress model

### ÖZ

**Amaç:** Bu çalışmada, sıçanlarda kronik öngörülemeyen depresyon modelinde propolisin antidepresan etkisi araştırıldı.

**Gereç ve Yöntem:** Çalışmada Wistar-Albino erkek ratlar kullanıldı ve propolis, stres, stres + propolis, ve kontrol olmak üzere 4 gruba ayrıldı. Her gruba sekiz hayvan atandı. Deney protokolü stres gruplarına 60 gün süreyle uygulandı ve hayvanlar farklı stresörlere maruz bırakıldı. Propolis ekstresi (100 mg/kg) propolis ve stres+propolis gruplarına deney protokolü boyunca oral yoldan verildi. Depresyon modellemesi sonucunda davranışsal değerlendirme için Zorunlu Yüzme Testi, Sükroz Tercih Testi ve Yükseltilmiş Artı Labirent Testi uygulandı. Sıvı kromatografi-tandem kütle spektrometresi yöntemi ile idrarda serotonin 5-hidroksi triptamin (5-HT) ve metaboliti 5-hidroksi indol asetik asidin (5-HIAA) miktarsal analizi için 24 saatlik idrar örnekleri toplandı. Deney işlemi sonucunda hayvanlar sakrifiye edildi.

**Bulgular:** Gruplar arasında davranış testleri açısından istatistiksel olarak anlamlı fark olduğu görüldü ($p<0,05$). Sıçanlara stres altında propolis verilmesinin sıçanlarda şeker tüketimini değiştirdiği gösterildi ($p<0,05$). Zorunlu Yüzme Testi için, stres grubu ile diğer gruplar arasında istatistiksel olarak fark vardı. 5-HT ve 5-HIAA düzeyleri için gruplar arasında anlamlı fark yoktu ($p>0,05$).

**Sonuç:** Bulgular, propolis özütünün antidepresan benzeri etkileri sayesinde depresyonu önlemeye yardımcı olabileceğini göstermiştir.

**Anahtar Kelimeler:** Serotonin, depresyon, propolis, kronik öngörülemeyen stres modeli

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## INTRODUCTION

Depression is one of the most common mood disorders in society. It is also a common health problem associated with a high threat of death from other attendant medical disorders. Depression is characterized by wakefulness or inordinate somnolence, fatigue or loss of energy, loss of sense of control, and private experience of great torture. It affects the thinking and performing processes of the existent, greatly reducing his social part and productivity[1].

When the basic physiology and pathophysiology of depression are examined, it has been seen that some neurotransmitters in the brain are directly active in this process. It is known that serotonin 5-hydroxytryptamine (5-HT), dopamine, and noradrenaline neurotransmitters fall to critical levels in depressive moods, and the working mechanisms of these chemicals are interrupted. Antidepressant drug treatments developed from this point of view are responsible for increasing the levels of these neurotransmitters in the brain to healthy mood levels and controlling various inhibition and activation processes[2].

In recent years, it has been seen that alternative medicine applications are used as treatment options as well as existing drug treatments. It is thought that the reasons for this situation are the reduction of chemical damage with alternative medicine applications, problems in accessing the drug required for treatment, and the treatment process with fewer drugs by using alternative medicine applications in diseases with high comorbidities such as depression[3].

Propolis, which is the most studied substance among bee products, is known as a product that benefits the body in many ways. Considering the usage areas of propolis as a treatment tool, it has been seen that it is used as a treatment option in cancer, neurological disorders, dentistry, cardiovascular, digestive, and dermatological diseases. Propolis components (caffeic acid phenethyl ester, flavonoids) are known to exhibit neuroprotective effects against oxidative damage in a model of induced ischemia and neurodegenerative disorders, including Alzheimer's disease[4]. Concerning its effect on the central nervous system (CNS), several studies have suggested that propolis has neuroprotective effects in both in vitro and in vivo models. However, the effect(s) of propolis on the CNS, such as depressant and anxiolytic effects, have been poorly reported[5]. Therefore, investigating the effect of propolis on changing behavior and mood and on neurotransmitter levels is considered substantial to define its relationship with depression. Since it is known that it is a crucial requirement for alternative medicine application areas such as apitherapy and phytotherapy to offer proven activities, it is scientifically invaluable that such studies both obtain new results and provide results that support existing research[6].

Considering this information, in this study, it is aimed to investigate the antidepressant effect of propolis using a chronic unpredictable depression model in rats. The behavior of animals exposed to the stressors presented in the experimental procedure, in proportion to their current mood, was examined with determined behavioral tests, and the levels of 5-HT and its metabolite, 5-hydroxy indole acetic acid (5-HIAA) were compared in urine samples collected from animals.

## MATERIALS AND METHODS

### Chemicals

The 12% sucrose solution used in the experimental process was obtained from Bilgi Kimyevi Laboratory Products Manufacturing Consultancy Analysis Services Industry and Trade Limited company (İstanbul, Turkey). To determine 5-HT and its metabolite 5-HIAA in urine, CE-IVD certified and validated Jasem HVA-VMA-5HIAA in Urine LC-MS/MS Analysis Kit was used (Sem Laboratuvar Cihazları Pazarlama San. ve Tic. Inc., İstanbul, Turkey). Glycerin (E422) and propolis extract (96 mg) were obtained from Aksu Vital Natural Products Joint Stock Company (İstanbul, Turkey) to prepare propolis extract in 50 mL glass bottles (192%; m/v) for the use in the experimental process. Other chemicals were obtained from Sigma-Aldrich, United States of America.

### Animals

Wistar albino rats aged 12-16 weeks and weighing 300-400 g were used in this study. The rats were housed in a temperature and light-controlled room (12 h dark-light cycles, 22±2 °C, and humidity 60±5%). All animals were free to access water and pellet food, and experiments were performed according to national laws and guidelines. The Laboratory Animal Care and Use Guidelines were taken into account. The protocol used in this study was approved by the Üsküdar University on the Ethics of Animal Experiments of (ÜÜ-HADYEK), İstanbul, Turkey (decision no: 2020-17, date: 22.01.2021).

### Experimental Design

In this study, 32 rats were divided into four groups, including eight in each group. For the control group, 0.4 mL of saline (0.9% NaCl) was administered to group animals by gavage for 60 days. In the propolis group, 0.4 ml/day (~ 100 mg/kg) propolis extract (192%; m/v) was administered by gavage to group animals daily for 60 days. The given propolis dose (100 mg/kg) was determined by taking into account a similar study[7]. In the stress group, the animals were exposed to various stressors for 60 days. At the same time, 0.4 mL of physiological saline (0.9% NaCl) was administered to the group animals by gavage. In the stress+propolis group, 0.4 mL/day

(~ 100 mg/kg) propolis extract (192%; m/v) was administered by gavage to the animals daily for 60 days. At the same time, group animals were exposed to various stressors for 60 days.

## Depression Model and Behavioral Tests

### Chronic Unpredictable Stress Model

As a depression model in the study, the chronic unpredictable stress procedure defined by López-López et al.[8] was applied as feed restriction (12 hours), water restriction (12 hours), permanent light (24 hours), crowded cage (24 hours), no stressor applied (24 hours), float in cold water (15 minutes), immobilization (1.5-2 hours), insulation (24-48 hours), wet sawdust (12 hours), lattice tilting 45° (5 hours), foreign object (5 hours), and changing animals between cages (12-24 hours).

The stress procedure mentioned above was applied to the stress and stress+propolis group experimental animals every day for 60 days. Each stress procedure was applied 8-10 times. To prevent the experimental animals from predicting the applied stress procedure, the same procedure was tried not to be applied consecutively. In addition, the stress procedure was applied at different times of the day. The body weights of the rats were determined before starting the stress model and after 60 days of exposure.

### Forced Swimming Test

The 40 cm high and 20 cm in diameter cylindrical glass container was filled with water up to 30 cm. The temperature of the water was kept at 24-26 °C. The animals in all groups were allowed to swim for 15 minutes on the first day to adapt to the experimental environment and learn, and then they were dried and placed back in their cages. After 24 hours, the subjects were allowed to swim for five minutes[9]. Video recording was made to score the animals' immobility (swimming periods where only the head is above the water but motionless), swimming, and climbing movements during the total time. Recordings were calculated by scoring (swimming, climbing, and immobility) at 5-second intervals by an unbiased observer[10]. The water in the bowl was changed after each animal. The animals taken from the lantern were dried and brought into a warm cage. Prolonged inactivity of rats is correlated with helplessness behavior, which is one of the important markers of depression, but it is a depression-like behavior. Studies have shown that the duration of inactivity is shortened as a result of antidepressant treatments applied[11].

### Sucrose Preference Test

The Sucrose Preference Test (SPT) measures aversion to pleasure (Anhedonia) in experimental animals. Anhedonia, one of the main symptoms of major depression, is measured with SPT, which is used to Anhedonia in experimental animals. Initially, two different water bottles were placed on the right and left sides of the cage. The experimental animals were allowed to drink water from both bottles for 24 hours, and the water bottles were changed every 12 hours. After two training days, 200 mL of water containing 2% sucrose was randomly placed in one of the bottles. The vials were weighed before and 24 hours after administration to the rats. Percent sucrose consumption was calculated according to the following formula in Equation 1[12,13].

% Sucrose Consumption=Sucrose consumption x100/Total consumption *Equation 1*

Total consumption: water and sucrose consumption was evaluated as the total consumption[12,13].

### The Elevated Plus Maze Test

The apparatus used for the elevated plus maze test is "+" shaped, perpendicular to two opposing open arms (25x5x0.5 cm) and a center and two closed arms (25x5x16 cm) with platform (5x5x0.5 cm). Open arms have a very small (0.5 cm) wall to reduce the number of falls, while closed arms have a high (16 cm) wall to surround the arm. The whole apparatus is 50 cm above the ground. The device is made of plastic materials. The platform is black, and the walls are opaque. All test rats were transferred to the behavioral test chamber 30 minutes before starting the first experiment to acclimate to the condition of the behavioral test chamber. A test trial using an application animal has two purposes. The first step is to ensure everything in the registry to be okay. Another important thing is to keep the test condition as monotonous as possible[14,15]. A rat was placed in the middle area of the maze, with its head directed towards a closed arm. The elevated plus maze test was recorded using a video camera connected to a computer controlled by a remote device. The number of entries in each arm (one entry is defined as the mouse's center of gravity entering the arm) and time spent in the open arms were recorded, and these measurements serve as indicators of anxiety-like behavior. Rats were allowed to move freely in the maze for 5 minutes. After each trial, all arms and core areas were cleaned with 70% alcohol, an effective deodorizing agent with a relatively weak odor compared to other cleaning solutions, to avoid bias based on olfactory cues[14].

### Sample Preparation Procedure for Quantitative Analysis of 5-HT and Its Metabolite 5-HIAA in Urine by LC-MS/MS Method

Twenty-four-hour urine samples were collected from rats in each group to analyze 5-HT and its metabolite 5-HIAA molecules in urine by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Urine samples were analyzed using

the colorimetric method to determine creatine levels. Since the urine samples of three rats in the experimental groups were insufficient, creatine and quantitative analyses could not be performed in these rats. Quantification of 5-HT and 5-HIAA in urine samples was carried out by Sem Laboratories using CE-IVD certified validated Jasem HVA-VMA-5HIAA in Urine LC-MS/MS Analysis Kit (Sem Laboratuvar Cihazları Pazarlama San. ve Tic. Inc., İstanbul, Turkey).

### Sample Preparation Procedure for Creatine Analysis in Urine Samples by Colorimetric -Jaffe Method

Urine samples were normalized via the creatinine level to provide a reduction for inter-individual variations in urine samples[16]. In order to evaluate the amount of 5-HT and its metabolite 5-HIAA in the urine, the creatine levels of the urine were determined, and the creatine amounts were included in the calculation. Using the formula in Equation 2 and Equation 3, normalized 5-HT and 5-HIAA values were calculated in the urine.

Normalized 5HT (mg/g crea)=5-HT (ppm)/ (Creatine(mg/dL)/100)
*Equation 2*

Normalized 5-HIAA (mg/g crea)=5-HIAA (ppm)/ (Creatine(mg/dL)/100)
*Equation 3*

Urine samples were centrifuged at 3500 rpm for 5 minutes. Urine samples given to the Cobas Integra 400 Plus biochemistry autoanalyzer device are automatically diluted with 1/25 distilled water in the device. The results are then obtained by reading at a 512/583 nm wavelength. After reading, the result is reached by automatic multiplication.

### Statistical Analysis

Data analysis was done using Statistical Package for the Social Sciences (SPSS) software. Considering the distribution of the data, they were found to be normally distributed ($p>0.05$). From this point of view, the t-test and one-way analysis of variance (ANOVA) test were used to examine the amount of variation between the groups. The Tukey and Tamhane tests, which are post-hoc tests, were used for the comparison between the groups in ANOVA tests that gave statistical significance, taking into account the homogeneity of population variances. All data were tested at a 95% confidence interval.

## RESULTS

### Body Weight

The bodies of the animals were planned to be ahead of the days when the training was scheduled. The groups were compared among themselves and the analysis was done using the t-test in Table 1. There was a significant difference between the initial and final weights in the control, propolis, and stress + propolis groups ($p<0.05$) (Figure 1).

### Evaluation of Behavioral Experiments

With the Forced Swimming Test, the immobility times of the animals were compared, and when the values in the groups were examined, there was a statistical difference between the stress group and the other groups (Figure 2).

Supportive periodontal therapy was applied to measure the anhedonia behavior, which is one of the depressive mood characteristics of animals exposed to stress procedures for 60 days, and the results were compared with a one-way ANOVA. Sucrose consumption was lower in the stress group than in all groups ($p<0.05$) (Figure 3).

In the elevated plus maze test performed to examine the effects of the animals' anxiety on their behaviors, the times of staying in the closed arm were compared between the groups and a significant difference was found between the stress group and the propolis and propolis + stress groups (Figure 4).

The open arm durations in the elevated plus maze test were compared between the groups and a significant difference was found between the propolis and stress groups, and between the stress + propolis group and the stress group (Figure 5).

### Quantitative Analysis of 5-HT and its Metabolite 5-HIAA in Urine

Chromatograms of 5-HT and its metabolite 5-HIAA analyzed in urine are given in Figure 6. The results of the quantitation of 5-HT and its metabolite 5-HIAA molecules in the urine, measured by the LC-MS/MS method and calculated according to the device analysis results and urinary creatinine levels, are given in Table 2. In each experimental group, three out of the rats, numbered from one to eight, could not have their creatinine and quantity determination analyses conducted due

**Table 1. Weights of the animals on the first and last days**

| Experimental groups | Day zero measurements (g) (mean ± standard error) | 60th day measurements (mean ± standard error) | p value (t-test) |
|---|---|---|---|
| Propolis | 354±8.88 | 402±10.17 | <0.05 |
| Stress | 384.37±13.85 | 390.37±13.87 | >0.05 |
| Stress+propolis | 349.37±10.78 | 391.31±8.96 | <0.05 |
| Control | 361.14±20.63 | 422.28±10.17 | <0.05 |

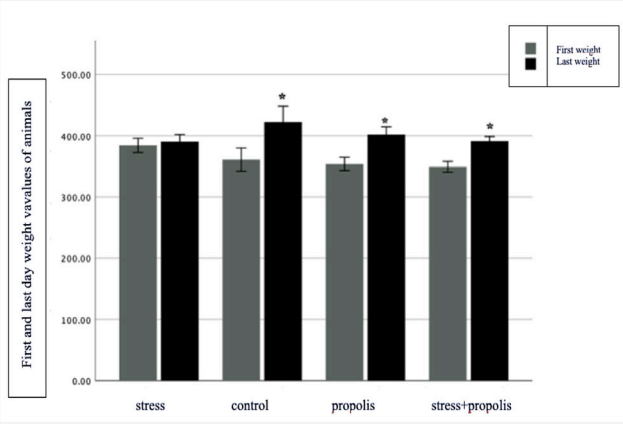


**Figure 1.** First and last day weight values of animals. *p<0.05 shows that there is a significant difference between the initial and final weights in the control, propolis and stress+propolis groups (t-test)

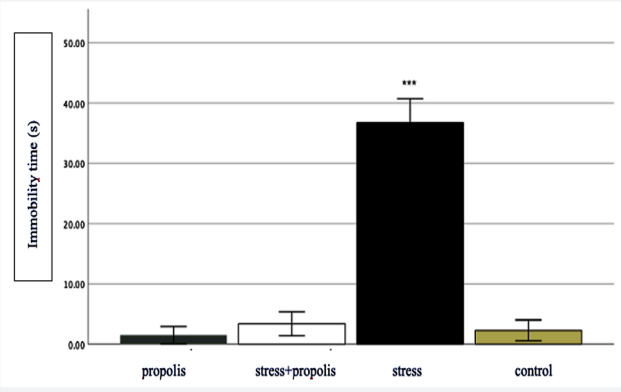


**Figure 2.** Immobility times in the forced swim test. ***p<0.05 shows a significant difference in the comparison of the stress group with the other groups (ANOVA). The differentiation status between the groups was analyzed by the Tamhane from Post-Hoc tests

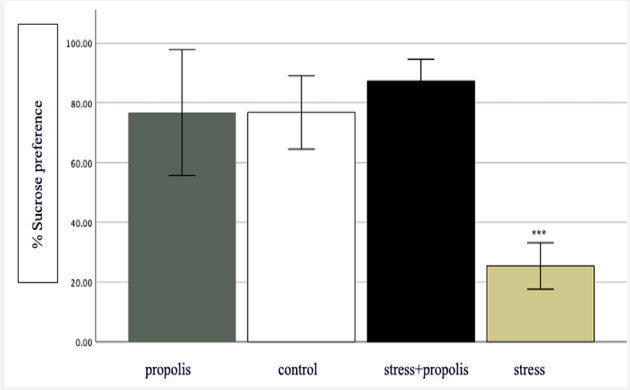


**Figure 3.** Sucrose consumption of two experimental groups. ***p<0.05 indicates that the stress group consumed significantly less sucrose than the other groups (ANOVA). The Tukey test was used as a Post-Hoc test

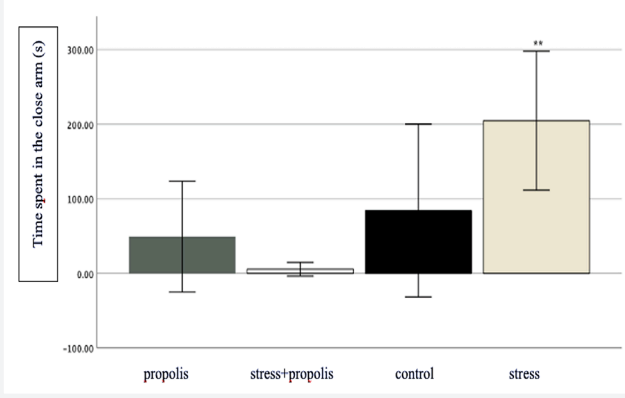


**Figure 4.** Time spent in the closed arm in the 4 elevated plus maze test. **p<0.05 shows that the duration of the stress group is significantly higher than the propolis and stress + propolis groups (ANOVA). The Tamhane test was used as a Post-Hoc test

to insufficient urine samples collected during the study. The analyses for the quantitative determination of rats labeled control 7, propolis 2, and propolis 5 were not performed and thus were not included in the calculations. The values for quantitative determination of the analyzed rats are presented in Table 2.

Figure 7 shows the calculated results of 5-HT and 5-HIAA levels. Table 2 and Figure 7 show that there is no significant difference between the groups (p>0.05).

## DISCUSSION

Whether propolis has a positive effect on mood disorders, especially depression, is one of the issues that researchers have focused on in recent years. It is thought that the potential antidepressant effect of propolis, as an apigenin-containing product, stems from this[17-20]. Studies have shown that apigenin has an antidepressant-like effect on dopamine and norepinephrine, and according to a study with mice, it has a reversal effect on decreased sucrose consumption due to depressed mood and increased inactivity times in floatation tests. In this study, which aimed to achieve supportive results for being an alternative and easily applicable treatment option, the effect of propolis on the 5-HT level was investigated in a model of chronic unpredictable mild stress-induced depression in rats by biochemical analysis. In addition to biochemical parameters, the effectiveness of propolis on behavioral tests was also examined, and in general, it was seen that propolis created differentiation in behavioral tests. Still, there was

no statistically significant difference despite observable differences in biochemical parameters.

In previous studies, it has been shown that rats exposed to chronic, unpredictable mild stress procedures have a decrease in body weight. Similarly, in this study, there was a stress-induced reduction in the weight of the animals, as seen in Table 1. The findings obtained were statistically significant, as shown in Figure 1. However, propolis, our focus of study, had a remarkably positive and significant effect on both the decrease in body weights and the recovery of the behaviors observed in the increased plus labyrinth test with the forced swimming test due to anhedonia and returning to normal values. This is a promising indication of the potential of propolis in stress management. In another study on the use of propolis in animals exposed to a chronic unpredictable stress procedure, a significant decrease was reported in the weight of stress group animals. It was noted that there was an increase in weight in the groups after applying of propolis[8], further reinforcing the positive effects of propolis.

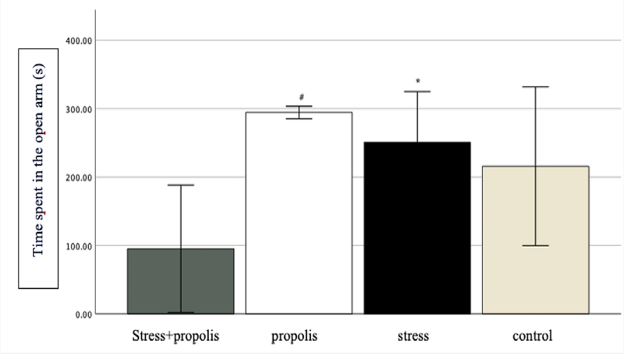


**Figure 5.** Open arm times in the elevated Plus Maze test, *p<0.05 propolis group spent longer time on the open arm than the stress group, #p<0.05 stress+propolis group spent longer time on the open arm compared to the stress group

The increase in the immobilization time in the forced swimming test is an indicator of depressive mood. Zangen et al.[21] studied the effect of antidepressant treatment on the change in 5-HT and 5-HIAA levels in genetically selected flinders susceptible strain (FSS) rats. In the selection of FSS rats, they exhibited the characteristic behavioral features of depression, such as decreased movement, increased anhedonia, increased amount of rapid eye movement (REM) sleep, decreased REM sleep onset, and cognitive difficulties in response to chronic mild stress[21]. In the study, animals were subjected to mandatory swimming tests before and after antidepressant treatment. It was observed that depressed animals remained inactive for a longer period compared to the control group, and this period was shortened after treatment. When the immobilization times in our study were evaluated, it was concluded that the stress group remained motionless in water significantly longer than the other groups. As shown in Figure 2, the results obtained are statistically significant (p<0.05). It was observed that animals in the stress group remained inactive longer than the control group did, which was shortened after propolis treatment (Figure 2). Our study results are similar to those in the literature. It has been demonstrated by the forced swimming test model that propolis has potential antidepressant activity. In addition,

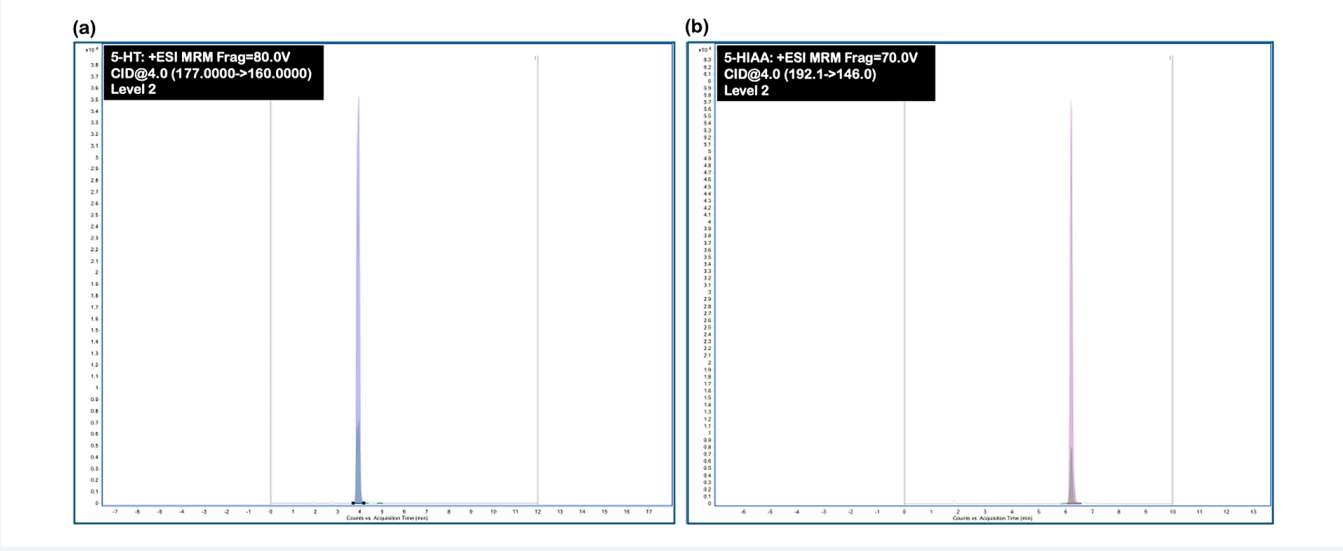


**Figure 6.** Chromatograms of serotonin 5-HT and its metabolite 5-HIAA in urine. (a) 5-HT (m/z: 177.0>160.0), (b) 5-HIAA (m/z: 192.1>146.0)
*5-HT: 5-hydroxytryptamine, 5-HIAA: 5-hydroxy indole acetic acid*

**Table 2. Results of 5-HT, 5-HIAA, and creatine assays**

| Sample | 5-HT (ppm) | 5-HIAA (ppm) | 5-HIAA/5-HT | Creatine (mg/dL) | Normalized 5-HT (mg/g crea) | Normalized 5-HIAA (mg/g crea) | Normalized (5-HIAA/5-HT) |
|---|---|---|---|---|---|---|---|
| Stress 1 | 0.386 | 4.656 | 0.01 | 93.25 | 0.414 | 4.993 | 12.06 |
| Stress 2 | 2.065 | 13.708 | 0.00 | 250.47 | 0.825 | 5.473 | 6.63 |
| Stress 3 | 0.373 | 9.065 | 0.02 | 195.88 | 0.191 | 4.628 | 24.23 |
| Stress 4 | 0.223 | 1.754 | 0.00 | 71.7 | 0.311 | 2.447 | 7.87 |
| Stress 5 | ND | 14.364 | NC | ND | NC | NC | NC |
| Stress 6 | 0.676 | 2.531 | 0.00 | 60 | 1.127 | 4.219 | 3.74 |
| Stress 7 | 0.879 | 8.992 | 0.01 | 156.25 | 0.563 | 5.755 | 10.22 |
| Stress 8 | 0.932 | 15.079 | 0.01 | 197.55 | 0.472 | 7.633 | 16.17 |
| Stress+propolis 1 | 0.465 | 7.890 | 0.01 | 129.14 | 0.360 | 6.110 | 16.97 |
| Stress+propolis 2 | ND | 9.788 | NC | ND | NC | NC | NC |
| Stress+propolis 3 | 0.287 | 10.264 | 0.03 | 128.41 | 0.224 | 7.993 | 35.68 |
| Stress+propolis 4 | 0.531 | 9.241 | 0.01 | 138.74 | 0.383 | 6.661 | 17.39 |
| Stress+propolis 5 | 0.586 | 7.617 | 0.01 | 134.98 | 0.434 | 5.643 | 13.00 |
| Stress+propolis 6 | 0.535 | 6.772 | 0.01 | 108.52 | 0.493 | 6.240 | 12.66 |
| Stress+propolis 7 | 0.662 | 9.577 | 0.01 | ND | NC | NC | NC |
| Stress+propolis 8 | 0.722 | 7.004 | 0.00 | 123.08 | 0.587 | 5.691 | 9.69 |
| Control 1 | 0.385 | 4.385 | 0.01 | 95.42 | 0.404 | 4.596 | 11.38 |
| Control 2 | 0.979 | 11.718 | 0.01 | 153.14 | 0.639 | 7.652 | 11.97 |
| Control 3 | 0.415 | 7.410 | NC | 111.79 | 0.372 | 6.628 | 17.82 |
| Control 4 | 1.194 | 2.693 | 0.00 | 62.5 | 1.911 | 4.309 | 2.26 |
| Control 5 | 0.150 | 3.402 | 0.02 | 74.37 | 0.203 | 4.574 | 22.53 |
| Control 6 | 1.138 | 1.062 | 0.00 | 35.65 | 3.194 | 2.980 | 0.93 |
| Control 8 | 0.382 | 5.157 | 0.01 | 107.02 | 0.357 | 4.818 | 13.50 |
| Propolis 1 | 0.603 | 2.181 | 0.00 | 66.86 | 0.903 | 3.262 | 3.61 |
| Propolis 3 | 0.440 | 3.333 | 0.00 | 195.88 | 0.225 | 1.702 | 7.56 |
| Propolis 4 | 0.304 | 5.889 | 0.01 | 71.7 | 0.425 | 8.214 | 19.33 |
| Propolis 7 | 0.294 | 3.254 | 0.01 | 156.25 | 0.188 | 2.083 | 11.08 |
| Propolis 8 | 0.216 | 5.752 | 0.02 | 197.55 | 0.109 | 2.912 | 26.71 |

Study groups in rats: Animals in each group are numbered from one to eight individually. The analyses for the quantitative determination of rats labeled Control 7, propolis 2, propolis 5, and propolis 6 were not performed due to insufficient urine samples collected during the study and thus were not included in the calculations.
5-HT: 5-hydroxytryptamine, 5-HIAA: 5-hydroxy indole acetic acid, ND: Not detected, NC: Not calculated, no significant results were obtained between the groups ($p>0.05$) (ANOVA)

as seen in Figure 3, low consumption of sucrose, one of the anhedonic behavioral characteristics, is consistent with the literature, especially in the stress group[22,23]. Sugar consumption in the propolis groups is similar to that in the control group. The administration of propolis to rats under stress has been shown to alter sugar consumption in rats (Figure 3).

The elevated plus maze test is one of the tests used to measure anxiety in experimental animals. According to the evidence presented in the literature, the time spent in the closed arm increases compared to the time spent in the open arm in rats with anxiety behavior[15]. When the rats stayed in the closed and open arms were compared in the study, results consistent with those in the previous study were obtained. According to the findings obtained in Figure 4 and Figure 5, there was a significant difference between the groups regarding duration ($p<0.05$). When the data of propolis groups were evaluated, it was found that propolis decreased anxiety-like behaviors in rats.

Studies examining the relationship between depression and serotonin metabolism have shown that elevated 5-HIAA levels may be associated with depression and that the 5-HT cycle in patients increases during depression, especially according to 5-HT conversion measurement results in patients with major depressive disorder[24,25]. 5-HT is found in various tissues and platelets of the digestive system and CNS and is widely distributed in our body. As a hydrophilic

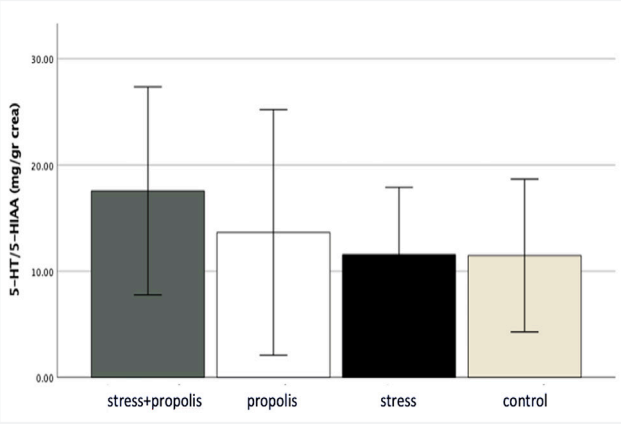


**Figure 7.** Average values of 5-HT/5-HIAA device result in urine. No significant results were obtained between the groups (p>0.05) (ANOVA)

*5-HT: 5-hydroxytryptamine, 5-HIAA: 5-hydroxy indole acetic acid*

substance, 5-HT cannot cross the blood-brain barrier[26,27]. Monoamine oxidase is primarily responsible for the metabolism of 5-HT, which is converted to 5-HIAA, the main metabolite, by the aldehyde dehydrogenase enzyme. It provides a stable means to measure the amount of serotonin in the body[28]. It has been shown in many studies that the change in serotonin levels is important, particularly for patients with depression, and that this condition is characterized by a decrease in serotonin levels. This decrease is thought to be due to the location of the raphe nuclei in the memory and cognition regions[29]. The measurement of serotonin levels and their metabolites in different biological fluids is given in the literature. Studies to identify possible biomarkers of depression have provided substantial evidence. To this end, Zhao et al.[30] compared the concentrations of monoamine neurotransmitters and amino acid neurotransmitters in plasma samples taken before and after fluoxetine administration in depression model rats. Considering the results, it was determined that the 5-HT, 5-HIAA concentrations of the depressive group were lower than those of the healthy controls, and it was emphasized that fluoxetine might have a crucial role in increasing the plasma concentrations of 5-HT, 5-HIAA[29]. However, in our study, no significant difference was found between the groups for 5-HT and 5-HIAA levels. The fact that the 5-HT and 5-HIAA levels shown in Table 2, which we obtained in our study, were not statistically significant (p>0.05), as seen in Figure 7, maybe because the sample we used was urine. Although there are consistent and reliable studies on using urine samples for metabolite determination, as a depression-related biochemical, 5-HT levels can be measured in blood and cerebrospinal fluid, and the tissues responsible for 5-HT release or inhibition can be directly examined. It may be possible to obtain a significant difference between the groups regarding 5-HT and 5-HIAA levels if they are examined in such different fluids and tissues. Some in the literature have obtained significant results from the studies conducted with these samples and tissues[27,31]. On the other hand, as is known, the monoamine hypothesis, which is the most accepted hypothesis regarding major depressive disorder, posits that the concentration of dopamine, 5-HT, and noradrenaline neurotransmitters in synaptic gaps decreases in the depressive state. This hypothesis is significant in the context of other hypotheses about major depressive disorder, as it provides a framework for understanding the role of neurotransmitters in depression[32]. Therefore, considering that multiple neurotransmitters are involved in the process, it is evident in our study that it is important to examine the levels of other neurotransmitters rather than just a single neurotransmitter when investigating the antidepressant effect of any substance. Although the findings obtained from behavioral tests in our study indicate that propolis extract may help prevent depression due to its antidepressant-like effects, the reason for these effects has not been explained solely based on serotonin levels.

The chronic unpredictable stress model is frequently used for depression modeling[23,31]. The use of natural stressors and making it possible to observe anhedonic behaviors in animals increase its validity. On the other hand, this model also has the disadvantage that it does not decrease the stress and anxiety levels of animals to the expected degree in case the animals adapt to stressors depending on their application for a certain period and routinely[33]. Therefore, different stressors were applied to the rats at different times during the experiment.

It is seen that the monoamine hypothesis is losing its validity with the studies conducted in recent years. The fact that one-third of people diagnosed with major depression do not respond to pharmacological treatments based on the current monoamine hypothesis shows that this hypothesis alone is not sufficient to explain depression, and other explanations are needed. These results may explain the significant behavioral results, although statistically significant results were not obtained in the chemical tests examined in our study. Considering that behaviors occur due to complex processes, especially in mammals, the meaningful results in behavioral tests show the effectiveness of propolis[23].

## Study Limitations

Due to the difficulties experienced at collecting urine samples from animals, a sufficient number of samples could not be taken from some animals, and these samples could not be included in the analysis. It is thought that the lost data caused by such reasons affect the results of the study.

## CONCLUSION

As a result, it was seen that the study created differentiation in terms of behavioral tests between animals exposed to chronic unpredictable stress due to propolis use and healthy subjects, and this literature supported this situation. As a result of behavioral tests, the findings showed that propolis extract might help prevent depression, thanks to its antidepressant-like effects. However, when the biochemical parameters in the urine samples were examined, no difference was found between the groups in the levels of serotonin and metabolites. The reasons for the lack of statistical significance in biochemical parameters were focused on, and in this direction, 5-HT, and 5-HIAA levels could be examined in different body fluids and tissues for future research. Considering that the results of the studies may have a lower efficiency due to the unpredictable conditions of the animals in the laboratory environment and controllable size, it is recommended to advance the experiments.

### Ethics

**Ethics Committee Approval:** The protocol used in this study was approved by the Üsküdar University on the Ethics of Animal Experiments of (ÜÜ-HADYEK), İstanbul, Turkey (decision no: 2020-17, date: 22.01.2021).

**Informed Consent:** Animal experiment.

### Authorship Contributions

Surgical and Medical Practices: A.T., F.C., S.N.Y., B.Ç., Concept: A.T., Design: A.T., Data Collection or Processing: A.T., S.N.Y., B.Ç., Analysis or Interpretation: A.T., F.C., Literature Search: A.T., Writing: A.T.

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# Prognostic Value of ALBI Score and Lymphocyte-Associated Inflammation Markers in Advanced Hepatocellular Carcinoma: A Single Centre Retrospective Cross-Sectional Study

## ALBI Skoru ve Lenfosit İlişkili Enflamasyon Belirteçlerinin İleri Evre Hepatoselüler Karsinomda Prognostik Değeri: Tek Merkez Retrospektif Kesitsel Çalışma

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### ABSTRACT

**Aim:** According to the information obtained from the World Health Organization database, the incidence of hepatocellular carcinoma (HCC) in Turkey increased by 17.78% between the years of 2018 and 2020. In this study, we investigated the prognostic value of albumin-bilirubin (ALBI) score and lymphocyte-associated inflammation markers on overall survival (OS) and progression-free survival (PFS) in advanced hepatocellular carcinoma.

**Materials and Methods:** Data of 141 patients with advanced HCC were included in this study. ALBI score and lymphocyte-associated inflammatory marker were calculated. As a result, the prognostic significance of these tests for survival were evaluated.

**Results:** The median age was 65 years (min: 26-max: 88). There were 58 (41.1%) hepatitis B virus (HBV) positive, 20 (14.2%) hepatitis C (HCV) positive and 63 (44.7%) patients with no history of hepatitis. Cut-off values of ALBI score and lymphocyte-associated inflammation markers were found by receiver operating characteristic analysis. ALBI (p<0.001), aspartate aminotransferase-to-lymphocyte ratio (ALRI) (p<0.001), prognostic nutritional index (PNI) (p=0.030), hemoglobin, albumin, lymphocyte, and platelet score (HALP) (p=0.003) scores were significantly associated with survival. In multivariate analysis, being ≥65 years old [hazard ratios (HR): 2.13; 95% confidence interval (CI): 1.44-3.17; p<0.001], ALRI≥30.79 (HR: 2.14; 95% CI: 1.20-3.82; p=0.009) predicted an increased risk of death and ALBI≥-2.54 (HR: 0.44, 95% CI: 0.29-0.69; p<0.001) predicted a decreased risk of death. Being ≥65 years old (HR: 174, 95% CI: 1.18-2.56; p=0.005) increased the risk of progression.

**Conclusion:** This study supports the statistically significant association of ALBI score and lymphocyte-associated inflammation markers (ALRI, PNI, HALP) with OS and PFS in advanced HCC patients. It is thought that this study will contribute to the literature and clinical practice.

**Keywords:** HCC, ALBI score, lymphocyte-associated inflammatory marker, ALRI, survival

### ÖZ

**Amaç:** Dünya Sağlık Örgütü veri tabanından edinilen bilgiye göre, Türkiye'de hepatosellüler karsinom (HCC) insidansı 2018-2020 yılları arasında %17,78 artmıştır. Bu çalışmada, ilerlemiş hepatosellüler karsinomda albümin-bilirubin (ALBI) skoru ve lenfosit ilişkili enflamasyon belirteçlerinin genel sağkalım (GS) ve progresyonsuz sağkalım (PFS) üzerindeki prognostik değerini araştırdık.

**Gereç ve Yöntem:** Bu çalışmaya 141 ileri evre HCC hastasının verileri dahil edildi. Tanı anındaki laboratuvar verileri kullanılarak ALBI skor ve lenfosit ilişkili enflamasyon belirteçleri hesaplandı. Sonuç olarak bu testlerin sağkalım için prognostik önemi değerlendirildi.

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**Bulgular:** Medyan tanı yaşı 65'ti (min: 26-max: 88). Hepatitis B virüs (HBV) pozitif 58 (%41,1), hepatitis C virüs (HCV) pozitif 20 (%14,2) ve hepatit öyküsü olmayan 63 (%44,7) hasta vardı. ALBI skoru ve lenfosit ilişkili enflamasyon belirteçlerinin alıcı çalışma karakteristiği analizi analizi ile cut-off değerleri bulundu. ALBI (p<0,001), aspartat aminotransferaz-lenfosit oranı (ALRI) (p<0,001), prognostik nutrisyonel indeks (PNI) (p=0,030) ve hemoglobin, albümin, lenfosit, trombosit skoru (HALP) (p=0,003) skoru ile sağkalım arasında anlamlı ilişki bulundu. Multivariate analizde ≥65 yaş olanların [hazard oranı (HR): 2,13; %95 güven aralığı (GA): 1,44-3,17; p<0,001], ALRI≥30,79 olmanın (HR: 2,14; %95 GA: 1,20-3,82; p=0,009) artmış ölüm riskini; ALBI≥-2,54 olmasının ise (HR: 0,44; %95 GA: 0,29-0,69; p<0,001) azalmış ölüm riskini predikte ettiği belirlendi. Altmış beş yaş üstü olmanın (HR: 1,74, %95 GA: 1,18-2,56; p=0,005) progresyon riskini artırdığı belirlendi.

**Sonuç:** Bu çalışma, ileri evre HCC hastalarında ALBI skoru ve lenfosit ilişkili enflamasyon belirteçlerinin (ALRI, PNI, HALP) GS ve PFS ile istatistiksel olarak anlamlı ilişkisini desteklemektedir. Bu çalışmanın literatüre ve klinik pratiğe katkı sağlayacağı düşünülmektedir.

**Anahtar Kelimeler:** HCC, ALBI skor, lenfosit ilişkili enflamatuar belirteç, ALRI, sağkalım

## INTRODUCTION

Liver cancer is the sixth most common type of cancer in the world. It is the fifth leading cause of death. Based on the World Health Organization (WHO) database GLOBOCAN, the incidence of hepatocellular carcinoma (HCC) in Turkey increased by 17.78% between the years of 2018 and 2020[1]. Differences in metabolic, endocrinological and behavioral factors in the etiology have been used to explain the frequency in men[2-4].

Risk factors for HCC, which is the most common histology in liver cancers, include alcohol, chronic viral hepatitis caused by hepatitis B virus (HBV) and hepatitis C virus (HCV), aflatoxin, obesity, non-alcoholic steatohepatitis (NASH) caused by metabolic syndrome, haemochromatosis and other rare causes. Chronic inflammation due to alcohol, chronic viral hepatitis and NASH triggers unbalanced cytokine release and hepatocarcinogenesis. Poor differentiation of hepatocytes initiates the process progressing to dysplastic nodules and HCC[1,5,6]. Immune and inflammatory responses are important in the prognosis of tumor progression[7].

In advanced hepatocellular carcinoma, immunotherapy, tyrosine kinase inhibitors and locoregional therapies added to these therapies are the treatment options offered according to patient characteristics. Many prognostic features and markers used in this treatment selection have been defined. The prognostic properties of the scores calculated by biochemical and hematological parameters were evaluated before the treatment plan. Albümin-bilirubin (ALBI) score provides the evaluation of liver function with an evidence-based, objective and simple method[8]. Lymphocyte-associated inflammation scores [ALRI[9], PNI[10], Hemoglobin, albümin, lenfosit, trombosit score (HALP)[11], systemic immune inflammation score (SII)[12], neutrophil/lymphocyte ratio (NLR)[13], platelet-lymphocyte ratio (PLR)[14], lymphocyte-monocyte ratio (LMR)[15] and systemic inflammation response index (SIRI)[16]] have been shown to predict survival in clinical studies in different solid tumors.

In this study, we analyzed the prognostic value of ALBI score and lymphocyte-associated inflammation markers calculated before the treatment plan on survival in advanced HCC.

## MATERIALS AND METHODS

This study included the data of 141 patients with advanced hepatocellular carcinoma who were followed up in Pamukkale University Faculty of Medicine, Department of Medical Oncology between July 2009 and March 2023. The approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Pamukkale University (approval number: E-60116787-020-476142, date: 15.01.2024). The entire clinic database was screened. Patients with missing clinicopathological data, severe infection, poor performance score (PS), second primary malignancy and chronic immunological disease were considered as exclusion criteria. Patients aged 18 years and older were included in the study. Age, chronic habits (smoking, alcohol), hepatitis markers and treatment history (systemic treatment, surgery, locoregional treatments) were recorded from patient files. Hematological and biochemical parameters measured before the treatment were retrospectively recorded from the hospital laboratory information system. Child, ALBI, ALRI, PNI, HALP, SII, NLR, PLR, LMR and SIRI scores were calculated using hematological and biochemical values. ALBI score=[log10 bilirubin (micromol/L) x 0.66]+[albumin (g/L)x-0.085] [ALBI grade 1 (score≤-2.60), grade 2 (score>-2.60 with≤-1.39) and grade 3 (>-1.39)], SII=platelet*neutrophil/lymphocyte, NLR=neutrophil/lymphocyte, PLR=platelet/lymphocyte, LMR=lymphocyte/monocyte, SIRI=neutrophil*monocyte/lymphocyte, ALRI=AST/lymphocyte, PNI=albumin(g/L)+5*lymphocyte($10^9$/L) and HALP score=hemoglobin(g/L)*albumin(g/L)*lymphocyte (*$10^9$/L)/platelet(*$10^9$/L) were calculated.

### Statistical Analysis

Statistical analyses were performed using "IBM SPSS Statistics for Windows version 25.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA)". Descriptive statistics are presented as median ± standard deviation for continuous variables, and as n and % for categorical variables. Receiver operating characteristic (ROC) analysis was used to find the cut-off value of prognostic scoring (ALBI, ALRI, PNI, HALP, SII, NLR, PLR, LMR, SIRI). The Kaplan-Meier method was used for survival [overall survival (OS), PFS] analyses. Univariate analysis

was performed. Finally, multivariate Cox regression results were given for the evaluation of statistically significant parameters in survival analysis. P<0.05 was considered statistically significant.

## RESULTS

One hundred-forty one patients were included. The median age at the time of diagnosis was 65 years (min: 26-max: 88). Eastern Cooperative Oncology Group PS was '0-1' in 118 (83.7%) patients. The mean body mass index was 25.90±5.62. Of patients, 126 were male (89.9%) and male/female ratio was 9:1. HBV positive 58 (41.1%), HCV positive 20 (14.2%) and 63 (44.7%) patients had no history of chronic hepatitis. Forty eight (77%) of the patients without chronic hepatitis had a history of diabetes mellitus and steatohepatitis. They were thought to be HCC cases developing on the background of non-alcoholic steatohepatitis. One patient had haemochromatosis in the etiology, and received only locoregional treatment and no systemic treatment (mOS: 5 months). Alcohol use was present in 20 (14.2%) patients. Asymptomatic patients comprised 51 (36.2%) of the cohort. The presenting complaints were abdominal pain in 60 (42.6%), fatigue in 20 (14.2%), abdominal distension in 17 (12.1%) and jaundice and nausea and vomiting in 4 (2.8%) patients. The primary localization of the tumor was right lobe in 72 (51.1%), left lobe in 18 (12.8%) and multifocal in 51 (36.2%) patients. Tumor size was ≤50 mm in 74 (52.5%) patients. The number of tumors was single lesion in 67 patients (47.5%) and multiple in 62 patients (50.0%). First-line treatment included 41 patients (28.7%) who received no treatment, 6 patients (4.2%) who received single agent doxorubusin, 93 patients (65%) who received sorafenib and 1 patient (0.7%) who received immunotherapy. First-line treatment response was complete response in 11 (7.7%) patients, stable disease in 4 (2.8%) patients and progressed disease in 85 (59.4%) patients. Sixty one (42.7%) patients had no side effects. The most common side effects were skin rash, diarrhea, mucositis and hypertension, which were observed in 39 (27.3%) patients. Very few patients received second-line treatment. Four patients (2.8%) received sorafenib and 11 patients (7.7%) received regorafenib. Thirteen of these patients (86.7%) developed progression and died. In the third-line setting, 1 patient received nivolumab. No local treatment was given to 73 patients (51%). Ablative treatments included TAKE: 49 patients (34.3%), TARE: 6 patients (4.2%) and RF: 9 patients (6.3%). After all treatments, progression developed in 118 (83.7%) patients and 116 (82.3%) patients died. The mean follow-up period of the patients was 19.99±25.26 months. Cut-off values were found by ROC analysis and cut-offs based on the state of exitus (Table 1). To assess the presence of a significant association that could predict overall survival, univariate analysis was performed. ALBI (p<0.001), ALRI (p<0.001), PNI (p=0.030) and HALP (p=0.003) parameters were statistically significant in the prediction of survival. OS and PFS at two and five years for ALBI score and lymphocyte-associated inflammatory markers according to ROC analysis cut-offs were evaluated. In the whole group, mPFS was 4.36 (95% CI: 2.98-5.75) months and mOS was 9.10 (95% CI: 5.60-12.58) months. Two and five-year OS (27.4%: 13.9%) and PFS (11.7%: 1.7%) values were found. Age (p=0.003), ALBI (p<0.001), ALRI (p<0.001), PNI (p=0.032) and HALP (p=0.021) groups showed significant association with mOS. Age (p=0.005), ALBI (p=0.014) and ALRI (p=0.017) groups showed significant association with mPFS (Table 2). In univariate analysis, the variables age, ALBI, ALRI, PNI and HALP had significant relationships to predict overall survival (p<0.05) (Table 3). These variables, which were found to be significant as a result of the univariate analysis, were included in the multivariate Cox regression model. After multivariable cox regression, age of 65 years and older [hazard ratios (HR): 2.13; 95% confidence interval (CI): 1.44 to 3.17; p<0.001], ALRI≥30.79 (HR: 2.14; 95% CI: 1.20 to 3.82; p=0.009) predicted an increased risk of death, and ALBI≥2.54 (HR: 0.44; 95% CI: 0.29-0.69; p<0.001) predicted a decreased risk of death (p<0.001, -2loglikelihood=896.27) (Figure 1).

**Table 1. ALBI score and lymphocyte-related inflammation markers cut-off values**

| Variables | AUC | 95% CI | Cut-off | Sensitivity (%) | Specificity (%) | p value |
|---|---|---|---|---|---|---|
| SII | 0.571 | 0.444-0.697 | ≥452.38 | 57.6 | 56.5 | 0.284 |
| ALBI | 0.789 | 0.702-0.876 | ≥-2.54 | 69.5 | 69.6 | **<0.001** |
| NLR | 0.581 | 0.447-0.714 | ≥2.97 | 56.8 | 56.5 | 0.222 |
| PLR | 0.627 | 0.504-0.750 | ≥113.01 | 56.8 | 56.5 | 0.054 |
| LMR | 0.566 | 0.436-0.697 | ≤3.23 | 52.5 | 52.2 | 0.315 |
| SIRI | 0.544 | 0.413-0.675 | ≥1.42 | 52.5 | 52.2 | 0.507 |
| ALRI | 0.722 | 0.618-0.825 | ≥30.79 | 65.3 | 65.2 | **<0.001** |
| PNI | 0.644 | 0.527-0.760 | ≤10.68 | 60.2 | 60.9 | **0.030** |
| HALP | 0.696 | 0.586-0.805 | ≤43 | 65.3 | 65.2 | **0.003** |

AUC: Area under the curve, 95% CI: Confidence interval, ALBI: Albumin-bilirubin score, SII: Systemic immune inflammation score, NLR: Neutrophil/lymphocyte ratio, LMR: Lymphocyte-monocyte ratio and SIRI: Systemic inflammation response index, ALRI: Aspartate aminotransferase-to-lymphocyte ratio, PLR: Platelet-lymphocyte ratio, PNI: Prognostic nutritional index, HALP: Hemoglobin, albumin, lymphocyte, and platelet score. p<0.05 was considered statistically significant

In the univariate analyses, age, ALBI, ALRI variables were significantly associated with PFS (p<0.05). These significant variables were included in the multivariate cox's regression model. According to multivariate Cox regression model results, it was determined that having over 65 years of age (HR: 1.74,95% CI: 1.18-2.56; p=0.005) increased the risk of progression (p=0.001,-2 loglikelihood=846.36) (Figure 2).

## DISCUSSION

In this study, we analyzed the predictive value of ALBI score and lymphocyte-associated inflammation markers on prognosis in advanced HCC. There are studies in the literature calculating different prognostic scores in HCC using laboratory values before or after surgery. We found that ALBI score≥-2.54 (p<0.001), ALRI<30.79 (p<0.001), PNI≤10.68 (p=0.030) and HALP score>43 (p=0.003) were associated with longer overall survival. In the ROC analysis of the prognostic markers evaluated in the study, ALBI score (AUC=0.789) and ALRI score (AUC=0.722) had the largest AUC for mOS. Our findings suggest that ALBI score and lymphocyte-associated inflammation markers (ALRI, PNI and HALP) at the time of diagnosis may be prognostic indicators.

In addition to the immortality of cancer cells, genomic instability and inflammation are facilitating factors for cancer

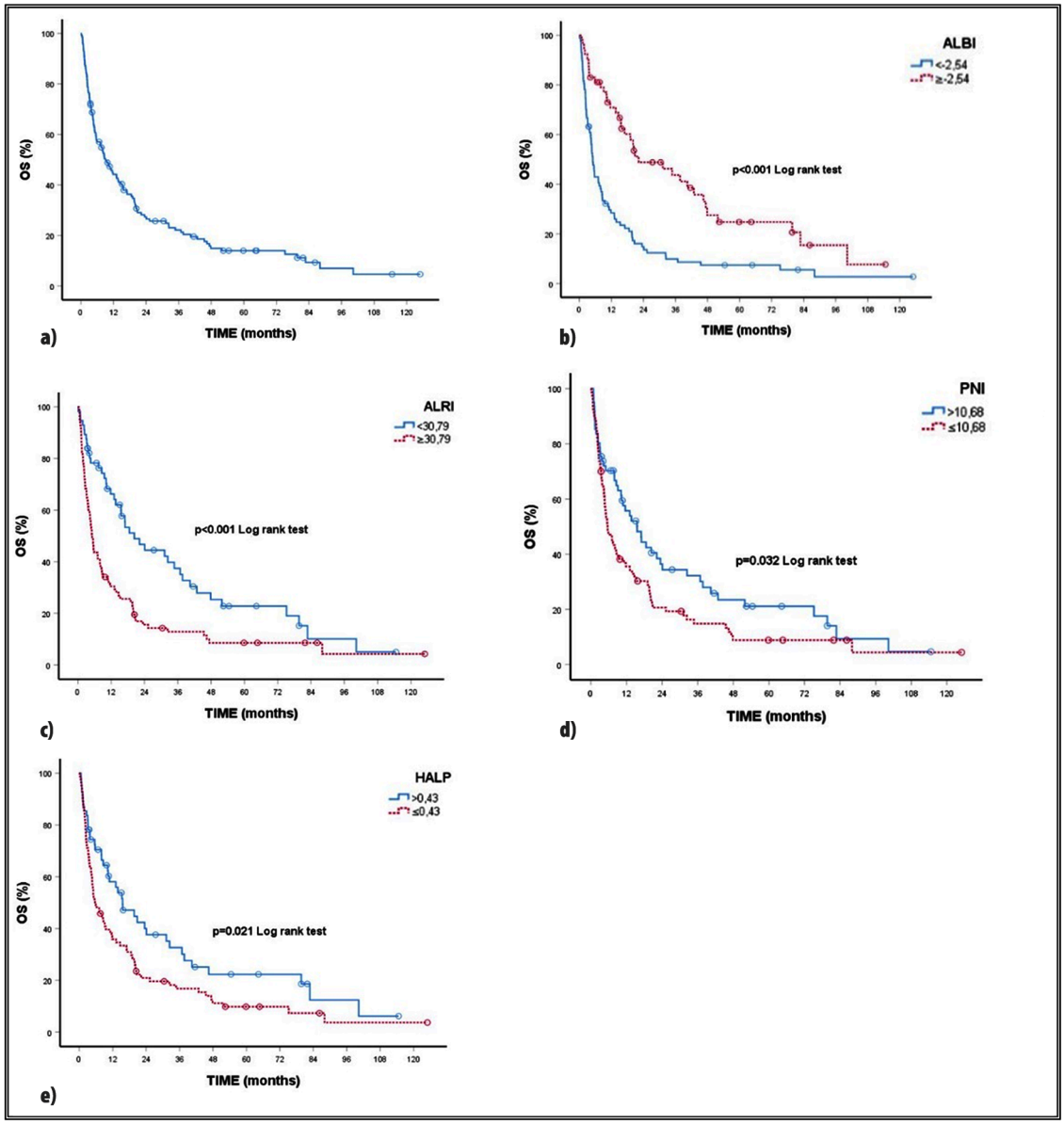


**Figure 1.** Graphical representation of multivariate Cox regression results on the effect of ALBI Score and Lymphocyte Related Prognostic Markers on survival

*ALBI: Albumin-bilirubin, OS: Overall survival, ALRI: Aspartate aminotransferase-to-lymphocyte ratio*

formation. The tumor microenvironment, which results from the interaction of cancer stem cells, cancer cells and stromal cells, is involved in tumor formation and progression[17].

Salazar-Onfray et al.[18] reported direct and indirect effects of cytokines. Cytokines may have direct effects by inhibiting and stimulating growth and indirect effects by triggering angiogenesis and causing inflammatory cell migration. Previously published studies have described that chronic inflammation is important in all processes of tumor formation, malignant transformation, invasion and metastasis. The inflammatory response in the tumor microenvironment provides escape from the immune response by causing neutrophilia, thrombocytosis, lymphopenia and lymphocyte dysfunction. It has been the subject of a large number of clinical studies that hematological and biochemical results of inflammatory reaction significantly predict the prognosis of solid tumors[17].

A systemic review about ALBI score has revealed that ALBI score better discriminates the prognosis in HCC patients compared to child-pugh, which is most frequently used by clinicians. This study suggests that the ALBI score alone is not sufficient to predict prognosis and that the predictive ability of ALBI should be improved. New algorithms are needed for prognosis prediction in HCC[8].

Zhao et al.[9] conducted a clinical study on 598 patients with HCC, who were receiving palliative care only, and the ALRI score was shown to be an independent prognostic factor in predicting OS (HR: 3.166, 95% CI: 1.411-7.103; p=0.005). In this study, two year survival was 20.0% in patients with ALRI<30.79 and 7.2% in patients with ALRI≥30.79 (HR: 2.14, 95% CI: 1.20-3.82; p=0.009). After multivariable analysis with patient age, ALBI, ALRI, HALP and PNI, survival was 7.0% for patients over 65 years old (HR: 2.13, 95% CI: 1.44-3.17; p<0.001) and ALRI≥30.79 (HR: 2.14, 95% CI: 1.20-3.82; p=0.009), while the risk of death increased in patients with ALBI≥-2.54 (HR: 0.44, 95% CI: 0.29-0.69; p<0.001). Multivariate analysis identified patient age, ALBI, and ALRI as independent prognostic factors for survival.

Chronic inflammation in the liver parenchyma due to different etiological causes triggers unbalanced cytokine release and hepatocarcinogenesis. The increase in AST seen in liver parenchymal damage is accompanied by loss of lymphocyte function in response to inflammation. The ALRI score obtained by formulating these variables is a prognostic marker. The ALBI score provides an evidence-based, objective and simple method for the evaluation of liver function. With its applicability to clinical practice, this result is considered a contribution to the literature.

Feng et al.[10] showed that PNI, GGT/ALT and tumor number evaluated before hepatectomy in 283 HCC patients were

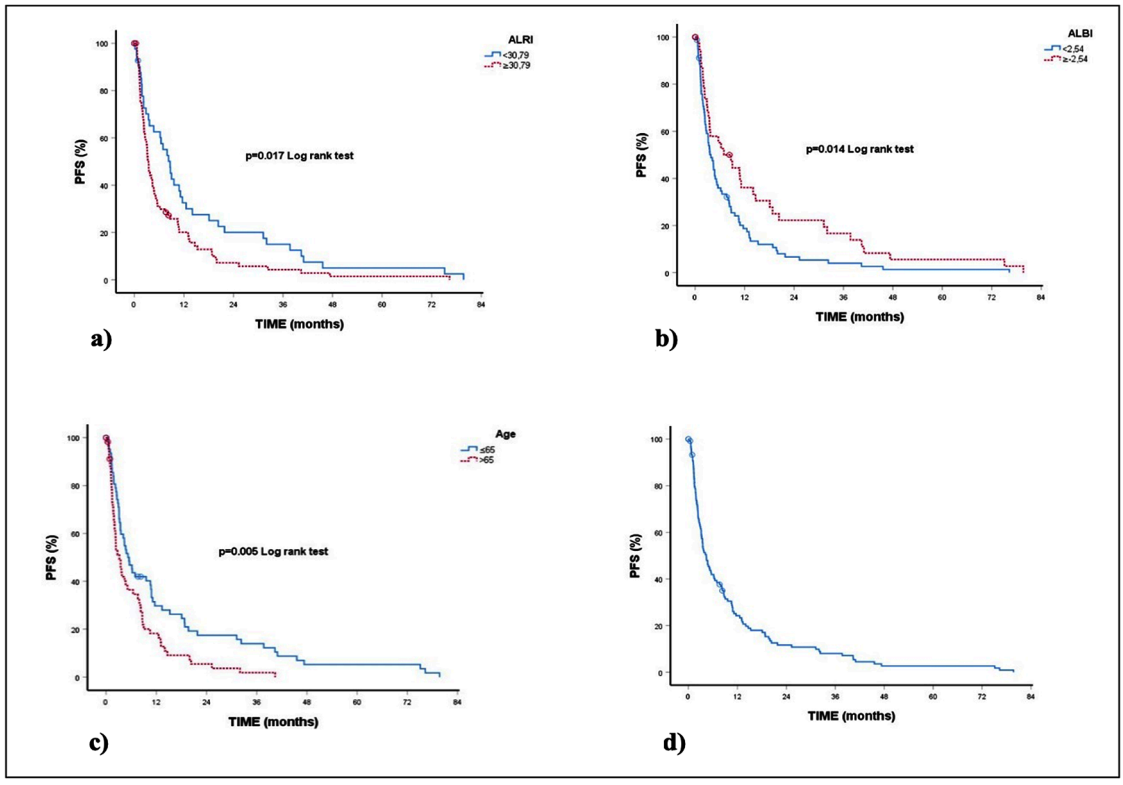


**Figure 2.** Multivariable Cox regression results of (a) ALRI, (b) ALBI, and (c) age variables and (d) progression-free survival curve in the entire patient group

*ALBI: Albumin-bilirubin, PFS: Progression-free survival, ALRI: Aspartate aminotransferase-to-lymphocyte ratio*

prognostic for OS in multivariate Cox regression analysis; PNI<48.48 (p=0.029) and gamma-glutamyl transferase (GGT)/alanine aminotransferase (ALT)≥1.65 (p=0.005) were associated with increasing OS and DFS. In this study, consistent results were obtained as reported in the literature. Two-year survival was 36.4% when PNI>10.68 and 20.6% when PNI≤10.68. Although a PNI≤10.68 was found to be significantly associated with increased survival in univariate analysis (HR: 5.63, 95% CI: 9.36-21.75; p=0.032); it was not found to be significant in multivariate analysis (HR: 0.65, 95% CI: 0.37-1.14; p=0.138). This marker, which assesses nutritional status and inflammation together, may be prognostic for survival.

HALP consists of hemoglobin, albumin, lymphocyte and platelet values. It provides evaluation of nutritional and inflammatory responses. In a clinical study with 273 HCC patients, Zhou and Yang[11] found that a preoperative HALP score below the cut-off value was significantly associated with a worse prognostic outcome (HR: 1.708, 95% CI: 1.192-2.448, p=0.004). In this study, the two-year survival rate in patients with a HALP score≤43 was 20.9% in a univariate analysis. In patients with HALP score >43, it was 40% (HR: 1.708, 95% CI: 7.30-23.76, p=0.021) and a significant relationship was found in accordance with the literature. The reason why HALP score >43 is associated with longer OS is based on the parameters in the formula. The absence of hypoxia due to anemia, adequate remaining liver parenchyma and absence of inflammation are associated with increased survival.

Katayama et al.[12] in a multi-center study on 1117 patients, a higher preoperative SII score was significantly associated with worse PFS in non-invasive bladder cancer (HR: 1.84, 95% CI: 1.23-2.77; p=0.003). In this study, high SII score was not significantly associated with survival prediction in HCC (95% CI: 0.444-0.697; p=0.222).

In another clinical study, in 166 non-small cell lung cancer patients with cranial metastases, survival was better in patients with NLR<5 calculated before radiosurgery (p=0.040). The increase in NLR value resulted in an increased risk of death (HR: 1.054, 95% CI: 1.024-1.085; p<0.001). However, it was reported that lymphocyte-based prognostic scoring (PLR, LMR) does not predict survival[13]. In this study, NLR rate did not predict OS (95% CI: 0.447-0.714, p=0.222), whereas lymphocyte-related inflammatory scores were significantly associated with survival (HR: 2.14, 95% CI: 1.20-3.82, p<0.001).

In a meta-analysis of 22 studies on PLR, the association between PLR and response to neoadjuvant chemotherapy was significant in 5533 breast cancer patients. It was found to be statistically significant (HR: 0.77, 95% CI: 0.67-0.88, p=<0.001) that high PLR value predicted low pathological complete response (PCR) and poor prognosis[14]. In this study, no significant association was found between high PLR score and survival in HCC (95% CI: 0.504-0.750, p=0.054). Negative results were thought to be due to the small patient numbers.

Neumann et al.[15] investigated prognostic scoring in 1294 pancreatic cancer patients using univariate and multivariate analyses. LMR≥1.6 (HR: 0.60; 95% CI: 0.61-0.79, p<0.001) and NLR<4 (HR: 1.5; 95% CI: 1.2-1.6; p=0.001) were found to significantly predict OS. In this study, no significant association was found with LMR and NLR cut-offs in survival analysis (95% CI: 0.436-0.697, p=0.315).

Another clinical study included 680 American prostate cancer patients of African and European descent. The median follow-up was 5.9 years and 194 deaths occurred. NLR>2.9 (HR: 1.23, 95% CI: 1.03-1.48; p=0.01), SII>430.8 (HR: 1.66, 95% CI: 1.06-2.60, p=0.01) and SIRI>0.9 (HR: 1.22, 95% CI: 1.02-1.46; p=0.01) were significantly associated with worse OS and DFS (prostate cancer-related mortality)[16]. In this study, NLR≥2.97 (95% CI: 0.447-0.714, p=0.222), SIRI≥1.42 (95% CI: 0.413-0.675, p=0.507) and SII≥452.38 (95% CI: 0.444-0.697, p=0.222) were not significantly associated with survival in HCC.

### Study Limitations

This study had some limitations that may have affected the results. Firstly, it included single center data and low number of patients. Moreover, since the data were collected retrospectively, all data that would increase inflammation may not have been recorded.

Unfortunately, these limitations were also present in the studies evaluating the effect of inflammation scores on survival in cancer patients in the literature. Although the data were collected retrospectively, inflammation scores were found to be statistically significantly associated with survival depending on cancer pathogenesis.

## CONCLUSION

In conclusion, there are studies on HCC and inflammation in preclinical, clinical and surgical areas with a large number of patients. In this study, we examined the predictive value of ALBI score calculated before the treatment plan and lymphocyte-associated inflammation markers (ALRI, PNI, HALP, SII, NLR, PLR, LMR and SIRI) on prognosis in advanced HCC. We found that ALBI score≥-2.54 and lymphocyte-associated inflammation markers ALRI<30.79, PNI≤10.68 and HALP>43 may be good prognostic indicators. In our clinical practice, the evaluation of patient age, ALBI score and ALRI score together will enable us to predict the survival in HCC patients. Multicenter prospective studies planned with a high number of patients may be recommended in the future.

| Table 2. Two-year and five-year OS and PFS values of ALBI score and lymphocyte-related inflammation markers | | | | |
|---|---|---|---|---|
| **OS (overall-survival) (months)** | **2 years %** | **5 years %** | **Median (95% CI)** | **p value** |
| **General** | 27.4 | 13.9 | 9.10 (5.60-12.58) | |
| **Age (years)** | | | | |
| ≤65 | 36.3 | 20.0 | 16.93 (9.04-24.82) | **0.003** |
| >65 | 17.8 | 6.8 | 5.86 (1.63-10.09) | |
| **Gender** | | | | |
| Male | 28.4 | 14.4 | 9.10 (5.45-12.75) | 0.786 |
| Female | 16.7 | - | 8.66 (2.87-14.46) | |
| **ALBI** | | | | |
| <-2.54 | 14.9 | 7.4 | 5.00 (3.93-6.06) | **<0.001** |
| ≥-2.54 | 48.8 | 24.8 | 22.20 (4.84-39.55) | |
| **ALRI** | | | | |
| <30.79 | 46.7 | 22.8 | 20.33 (11.29-29.36) | **<0.001** |
| ≥30.79 | 15.6 | 8.6 | 5.13 (4.09-6.17) | |
| **PNI** | | | | |
| >10.68 | 36.4 | 21.1 | 15.56 (9.36-21.75) | **0.032** |
| ≤10.68 | 20.6 | 8.9 | 5.63 (3.42-7.84) | |
| **HALP** | | | | |
| >43 | 40.0 | 22.3 | 15.53 (7.30-23.76) | **0.021** |
| ≤43 | 20.9 | 9.8 | 5.63 (2.33-8.93) | |
| **PFS (months)** | **2 years %** | **5 years %** | **Median (95% CI)** | **p** |
| **General** | 11.7 | 2.7 | 4.36 (2.98-5.75) | |
| **Age** | | | | |
| ≤65 | 17.5 | 5.2 | 5.26 (3.43-7.09) | **0.005** |
| >65 | 5.5 | - | 5.63 (1.78-4.61) | |
| **Gender** | | | | |
| Male | 12.8 | 2.9 | 4.13 (2.67-5.59) | 0.925 |
| Female | - | - | 4.36 (0.00-12.44) | |
| **ALBI** | | | | |
| <-2.54 | 6.7 | 1.3 | 3.80 (2.43-5.16) | **0.014** |
| ≥-2.54 | 22.2 | 5.6 | 6.93 (1.93-11.93) | |
| **ALRI** | | | | |
| <30.79 | 20.0 | 5.0 | 8.66 (6.11-11.22) | **0.017** |
| ≥30.79 | 7.2 | 1.4 | 3.43 (2.76-4.10) | |
| **PNI** | | | | |
| >10.68 | 13.1 | 4.4 | 6.26 (1.73-10.79) | 0.474 |
| ≤10.68 | 23.1 | 1.5 | 3.80 (2.75-4.84) | |
| **HALP** | | | | |
| >43 | 12.9 | 5.1 | 8.66 (5.57-11.75) | 0.135 |
| ≤43 | 11.7 | 1.5 | 3.43 (2.64-4.21) | |

OS: Overall survival, PFS: Progression-free survival, 95% CI: Confidence interval, ALBI: Albumin-bilirubin score, ALRI: Aspartate aminotransferase-to-lymphocyte ratio, PNI: Prognostic nutritional index, HALP: Hemoglobin, albumin, lymphocyte, and platelet score. p<0.05 was considered statistically significant

**Table 3. Multivariate Cox regression results for various clinical variables**

| | OS | | PFS | |
|---|---|---|---|---|
| | Multivariate | | Multivariate | |
| Variables | HR (95% CI) | p value | HR (95% CI) | p value |
| **Age (Ref:≤65)** | 2.13 (1.44-3.17) | **<0.001** | 1.74 (1.18-2.56) | **0.005** |
| **ALBI (Ref:<-2.54)** | 0.44 (0.29-0.69) | **<0.001** | 0.71 (0.46-1.12) | 0.145 |
| **ALRI (Ref:<30.79)** | 2.14 (1.20-3.82) | **0.009** | 1.47 (0.95-2.29) | 0.080 |
| **PNI (Ref:>10.68)** | 0.65 (0.37-1.14) | 0.138 | - | |
| **HALP (Ref:>43)** | 1.25 (0.80-1.94) | 0.312 | - | |
| | p<0.001; -2Log likelihood=896.27 | | p=0.001;-2Log Likelihood=846.36 | |

OS: Overall survival, PFS: Progression-free survival, 95% CI: Confidence interval, ALBI: Albumin-bilirubin score, ALRI: Aspartate aminotransferase-to-lymphocyte ratio, HALP: Haemoglobin, albumin, lymphocyte, and platelet score, PNI: Prognostic nutritional index, p<0.05 was considered statistically significant

## Ethics

**Ethics Committee Approval:** The approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Pamukkale University. (approval number: E-60116787-020-476142, date: 15.01.2024).

**Informed Consent:** Retrospective study.

## Authorship Contributions

Concept: M.Ö., G.G.D., Design: M.Ö., Data Collection or Processing: M.Ö., Analysis or Interpretation: M.Ö., G.G.D., B.Y.T., A.G.D., B.Ç.D., T.D., T.G.K., A.Y., S.D., S.T., B.A.Y., G.S.Ö., Literature Search: M.Ö., Writing: M.Ö.

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

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# Analysis of Epidemiological, Clinical, and Laboratory Characteristics of Patients Diagnosed with Brucellosis: A Comprehensive Study

## Bruselloz Tanılı Hastaların Epidemiyolojik, Klinik ve Laboratuvar Özelliklerinin Analizi: Kapsamlı Bir Çalışma

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**ABSTRACT**

**Aim:** Brucellosis, an endemic zoonotic disease within our nation, exhibits a notably high prevalence in the Southeastern, Eastern, and Central Anatolia regions. This study aims to assess the epidemiological, clinical, and laboratory characteristics, along with the complications, among both outpatient and inpatient cases diagnosed with brucellosis in Ağrı province.

**Materials and Methods:** This retrospective study analyzed 121 patients under the care of the Clinic of Infectious Diseases and Clinical Microbiology at Ağrı Training and Research Hospital between January 2022 and March 2024. Diagnosis of brucellosis was established based on clinical manifestations indicative of the disease, standard tube agglutination test titers of >=1/160, and/or isolation of *Brucella* spp./*Brucella melitensis* from blood cultures. Patients were categorized into acute, subacute, chronic (newly diagnosed), and relapsed groups based on their clinical presentations. Epidemiological, clinical, and laboratory parameters were evaluated across these patient groups.

**Results:** Among the 121 patients analyzed, 73 (60.3%) were female and 48 (39.7%) were male, with a mean age of 40.69 (±14.3) years. Of these patients, 87 (72%) were newly diagnosed, while 34 (28%) had experienced a relapse. Newly diagnosed patients exhibited notably higher rates of blood culture positivity and focal involvement compared to relapsed individuals (p=0.000, p=0.049, respectively). Elevated levels of C-reactive protein (CRP), sedimentation rate, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were observed among patients with organ involvement in comparison to those without organ involvement (p=0.001, p=0.022, p=0.013, p=0.035, respectively).

**Conclusion**: In regions where brucellosis is endemic, it should be considered among the primary differential diagnoses in patients presenting with fever. Biochemical markers such as CRP, sedimentation rate, ALT, and AST should be taken into consideration for assessing organ involvement in patients diagnosed with brucellosis. Combatting the disease requires a multidisciplinary approach, and healthcare professionals along with the local population should be educated about the disease and preventive measures.

**Keywords:** Brucellosis, epidemiologia, focal involvement, fever

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**ÖZ**

**Amaç:** Bruselloz ülkemizde endemik olarak görülen zoonotik bir hastalık olup, özellikle Güneydoğu, Doğu ve İç Anadolu bölgesinde yaygındır. Bu çalışmanın amacı Ağrı ilinde ayaktan ya da yatarak bruselloz tanısı alan hastaların epidemiyolojik, klinik ve laboratuvar bulgularını, komplikasyonlarını değerlendirmektir.

**Gereç ve Yöntem:** Bu çalışmada 2022 Ocak ve 2024 Mart tarihleri arasında Ağrı Eğitim ve Araştırma Hastanesi, Enfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Kliniği'nde takip edilen 121 hasta geriye dönük incelendi. Bruselloz tanısı, bruselloz düşündüren klinik bulgularla birlikte standart tüp aglütinasyon testi titre >=1/160 olan ve/veya kan kültüründe *Brusella* spp./*Brusella melitensis* üreyen hastalara konuldu. Hastalar klinik durumuna göre, akut, subakut, kronik hasta grupları (yeni tanı alanlar) ve relaps olarak gruplara ayrıldı. Hasta grupları epidemiyolojik, klinik ve laboratuvar değerleri ile değerlendirildi.

**Bulgular:** Olguların 73'ü (%60,3) kadın, 48'i (%39,7) erkekti, yaş ortalamaları 40,69 (±14,3) idi. Hastaların 87'si (%72) yeni tanı, 34'i (%28) relaps olarak değerlendirildi. Yeni tanı alanlarda relaps hastalara göre kan kültür pozitifliği ve fokal tutulum açısından anlamlı yükseklik saptandı (p=0,000, p=0,049). Organ tutulumu olan hastalarda olmayan hastalara göre C-reaktif protein (CRP), sedimantasyon, alanin aminotransferaz (ALT), aspartat aminotransferaz (AST) anlamlı şekilde yüksek saptandı (p=0,001, p=0,022, p=0,013, p=0,035).

**Sonuç:** Brusellozun endemik olduğu bölgelerde ateş varlığında ilk akla gelecek hastalıklardan biri bruselloz olmalıdır. Bruselloz tanısı konulan hastalarda CRP, sedimantasyon, ALT, AST gibi biyokimyasal belirteçler organ tutulumu açısından dikkate alınmalıdır. Hastalıkla mücadele multidisipliner olmalı ve sağlık çalışanları ve yerel halk hastalık ve önleme yöntemi hakkında bilgilendirilmelidir.

**Anahtar Kelimeler:** Bruselloz, epidemiyoloji, fokal tutulum, ateş

## INTRODUCTION

Brucellosis is a widespread zoonotic disease transmitted through the consumption of unpasteurized dairy products obtained from infected animals (such as cattle, sheep, goats, camels, and pigs), or through contact with the tissues or secretions of these animals[1]. Rare cases of transmission have been reported via blood transfusion, tissue transplantation, nosocomial infection, and sexual contact[2,3].

Brucellosis poses a significant threat to both human and animal health and imposes a substantial burden on national economies. The prevalence of brucellosis prevalence is closely associated with local livestock activities, with higher rates observed in rural areas known for intensive animal husbandry, particularly in regions such as Southeastern Anatolia, Eastern Anatolia, and Central Anatolia in Turkey. Individuals most commonly affected by the disease include those engaged in livestock farming, veterinarians, and laboratory workers[4].

The causative agent of brucellosis, *Brucella* spp., is a small, non-motile, facultative aerobic, intracellular bacterium that appears as Gram-negative coccobacilli in Gram staining. Among humans, *Brucella melitensis* is the most frequently encountered species[5].

The disease typically presents with symptoms such as fever, night sweats, and muscle and joint pain. Additionally, weight loss, headache, dizziness, loss of appetite, back pain, abdominal pain, and depression may also be present[6].

The incubation period of brucellosis is approximately 2-4 weeks. Based on the duration of symptoms, the disease is classified as acute if symptoms persist for the first 8 weeks, subacute if they last between 8 and 52 weeks, and chronic if symptoms persist for more than 52 weeks[7]. Recurrence of the disease within the first 6-12 months after treatment is classified as relapse[8].

Brucellosis can involve multiple tissues and organs. The most common manifestations include osteoarticular involvement, encompassing peripheral arthritis, sacroiliitis, and spondylodiscitis[9]. Additionally, it may affect the genitourinary system, central nervous system, cardiovascular system, ocular system, and skin[10,11].

Definitive diagnosis of brucellosis is established by isolating the causative agent from blood or other sterile body fluids through culture, or by observing a fourfold or greater increase in Brucella antibody titers between the acute and convalescent phases. A diagnosis may also be presumed if the standard tube agglutination (STA) test yields a titer of 1/160 or higher after the onset of symptoms[12].

Combination therapies form the cornerstone of brucellosis treatment. Nevertheless, despite treatment, relapse, chronicity, and organ involvement may occur, and there is no optimal recommendation for treatment regimen and duration in certain patient groups[13].

Brucellosis encompasses a wide range of clinical manifestations, from non-specific symptoms to severe organ involvement, mimicking many other diseases. This variability can lead

to delays in diagnosis and misdiagnosis[14]. Being the most common zoonotic disease worldwide, brucellosis continues to be of significance due to its impact on animal and human morbidity, reduction in animal productivity, and considerable economic burden, especially in endemic countries. Therefore, besides diagnosis and treatment, preventive measures to prevent disease transmission are equally important[15].

## MATERIALS AND METHODS

This retrospective study analyzed 121 patients who were either seen as outpatients or admitted to the Clinic of Infectious Diseases and Clinical Microbiology at Ağrı Training and Research Hospital between January 2022 and March 2024. Patients aged 18 years and above were included in the study.

The diagnosis of brucellosis was established in patients presenting with clinical manifestations suggestive of the disease, along with a STA test titer of ≥1/160 and/or isolation of *Brucella* spp./*Brucella melitensis* from blood cultures. Patient demographics, including age, gender, presence of comorbidities, occupational exposure to livestock, initial symptoms, physical examination findings, previous diagnosis of brucellosis, routine laboratory results, rose bengal and STA test results, blood culture results, hemogram, and biochemical data, were recorded.

Medical records pertaining to clinical follow-ups were scrutinized for evidence of systemic involvement, relapse, and development of complications. Patients with symptoms lasting less than 8 weeks were categorized as acute, those lasting between 8 and 52 weeks as subacute, and those lasting more than 52 weeks as chronic brucellosis cases. Within one year after the completion of treatment, patients exhibiting recurrent symptoms supported by physical examination and laboratory findings were classified as relapsed cases.

Diagnosis of brucellosis relied on either serological or culture positivity in conjunction with clinical findings. Serological test positivity was defined as an STA test titer ≥1/160 using specific antiserum (Ankara Public Health Laboratory, Turkey) or a ≥4-fold increase in STA test titer repeated 2-3 weeks apart. Detection of *Brucella* spp. and *Brucella melitensis* was performed using VITEK2 Compact (BioMérieux, France) and VITEK MS (BioMérieux, France) devices.

### Statistical Analysis

Descriptive statistics, including mean or median values for continuous variables and count (n) and percentage (%) values for categorical variables, were provided. The normality of continuous variables was assessed using the Shapiro-Wilk test. For normally distributed variables, independent samples t-test was utilized for between-group comparisons, while the Mann-Whitney U test was employed for non-normally distributed variables. The chi-square test was applied for comparisons between categorical variables. Statistical analyses were conducted using SPSS version 26 for Windows. Results were considered significant at $p<0.05$ level.

The study was conducted after obtaining the necessary permissions from Ağrı İbrahim Çeçen University Scientific Research Ethics Committee (decision no: E-95531838-050.99-98272, date: 29.03.2024).

## RESULTS

Of the cases, 73 (60.3%) were female and 48 (39.7%) were male, with a mean age of 40.69 (±14.3) years. Evaluation based on the place of residence revealed that 73 (60.3%) resided in rural areas, with 79 (65.3%) engaged in livestock farming, 104 (86%) consuming raw milk and dairy products, 3 (2.5%) exposed to laboratory hazards, and 4 (3.3%) with unidentified exposure. The most commonly observed comorbidities among patients were hypertension in 11 (9.1%) cases, coronary artery disease in 7 (5.8%) cases, and hyperlipidemia in 7 (5.8%) cases. Upon examining the demographic data of patients, no statistically significant differences were found between newly diagnosed and relapsed patients in terms of mean age, gender, comorbidities, place of residence, and exposure. The epidemiological data of patients are presented in Table 1.

The distribution of patients diagnosed with brucellosis by place of residence revealed the following proportions: city center 48 (39.7%), Diyadin 30 (24.8%), Taşlıçay 11 (9.1%), Hamur 9 (7.4%), Doğubayazıt 6 (5%), Patnos 6 (5%), Eleşkirt 6 (5%), and Tutak 5 (4.1%). The distribution of patients' places of residence throughout the province is depicted in Figure 1.

When patients were evaluated based on their clinical status at the time of diagnosis, 73 (60.3%) were diagnosed with acute brucellosis, 8 (6.6%) with subacute brucellosis, 34 (28%) with recurrent brucellosis, and 6 (5%) with chronic brucellosis. Assessment of presenting symptoms revealed that the predominant symptoms were joint pain in 113 (93.4%) cases, night sweats in 96 (79.3%), fatigue in 96 (79.3%), fever in 82 (67.8%), lower back pain in 80 (66.1%), and loss of appetite in 72 (59.5%). Fever was significantly more prevalent in patients with newly diagnosed brucellosis, while headache was more pronounced in relapsing cases. The presenting symptoms of patients are provided in Table 2.

Significant differences were observed in terms of blood culture positivity between newly diagnosed and relapse patients (p=0.000). All positive blood cultures were detected in newly diagnosed patients. Furthermore, a significant difference was found in terms of organ involvement between newly diagnosed and relapse patients (p=0.049-0.059), with a higher incidence of organ involvement observed in newly diagnosed cases. Focal

**Table 1. Demographic characteristics of patients diagnosed with brucellosis**

| | All cases (n=121) | New diagnoses (n=87) | Relapses (n=34) | p value |
|---|---|---|---|---|
| Mean age | 40.69 (±14.3) | 39.3(±14.4) | 44.2(±13.6) | 0.074 |
| Gender | | | | |
| Female | 73 (60.3%) | 54 (62.1%) | 19 (55.9%) | 0.542 |
| Male | 48 (39.7%) | 33 (37.9%) | 15 (44.1%) | 0.542 |
| Comorbidity | | | | |
| DM | 6 (5%) | 5 (5.8%) | 1 (2.9%) | 0.674 |
| HT | 11 (9.1%) | 9 (10.3%) | 2 (5.9%) | 0.726 |
| Autoimmune disease | 2 (1.7%) | 2 (2.3%) | 0 | 1 |
| CAD | 7 (5.8%) | 4 (4.6%) | 3 (8.8%) | 0.4 |
| HL | 7 (5.8%) | 3 (3.4%) | 4 (118%) | 0.096 |
| Osteoporosis | 2 (1.7%) | 0 | 2 (5.9%) | 0.079 |
| Asthma | 4 (3.3%) | 3 (3.4%) | 1 (2.9%) | 1 |
| Place of residence | | 35 (40.2%) | | |
| City center | 48 (39.7%) | 23 (26.4%) | 13 (38.2%) | 1 |
| Diyadin | 30 (24.8%) | 7 (8%) | 7 (20.6%) | 0.641 |
| Taşlıçay | 11 (9.1%) | 6 (6.9%) | 4 (11.8%) | 0.501 |
| Hamur | 9 (7.4%) | 5 (5.7%) | 3 (8.8%) | 0.710 |
| Doğubayazıt | 6 (5%) | 3 (3.4%) | 1 (2.9%) | 1 |
| Patnos | 6 (5%) | 5 (5.7%) | 3 (3.8%) | 0.348 |
| Eleşkirt | 6 (5%) | 3 (3.4%) | 1 (2.9%) | 1 |
| Tutak | 5 (4.1%) | 35 (40.2%) | 2 (5.9%) | 0.619 |
| Exposure | | | | |
| Livestock | 79 (65.3%) | 56 (64.4%) | 23 (67.6%) | 0.833 |
| Dairy products | 104 (86%) | 77 (88.5%) | 27 (79.4%) | 0.245 |
| Lab exposure | 3 (2.5%) | 3 (3.4%) | 0 | 0.558 |
| Undetermined | 4 (3.3%) | 2 (2.3%) | 2 (5.9%) | 0.314 |

DM: Diabetes mellitus, HT: Hypertension, CAD: Coronary artery disease, HL: Hyperlipidemia

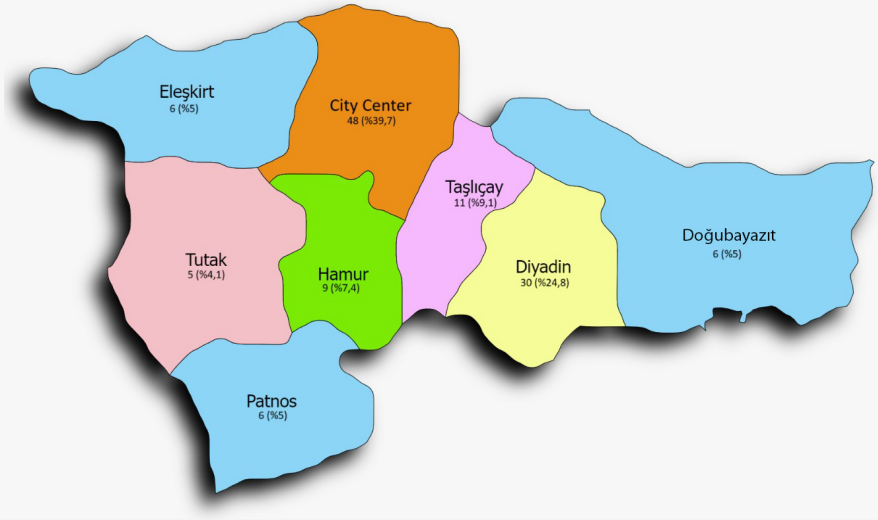


**Figure 1.** Distribution of patients throughout the province

involvement was present in 29 patients (24% of all cases), with the most common manifestations being sacroiliitis in 12 cases (41.3%), spondylodiscitis in 7 cases (24.1%), and peripheral arthritis in 9 cases (31%). Blood cultures were obtained from 84 patients (69.4%), with *Brucella melitensis* or *Brucella* spp. isolated in 24 cases. The results of blood cultures and organ involvement are presented in Table 3.

Out of the 84 patients from whom blood cultures were obtained, *Brucella* spp. or *Brucella melitensis* growth was observed in 24 cases. It was noted that blood culture positivity was mostly prevalent during the autumn season. The distribution of blood culture positivity according to months is presented in Figure 2.

Notably, when evaluating laboratory results, C-reactive protein (CRP), sedimentation rate, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were found to be significantly higher in complicated cases, with p values of 0.001, 0.002, 0.013, and 0.035, respectively. The laboratory values of patients with and without complications are presented in Table 4.

## DISCUSSION

Brucellosis continues to be endemic in areas where economic resources are limited, sanitation measures are lacking, and veterinary services are insufficient. Globally, it persists as an endemic disease in regions such as the Middle East, the Mediterranean, and Central and South America[16]. In Turkey, it is most commonly observed in rural areas where livestock farming is prevalent, particularly in the Southeastern Anatolia, Eastern Anatolia, and Central Anatolia regions[4]. In our study, we evaluated patients diagnosed with brucellosis residing in Ağrı province, and it was observed that the majority of patients lived in districts. This observation is consistent with the tendency for livestock farming activities to occur in rural areas. The higher number of diagnosed patients in Diyadin district may indicate the intense livestock farming and insufficient veterinary services in the area. Despite being the largest districts in the city, the lower number of diagnosed patients residing in Doğubayazıt and Patnos suggests that brucellosis diagnosis and treatment may be conducted in district hospitals within these regions.

**Table 2. Distribution of symptoms in patients diagnosed with brucellosis**

| Symptoms | All cases (n=121) | Newly diagnosed (n=87) | Relapse (n=34) | p value |
|---|---|---|---|---|
| Joint pain | 113 (93.4%) | 81 (93.1%) | 32 (94.1%) | 1 |
| Night sweats | 96 (79.3%) | 69 (79.3%) | 27 (79.4%) | 1 |
| Fatigue | 96 (79.3%) | 66 (75.9%) | 30 (88.2%) | 0.210 |
| Fever | 82 (67.8%) | 64 (73.6%) | 18 (52.9%) | **0.049** |
| Lower back pain | 80 (66.1%) | 55 (63.2%) | 23 (73.5%) | 0.393 |
| Anorexia | 72 (59.5%) | 53 (60.9%) | 19 (55.9%) | 0.682 |
| Headache | 63 (52.1%) | 39 (44.8%) | 24 (70.6%) | **0.015** |
| Weight loss | 55 (45.5%) | 41 (47.1%) | 14 (41.2%) | 0.685 |
| Abdominal pain | 35 (28.9%) | 24 (27.6%) | 11 (32.4%) | 0.658 |

**Table 3. Distribution of blood culture results and organ involvement in brucellosis patients**

| Blood culture | All cases (n=121) | Newly diagnosed (n=87) | Relapse (n=34) | p value |
|---|---|---|---|---|
| Collection frequency | 84 (69.4%) | 63 (72.4%) | 21 (61.7%) | 0.114 |
| Positivity | 24 (32.6%) | 24 (38%) | 0 | **0.000** |
| *Brucellosis* spp. | 7 (29.2%) | 7 (29.2%) | 0 | **0.000** |
| *Brucellosis melitensis* | 17 (70.8%) | 17 (70.8%) | 0 | **0.000** |
| **Organ involvement** | | | | |
| Focal brucellosis | 29 (24%) | 25 (28.7%) | 4 (11.8%) | **0.049** |
| Peripheral LAP | 1 (3.4%) | 1 (4%) | 0 | 1 |
| Splenic involvement | 3 (10.3%) | 2 (8%) | 1 (25%) | 1 |
| Sacroiliitis | 12 (41.3%) | 10 (40%) | 2 (50%) | 0.506 |
| Spondylodiscitis | 7 (24.1%) | 7 (28%) | 0 | 0.189 |
| Arthritis | 9 (31%) | 7 (28%) | 2 (50%) | 1 |
| Epididymo-orchitis | 2 (6.8%) | 2 (8%) | 0 | 1 |

LAP: Lymphadenopathy

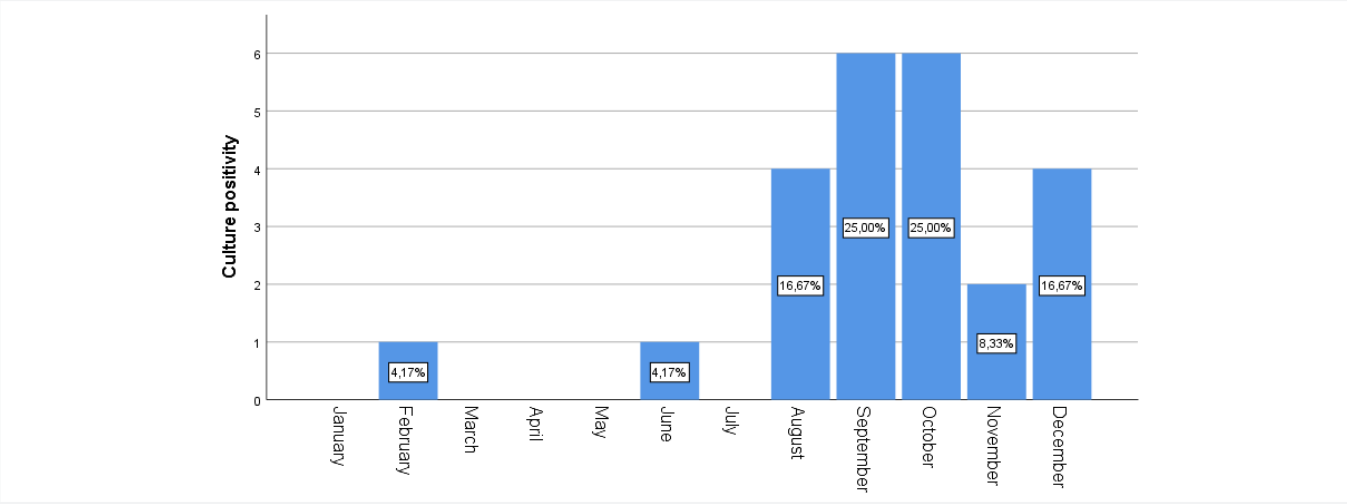


**Figure 2.** Distribution of blood culture positivity by month

**Table 4. Median laboratory results of complicated and non-complicated patients**

| Parameter | Non-complicated (n=78) | Complicated (n=26) | p value |
|---|---|---|---|
| WBC | 6595 | 7050 | 0.118 |
| NEU | 3885 | 3740 | 0.362 |
| LYM | 2125 | 2330 | 0.129 |
| MONO | 400 | 455 | 0.292 |
| HB | 13.8 | 13.7 | 0.550 |
| PLT | 261 | 258 | 0.993 |
| CRP | 4 | 13.5 | **0.001** |
| SED | 18.5 | 34.5 | 0.022 |
| ALT | 22 | 29 | 0.013 |
| AST | 22.5 | 24.5 | 0.035 |

WBC: White blood cell, NEU: Neutrophile, LYM: Lymphocyte, MONO: Monocyte, HB: Hemoglobin, PLT: Platelet, CRP: C-reactive protein, SED: Sedimentation, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

In our study, 73 (60.3%) of the cases were female, and 48 (39.7%) were male, with a mean age of 40.69 (±14.3) years. In a study conducted by Turkoglu-Yilmaz and Arslan[17], a retrospective evaluation of brucellosis patients over a 5-year period was conducted, revealing that 170 (72%) of 236 patients were male. In a meta-analysis comprising 57 studies examining the clinical manifestations of human brucellosis, it was found that 55% of the patients were male across all participant groups[18]. The higher number of female patients in our study, contrary to the literature, can be attributed to the smaller sample size.

In a study conducted by Almuneef et al.[19], consumption of unpasteurized raw milk was reported as the source of brucellosis in 75% of cases, while 45% were attributed to livestock handling. In our study, when analyzed in terms of transmission routes, it was found that the disease was most commonly transmitted through the consumption of raw milk and dairy products, accounting for 86% of cases. Secondly, 65.9% of cases were associated with occupational livestock handling. This suggests that even if local residents do not engage in livestock farming themselves, they obtain raw milk and dairy products and use them without pasteurization.

In a study conducted by Kuruoglu et al.[20], significantly elevated fever was observed in patients diagnosed with brucellosis across acute, subacute, chronic, and relapse patient groups. Fever was found in 79.2% of patients diagnosed with acute brucellosis. In a retrospective study by Buzgan et al.[21], encompassing the last 10 years and evaluating 1028 brucellosis patients, acute, subacute, and chronic brucellosis patients comprising the newly diagnosed group accounted for 96.8% of all patients, while relapse patients constituted 3.2% of the total. The most common symptoms observed in these patients were arthralgia

(73.7%) and fever (72.2%). In our study, the number of newly diagnosed brucellosis patients was 87 (72%), while the number of relapse brucellosis cases was 34 (28%). The most common symptom observed was arthralgia in 113 patients (93.4%), followed by night sweats in 96 patients (79.3%), fatigue in 96 patients (79.3%), and fever in 82 patients (67.8%). Regarding laboratory findings, elevated levels of CRP sedimentation, and anemia were prominent. However, in our study, while elevated CRP and sedimentation levels were observed in the group with focal involvement, elevated ALT and AST levels were also detected. Anemia was rarely observed. The absence of anemia may be attributed to the high altitude of the city, which predisposes individuals to polycythemia.

In a study conducted by Özdem et al.[22] from Turkey, which included 189 patients, a comparison was made between bacteremic and non-bacteremic brucellosis cases. It was found that organ involvement was significantly higher in the group with positive blood cultures. However, in our study, no significant relationship was found between culture positivity and organ involvement (p=0.391). This may be attributed to the small number of patients in our study.

Large and small ruminants are most reproductively active during the spring season, coinciding with the production of fresh cheese during this period[23,24]. In our study, it was observed that the positivity of blood cultures in patients was lowest in the spring months and highest in the summer and autumn months. The most common exposure factor identified in our study was the consumption of fresh cheese. The higher number of blood culture isolates in the autumn and winter months may be explained by the incubation period of brucellosis.

In our study, CRP, sedimentation rate, ALT, and AST levels were found to be significantly higher in patients with complicated disease who had organ involvement compared to non-complicated patients. Elevated CRP and sedimentation levels in patients with organ involvement may be considered as indicators of inflammation. The elevation of ALT and AST levels can be explained by brucellosis being a disease that affects the reticuloendothelial system, with the liver being a part of this system.

In conclusion, brucellosis is a zoonotic disease with significant public health implications, mimicking various illnesses, and often leading to suboptimal diagnosis and treatment management, thereby increasing the economic burden on countries. It can cause morbidity in both animals and humans. Understanding the epidemiological data of countries and regions, maintaining veterinary services, increasing knowledge among healthcare workers and local populations about the disease are crucial in combating brucellosis. This necessitates interdisciplinary collaboration.

### Study Limitations

The limited number of included patients and the inability to obtain blood cultures from every patient due to technical reasons are the primary limitations of this study.

## CONCLUSION

Brucellosis continues to be of global significance, necessitating further research on the epidemiological data of countries and regions. In regions where brucellosis is endemic, it should be considered as one of the primary differential diagnoses in the presence of fever. Biochemical markers such as CRP, sedimentation rate, ALT, and AST should be considered for organ involvement in patients diagnosed with brucellosis. Combatting the disease requires a multidisciplinary approach, and healthcare professionals along with the local population should be educated about the disease and preventive measures.

### Ethics

**Ethics Committee Approval:** The study was conducted after obtaining the necessary permissions from Ağrı İbrahim Çeçen University Scientific Research Ethics Committee (decision no: E-95531838-050.99-98272, date: 29.03.2024).

**Informed Consent:** Retrospective study.

### Authorship Contributions

Surgical and Medical Practices: M.K.T., E.E., K.Ş., Concept: M.K.T., E.E., K.Ş., Design: M.K.T., Data Collection or Processing: M.K.T., E.E., K.Ş., Analysis or Interpretation: M.K.T., K.Ş., Literature Search: M.K.T., Writing: M.K.T., E.E.

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

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# Predictors of Severity of Coronary Artery Disease in Patients with Acute ST-elevation Myocardial Infarction

## Akut ST-elevasyonlu Miyokard Enfarktüsü Olan Hastalarda Koroner Arter Hastalığının Ciddiyetinin Belirleyicileri

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**ABSTRACT**

**Aim:** Our study aims to investigate the relationship between platelet-lymphocyte ratio (PLR), neutrophil/lymphocyte ratio (NLR), and the correlation between percutaneous coronary intervention with taxus and cardiac surgery (SYNTAX) scores.

**Materials and Methods:** The patients were divided into three groups according to their SYNTAX score. Group 1 included patients with a SYNTAX score <23, group 2 with a SYNTAX score of 23-32, and group 3 with a SYNTAX score of >33. PLR was calculated as the ratio of platelet count to lymphocyte count.

**Results:** In multivariate logistic regression analysis, high PLR and NLR ratios and older age were independent predictors of high SYNTAX score II [odds ratio (OR): 1.052; 95% confidence interval (CI): (0.998-1.119), p=0.011; OR: 1.093; 95% CI: (1.016-1.175), p=0.016; OR:1.023; 95% CI: (1.010-1.038), p=0.001, respectively. Patients with high PLR had significantly higher SYNTAX scores [121.7 (114.2-129.3) in group 1; 139.4 (125.9-153.0) in group 2, 187.0 (141.8-232.2) in group 3; p<0.001], and there was a positive correlation between PLR, NLR and SYNTAX scores (r=0.52, p<0.001; r=0.58, p<0.001, respectively).

**Conclusion:** PLR and NLR were associated with the severity and complexity of coronary artery disease in patients with acute ST-elevation myocardial infarction.

**Keywords:** Lymphocytes, neutrophils, coronary artery disease, inflammation

**ÖZ**

**Amaç:** Trombosit-lenfosit oranı (PLR) ve nötrofil/lenfosit oranı (NLR), olumsuz kardiyovasküler sonuçlarla korelasyon gösteren sistemik enflamatuvar belirteçlerdir. Çalışmamız PLR, NLR ve SYNTAX skoru arasındaki ilişkiyi araştırmayı amaçlamaktadır.

**Gereç ve Yöntem:** Hastalar SYNTAX skorlarına göre üç gruba ayrıldı. Grup 1'de SYNTAX skoru <23 olan hastalar, grup 2'de SYNTAX skoru 23-32 olan hastalar ve grup 3'te SYNTAX skoru >33 olan hastalar yer aldı. PLR, trombosit sayısının lenfosit sayısına oranı olarak hesaplandı.

**Bulgular:** Çok değişkenli lojistik regresyon analizinde yüksek PLR ve NLR oranları ve ileri yaş, yüksek SYNTAX skoru 2'nin bağımsız belirleyicileriydi [odds oranı (OR): 1,052; %95 güven aralığı (GA): (0,998-1,119), p=0,011; OR: 1,093; %95 GA: (1,016-1,175), p=0,016, OR: 1,023, %95 GA: (sırasıyla 1,010-1,038), p=0,001]. Yüksek PLO'lu hastaların SYNTAX skorları anlamlı derecede yüksekti [grup 1'de 121,7 (114,2-129,3); grup 2'de 139,4 (125,9-153,0); grup 3'te 187,0 (141,8-232,2); p<0,001] ve PLR, NLR ve SYNTAX puanları arasında pozitif korelasyon vardı (sırasıyla r=0,52, p<0,001; r=0,58, p<0,001).

**Sonuç:** PLR ve NLR, koroner arter hastalığının ciddiyeti ve karmaşıklığı ile ilişkiliydi.

**Anahtar Kelimeler:** Lenfosit, nötrofil, koroner arter hastalığı, enflamatuvar

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## INTRODUCTION

Platelets significantly influence local inflammation at the site of atherosclerotic plaques. It has been noted that platelets play a significant role in mediating the relationship between the formation of an atherogenic milieu and the local inflammatory response at the vascular wall. When activated, platelets release a range of proinflammatory substances and chemokines to coordinate pro-atherogenic interactions between activated endothelial cells and circulating immune cells. Stromal cell-derived factor-1a and platelet factor 4 are two well-known instances of platelet chemokines. Both have been identified as pro-atherogenic elements that encourage endothelial monocyte adhesion and are found in atherosclerotic plaques[1]. According to recent research, inflammation is a major factor in atherosclerosis. Elevated platelet counts are associated with the long-term incidence of coronary artery disease and accelerate the formation, advancement, and destabilization of atherosclerotic plaques[2]. The development of atherosclerotic plaque is significantly influenced by the immune system. Poor outcomes are associated with inflammation, which is indicated by low lymphocyte levels in individuals with acute coronary syndrome[3]. Patients with acute coronary syndrome who had a high synergy between percutaneous coronary intervention with taxus and cardiac surgery (SYNTAX) score also had a significantly higher platelet-lymphocyte ratio (PLR)[4]. Atherosclerotic plaque contains a large number of neutrophils. Biomarkers such as PLR and neutrophil/lymphocyte ratio (NLR) are correlated with the severity of coronary artery disease in many previous studies[3,4].

The SYNTAX score, which shows the severity of coronary artery disease, not only determines the method of revascularization but also predicts mortality[5]. To predict the complexity of coronary artery disease, simple, practical, non-invasive biomarkers are required. PLR has been reported to correlate with the severity of the SYNTAX score in patients with non-ST-segment elevation myocardial infarction[6]. We, therefore, aimed to investigate the relationship between PLR, NLR, and the SYNTAX score in patients with acute ST-elevation myocardial infarction (STEMI).

## MATERIALS AND METHODS

### Study Population

Patients with acute STEMI between June 2022 and December 2023 were included in our study. Our study is a retrospective study conducted by scanning hospital data. The written informed consent form was obtained from all patients. The following criteria were used to diagnose STEMI: ST-segment elevation in at least two contiguous leads and typical chest pain lasting longer than 20 minutes, with the cut-off points being ≥0.2 mV in men aged 40 years or older, ≥0.25 mV in men under 40 years, or ≥0.15 mV in women, in leads V2 to V3 and/or ≥0.1 mV in the other leads, as well as posterior (V7-V9) and right derivations (V3R-V4R).

Patients with severe renal failure (creatinine >2 mg/dL) and active infection, being under the age of 18 years, and having an inflammatory disease or cancer were excluded from the study. Blood samples for PLR were contained at the time of arrival at the hospital. The blood was tested using an automated device that measured biochemical levels and performed a full blood count. Hyperlipidemia was defined as receiving treatment for high cholesterol or having a total cholesterol level of more than 220 mg/dL. Blood pressure more than 140/90 mmHg or the usage of antihypertensive drugs was referred to as hypertension. The use of antidiabetic medications, fasting plasma glucose levels of 7.0 mmol/L (126 mg/dL), or glycated hemoglobin A1c readings of 6.5% were all considered the indicators of diabetes mellitus. The study was approved by the Tekirdağ Dr. İsmail Fehmi Cumalıoğlu City Hospital Clinical Research Ethics Committee (decision no: 60, date: 01.09.2023).

Angiography was carried out utilizing the Judkins method, using multiple projections in all patients. All of the patients had their 300 mg of aspirin loaded with either 180 mg of ticagrelor or 600 mg of clopidogrel before pPCI. Following the decision to do a coronary intervention, bolus heparin at a dosage of 50-70 units/kg was given to each patient. Coronary angiograms were analyzed by two independent cardiologists after the vessel responsible for STEMI was revascularized and SYNTAX II scores were calculated[7]. The patients were divided into three groups, respectively, according to their SYNTAX score; group 1: SYNTAX score <23, group 1: SYNTAX score between 23-32, and group 3: SYNTAX score >33.

### Statistical Analysis

For statistical analysis, SPSS 22.0 (SPSS Inc., Chicago, IL) was utilized. The median or mean ± standard deviation was used to express continuous variables. The chi-square or Fisher's exact tests were employed to compare categorical variables that were given as percentages. To determine the normality of data distributions, the Kolmogorov-Smirnov test was used. The one-way ANOVA was used for continuously distributed data with a normal distribution. Non-normally distributed data were examined with the Kruskal-Wallis test. The Mann-Whitney U test was applied in post-hoc analysis of non-parametric data. To evaluate data conforming to normal distribution, the Tukey or Tamhane's test was used in post-hoc analysis, depending on the equality of variances. The PLR cut-off value was calculated using receiver-operating characteristic analysis to predict the severity and complexity of coronary artery disease in STEMI patients. For determining the appropriate cut-off value, Youden's J statistic was performed. For independent

characteristics predicting the high SYNTAX score in patients with STEMI, univariate and multivariate regression analyses were used. The Spearman correlation test was used to investigate the relationship between the PLR and the severity of the coronary arteries. Statistics with p values <0.05 were considered significant.

## RESULTS

This study consisted of 613 patients who underwent coronary angiography for STEMI. Group 1 included patients with a SYNTAX score of <23, group 2 included patients with a SYNTAX score between ≥23-32, and group 3 included those with a SYNTAX score of >33. Table 1 summarizes the basic characteristics and laboratory findings. Group 3 had higher rates of multivessel disease and chronic total occlusion, and left ventricular ejection fraction was lower in this group compared to other groups. Patients in group 3 were older and had a higher rate of diabetes mellitus. There was no difference between the two groups in terms of other demographic characteristics. When we examined the biochemical and hematological measurements Table 2, neutrophil levels were detected to be significantly higher in group 3, whereas lymphocyte rates were lower in this group. PLR and NLR were significantly higher in group 3 [PLR, group 1: 121.7 (114.2-129.3), group 2: 139.4 (125.9-153.0), group 3: 187.0 (141.8-232.2), p<0.001; NLR, group 1: 4.1 (3.8-4.5), group 2: 5.4 (4.7-6.2), group 3: 6.8 (5.0-8.7), p<0.001, respectively].

**Table 1. Baseline clinical and angiographic characteristics of the study population according to the severity of coronary artery disease**

| Variables | Group 1 (n=406) | Group 2 (n=173) | Group 3 (n=34) | p value |
|---|---|---|---|---|
| Male | 306 (75.4%) | 124 (71.7%) | 24 (70.6%) | 0.581 |
| Age (years) | 57±12 | 60±13 | 66±11 | **<0.001** |
| Hypertension | 145 (35.7%) | 67 (38.7%) | 19 (55.9%) | 0.062 |
| Diabetes mellitus | 109 (26.8%) | 47 (27.2%) | 18 (52.9%) | **0.005** |
| Hypercholesterolemia | 100 (24.6%) | 36 (20.8%) | 8 (23.5%) | 0.082 |
| Smoking | 225 (55.4%) | 88 (50.9%) | 17 (50%) | 0.432 |
| Body mass index (kg/m$^2$) | 28.2±4.6 | 27.6±3.8 | 26.7±4.6 | 0.102 |
| Systolic blood pressure (mmHg) | 129.6±24.0 | 127.6±24.3 | 126.8±27.4 | 0.07 |
| Diastolic blood pressure (mmHg) | 78.9±14.0 | 77.3±14.3 | 76.9±15.8 | 0.09 |
| Left ventricular ejection fraction (%) | 48±9 | 41±9 | 37±11 | **<0.001** |
| Multi-vessel disease | 154 (37.9%) | 119 (68.8%) | 33 (97.1%) | **<0.001** |
| Chronic total occlusion | 19 (4.7%) | 33 (19.1%) | 20 (58.8%) | **<0.001** |

**Table 2. Biochemical and hematological measurements of the study population**

| Variables | Group 1 (n=406) | Group 2 (n=173) | Group 3 (n=34) | p value |
|---|---|---|---|---|
| Hemoglobin (g/dL) | 14.3±1.7 | 14.2±1.9 | 13.4±1.9 | 0.008 |
| WBC (X10$^9$/L) | 11.4±3.2 | 12.1±3.5 | 11.9±4.2 | 0.066 |
| Neutrophil (X10$^9$/L) | 7.8±2.9 | 8.7±3.6 | 9.0±4.2 | **0.002** |
| Lymphocyte (X10$^9$/L) | 2.6±1.3 | 2.3±1.3 | 1.8±1.2 | **0.002** |
| Monocyte (X10$^9$/L) | 0.7±0.3 | 0.7±0.3 | 0.7±0.3 | 0.95 |
| Platelet (X10$^9$/L) | 249±74 | 243±59 | 248±65 | 0.60 |
| NLR | 4.1 (3.8-4.5) | 5.4 (4.7-6.2) | 6.8 (5.0-8.7) | **<0.001** |
| PLR | 121.7 (114.2-129.3) | 139.4 (125.9-153.0) | 187.0 (141.8-232.2) | **<0.001** |
| Glucose (mg/dL) | 114 (110-140) | 122 (116-152) | 128 (122-164) | 0.06 |
| Creatinine (mg/dL) | 1.1±0.2 | 1.2±0.2 | 1.2±0.4 | 0.74 |
| Total cholesterol (mg/dL) | 193±45 | 190±48 | 192±68 | 0.81 |
| Triglycerides (mg/dL) | 154 (146-163) | 159 (143-175) | 134 (108-160) | 0.37 |
| LDL-C (mg/dL) | 124±38 | 121±44 | 128±52 | 0.64 |
| HDL-C (mg/dL) | 40±8 | 40±9 | 42±11 | 0.38 |
| Hs-CRP (mg/L) | 6.5 (6.0-7.1) | 8.5 (7.0-10.0) | 8.8 (4.8-12.7) | 0.006 |

NLR: Neutrophil/lymphocyte ratio, PLR: Platelet-lymphocyte ratio, Hs-CRP: High-sensitivity C-reactive protein, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, WBC: White blood cell

The following receiver operating characteristic analysis of PLR and NLR values was used to predict the SYNTAX >33 [NLR cut-off ≥3.28, area under the curve (AUC): 0.605; 95% confidence interval (CI): (0.557-0.653) with 61.4% sensitivity and 55.4% specificity, p<0.001, and PLR cut-off ≥100.8, AUC: 0.587; 95% CI: (0.538-0.635) with 62.8% sensitivity and 50.2% specificity, p<0.001] Figure 1. A positive correlation between PLR, NLR, and SYNTAX score II was found (r=0.52, p<0.001 and r=0.58, p<0.001, respectively). High PLR and NLR ratios and older age were independent predictors of high SYNTAX score II [odds ratio (OR): 1.052; 95% CI: (0.998-1.119), p=0.011; OR: 1.093;95% CI: (1.016-1.175), p=0.016; OR: 1.023; 95% CI: (1.010-1.038), p=0.001, respectively] Table 3.

## DISCUSSION

This is the first study to investigate the relationship between NLR, PLR, and SYNTAX scores in patients with STEMI. We found that NLR, PLR, and age were associated with high SYNTAX scores. These practical markers may be capable of predicting the high SYNTAX score. Platelets have an important role in the atherosclerosis. They produce anti-inflammatory and immunomodulatory chemicals during the rupture of plaque[8,9]. Neutrophils secrete pro-inflammatory mediators. Extracellular neutrophil traps may contribute to the formation of atherosclerotic plaque. The role of neutrophils has been examined in many studies of myocardial ischemia-reperfusion and absence of myocardial reflow.

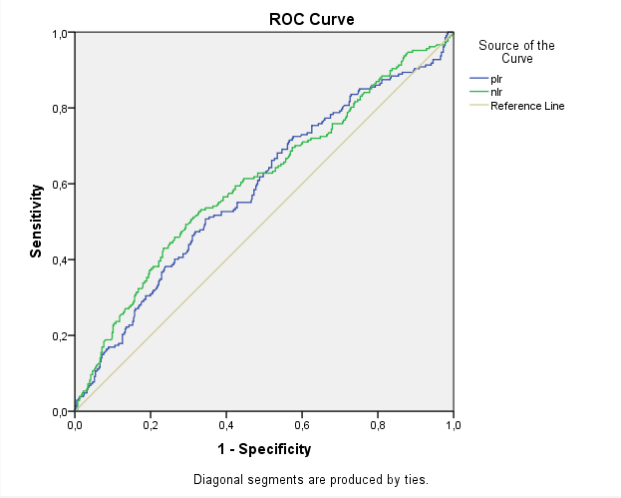


**Figure 1.** The receiver operating characteristic (ROC) curve analysis for cut-off values of plr and nlr for predicting predicting a high SYNTAX score II

*AUC: Area under the curve, plr: Platelet-to lymphocyte ratio, NLR: Neutrophil/lymphocyte ratio, ROC: Receiver operating characteristic*

It was concluded that neutrophils have a detrimental effect on the myocardium where ischemia-reperfusion is forced. Acute stress and inflammation with coronary disease can raise plasma cortisol levels, leading to more neutrophils and fewer lymphocytes in circulation. In studies conducted on patients with acute coronary syndrome, a higher number of active neutrophils was observed in blood samples taken from the plaque-ruptured area compared to samples taken from the peripheral arterial region[10].

The SYNTAX score is an angiographic risk stratification score used to measure coronary involvement and choose the best revascularization strategy for each patient depending on their clinical setting. The effect on mortality may be related to the SYNTAX score.

In our study, we found a correlation between increased PLR and NLR and high SYNTAX scores. A high PLR ratio was found in patients with coronary artery disease as a prognostic factor in numerous previous studies[11]. PLR has also been used to predict the prognosis of coronary slow flow[12] and it has also been found to be an indicator of plaque burden[13]. In addition, high levels of PLR ratio have been reported to be related to in-stent restenosis, saphenous vein graft disease, atrial fibrillation, heart failure, and poor coronary collateral circulation[13]. Sari et al.[14] found that the NLR and PLR may be used to predict the high SYNTAX score before coronary angiography. Systemic immune-inflammation index, which comprises NLR and PLR, has been reported to be associated with coronary artery disease in many studies[15]. By managing hyperlipidemia and thrombosis, atherosclerosis can be slowed down and cardiovascular events can be reduced by lowering the inflammatory response. Examining these biomarkers in patients with acute coronary

**Table 3. Univariate and multivariate regression analysis of independent variables in predicting a high SYNTAX score**

| | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| **Variables** | **OR** | **95% CI** | **p value** | **OR** | **95% CI** | **p value** |
| Age | 1.027 | 1.014-1.041 | **0.001** | 1.023 | 1.010-1.038 | **0.001** |
| Hypertension | 1.279 | 0.908-1.803 | 0.159 | - | - | - |
| Diabetes mellitus | 1.247 | 0.865-1.799 | 0.237 | - | - | - |
| PLR | 1.003 | 1.001-1.005 | **0.001** | 1.052 | 0.998-1.119 | **0.011** |
| NLR | 1.087 | 1.043-1.132 | **<0.001** | 1.093 | 1.016-1.175 | **0.016** |

NLR: Neutrophil/lymphocyte ratio, PLR: Platelet-lymphocyte ratio, OR: Odds ratio, CI: Confidence interval

syndrome will aid in both diagnosing high SYNTAX scores and predicting the patient's prognosis. The high SYNTAX score was indicated by higher PLR and NLR levels, as we observed. Before invasive angiography, PLR and NLR, which are readily obtained from routine blood tests, can be computed rapidly.

### Study Limitations

The present investigation has various constraints. First of all, the PLR and NLR were assessed as potential predictors of the high SYNTAX score in our small, single-center retrospective study. By drawing additional blood samples from the patients who were part of our study, an average calculation for biomarkers could be made. Finally, there are significant confounders that we do not know, like medication previously taken by patients, which can affect the inflammatory process. Larger, multi-center prospective studies are needed.

## CONCLUSION

The strategy to be followed for subsequent coronary lesions is as important as a primary percutaneous intervention in the early period of STEMI. New biomarkers and scoring systems are parameters that can help in the treatment and prediction of prognosis. PLR and NLR may help classify high-risk patients with STEMI as they are practical, cost-effective biomarkers.

### Ethics

**Ethics Committee Approval:** This study was conducted according to the guidelines of the Declaration of Helsinki and approved by Tekirdağ Dr. İsmail Fehmi Cumalıoğlu City Hospital Clinical Research Ethics Committee (decision no: 60, date: 01.09.2023).

**Informed Consent:** Retrospective study.

### Authorship Contributions

Surgical and Medical Practices: M.K., Ç.A., Concept: C.A., A.D., Design: C.A., Data Collection or Processing: M.K., Ç.A., Analysis or Interpretation: A.D., Literature Search: C.A., Writing: C.A., A.D.

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

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# The Use of Neutrophil/Lymphocyte Ratio and Prognostic Nutritional Index for Predicting Mortality in COVID-19 Patients: A Retrospective Study

## COVID-19 Hastalarında Nötrofil/Lenfosit Oranının ve Prognostik Nutrisyonel İndeksinin Mortalite Öngördürücülüğü: Retrospektif Bir Çalışma

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**ABSTRACT**

**Aim:** Various prognostic risk-scoring systems and parameters have been investigated to predict mortality in patients with coronavirus disease-2019 (COVID-19). In this study, we aimed to evaluate the neutrophil/lymphocyte ratio (NLR) and prognostic nutritional index (PNI) scores to evaluate their predictive value on COVID-19-related mortality.

**Materials and Methods:** A total of 1239 patients were admitted in the study. Patients were followed up for 34 months. Specific variables and biochemical parameters were recorded. NLR and PNI values of the participants were calculated and divided into three equal groups from smallest to largest according to these values (NLR and PNI incrementally from 1 to 3). The terciles of NLR and PNI levels were compared between the survival and non-survival groups.

**Results:** The study included 1.239 patients. The patients were followed up for an average of 22 months (range, 0-34 months). Compared to surviving patients in the mortality group, NLR was also significantly higher and the PNI score was significantly lower ($p<0.001$).

**Conclusion:** Lower PNI and higher score NLR were found to be independent risk factors for mortality in hospitalized COVID-19 patients.

**Keywords:** Prognostic nutritional index, neutrophil/lymphocyte ratio, mortality, COVID-19

**ÖZ**

**Amaç:** Koronavirüs hastalığı-2019 (COVID-19) hastalarında mortaliteyi tahmin etmek için çeşitli prognostik skorlama sistemleri ve parametreleri üzerine araştırmalar yapılmıştır. Bu çalışmada nötrofil/lenfosit oranı (NLR) ve prognostik nutrisyonel indeks (PNI) skorlarının, COVID-19'a bağlı mortalite üzerindeki öngördürücülüğünün değerlendirilmesi amaçlandı.

**Gereç ve Yöntem:** Çalışmaya toplam 1239 hasta dahil edildi. Hastalar taburculuk sonrası 34 ay boyunca takip edildi. Hastaların spesifik değişkenler ve biyokimyasal parametreleri kaydedildi. Tüm hastaların NLR ve PNI değerleri hesaplandı. Katılımcıların NLR ve PNI değerleri hesaplandı. Küçükten büyüğe sıralandı ve 1. gruptan 3. gruba artacak şekilde 3 eşit gruba ayrıldı (NLR ve PNI'nın 1. gruptan 3'e kadar artış gösteren üçte birlik dilimler halinde). NLR ve PNI düzeylerinin üçte birlik dilimleri hayatta kalan ve hayatta kalmayan gruplar arasında karşılaştırıldı.

**Bulgular:** Çalışmaya 1.239 hasta dahil edildi. Hastalar ortalama (minimum 0 - maksimum 34) 22 ay takip edildi. Mortalite grubunda hayatta kalan hastalarla karşılaştırıldığında, NLR düzeyleri anlamlı derecede yüksek ve PNI skoru anlamlı derecede düşük saptandı.

**Sonuç:** Hastanede yatan COVID-19 hastalarında daha düşük PNI ve daha yüksek NLR skoru mortalite için bağımsız risk faktörleri olarak bulundu.

**Anahtar Kelimeler:** Prognostik nutrisyonel indeks, nötrofil/lenfosit oranı, mortalite, COVID-19

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## INTRODUCTION

Coronavirus disease-2019 (COVID-19), which the World Health Organization designated as a pandemic in March 2020, has maintained its importance, even though its effect has decreased recently, and it has been one of the causes of increased mortality[1]. One of the causes of increased mortality in coronavirus disease-19 (COVİD-19) disease is severe lung parenchyma disease and respiratory failure, which require hospitalization in the intensive care unit [2]. There is no standardized treatment and medication for those with COVID-19, therefore, determining the risk factors for prognosis and mortality is important[3]. Several prognosis and risk parameters have been investigated for their use in mortality prediction for those with COVID-19[4]. The aim of estimating mortality is the early detection and treatment of patients with a poor prognosis. In particular, markers of inflammation have frequently been used to make such predictions[5]. Inflammatory parameters in peripheral blood cell composition are important predictive inflammatory response indicators, and their use as mortality indicators is increasing[6]. This measurement method is inexpensive, easy, and cost-effective. The neutrophil and lymphocyte counts are hemogram parameters, and this study calculates the neutrophil/lymphocyte ratio (NLR). NLR is shown to be an important parameter in showing the inflammatory status of patients. NLR is a risk factor that increases mortality in infectious diseases, malignancy, and ischemic heart diseases[7-9]. Malnutrition refers to a chronic imbalance between the intake of consumed nutrients (protein, energy, and other nutrients) and meeting changing metabolic needs. Malnutrition causes some changes in the immune system. High degrees of malnutrition have been shown to be associated with increased inflammation[10]. Moreover, malnutrition suppresses the immune system response and increases susceptibility to COVID-19 and similar infections[11,12]. There is no specific screening method for nutrition in inpatients with COVID-19. Recently, researchers have proposed a prognostic nutritional index (PNI) for detecting malnutrition and calculated it based on the serum albumin value and lymphocyte count. Previous studies have reported that the PNI is significantly associated with increased mortality and morbidity in patients with various gastrointestinal malignancies[13,14]. Therefore, PNI may indicate nutrition and inflammation in COVID-19 patients. This study has the aim of determining the effects that the NLR and PNI score have on predicting COVID-19-related mortality.

## MATERIALS AND METHODS

### Data-Sources

This research was conducted using the data of patients hospitalized University of Health Sciences Turkey, İstanbul Haseki Training and Research Hospital Internal Medicine Units for the Pandemic. The Ethics Committee of the University of Health Sciences, İstanbul Haseki Training and Research Hospital approved the study (decision no: 253-2023, date: 27.12.2023), which has been performed in line with guidelines from the National Institutes of Health and the Declaration of Helsinki principles regarding proper clinical practices. Patient data were all gathered without including any patient-identifying information and without permitting investigation of the patients' medical data. Consent was obtained from the inpatients as a matter of routine upon admission.

### Population of the Study and Data Collection

Data were collected from all adult patients hospitalized in internal medicine pandemic services with a COVID-19 diagnosis between April 2020 and March 2021. The study had 1.239 enrolled patients. Only those with positive swab test results and clinical and/or radiological findings were considered to have COVID-19 disease and were included in this study. Patients who had fully recovered as well as those who had passed away were also included. Patients who had negative swab tests and/or serological tests, those hospitalized for other diagnoses, and re-admissions were excluded from the study. Patients were followed up for 34 months, including primary endpoints and all-cause mortality. The study obtained all follow-up data from the University of Health Sciences Turkey, İstanbul Haseki Training and Research Hospital, IT Department, with death reports verified using the Turkish National Death Registry. The specific variables of age and gender, as well as glucose, white blood cell, neutrophil, lymphocyte, hemoglobin, platelet, uric acid, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, alkaline phosphatase, C-reactive protein, procalcitonin, ferritin, thyroid stimulant hormone, and albumin levels were recorded. Blood samples were obtained immediately after hospitalization and before the treatment. The NLR and PNI / 10 × serum albumin [g/dL] + 0.005 × lymphocytes per µL were formulated for all participants. NLR and PNI values of the participants were calculated and divided into three equal groups from smallest to largest according to these values (NLR and PNI incrementally from 1 to 3). These terciles were then compared in terms of survival and non-survival groups.

### Statistical Analysis

The study benefitted from IBM SPSS Statistics for Windows (version 25.0; IBM Corp., Armonk, NY, USA) for performing the statistical analyses, presenting continuous data as mean and standard deviation values regarding groups T1–T3, and all categorical variables as percentages. The study implemented the chi-square test to compare the groups' ratios and the Student's t-test to examine the normally distributed numerical data. The Mann-Whitney U test was used to compare the two groups

regarding their non-normally distributed numerical data and the Cox regression model was used to analyze the variables' effects on event-free survival. Moreover, the Cox regression was employed to assess the predictive performance of age, gender, hemoglobin and uric acid levels, NLR, and PNI. The study included parameters that were found to vary between the different outcomes (survivor or non-survivor) in the regression models to reveal which ones showed independent relationships with these outcomes. Statistical significance was set at $p<0.05$. The study used the Kaplan-Meier method to examine time-to-event data and the log rank test to determine differences between groups.

## RESULTS

The study included 1.239 patients (565 women and 674 men) admitted to the clinic and given treatment. The patients were followed up for a median (minimum 0 - maximum 34) 22 months. The main characteristics of the surviving and non-surviving patient groups are shown in Table 1. When compared to the survivor group, the mortality risk for the non-surviving group was significantly higher among older patients and male gender ($p<0.001$). The PNI levels were less than 36.95 for tercile 1, between 36.95 and 42.5 for tercile 2, and greater than 42.5 for tercile 3. The NLR levels were less than 3.26 for tercile 1, between 3.26 and 7.4 for tercile 2, and greater than 7.4 for tercile 3.

In terms of the NLR, terciles 1 and 2 had fewer non-surviving patients than surviving patients, and a greater number of non-surviving patients from tercile 3. Regarding PNI, the rate of survival was lower for tercile 1 while it was higher for terciles 2 and 3 (Table 1). The study conducted a multivariable cox regression to assess the mortality risk between the groups, with Table 2 showing these results with regard to age, male sex, hemoglobin level, uric acid level, NLR, and PNI. The NLR tertiles 3 and 2 showed a very close relationship in terms of mortality risk. [NLR: (tercile 1 and tercile 2) odds ratio (OR): 1.63, 95% confidence interval (CI): 1.14-2.32, p=0.007; NLR:

**Table 1. Patients' baseline clinical and laboratorial characteristics**

| | Survivor | Non-survival | p value |
|---|---|---|---|
| Gender<br>Female: n (%)<br>Male: n (%) | <br>416 (73.6%)<br>442 (65.5%) | <br>149 (26.4%)<br>232 (34.5%) | <br>0.002 |
| Age (years) | 57.5±15.1 | 70.9±13.1 | 0.001 |
| Glucose (mg/dL) | 160.9±82.3 | 173.6±101.8 | 0.021 |
| White blood cell count $\times10^9$/L | 7,6±4.9 | 10.1±22.2 | 0.02 |
| Neutrophils $\times10^9$/L | 5.4±3.6 | 7.7±5.4 | 0.001 |
| Lymphocytes $\times10^9$/L | 1.3±3.1 | 1.1±2.3 | 0.095 |
| Hemoglobin (g/dL) | 12.2±1.9 | 10.9±2.4 | 0.001 |
| Platelet ($\times1000/mm^3$) | 214.3±89.1 | 205.7±116.4 | 0.161 |
| Uric acid (mg/dL) | 4.9±2 | 6.5±2.7 | 0.001 |
| ALT (U/L) | 30.8±31.1 | 34.6±113.2 | 0.005 |
| AST (U/L) | 37.8±41.7 | 58.3±344.5 | 0.005 |
| GGT (U/L) | 52.3±73.8 | 72.52±145.4 | 0.001 |
| ALP (U/L) | 76.9±64.1 | 107.9±106.5 | 0.001 |
| Albumin (g/dL) | 3.4±6.4 | 3.1±6.1 | 0.001 |
| Hs-CRP (mg/L) | 74.3±70.9 | 110.6±92.3 | 0.001 |
| Procalcitonin (ug/L) | 0.9±6.7 | 8.8±53.3 | 0.001 |
| Ferritin (ug/dL) | 406.3±558.7 | 623.9±934.4 | 0.001 |
| TSH (mU/L) | 1.2±8.5 | 0.8±1.6 | 0.325 |
| NLR tercile 1 (n) | 354 (29%) | 53 (4.3%) | |
| NLR tercile 2 (n) | 295 (24.2%) | 112 (9.2%) | 0.001 |
| NLR tercile 3 (n) | 202 (16.5%) | 205 (16.8%) | |
| PNI tercile 3 (n) | 351 (28.9%) | 55 (4.5%) | |
| PNI tercile 2 (n) | 298 (24.5%) | 107 (8.8%) | 0.001 |
| PNI tercile 1 (n) | 199 (16.4%) | 206 (16.9%) | |

Statistically significant variables ($p<0.05$), ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferase, ALP: Alkaline phosphatase, CRP: C-reactive protein, TSH: Thyroid stimulant hormone, NLR: Neutrophil/lymphocyte ratio, PNI: Prognostic nutritional index

(tercile 1 and tercile 3)] OR: 2.21, 95% CI: 1.55-3.17, ≤0.001 (Table 2). PNI's first tertile indicates a close relationship with a mortality risk [PNI between terciles 3 and 1 OR: 1.51, 95% CI: (1.06, 2.17), p=0.022]. In addition, the mortality risk was higher in patients with high uric acid levels and low hemoglobin levels, and in those who were male and older in age (p≤0.001 for each).

The Kaplan-Meier curves illustrate the incidences for all-cause mortality as categorized by the NLR (Figure 1) and by PNI (Figure 2) in the COVID-19 patients. The long-term survival rates of inpatients with elevated NLR and lower PNI values were significantly worse than for patients with lower NLR and elevated PNI levels. All-cause mortality had a significantly higher cumulative incidence in patients with a lower PNI score (log rank=154.2; p<0.001) and higher NLR (log rank=149.1; p<0.001).

## DISCUSSION

Blood tests and simple scoring systems provide the physician with information about the inflammatory process and may also provide important clues about the prognosis of the disease. This study has found higher NLR and lower PNI to be significantly associated with increased mortality. Moreover, the results indicate that low hemoglobin, uric acid level and male gender were independent predictors of mortality. Studies have shown that advanced age, male sex, and uric acid level are strongly associated with mortality, as in our study, apart from these known factors, various markers have been put forward to predict mortality[15,16].

**Table 2. Cox regression anaysis for neutrophil/lymphocyte ratio and prognostic nutritional index**

| Factor | Hazard ratio 95% CI [lower, upper] | p value |
|---|---|---|
| Age | 1.04 [1.03, 1.05] | 0.001 |
| Gender (male) | 1.59 [1.27, 1.99] | 0.001 |
| Hemoglobin (g/dL) | 0.89 [0.85, 0.94] | 0.001 |
| Uric acid (mg/dL) | 1.10 [1.06, 1.15] | 0.001 |
| NLR (terciles 1 and 2) | 1.63 [1.14, 2.32] | 0.007 |
| NLR (terciles 1 and 3) | 2.21 (1.55-3.17) | 0.001 |
| PNI (terciles 3 and 2) | 1.18 (0.83-1.67) | 0.363 |
| PNI (terciles 3 and 1) | 1.51 (1.06-2.17) | 0.022 |

Statistically significant variables (p<0.05), CI: Confidence interval, NLR: Neutrophil/lymphocyte ratio, PNI:Prognostic nutritional index

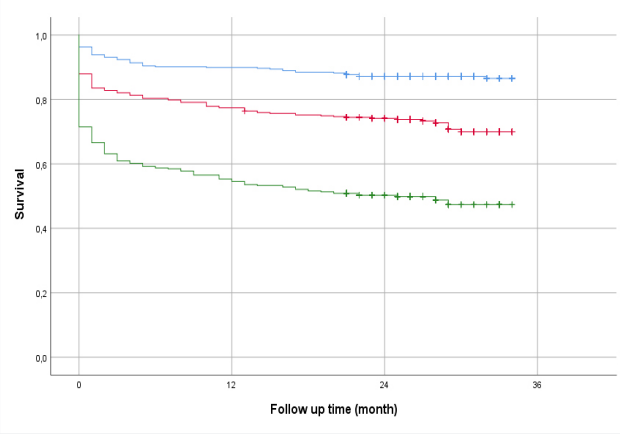


**Figure 1.** Kaplan-Meier curve for all-cause mortality by tercile with regard to NLR.

Blue line = tercile 1 of NLR (=407; NLR ≤3.26). Red line = tercile 2 of NLR (n=407; 3.26< NLR <7.40). Green line = tercile 3 of NLR (n=407 patients; 7.40 ≤ NLR)

*NLR: Neutrophil/lymphocyte ratio*

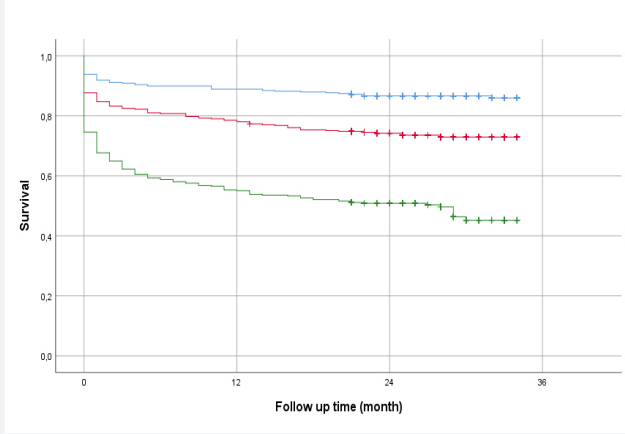


**Figure 2.** Kaplan-Meier curve for all-cause mortality by terciles with regard to PNI

Blue line = tercile 3 of PNI (n=406; 42.5≤ PNI). Red line = tercile 2 of PNI (n=405; 36.95< PNI <42.5). Green line = tercile 1 of PNI (n=405; PNI ≤36.95)

*PNI: Prognostic nutritional index*

The results also reveal PNI to be an independent predictor of COVID-19 patients' mortality. Malnutrition is known to be associated with increased morbidity and mortality[17]. Studies have shown PNI, being a simple and easily applied scoring system to determine malnutrition levels, to be associated with poor prognosis regarding various diseases, with a low PNI score having been reported as being significantly associated with poor prognosis and increased complications in patients with malignant gastrointestinal tumors[18].

Albumin, a PNI component, is synthesized by the liver and widely used as a nutritional indicator. Systemic inflammation resulting from acute or chronic diseases reduces albumin synthesis and increases its degradation. Studies have shown an increased cytokine storm occurring alongside COVID-19 to be associated with albumin depletion[19]. Previous studies have shown low albumin levels to be associated with poor outcomes in COVID-19 patients[20]. Additionally, decreased serum albumin levels that occur during an infection and systemic inflammation may be indicators of liver function due to inflammatory cytokines' ability to reduce hepatocytes' albumin synthesis capacity[21]. Moreover, Wei et al.[22] found in their study that PNI had the strongest relationship with COVID-19 inpatients' NLRs and lactate dehydrogenase levels. In parallel with all these findings, the current study found a low PNI to be closely related to mortality in COVID-19 patients. Therefore, PNI can be used as a new biomarker to predict mortality in COVID-19 patients.

NLR has been demonstrated as a new biomarker indicative of systemic inflammation. The inflammatory response stimulates neutrophil production and increases the apoptosis of lymphocytes. This causes an increase in NLR. Recent studies have shown that increased levels of inflammatory cytokines, chemokines, and NLRs in patients with infection correlate with disease severity due to cytokine storms[23]. Therefore, NLR can be used as a predictive indicator of mortality in diseases. In line with this information, the study has found high NLR to be an independent risk factor for COVID-19 mortality. However, research on this subject is new, and studies continue to be conducted in this field regarding COVID-19 and other diseases. When the literature was examined, studies on this subject have generally involved other diseases. The strong features that distinguish the current study from others are its long examination and follow-up periods, large number of cases, and case diversity. In addition, this study was conducted using data from patients managed by a team equipped and experienced in the treatment of COVID-19 in a reference pandemic hospital. This study has also found all-cause mortality to be significantly higher in patients with low PNI and high NLR. These findings may contribute to the development of new scoring systems for predicting mortality in the future.

### Study Limitations

This study is found to have several limitations. Firstly, it is a retrospective study. Secondly, the hematological and biochemical markers examined limited the implications of the findings.

## CONCLUSION

This study has found lower PNI and higher NLR values to be independent risk factors for COVID-19 mortality regarding hospitalized COVID-19 inpatients. As long as COVID-19 remains a part of life, research in line with it will continue to attract attention.

### Ethics

**Ethics Committee Approval:** The study was conducted after obtaining the necessary permissions from University of Health Sciences, İstanbul Haseki Training and Research Hospital Ethics Committee (decision no: 253-2023, date: 27.12.2023).

**Informed Consent:** Retrospective study.

### Authorship Contributions

Surgical and Medical Practices: B.Ç.T., E.H., E.Ç.Ö., F.T., H.E.A., Concept: E.H., Design: E.Ç.Ö., Data Collection or Processing: H.E.A., Analysis or Interpretation: H.E.A., Literature Search: F.T., Writing: B.Ç.T.

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

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# The Effect of Sepsis Associated Encephalopathy on One-Year Mortality in Patients Aged 65 Years and Over After Discharge: A Retrospective Cohort Study

## Sepsis İlişkili Ensefalopatinin 65 Yaş ve Üzeri Hastalarda Taburculuk Sonrası Bir Yıllık Mortalite Üzerine Etkisi: Retrospektif Bir Kohort Çalışması

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### ABSTRACT

**Aim:** Sepsis remains a leading cause of mortality among the older hospitalized patients, particularly those with complex comorbidities. This study investigates the prognostic factors influencing one-year mortality in patients aged 65 years and over, who were hospitalized with sepsis, emphasizing the role of sepsis-associated encephalopathy (SAE) in long-term outcomes.

**Materials and Methods:** In a retrospective cohort of 207 older patients treated for sepsis, clinical and laboratory data were meticulously recorded. Demographic details, comorbidity indices, and specific treatment interventions were analyzed. The association between these variables and one-year mortality was evaluated using univariate and multivariate Cox regression models. The Kaplan-Meier curves, complemented by the Log-rank test, assessed the survival probabilities.

**Results:** The cohort consisted of patients with a nearly equal gender distribution, with a mean age of 73.7 years. The study found that SAE, increased international normalized ratio (INR), and advanced age were significantly associated with higher one-year mortality ($p<0.05$). Notably, SAE presented a hazard ratio of 3.41 in the multivariate analysis. Other factors such as gender, Charlson Comorbidity Index, Sequential Organ Failure Assessment score and various laboratory markers did not show significant prognostic value.

**Conclusion:** SAE and elevated INR are potent predictors of one-year mortality in older sepsis survivors. These findings highlight the importance of close neurological assessment and monitoring of coagulation parameters in this population. Focused strategies on these elements could potentially improve the management and outcomes of sepsis in the older patients.

**Keywords:** Sepsis, encephalopathy, mortality

### ÖZ

**Amaç:** Sepsis, hastanede yatan yaşlı hastalar arasında, özellikle de karmaşık komorbiditeleri olanlarda önde gelen bir mortalite nedeni olmaya devam etmektedir. Bu çalışmada, sepsis ile hastaneye yatırılan 65 yaş ve üzeri hastalarda bir yıllık mortaliteyi etkileyen prognostik faktörlerin araştırılması ve uzun vadeli sonuçlarda sepsis ilişkili ensefalopatinin (SAE) rolününün vurgulanması amaçlanmaktadır.

**Gereç ve Yöntem:** Sepsis nedeniyle tedavi edilen 207 yaşlı hastadan oluşan retrospektif bir kohortta, klinik ve laboratuvar verileri kaydedilmiştir. Demografik ayrıntılar, komorbiditeler ve spesifik tedavi müdahaleleri analiz edilmiştir. Bu değişkenler ile bir yıllık mortalite arasındaki ilişki tek değişkenli ve çok değişkenli Cox regresyon modelleri kullanılarak değerlendirilmiştir. Log-rank testi ile tamamlanan Kaplan-Meier eğrileri sağkalım olasılıklarını değerlendirmiştir.

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**Bulgular:** Kohort, ortalama yaşı 73,7 olan ve cinsiyet dağılımı neredeyse eşit olan hastalardan oluşmuştur. Çalışmada ensefalopati, artmış uluslararası normalleştirilmiş oran (INR) ve ileri yaşın daha yüksek bir yıllık mortalite ile anlamlı şekilde ilişkili olduğu bulunmuştur (p<0,05). Özellikle, sepsis ilişkili ensefalopati çok değişkenli analizde 3,41'lik bir oranı sunmuştur. Cinsiyet, Charlson Komorbidite İndeksi, Ardışık Organ Yetmezliği Değerlendirme skoru ve çeşitli laboratuvar belirteçleri gibi diğer faktörler anlamlı prognostik değer göstermemiştir.

**Sonuç:** Sepsis ilişkili ensefalopati ve yüksek INR, sepsis sonrası sağ kalan yaşlı hastalarda bir yıllık mortalitenin güçlü belirleyicileridir. Bu bulgular, bu popülasyonda yakın nörolojik değerlendirme ve koagülasyon parametrelerinin izlenmesinin önemini vurgulamaktadır. Bu unsurlara odaklanan stratejiler, yaşlı hastalarda sepsis yönetimini ve sonuçlarını potansiyel olarak iyileştirebilir.

**Anahtar Kelimeler:** Sepsis, ensefalopati, mortalite

## INTRODUCTION

Sepsis is a life-threatening organ dysfunction due to dysregulated host response to infection[1]. Atypical presentations can be particularly more common in older patients[2-5]. Although sepsis affects all age groups, older patients are at greater risk as a result of increased frequency of comorbidities, malnutrition, polypharmacy, immunosenescence and inflammaging[6]. Sepsis-related in-hospital mortality varies between 30% and 60% in older patients[3]. Besides that, with the advances in treatment, a decrease in sepsis-related mortality has been reported in older patients likewise the patients under the age of 65 years[7]. Poor prognosis in patients with sepsis is closely associated with advanced age, disease severity, organ failures, and comorbidities[8,9]. Sepsis-associated encephalopathy (SAE) is a diffuse cerebral dysfunction resulted from a dysregulated host response without central nervous system infection[10]. Although the mechanisms are incompletely understood, compromised blood brain barrier, increased central nervous cytokine levels and microglial and astrocytic activation which results in neuroinflammation are the probable pathophysiological and molecular alterations[11]. The clinical course of the patient with SAE is characterized by the changes in patient's consciousness[12]. In the study by Young et al.[13], SAE was reported to be associated with poor prognosis and also mortality was correlated with the severity of SAE. Survivors of the sepsis have been reported to have a tendency to develop cognitive alterations[14,15], however, the data about the long-term survival after an episode of sepsis are limited among older adults. In this retrospective cohort study, we aimed to determine the factors influencing one-year mortality in patients aged 65 years and over, who were hospitalized with sepsis, emphasizing the role of SAE.

## MATERIALS AND METHODS

### Patient Selection

This retrospective study included patients aged 65 years and above, with a diagnosis of sepsis in accordance with the Sepsis-3 definition[3], who were admitted to the intensive care unit (ICU) of Ege University Faculty of Medicine, Department of Internal Medicine between January 2013 and January 2023. The study was performed with the permission of Ege University Medical Research Ethics Committee (decision no: 24-3.1T/77, date: 21.03.2024), and adhered to the principles of the Declaration of Helsinki. For each patient included in the present study, we retrospectively collected the following data: the medical history, general data including age, gender, comorbidities and laboratory parameters of the patients during ICU admission. The Charlson Comorbidity Index (CCI) was calculated for comorbidity assessment[16]. For detecting acute kidney injury at any stage, the Kidney Disease Improving Global Outcomes criteria were used[17]. The sequential organ failure assessment (SOFA) score on ICU admission was recorded. SAE identification was defined as a Glagow coma score <15 or the presence of delirium confirmed by the confusion assessment method for the intensive care unit (CAM-ICU)[18,19]. Exclusion criteria were as follows: having a history of advanced stage of malignancy, being on palliative care, having central nervous system infections, having sedative-related cognitive effects, the presence of chronic alcohol or drug abuse, and having severe electrolyte imbalances.

### Statistical Analysis

In this study, descriptive statistics were used to summarize the collected data. For continuous variables, the data obtained were presented considering their distribution: mean ± standard deviation or median with minimum and maximum values were used and displayed in tables. Categorical variables were represented as counts and percentages. The normality of numerical variables was checked using the Shapiro-Wilk, Kolmogorov-Smirnov, and Anderson-Darling tests.

For the analysis of categorical variables between the groups, the Pearson chi-square test was applied to 2x2 tables with expected observations of five or more. The Fisher's exact test was used for tables where expected observations were fewer than five. For RxC tables with small expected observations, the Fisher Freeman Halton test was utilized.

In comparisons of two independent groups, the Independent Samples t-test was employed for numerical variables with a normal distribution. The Mann-Whitney U test was used when the distribution was not normal.

Cox regression analysis was used to identify factors affecting 1-year mortality, with both univariable and multivariable analyses performed. The univariable Cox regression analysis examined the effect of each independent variable such as gender, encephalopathy, bacteremia, age, CCI, SOFA score, albumin, lactate, hemoglobin, neutrophil/lymphocyte ratio (NLR), international normalized ratio (INR), activated partial thromboplastin time (APTT), and procalcitonin levels on 1-year mortality. Hazard ratio (HR) with 95% confidence intervals (CI) and p values were calculated for each factor. In the multivariable analysis, the combined effect of significant variables from the univariable analysis was evaluated, adjusting for other factors, and HR, 95% CI, and p values were presented.

The Kaplan-Meier survival analysis was used to examine the factors affecting 1-year mortality among patients who survived during the hospital stay. The analysis assessed the impact of variables such as the presence or absence of encephalopathy on 1-year mortality. The log-rank test determined the significance of differences in survival times between the groups.

Statistical analyses were conducted using Jamovi (version 2.3.28) and JASP (version 0.18.3) software. The significance level for all statistical tests was set at 0.05 (p value).

## RESULTS

Of the 207 participants, 50.7% were female (n=105) and 49.3% were male (n=102), with a mean age of 73.7±7.2 years. Prevalence of comorbidities was as follows: diabetes mellitus (DM) 35.7% (n=74), hypertension 60.9% (n=126), chronic renal failure 22.7% (n=47), cardiovascular diseases 27.1% (n=56), chronic obstructive pulmonary disease (COPD)/asthma 15.0% (n=31), chronic kidney disease 17.4% (n=36), malignancy 21.3% (n=44). Clinical outcomes during ICU follow-up included a median CCI of 5 points for sepsis patients and a median hospital stay of eight days. Encephalopathy was observed in 40.6% of the patients (n=84). The predominant sources of sepsis were respiratory system infections at 24.6% (n=51), followed by urinary tract infections at 20.3% (n=42), skin and soft tissue infections at 11.1% (n=23), and blood-catheter-related sepsis also at 11.1% (n=23). Other sources included abdominal infections at 8.7% (n=18), multiple foci at 8.7% (n=18), and various other causes at 8.2% (n=17). During ICU follow-up, mortality was recorded at 49.8% (n=103) among hospitalized sepsis patients. At the one-year follow-up of discharged patients, mortality was 25% (n=26). The median survival time over one year was 12 months. In intensive care, 55.6% of patients (n=115) required vasopressor support, and 62.8% (n=130) developed acute kidney injury. Hemodialysis was necessary for 20.3% of patients (n=42). The median SOFA score at the onset of sepsis was 7 points. Bacteremia was identified in 33.2% (n=68) of the cases Table 1.

During the ICU follow-up, analyses of hematologic and biochemical parameters are shown in Table 2.

**Table 1. Demographic and clinical variables for patients admitted to intensive care unit for sepsis**

| | Overall (n=207) |
|---|---|
| Age† | 73.7±7.2 |
| Gender‡ | |
| Female | 105 (50.7) |
| Male | 102 (49.3) |
| Diabetes mellitus, present‡ | 74 (35.7) |
| Hypertension, present‡ | 126 (60.9) |
| Heart failure, present‡ | 47 (22.7) |
| Cardiovascular disease, present‡ | 56 (27.1) |
| Chronic obstructive pulmonary disease, present‡ | 31 (15.0) |
| Chronic kidney disease, present‡ | 36 (17.4) |
| Malignancy, present‡ | 44 (21.3) |
| Charlson comorbidity index§ | 5.0 [1.0-10.0] |
| Encephalopathy, present‡ | 84 (40.6) |
| Source of sepsis‡ | |
| Respiratory system | 51 (24.6) |
| Urinary system | 42 (20.3) |
| Hepatobiliary system | 15 (7.2) |
| Skin/soft tissue | 23 (11.1) |
| Abdomen | 18 (8.7) |
| Blood-catheter | 23 (11.1) |
| Multiple focus | 18 (8.7) |
| Other | 17 (8.2) |
| Length of stay, days§ | 8.0 [1.0-80.0] |
| In hospital mortality, yes‡ | 103 (49.8) |
| 1-year mortality, yes‡ | 26 (25.0) |
| Post discharge survival time (months)§ | 12.0 [1.0-12.0] |
| Vasopressor support, present‡ | 115 (55.6) |
| Acute kidney injury, present‡ | 130 (62.8) |
| Need for hemodialysis, present‡ | 42 (20.3) |
| SOFA score at onset of sepsis§ | 7.0 [0.0-16.0] |
| Bacteremia, present‡ | 68 (33.2) |

†: Values are presented as mean ± standard deviation for continuous variables, ‡: Values for categorical variables are presented as number (percentage) of patients, §: Median values with range (minimum-maximum) are shown for continuous variables, SOFA: Sequential organ failure assessment

In the analysis of demographic and clinical variables between patients with and without mortality at the 1-year follow-up post-discharge, significant findings included a higher mean age in the mortality group (p<0.001). The incidence of COPD/asthma was also significantly greater in the mortality group at 30.8% compared to those without mortality (p=0.011). No statistically significant differences were found between the groups regarding gender, diabetes mellitus, hypertension, chronic renal failure, cardiovascular diseases, chronic kidney disease, and malignancy (p>0.05 for each). At the 1-year follow-up after discharge, patients who died had significantly higher CCI and SOFA scores compared to survivors (p=0.002 and p=0.047, respectively). Additionally, a higher prevalence of encephalopathy was observed among deceased patients (p<0.001), along with longer hospital stays (p=0.013). Formun Üstü On the other hand, no significant differences were observed between the groups regarding the sources of sepsis, the need for vasopressor support, acute kidney injury, the need for hemodialysis, and bacteremia (p>0.05 for each) Table 3.

Laboratory values were analyzed in 104 patients followed up post-discharge. The bilirubin level was 0.6 mg/dL, aspartate aminotransferase 28.5 U/L, INR 1.1, prothrombin time (PT) 13.4 seconds, and APTT 27.3 seconds. Fibrinogen level was 543.0 mg/dL, and baseline creatinine 3.0 mg/dL. The C-reactive protein (CRP) level was 196.0 mg/L, and albumin 3.1 g/dL with a standard deviation of 0.6 at baseline. Hematologic parameters included a neutrophil count of 9535 cells/mm$^3$, lymphocyte count of 815 cells/mm$^3$, hemoglobin level of 10.9 g/dL, platelet count of 165.000 cells/mm$^3$, NLR of 12.1, lactate level of 1.9 mmol/L, and procalcitonin of 5.5 ng/mL. At discharge, CRP and albumin levels were 15.0 mg/L and 2.9±0.7 g/dL, respectively.

According to pairwise comparisons, INR (p=0.031), PT (p=0.038), and lactate levels (p=0.012) were significantly higher in patients with mortality at the 1-year follow-up. Conversely, albumin levels measured at baseline and discharge were significantly lower in patients with mortality (p=0.032 and p=0.004, respectively). Other laboratory parameters, including fibrinogen, creatinine, CRP, neutrophils, lymphocytes, hemoglobin, platelets, NLR, and procalcitonin, values did not show significant differences between the groups (p>0.05 for each) Table 4.

When analyzing factors that might affect 1-year mortality rates post-discharge, univariate Cox regression analysis revealed significantly higher mortality risk in patients with encephalopathy, with a HR of 9.69 (CI: 1.94-48.45, p=0.006). Additionally, each unit increase in INR values raised the mortality risk by nearly threefold (HR: 2.97, CI: 1.20-7.36, p=0.019). An increase in APTT value by each unit was associated with an 8% increase in the 1-year mortality risk (HR: 1.08, CI: 1.01-1.15, p=0.017). However, other variables such as gender, bacteremia, CCI, SOFA score, albumin, lactate, hemoglobin,

**Table 2. Laboratory parameters in patients admitted to intensive care unit for sepsis**

| | Overall (n=207) |
|---|---|
| Bilirubin[§] | 0.7 [0.1-11.0] |
| INR[§] | 1.2 [0.8-4.2] |
| PT[§] | 13.9 [9.9-41.7] |
| APTT[§] | 28.6 [17.0-80.0] |
| Fibrinogen[§] | 483.0 [40.0-1044.0] |
| Creatinine[§] | 2.5 [0.2-9.6] |
| CRP[§] | 184.0 [1.0-619.0] |
| Albumin[§] | 2.9 [1.2-9.0] |
| Neutrophils[§] | 9380.0 [20.0-46600.0] |
| Lymphocyte[§] | 760.0 [9.4-87520.0] |
| Hemoglobin[§] | 10.1 [6.3-570.0] |
| Platelet count[§] | 144000.0 [70.0-570000.0] |
| NLR[§] | 12.2 [0.1-278.0] |
| Lactate[§] | 2.3 [0.5-15.0] |
| Procalcitonin[§] | 4.7 [0.0-100.0] |

[§]: Data represents median values with the range (minimum-maximum) for key biochemical and hematologic parameters monitored during the intensive care unit stay of sepsis patients. INR: International normalized Ratio, PT: Prothrombin time, APTT: Activated partial thromboplastin time, CRP: C-reactive protein, NLR: Neutrophil/lymphocyte ratio

NLR, and procalcitonin did not significantly affect mortality risk (p>0.05 for each).

In multivariate analysis, encephalopathy, INR values, and age significantly influenced mortality risk. The 1-year mortality risk in patients with encephalopathy was 3.41 times higher than in those without (HR: 3.41, CI: 1.46-7.95, p=0.005). Each unit increase in age led to a 6% increase in mortality risk (HR: 1.06, CI: 1.01-1.11, p=0.018). Increases in INR levels resulted in a 2.42-fold increase in mortality risk (HR: 2.42, CI: 1.28-4.58, p=0.007) Table 5. Post discharge mortality outcomes in sepsis patients with and without encephalopathy are shown in Figure 1.

## Discussion

In the current study, we found that patients with encephalopathy exhibited significantly higher one-year mortality rates, underscoring the need for intensive neurological monitoring and management in older sepsis survivors. Elevated INR levels were associated with increased one-year mortality,

**Table 3. Comparison of demographic and clinical characteristics in terms of 1-year mortality in discharged sepsis patients**

| | Overall (n=104) | 1-year mortality | | p value |
|---|---|---|---|---|
| | | Yes (n=26) | No (n=78) | |
| Age† | 73.6±7.7 | 78.7±6.2 | 71.9±7.4 | **<0.001** |
| Gender‡ | | | | |
| Female | 49 (47.1) | 15 (57.7) | 34 (43.6) | 0.307 |
| Male | 55 (52.9) | 11 (42.3) | 44 (56.4) | |
| Diabetes mellitus, present‡ | 40 (38.5) | 10 (38.5) | 30 (38.5) | 0.999 |
| Hypertension, present‡ | 66 (63.5) | 13 (50.0) | 53 (67.9) | 0.158 |
| Heart failure, present‡ | 25 (24.0) | 9 (34.6) | 16 (20.5) | 0.233 |
| Cardiovascular disease, present‡ | 30 (28.8) | 11 (42.3) | 19 (24.4) | 0.134 |
| Chronic obstructive pulmonary disease, present‡ | 15 (14.4) | 8 (30.8) | 7 (9.0) | **0.011** |
| Chronic kidney disease, present‡ | 17 (16.3) | 7 (26.9) | 10 (12.8) | 0.125 |
| Malignancy, present‡ | 15 (14.4) | 2 (7.7) | 13 (16.7) | 0.346 |
| Charlson comorbidity index§ | 5.0 [1.0-10.0] | 6.0 [3.0-9.0] | 5.0 [1.0-10.0] | **0.002** |
| Encephalopathy, present‡ | 29 (27.9) | 15 (57.7) | 14 (17.9) | **<0.001** |
| Source of sepsis‡ | | | | |
| Respiratory system | 21 (20.2) | 7 (26.9) | 14 (17.9) | 0.780 |
| Urinary system | 29 (27.9) | 9 (34.6) | 20 (25.6) | |
| Hepatobiliary system | 11 (10.6) | 2 (7.7) | 9 (11.5) | |
| Skin/soft tissue | 8 (7.7) | 2 (7.7) | 6 (7.7) | |
| Abdomen | 7 (6.7) | 1 (3.8) | 6 (7.7) | |
| Blood-catheter | 11 (10.6) | 3 (11.5) | 8 (10.3) | |
| Multiple focus | 10 (9.6) | 2 (7.7) | 8 (10.3) | |
| Other | 7 (6.7) | 0 (0.0) | 7 (9.0) | |
| Length of stay, days§ | 10.0 [3.0-80.0] | 13.0 [3.0-58.0] | 9.0 [3.0-80.0] | **0.013** |
| Vasopressor support, present‡ | 47 (45.2) | 15 (57.7) | 32 (41.0) | 0.211 |
| Acute kidney injury, present‡ | 66 (63.5) | 18 (69.2) | 48 (61.5) | 0.638 |
| Need for hemodialysis, present‡ | 17 (16.3) | 3 (11.5) | 14 (17.9) | 0.552 |
| SOFA score at onset of sepsis§ | 7.0 [0.0-14.0] | 7.5 [2.0-10.0] | 6.0 [0.0-14.0] | **0.047** |
| Bacteremia, present‡ | 26 (25.0) | 7 (26.9) | 19 (24.4) | 0.999 |

†: Mean ± standard deviation, ‡: n (%), §: Median (min.-max.), SOFA: Sequential organ failure assessment

emphasizing the importance of monitoring coagulation states as part of post-sepsis patient care. Advanced age was found to incrementally increase the risk of mortality within one year after sepsis, highlighting the necessity for age-adapted therapeutic strategies in elderly patients. Common comorbidities such as DM, hypertension, along with traditional inflammatory markers like CRP and procalcitonin, did not significantly predict mortality, suggesting that specific post-sepsis conditions such as encephalopathy and coagulation disturbances may be more relevant predictors in this population. Kaplan-Meier analysis revealed that variables such as the presence or absence of encephalopathy distinctly affected survival outcomes, providing a clear direction for targeted interventions. The use of both univariate and multivariate Cox regression analyses helped to identify the most critical determinants of mortality, offering actionable insights for clinicians focusing on the long-term recovery and management of older sepsis patients.

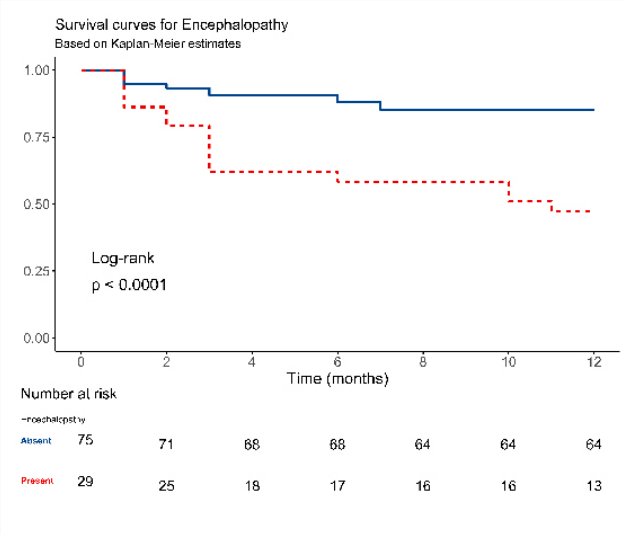


**Figure 1.** 1-Year post-discharge mortality outcomes in sepsis patients with and without encephalopathy

Sepsis can be complicated with SAE. In the current literature, SAE was reported in up to 40-70% of patients[20,21]. In our study, SAE frequency was 40.6% which is consistent with literature. In hospital mortality was 49.8%, which is also similar with reports in the current data[3].

When compared in terms of one-year mortality, SAE was significantly higher in non-survivors. Long-term sequel including neurocognitive dysfunction was reported in many studies[22-24], however, data about the mortality are limited. In our study, one-year mortality was 25% and presence of SAE during ICU increased the one-year mortality. Many older sepsis survivors develop chronic critical illness, which results in hospital readmissions and possibly death[25]. In a prospective cohort study, one-year mortality was 63.3% and severely frail group was associated with worse outcomes[26]. Our mortality results were lower than the literature data. Although it is difficult to compare the populations, sociocultural characteristics may be one of the reasons. We did not compare the frailty, which may be an also possible explanation.

**Table 4. Comparison of biochemical and hematologic characteristics in terms of 1-year mortality in discharged sepsis patients**

| | Overall (n=104) | 1-Year Mortality | | p value |
|---|---|---|---|---|
| | | Yes (n=26) | No (n=78) | |
| Bilirubin§ | 0.6 [0.2-10.1] | 0.6 [0.2-10.1] | 0.6 [0.2-10.1] | 0.895 |
| INR§ | 1.1 [0.8-3.9] | 1.2 [0.9-3.9] | 1.1 [0.8-2.0] | **0.031** |
| PT§ | 13.4 [9.9-39.0] | 14.9 [10.7-39.0] | 13.2 [9.9-23.3] | **0.038** |
| APTT§ | 27.3 [17.0-64.9] | 28.8 [21.0-64.9] | 27.0 [17.0-47.0] | 0.091 |
| Fibrinogen§ | 543.0 [40.0-900.0] | 503.0 [40.0-610.0] | 577.0 [214.0-900.0] | 0.263 |
| Creatinine§ | 3.0 [0.4-9.6] | 2.8 [1.0-8.2] | 3.2 [0.4-9.6] | 0.647 |
| CRP§ | 196.0 [1.0-619.0] | 208.5 [24.0-550.0] | 184.0 [1.0-619.0] | 0.785 |
| Albumin† | 3.1±0.6 | 2.9±0.4 | 3.2±0.6 | **0.032** |
| Hemoglobin§ | 10.9 [6.6-124.0] | 11.3 [7.3-14.0] | 10.6 [6.6-124.0] | 0.893 |
| NLR§ | 12.1 [0.1-109.6] | 15.2 [3.3-46.9] | 11.8 [0.1-109.6] | 0.331 |
| Lactate§ | 1.9 [0.5-9.4] | 2.5 [0.6-9.4] | 1.5 [0.5-8.4] | **0.012** |
| Procalcitonin§ | 5.5 [0.0-100.0] | 2.6 [0.3-100.0] | 8.3 [0.0-100.0] | 0.391 |
| Discharge CRP§ | 15.0 [0.4-110.0] | 22.0 [2.3-110.0] | 12.5 [0.4-100.0] | 0.157 |
| Discharge albumin† | 2.9±0.7 | 2.5±0.7 | 3.0±0.7 | **0.004** |

†: Mean ± standard deviation, §: Median (min.-max.), INR: International normalized ratio, PT: Prothrombin time, APTT: Activated partial thromboplastin time, CRP: C-reactive protein, NLR: Neutrophil/lymphocyte ratio

**Table 5. Factors affecting 1-year survival in discharged sepsis patients**

| Dependent: 1-year Mortality, Time: Survival time (Months) | HR (univariable) | HR (multivariable) |
|---|---|---|
| Gender: Male vs. Female | 0.84 (0.20-3.52, p=0.813) | - |
| Encephalopathy: Present vs. absent | 9.69 (1.94-48.45, p=0.006) | 3.41 (1.46-7.95, **p=0.005**) |
| Bacteremia: Present vs. absent | 0.84 (0.17-4.19, p=0.836) | - |
| Age | 1.08 (1.00-1.16, p=0.065) | 1.06 (1.01-1.11, **p=0.018**) |
| Charlson comorbidity index | 1.09 (0.78-1.53, p=0.602) | - |
| SOFA score | 1.08 (0.88-1.33, p=0.481) | - |
| Albumin | 0.72 (0.17-2.99, p=0.655) | - |
| Lactate | 0.96 (0.63-1.47, p=0.842) | - |
| Hemoglobin | 1.15 (0.82-1.60, p=0.423) | - |
| NLR | 0.99 (0.96-1.03, p=0.728) | - |
| INR | 2.97 (1.20-7.36, **p=0.019**) | 2.42 (1.28-4.58, **p=0.007**) |
| APTT | 1.08 (1.01-1.15, **p=0.017**) | - |
| Procalcitonin | 0.98 (0.96-1.01, p=0.218) | - |

Depicts hazard ratios (HR) from univariable and multivariable Cox regression analyses assessing factors influencing 1-year mortality among discharged sepsis patients. SOFA: Sequential organ failure assessment, NLR: Neutrophil/lymphocyte ratio, INR: International normalized ratio, APTT: Activated partial thromboplastin time, HR: Hazard ratios

Age was associated with increased in-hospital mortality in older sepsis patients[27,28]. Additionally, we found that increased age was a risk factor for one-year mortality. Albumin, both on admission and at discharge, were significantly higher in survivors. Low albumin is a well-known poor prognostic factor in sepsis[29]. Our study also reveals the effect of discharge albumin value on one-year mortality, for which there are limited data in the literature.

The presence of sepsis-associated coagulopathy predicts hospital mortality, with increasing INR values at higher risk[30]. In addition to current literature, we determined that INR level was also associated with long-term mortality.

### Study Limitations

This study, while shedding light on significant prognostic factors for one-year mortality post-sepsis, is not without limitations. The retrospective design limits the ability to infer causality from the associations found. Data were derived from a single-center, which may limit the generalizability of the findings to different healthcare settings or populations. Another limitation lies in the variability of clinical presentations and the complexity of sepsis, which may influence the recorded variables. The potential for unrecognized confounders, despite meticulous data collection and analysis, cannot be entirely ruled out. Moreover, some variables of interest, such as detailed functional status post-discharge or quality of life assessments, were not available for analysis.

Future studies should aim to include prospective designs, multi-center data, and a broader patient demographics to validate and expand upon these findings. Longitudinal studies that track the recovery trajectory of sepsis survivors beyond the one-year mark could provide further insights into the chronic impact of sepsis and its management. Additionally, the integration of qualitative data reflecting patient and caregiver experiences could offer a more holistic understanding of the post-discharge journey for sepsis survivors.

## CONCLUSION

The present study comprehensively explored the factors influencing one-year mortality in patients over the age of 65 years, who were hospitalized with sepsis, with a particular focus on the role of encephalopathy. The findings reveal that encephalopathy is an independent predictor of mortality within one year following hospital discharge. This association highlights the critical importance of neurological assessment in the management of sepsis, which could significantly affect patient outcomes. Age, a non-modifiable risk factor, was also found to be incrementally associated with higher mortality, underscoring the need for heightened vigilance and potentially different therapeutic strategies in the older sepsis patient population. The study's findings support the necessity for a multidisciplinary approach to sepsis treatment, considering both the immediate and long-term implications of this condition in the older sepsis patients.

These insights are instrumental for clinicians who are tasked with providing care for this vulnerable patient population. They indicate a pivotal shift towards including comprehensive neurological evaluations in routine sepsis management protocols. Integrating such parameters into the prognostic models for sepsis may improve the ability to identify high-risk patients and tailor interventions more effectively, potentially improving survival outcomes.

## Ethics

**Ethics Committee Approval:** The study was conducted after obtaining the necessary permissions from Ege University Medical Research Ethics Committee (decision no: 24-3.1T/77, date: 21.03.2024). The study was conducted in accordance with Good Clinical Practice Guidelines and adhered to the principles of the Declaration of Helsinki.

**Informed Consent:** Retrospective study.

## Authorship Contributions

Surgical and Medical Practices: Ş.M.K.B., İ.A.K., C.A., Z.T.K., R.Y., H.D.A., M.D.E., D.B., Concept: M.K.B., İ.A.K., C.A., D.B., Design: M.K.B., İ.A.K., C.A., D.B., Data Collection or Processing: Z.T.K., R.Y., H.D.A., M.D.E., D.B., Analysis or Interpretation: Z.T.K., R.Y., H.D.A., M.D.E., D.B., Literature Search: Z.T.K., R.Y., H.D.A., M.D.E., D.B., Writing: Ş.M.K.B., İ.A.K., C.A., D.B.

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# Brucellosis Awareness and Knowledge: A Single Center Study From Eastern Anatolia

## Bruselloz Farkındalığı ve Bilgi Düzeyi: Doğu Anadolu'dan Tek Merkez Çalışması

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**ABSTRACT**

**Aim:** Brucellosis is a zoonotic disease prevalent in Turkey. The aim of this study is to assess the knowledge level about brucellosis and to lay the groundwork for necessary preventive measures.

**Materials and Methods:** This study was conducted over a six-month period at a single state hospital's infectious diseases outpatient clinic. Volunteers who met the inclusion criteria completed a questionnaire. Participation were grouped into those involved in animal husbandry, healthcare workers, and others. Scores were assigned based on responses to the questionnaire.

**Results:** The study included a total of 400 participation, of which 191 (47.8%) were female and 209 (52.2%) were male. The most common occupation was animal husbandry [88 (22%)]. The majority of participation [246 (61.5%)] resided in the provincial capital. Healthcare workers exhibited the highest awareness of brucellosis (93.1%). Seminars were identified as the most important source of information for healthcare workers (72.2%), while personal experience with the disease was most common among those involved in animal husbandry (15.4%). University graduates demonstrated higher awareness levels. Those who had experienced brucellosis, seminars, the internet, and television were identified as more accurate sources of information.

**Conclusion:** In the face of increasing global zoonotic diseases, raising awareness within the framework of the one health concept is essential. Measures such as vaccination, isolation, and waste control should be emphasized for brucellosis prevention in animals. The importance of consuming pasteurized dairy products should be emphasized. Correct information should be disseminated through platforms such as seminars, the internet, and television.

**Keywords:** Brucellosis, livestock, One Health

**ÖZ**

**Amaç:** Bruselloz zoonotik bir hastalıktır. Türkiye endemik bölgede yer almaktadır. Amacımız bruselloz hakkında bilgi düzeyini ölçmek ve önlem amacıyla gereken faaliyetler için zemin hazırlamaktır.

**Gereç ve Yöntem:** Çalışma altı aylık periyodda tek merkez devlet hastanesinde enfeksiyon hastalıkları polikliniğinde, dahil edilme kriterlerini karşılayan gönüllülerle anket doldurularak yapıldı. Katılımcılar hayvancılıkla uğraşanlar, sağlık çalışanları ve diğerleri olacak şekilde gruplara ayrıldı. Anket sorularına verilen cevaplara göre puanlama yapıldı.

**Bulgular:** Çalışma 191'i (%47,8) kadın, 209'u (%52,2) erkek toplam 400 kişi ile yapıldı. En sık meslek grubu 88 (%22) kişi ile hayvancılıkla uğraşanlardı. En sık katılım 246 (%61,5) kişi ile il merkezindendi. Sağlık çalışanlarında (%93,1) brusellozu duyma oranı en yüksekti. Sağlık çalışanlarında en önemli bilgi kaynağı seminerler (%72,2), hayvancılıkla uğraşanlarda ise hastalığı geçirmekti (%15,4). Puanlama sonucunda sağlık çalışanları en farkında olan grup olarak tespit edildi. Üniversite mezunlarının farkındalığının daha yüksek olduğu tespit edildi. Brusellozun bilgi kaynakları arasında hastalığı geçiren kişiler, seminerler, internet ve televizyon daha doğru kaynaklar olarak tespit edildi.

**Sonuç:** Küreselleşen dünyada artan zoonotik hastalıklarla mücadele için Tek Sağlık kavramı çerçevesinde farkındalığın artırılması gerekmektedir. Hayvanlarda brusellozu önlemek amacıyla gereken aşılama, izolasyon önlemleri, atık kontrolü tedbirleri anlatılmalı ve enfekte hayvan bildirimleri eksiksiz yapılmalıdır. Süt ve süt ürünlerinin pastörize edilmeden tüketilmemesi gerektiği vurgulanmalıdır. Doğru bilginin aktarılması için seminer, internet ve televizyon gibi platformlar kullanılmalıdır.

**Anahtar Kelimeler:** Bruselloz, hayvancılık, Tek Sağlık

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## INTRODUCTION

Brucellosis is a zoonotic disease primarily hosted by animals including cattle, sheep, goats, and pigs, with humans serving as incidental hosts[1]. Brucellosis is caused by Gram-negative, facultative, intracellular *Coccobacilli* belonging to the *genus brucella*. There are basically four types that cause the disease in humans, *Brucella abortus, Brucella melitensis, Brucella suis and Brucella canis*[2]. The most common species in Turkey is *Brucella melitensis,* which is found in goats and sheep. Between 150,000 to 250,000 human brucellosis cases are reported globally each year[3]. The Mediterranean basin countries are among the regions where the disease is endemic, and Turkey is situated within this region[4]. In our country, it is most frequently observed in Southeastern and Eastern Anatolia[5].

The routes of transmission to humans often involve the consumption of raw or unpasteurized milk and dairy products, direct contact of skin or mucous membranes with infected animal tissues and fluids, or inhalation of infected aerosols[6]. *Brucella* species can survive for 2-6 weeks in raw milk, 6 weeks in cream at 4 °C, 30 day in ice cream, and 15-100 days in fresh cheese. Contamination can be prevented by boiling milk and dairy products, as well as thoroughly cooking meat. Human-to-human transmissions are rare. The clinical symptoms include fever, chills, headache, myalgia, arthralgia, night sweats, fatigue, anorexia, and weight loss. Depending on organ involvement, symptoms may develop in the relevant region[7].

Diagnosis of brucellosis involves blood, tissue, and bone marrow cultures, along with serological tests such as rose bengal, wright tube agglutination, and coombs agglutination. Due to its intracellular location, treatment is challenging, necessitating the use of combination antibiotics for at least six weeks. Prolonged organ involvement may require an extended treatment duration[8].

The concept of One Health, which highlights the necessity of various professional groups working together to combat the increasing zoonotic diseases in a globalizing world, signifies the holistic approach of addressing humans, animals, and the environment together. To control brucellosis in animals, screening should be performed, susceptible animals should be vaccinated, and the infected animals should be slaughtered. Controlling brucellosis in animals is essential for mitigating the disease in humans. Pasteurization processes and class II and III biosafety cabinet precautions should be taken in laboratories[9].

The aim of our study was to assess the level of knowledge regarding brucellosis, a prevalent disease in the region, and to pave the way for essential preventive measures based on the findings obtained.

## MATERIALS AND METHODS

### Selection and Description of the Cases

This study was conducted with individuals in the infectious diseases outpatient clinic at a single-center hospital between October 1, 2023, and March 31, 2024. Participants who were literate and voluntary were included in the study. A cross-sectional study was conducted employing a survey methodology. Participants were requested to complete the survey within approximately 10-15 minutes. The person's identity was kept confidential. Non-voluntary people were not included in the study. Informed consent was obtained from all participants who volunteered for the study. The study was conducted after obtaining the necessary permissions from Ağrı İbrahim Çeçen University Ethics Committee (decision no: E-95531838-050.99-83962, date: 06.10.2023).

### Technical Information

The survey comprised 20 questions assessing participants' demographic information, status, and knowledge of brucellosis. Additionally, individuals engaged in animal husbandry were presented with a supplementary form containing 12 questions. The survey results were summarized out of 17 full points, with 1 point given for correct answers and 0 points given for wrong answers and answers without information. Participants were categorized into groups such as healthcare workers, livestock workers, and others.

### Statistical Analysis

Based on a power analysis utilizing 2017 brucellosis incidence data, the sample size was determined to be 384 individuals, and ultimately, 400 participants were included in the study. Descriptive statistics, including mean or median values for continuous variables and number (n) and percentage (%) values for categorical variables, were utilized for data presentation. The Shapiro-Wilk test assessed the normality of numerical variables. For normally distributed variables, independent group t-tests were employed for between-group comparisons, while the Mann-Whitney U and Kruskal-Wallis tests were utilized for variables not adhering to normal distribution. Categorical variables were compared using the chi-square test. Statistical analyses were conducted using the SPSS 26 Windows version program, with results considered statistically significant at $p<0.05$.

## RESULTS

A total of 400 people who met the inclusion criteria were included in this study. Of the participants, 191 (47.8%) were women and 209 (52.2%) were men, and the mean age was 35.7 (±12) years. 33 (8.2%) participants had no formal education, 97 (24.3%) were primary school graduates,

49 (12.2%) had completed middle school, 89 (22.3%) were high school graduates, and 132 (33%) were university graduates. Regarding occupation, 88 (22%) participants were involved in animal husbandry, 86 (21.5%) were housewives, and 64 (16%) were blue-collar workers. In Table 1, the demographic data of the participants are provided by dividing them into groups.

It was determined that the most frequent application was from the city center with 246 (61.5%) participants, followed by Diyadin district with 47 (11.8%) participants, and Taşlıçay district with 25 (6.3%) participants. The participants' places of residence are listed in Table 2. Participants' answers to the survey questions are presented in Table 3.

Among the participants, 86.3% had heard of brucellosis. The rate of hearing about brucellosis was higher among healthcare (93.1%) and livestock workers (90.9%) (p=0.000). When information sources were compared between the groups, it was found that the most significant source of information for healthcare professionals (72.2%) was learning through seminars (p=0.000). The most important source of information for those involved in animal husbandry (15.4%) was learning about the disease through experience (p=0.000). The rate of knowledge about animal-to-animal transmission was higher among those engaged in animal husbandry (43.2%) (p=0.000).

It was found that individuals involved in animal husbandry were more likely to believe that yogurt (38.6%) and Kashar cheese (23.9%) were contaminated (p=0.027, p=0.042), compared to other groups. Additionally, healthcare workers

**Table 1. Demographic data of participants**

| Groups | All participants | Husbandry | Healthcare workers | Others |
|---|---|---|---|---|
| **n (%)** | 400 (100%) | 88 (22%) | 29 (7.3%) | 283 (70.7%) |
| **Age mean (ss)** | 35.76 (±12) | 38.98 (±12.2) | 23.83 (±6.8) | 35.98 (±11.7) |
| **Gender (n, %)** | | | | |
| Female | 191 (47.8%) | 31 (35.2%) | 19 (65.5%) | 141 (49.8%) |
| Male | 209 (52.2%) | 57 (64. 8%) | 10 (34.5%) | 142 (50.2%) |
| **Education (n, %)** | | | | |
| No education | 33 (8.2%) | 9 (10.2%) | | 24 (8.5%) |
| Primary school | 97 (24.3%) | 40 (45.5%) | | 57 (20.1%) |
| Middle school | 49 (12.2%) | 12 (13.6%) | | 37 (13.1%) |
| High school | 89 (22.3%) | 22 (25%) | 12 (41.4%) | 55 (19.4%) |
| University | 132 (33%) | 5 (5.7%) | 17 (58.6%) | 110 (38.9%) |
| **Occupation (n, %)** | | | | |
| Animal husbandry | 88 (22%) | 88 (100%) | | |
| Housewife | 86 (21.5%) | | | 86 (30.3%) |
| White-collar | 64 (16%) | | | 64 (22.7%) |
| Blue-collar | 62 (15.5%) | | | 62 (21.9%) |
| Student | 32 (8%) | | | 32 (11.3%) |
| Healthcare worker | 29 (7.3%) | | 29 (7.3%) | |
| Retired | 8 (2%) | | | 8 (2.9%) |
| Unknown | 31 (7.7%) | | | 31 (10.9%) |

**Table 2. Residence of participants**

| Groups | All participants | Husbandry | Healthcare workers | Others |
|---|---|---|---|---|
| n (%) | 400 (100%) | 88 (22%) | 29 (7.3%) | 283 (70.7%) |
| City center | 246 (61.5%) | 33 (37.5%) | 26 (89.8%) | 187 (66.1%) |
| Diyadin | 47 (11.8%) | 18 (20.5%) | 1 (3.4%) | 28 (9.9%) |
| Taşlıçay | 25 (6.3%) | 13 (14.8%) | 1 (3.4%) | 11 (3.9%) |
| Hamur | 22 (5.4%) | 9 (10.2%) | | 13 (4.5%) |
| Doğubayazıt | 19 (4.7%) | 3 (3.4%) | | 16 (5.7%) |
| Eleşkirt | 15 (3.7%) | 6 (6.8%) | | 9 (3.2%) |
| Tutak | 11 (2.8%) | 6 (6.8%) | | 5 (1.8%) |
| Neighboring province | 9 (2.3%) | | 1 (3.4%) | 8 (2.8%) |
| Distant province | 6 (1.5%) | | | 6 (2.1%) |

**Table 3. Answers to survey questions**

| Groups | All participants | Husbandry | Healthcare workers | Others |
|---|---|---|---|---|
| **n (%)** | 400 (100%) | 88 (22%) | 29 (7.3%) | 283 (70.7%) |
| **Heard of brucellosis** | 345 (86.3%) | 80 (90.9%) | 27 (93.1%) | 238 (84.1%) |
| **Information source** | | | | |
| Respondents | 265 (100%) | 65 (24.5%) | 18 (6.8%) | 182 (68.7%) |
| Seminar | 29 (10.9%) | 1 (1.5%) | 13 (72.2%) | 15 (8.2%) |
| Relatives | 62 (23.4%) | 13 (20%) | 3 (16.7%) | 46 (25.3%) |
| Public | 122 (46%) | 32 (49.2%) | | 90 (49.6%) |
| Family | 21 (7.9%) | 4 (6.2%) | | 17 (9.3%) |
| I had the disease | 18 (6.8%) | 10 (15.4%) | 2 (11.1%) | 8 (4.4%) |
| Internet, TV | 12 (4.6%) | 5 (7.7%) | | 5 (2.7%) |
| Public education | 1 (0.4%) | | | 1 (0.5%) |
| **Does it cause disease in humans?** | | | | |
| Yes | 273 (68.3%) | 59 (67%) | 21 (72.4%) | 193 (68.2%) |
| No | 11 (2.8%) | 4 (4.5%) | | 7 (2.5%) |
| No idea | 116 (29%) | 25 (28.4%) | 8 (27.6%) | 83 (29.3%) |
| **Is it transmitted from animals to humans?** | | | | |
| Yes | 252 (63%) | 61 (69.3%) | 23 (79.3%) | 168 (59.4%) |
| No | 11 (2.8%) | 3 (3.4%) | | 8 (2.8%) |
| No idea | 137(34.3%) | 24 (27.3%) | 6 (20.7%) | 107 (37.8%) |
| **Is it transmitted between animals?** | | | | |
| Yes | 96 (24%) | 38 (43.2%) | 7 (24.1%) | 51 (18%) |
| No | 28 (7%) | 4 (4.5%) | 6 (20.7%) | 18 (6.4%) |
| No idea | 276 (69%) | 46(52.3%) | 16 (55.2%) | 214 (75.6%) |
| **Can it be transmitted between people?** | | | | |
| Yes | 70 (17.5%) | 18 (20.5%) | 11 (37.9%) | 41 (14.5%) |
| No | 90 (22.5%) | 22 (25%) | 7 (24.2%) | 61 (21.5%) |
| No idea | 240 (60%) | 48 (54.5%) | 11 (37.9%) | 181 (64%) |
| **Which animals transmit it?** | | | | |
| Cattle, sheep, goat | 258 (64.5%) | 59 (67%) | 20 (69%) | 179 (63.3%) |
| Poultry | 19 (4.7%) | 6 (6.9%) | 4 (13.9%) | 9 (3.2%) |
| Bee | 4 (1%) | 1 (1.1%) | | 3 (1.1%) |
| Aquatic | 20 (5%) | 5 (5.7%) | 1 (3.4%) | 14 (4.9%) |
| No idea | 124 (31%) | 26 (29.5%) | 7 (24.1%) | 91 (32.2%) |
| **Ways of transmission** | | | | |
| Raw milk | 223 (55.8%) | 44 (50%) | 22 (75.9%) | 157 (55.5%) |
| Raw dairy products | 191 (47.8%) | 40 (45.5%) | 17 (58.6%) | 134 (47.3%) |
| Yogurt | 116 (29%) | 34 (38.6%) | 11 (37.9%) | 71 (25.1%) |
| Kashar cheese | 64 (16%) | 21 (23.9%) | 2 (6.9%) | 41 (14.5%) |
| Animal waste | 94 (23.5%) | 21 (23.9%) | 11 (37.9%) | 62 (21.9%) |
| Abort material | 72 (18%) | 18 (20.5%) | 10 (34.5%) | 44 (15.5%) |
| Undercooked meet | 97 (24.3%) | 19 (21.6%) | 12 (41.4%) | 66 (23.3%) |
| Laboratory | 34 (8.5%) | 4 (4.5%) | 7 (24.1%) | 23 (8.1%) |
| No idea | 113 (28.3%) | 27 (30.7%) | 4 (13.8%) | 82 (29%) |
| **Symptoms** | | | | |
| Fever | 120 (30%) | 21 (23.9%) | 18 (62.1%) | 81 (28.6%) |
| Sweating | 120 (30%) | 32 (36.4%) | 7 (24.1%) | 59 (20.8%) |
| Muscle/joint pain | 183 (45.8%) | 44 (50%) | 18 (62.1%) | 120 (42.4%) |
| Back pain | 84 (21%) | 28 (31.8%) | 10 (34.5%) | 45 (15.9%) |
| Jaundice | 30 (7.5%) | 6 (6.8%) | 4 (13.8%) | 20 (7.1%) |
| No idea | 178 (44.5%) | 34 (38.6%) | 9 (31%) | 135 (47.7%) |

**Table 3. Continued**

| Groups | All participants | Husbandry | Healthcare workers | Others |
|---|---|---|---|---|
| **Can contamination be prevented by boiling milk?** | | | | |
| Yes | 162 (40.5%) | 32 (36.4%) | 16 (55.2%) | 114 (40.3%) |
| No | 27 (6.8%) | 9 (10.2%) | 2 (6.9%) | 16 (5.7%) |
| No idea | 211 (52.7%) | 47 (53.4%) | 11 (37.9%) | 153 (51%) |
| **Can brucellosis be treated?** | | | | |
| Yes | 276 (69%) | 62 (70.5%) | 21 (72.4%) | 193 (68.2%) |
| No | 4 (1%) | 3 (3.4%) | | 1 (0.4%) |
| No idea | 120 (30%) | 23 (26.1%) | 8 (27.6%) | 89 (31.4%) |
| **Is there a vaccine for animals?** | | | | |
| Yes | 142 (35.5%) | 48 (54.5%) | 11 (37.9%) | 83 (29.3%) |
| No | 7 (1.8%) | 2 (2.3%) | 1 (3.4%) | 4 (1.4%) |
| No idea | 251 (62.8%) | 38 (43.2%) | 17 (58.6%) | 196 (69.3%) |
| **Is there a vaccine for humans?** | | | | |
| Yes | 82 (20.5%) | 25 (28.4%) | 6 (20.7%) | 51 (18%) |
| No | 19 (4.8%) | 2 (2.3%) | 4 (13.8%) | 13 (4.6%) |
| No idea | 299 (74.8%) | 61 (69.3%) | 19 (65.5%) | 219 (77.4%) |

demonstrated greater awareness of contamination risks in laboratory environments (24.1%) (p=0.004).

It was found that individuals working in animal husbandry (54.5%) were significantly more knowledgeable about animal vaccination (p=0.000), whereas healthcare professionals (13.8%) were more aware of the absence of vaccines for humans (p=0.031).

The answers to supplementary survey questions from participants involved in animal husbandry are presented in Table 4.

After scoring the answers provided by participants, the average score for healthcare workers was determined to be 8.66±0.9 out of 17 points, 7.16±0.4 for those engaged in animal husbandry, and 6.26±0.2 for the other group. A comparison between the groups revealed that healthcare workers (median 9) achieved higher scores compared to the other groups (median 6) (p=0.012).

Analyzing the impact of educational status on survey scores, it was found that university graduates (median 8) attained higher scores compared to high school graduates (median 7) and middle school graduates (median 4) (p=0.020, p=0.000), which indicated a significant difference.

When examining the influence of the information source on brucellosis on survey scores, individuals whose relatives had brucellosis (median 9), those who had the disease themselves (median 11), those who obtained information from media sources such as the internet and television (median 9), and those who attended seminars (median 10) tended to score higher compared to those who learned about the disease from their family (median 6) (p=0.009, p=0.000, p=0.040, p=0.003). Moreover, individuals with relatives affected by brucellosis (median 9), those who had the disease themselves (median 11), and those who attended seminars (median 10) achieved higher scores than individuals who learned about the disease from public awareness (median 7) (p=0.001, p=0.000, and p=0.001, respectively).

## DISCUSSION

Brucellosis, which is endemic in our country, continues to be a serious public health problem. In the study by Hull et al.[10] in 2018, the incidence of human brucellosis in Turkey was determined as 49.5 per 1,000,000, which was above the world average. According to 2017 Ministry of Health data, the incidence of brucellosis in Ağrı was 19 per 100,000[11]. The aim of our study was to measure the level of knowledge about brucellosis and to prepare the ground for the necessary activities for prevention.

In the study conducted by Alkan et al.[12] in 2022, it was found that 95.6% of individuals living in rural areas had heard about brucellosis. Similarly, Avcı et al.[13] 2017 study reported a rate of 98.5% among residents of a village settlement in Van province. Özen et al.[14], in 2020, found that 79.6% of hospital employees had heard about brucellosis. In contrast, Akkuş et al.[15] 2011 study reported a lower rate of 66% among individuals engaged in animal husbandry, while Babaoğlu et al.[16] 2017 study found a rate of 65.2% among those living in semi-urban areas. Notably, in the same study, the rate of awareness about brucellosis was significantly higher among high-risk professional groups such as veterinarians, agricultural engineers, and farmers (p=0.010). Additionally, awareness of brucellosis increased with the level of education (p=0.010)[16]. In our study, the rates of having heard about brucellosis were 90.9% among livestock workers, 93.1% among healthcare workers, and 86.3% overall. As a result of the survey scoring, the highest scores were received by healthcare workers, followed by those dealing with animal

**Table 4. Husbandry survey questions**

| | Husbandry |
|---|---|
| **n (%)** | 88 (22%) |
| **Owned animals** | |
| Cattle | 51 (58%) |
| Sheep | 40 (45.5%) |
| Others | 7 (7.8%) |
| **Use of protective equipment** | |
| Glass | 7 (8%) |
| Glove | 67 (76.1%) |
| Mask | 27 (30.7%) |
| Boot | 34 (38.6%) |
| Apron | 22 (25%) |
| No precautions required | 12 (13.6%) |
| **Approach to animal waste** | |
| Bury | 10 (11.4%) |
| Throwing waste to a distant place | 29 (33%) |
| Incinerating waste | 28 (31.8%) |
| Make fertilizer | 31 (35.2%) |
| Throwing waste away | 15 (17%) |
| Giving it to stray animals | 6 (6.8%) |
| **What do you do about the membrane that grows after birth?** | |
| Bury | 32 (36.4%) |
| Throwing waste to a distant place | 22 (25%) |
| Incinerating waste | 3 (3.4%) |
| Making fertilizer | 1 (1.1%) |
| Throwing waste away | 22 (25%) |
| Giving it to stray animals | 32 (36.4%) |
| **How do you clean up animal waste?** | |
| Water | 51 (58%) |
| Lime | 34 (38.6%) |
| Detergent | 10 (11.4%) |
| **Abortion** | 55 (62.5%) |
| **Separating the animal that had an abortion from the herd** | 39 (44.3%) |
| **Consuming the milk/meat of an animal that has had an abortion** | 35 (39.8%) |
| **Giving milk from an animal that has had an abortion to a calf** | 33 (37.5%) |
| **What do you do to a sick animal?** | |
| Treat it myself | 15 (17%) |
| Call the vet | 78 (88.6%) |
| Slaughter it for its meat | 1 (1.1%) |
| Nothing | 2 (2.3%) |
| **Getting vaccinated against brucellosis** | 60 (68.2%) |
| **What is your reason for not getting the brucellosis vaccine?** | 28 (100%) |
| Couldn't find the vaccine | 8 (28.5%) |
| Vaccine side effects | 7 (25%) |
| It doesn't work | 4 (14.3%) |
| Expensive | 9 (32.2%) |

husbandry. The results were interpreted as those who received training on the subject and those in the risk group were more conscious.

When examining the sources of information about brucellosis, Alkan et al.[12] found that 62.4% relied on information from relatives, neighbors, or families. Avcı et al.[13] reported that 36.4% obtained information from healthcare workers, 24.2% from neighbors, and 19.7% from family members or friends. Özen et al.[14] found that 30.8% received information from friends, relatives, and neighbors, and 20.93% from television, radio, and newspapers. In Babaoğlu et al.[16] study, 45.6% relied on relatives or neighbors. In our study, participants accessed information through various channels, with 46% obtaining it publicly, 23.4% through a relative who had the disease, 10.9% through seminars, 7.9% within the family, and 4.6% through the internet or television. Healthcare professionals primarily relied on seminars, while individuals involved in animal husbandry relied on their own experiences with the disease. In terms of survey scoring, obtaining information from individuals having experienced the disease, learning through seminars, and accessing information via the Internet or television yielded better results compared to learning from public sources or within the family. We believe that in order to raise public awareness and combat misinformation, media platforms such as the Internet and television should be utilized, and awareness-raising events should be organized.

The main hosts for *Brucella* are animals, such as cattle, sheep, goats, and pigs, and humans are incidental hosts. Transmission occurs between animals, but transmission between humans is rare[1]. According to 2022 data, Ağrı Province is Turkey's fourth-largest province with a meadow-pasture area and it ranks 14$^{th}$ in the presence of cattle and 11$^{th}$ in the presence of small ruminants[17]. In terms of awareness of zoonotic transmission, Akkuş et al.[15] reported a rate of 49.5%, while Babaoğlu et al. reported a rate of 29.4%[16]. Additionally, Babaoğlu et al.[16] reported animal-to-animal transmission at 19.1%, while Akkuş et al. reported that 36.1% of cases were transmitted from person to person[15]. In our study, the rate was 63% for the awareness of zoonotic transmission, 24% for the transmission between animals, and 17.5% for human-to-human transmission. Notably, awareness of zoonotic contamination was higher among individuals engaged in animal husbandry.

In a meta-analysis conducted by Zhang et al.[18], the main route of transmission in endemic countries is unpasteurized milk and dairy products, and in developed countries, it has been shown to be more common through contact and inhalation. In a study by Özen et al.[14], the mode of disease transmission was identified as follows: 49% through the consumption of unboiled milk, 20% through the raw or undercooked consumption of red meat or offal products, 18% through direct contact with animals such as sheep, goats, and cows, and 12% through yoghurt. Furthermore, the study found that the awareness of contamination risk from raw or

undercooked red meat increased with education level (p=0.036). In the same study, when participants were asked if the disease could be transmitted through consuming unboiled milk, 35.7% of those with a primary school education or lower, 25% of those with a middle school education, and 47.2% of those with a high school education and above reported contamination (p=0.038). In a study by Akkuş et al.[15], it was reported that 32% of transmissions occurred through contact with raw milk and dairy products during animal birthing, while 44.3% were attributed to the consumption of raw milk and dairy products. In our study, the transmission routes for the disease were identified as follows: consumption of raw milk (55.8%), consumption of products produced from raw milk (47.8%), consumption of raw or undercooked meat (24.3%), contact with animal abort material (18%), direct contact with animal excretions (23.5%), and laboratory exposure (8.5%). Participants who believed contamination could occur with kashar cheese accounted for 16%, while those who expressed concern about contamination with yogurt were 29%. Furthermore, it was observed that individuals working in animal husbandry were more likely to believe they could be infected by consuming yogurt and kashar cheese. Healthcare workers demonstrated higher awareness regarding laboratory contamination. It is important to emphasize that the pathogenicity of the agent diminishes during the production stages of yogurt and kashar cheese, thereby mitigating the risk of contamination[19]. Upon evaluating survey scores for brucellosis awareness, it was noted that university graduates exhibited a higher level of awareness compared to individuals with high school or middle school education.

Animal vaccines are available for brucellosis; however, there is currently no vaccine available for human use[20]. In Özen et al.[14] study, the awareness of animal vaccines was reported to be 5.8%. In our study, we found that 35.5% of participants were aware of animal vaccines, while only 4.8% were aware that there was no vaccine available for humans. Notably, individuals involved in animal husbandry demonstrated higher awareness regarding animal vaccinations compared to other groups. This finding underscores the importance of informing those engaged in animal husbandry about the significance of vaccination in preventing zoonotic transmission. By educating this group about protective measures, we can potentially mitigate the risk of zoonotic disease transmission.

The control of brucellosis in humans entails several measures, including controlling brucellosis in animals, ensuring the pasteurization of milk and dairy products before consumption, and preventing laboratory-borne contamination[21]. Treatment is not recommended for animal brucellosis due to economic concerns and the inability to prevent carriage. Instead, the primary focus of managing animal brucellosis is prevention. Key prevention methods include the use of protective equipment such as gloves, glasses, and aprons, maintaining good hygiene practices, isolating infected animals from the herd, and implementing vaccination programs[22]. All waste from animals giving birth and the feed they come into contact with should be buried deeply, with unburnt lime poured on it or destroyed by burning. Consequently, animals diagnosed with brucellosis should not be sold. Additionally, new animals introduced to the herd should undergo testing for brucellosis and, if necessary, be vaccinated[23]. In Özen et al.[14] study, 58.3% of participants reported taking contact precautions. In our study, 76.1% of participants believed that gloves should be used as a precaution, while 38.6% thought boots should be used. Only 13.6% expressed the belief that pre-contact precautions were unnecessary.

In Alkan et al.[12] study, 55.8% of participants reported burying animal waste, while in Babaoğlu et al.[16] study, this figure was 69.3%. In the study by Babaoğlu et al.[16], 15.9% of participants reported throwing away animal waste. Additionally, the abortion rate in the same study was 40.7%. In the present study, the abortion rate was found to be 62.5%. Waste management practices reported by participants included making waste into fertilizer (35.2%), throwing waste to a distant place (33%), incinerating waste (31.8%), throwing waste away (17%), and burying waste (11.4%). Additionally, 58% of respondents reported treating waste with water, while 38.6% used the liming method. These findings suggest that awareness of waste management may be insufficient, particularly in environments with high abortion rates. There is a need for improvement in waste management approaches for animal waste infected with *Brucella* beyond burying, lime pouring, and burning.

Alkan et al.[12] reported that 40.9% of participants preferred to slaughter and eat infected animals, whereas Avcı et al.[13] found this percentage to be 77.2%. Additionally, in the same study, the veterinarian's infection rate was determined to be 7.6%. In our study, it was found that 44.3% of participants separated infected animals from the herd, while 39.8% consumed the meat or milk of infected animals, and 37.5% fed the milk of infected animals to calves. Only 1.1% preferred to slaughter and eat infected animals. The rate of animals being taken to the veterinarian was found to be 88%. These findings underscore the importance of emphasizing isolation in the fight against brucellosis in animals and avoiding the consumption of infected animals and their products. Collaboration with veterinarians is crucial for implementing correct approaches, notifications, and raising awareness about the current situation.

Alkan et al.[12] reported a vaccination rate of 59.7% in animals, while Avcı et al.[13] reported it as 20%. In our study, the animal vaccination rate was found to be 68.2%. Among participants who did not receive the vaccine, 32.2% cited cost as the reason, 28.5% mentioned difficulty in finding the vaccine, 25% expressed concerns about vaccine side effects, and 14.3% believed the vaccine to be ineffective. The continued endemic status of the disease suggests that vaccination rates remain insufficient. It is crucial to provide necessary information about vaccination and encourage its uptake, while simultaneously ensuring that vaccines are affordable and accessible.

### Study Limitations

The main limitations of our study include the inability to perform parametric analyses due to a decrease in participant numbers when grouping patients. There is a need for future studies with more homogeneous participant groups and larger populations. Additionally, the lack of a standard scale for measuring brucellosis awareness led us to create our survey form based on data from the literature review. Moreover, constraints such as time and financial limitations prevented us from conducting fieldwork, limiting our study to participants who visited the outpatient clinic. As a result, we were unable to provide information regarding seroprevalence.

## CONCLUSION

Under the framework of One Health, the integration of human, animal, and environmental health is essential. To prevent brucellosis in animals, attention should be paid to necessary vaccination, hygiene conditions, isolation measures, waste control, and complete reporting of infected animals. Moreover, it is essential to emphasize the importance of pasteurization for milk and dairy products to prevent transmission. Raising awareness about the disease is paramount to mitigate workforce and economic losses associated with brucellosis. This can be achieved through educational initiatives delivered via various platforms such as seminars, the Internet, and television. It is imperative to prevent the propagation of incorrect attitudes and behaviors through social learning and to provide accurate information to the public.

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### Ethics

**Ethics Committee Approval:** The study was conducted after obtaining the necessary permissions from Ağrı İbrahim Çeçen University Ethics Committee (decision no: E-95531838-050.99-83962, date: 06.10.2023).

**Informed Consent:** Informed consent was obtained from all participants who volunteered for the study.

### Authorship Contributions

Surgical and Medical Practices: E.E., M.K.T., Concept: E.E., Design: E.E., M.K.T., Data Collection or Processing: E.E., M.K.T., Analysis or Interpretation: E.E., Literature Search: E.E., M.K.T., Writing: E.E., M.K.T.

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# Evaluation of Post-Infectious Glomerulonephritis: Single Center Experience

## Post-Enfeksiyöz Glomerülonefrit Değerlendirilmesi: Tek Merkez Deneyimi

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### ABSTRACT

**Aim:** Post-infectious glomerulonephritis (PIGN) is an acute glomerulonephritis, often develops after infections with nephritogenic strains of group A beta hemolytic streptococcus. Patients may present with mild findings such as asymptomatic microscopic hematuria and non-nephrotic proteinuria or they may present with severe findings such as macroscopic hematuria, nephrotic range proteinuria, edema, hypertension, and acute kidney injury. In this paper, we aimed to present the laboratory and clinical findings of patients who applied to pediatric nephrology clinic in a short period of six months and were followed up with the diagnosis of PIGN.

**Materials and Methods:** The medical records of 30 patients who were followed up in Adana City Training and Research Hospital with the diagnosis of PIGN in a six-month period (between October 2022 and March 2023) were evaluated retrospectively. The clinical, laboratory and treatment data of the patients were recorded.

**Results:** Acute nephritic syndrome was the most common clinical presentation with 22 patients (73.3%) followed up with PIGN. Four (13.3%) of the patients presented with nephrotic syndrome, two (6.7%) with rapidly progressive glomerulonephritis, and two (%6.7) with hypertensive encephalopathy. Kidney biopsy was performed in 3 patients, two of whom presented with rapidly progressive glomerulonephritis and one whose complement 3 level remained low beyond 8 weeks. The pathological diagnosis of these patients was reported as diffuse proliferative glomerulonephritis.

**Conclusion:** Although PIGN usually occurs as a benign nephritis, it should be kept in mind that patients whose diagnosis is delayed and not treated appropriately may present with poor clinical

**Keywords:** Glomerulonephritis, post-streptococcus glomerulonephritis, children, nephrotic syndrome, acute nephritic syndrome

### ÖZ

**Amaç:** Post-enfeksiyöz glomerülonefrit (PIGN), sıklıkla A grubu beta hemolitik streptokokların nefritojenik suşları ile enfeksiyonlardan sonra gelişen akut bir glomerülonefrittir. Hastalar asemptomatik mikroskobik hematüri ve nefrotik olmayan proteinüri gibi hafif bulgularla başvurabilecekleri gibi makroskopik hematüri, nefrotik düzeyde proteinüri, ödem, hipertansiyon ve akut böbrek hasarı gibi ciddi bulgularla da başvurabilirler. Bu yazıda altı ay gibi kısa bir sürede çocuk nefroloji kliniğine başvuran ve PIGN tanısıyla takip edilen hastaların laboratuvar ve klinik bulgularını sunmayı amaçladık.

**Gereç ve Yöntem:** Adana Şehir Eğitim ve Araştırma Hastanesi'nde PIGN tanısıyla altı aylık dönemde (Ekim 2022 ile Mart 2023 tarihleri arasında) takip edilen 30 hastanın tıbbi kayıtları retrospektif olarak değerlendirildi. Hastaların klinik, laboratuvar ve tedavi bilgileri kaydedildi.

**Bulgular:** Akut nefritik sendrom, enfeksiyon sonrası glomerülonefrit ile takip edilen 22 hasta (%73,3) ile en sık görülen klinik tabloydu. Hastaların 4'ü (%13,3) nefrotik sendrom, 2'si (%6,7) hızlı ilerleyen glomerülonefrit ve 2'si (%6,7) hipertansif ensefalopati ile başvurdu. İkisi hızlı ilerleyen glomerülonefrit ile başvuran ve kompleman 3 düzeyi 8 haftadan fazla düşük kalan bir hasta olmak üzere 3 hastaya böbrek biyopsisi yapıldı. Bu hastaların patolojik tanısı diffüz proliferatif glomerülonefrit olarak bildirildi.

**Sonuç:** PIGN genellikle benign nefrit şeklinde ortaya çıksa da tanısı geciken ve uygun şekilde tedavi edilmeyen hastaların kötü klinik tabloyla başvurabileceği akılda tutulmalıdır.

**Anahtar Kelimeler:** Glomerülonefrit, post-streptokoksik glomerülonefrit, çocuk, nefrotik sendrom, akut nefritik sendrom

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## INTRODUCTION

Post-infectious glomerulonephritis (PIGN) is an immune-mediated glomerular damage occurring as a result of the host response to an extrarenal infection[1]. The most prominent mechanism of glomerular damage in PIGN is the development of an autoimmune response against nephritogenic streptococcal antigens.

The development of the autoimmune response leads to immune complex formation and activation of the alternative complement pathway, causing glomerular inflammation and damage[2].

PIGN can be seen after viral, bacterial and fungal infections[3]. Group A beta hemolytic streptococcal (GAS) infections are the most common cause of acute nephritis. Of the estimated 470 000 new post-streptococcal glomerulonephritis (PSGN) cases annually, 97% occur in countries with poor socioeconomic status[4,5].

Patients usually have a history of previous skin or throat infection with GAS[6]. There is a latent period of 1-3 weeks following GAS pharyngitis and 3-6 weeks following GAS skin infection. Clinical presentation varies widely, from asymptomatic microscopic hematuria to acute kidney injury[2].

Approximately two-thirds of the patients have widespread edema and hypervolemia due to water and sodium retention. In severe cases, fluid overload can lead to pulmonary edema, causing respiratory distress. Macroscopic hematuria is present in approximately 30-50% of patients. Hypertension is present in 50-90% of patients, and hypertensive encephalopathy is a rare but serious complication[7,8]. Acute kidney injury develops in approximately 20% of the cases but rarely requires dialysis[9].

Complement 3 (C3) level is low in the first two weeks of the disease course and complement 4 (C4) level is generally normal in approximately 90% of the patients. However, in some patients, C4 and complement 2 (C2) levels may be low, suggesting the activation of both classical and alternative pathways. C3 level returns to normal within 4-8 weeks after the onset of the disease[2,10].

The presence of a previous history of GAS infection is important for the diagnosis of PSGN. Although throat culture or rapid antigen test shows GAS infection during acute infection, throat culture is positive in only 20-25% of patients when nephritic symptoms begin. Since the diagnosis of impetigo is made with clinical findings without taking a wound culture, culture results are not required for the diagnosis of PSGN. In the absence of a positive culture result, anti-streptolysin O (ASO) and anti-DNase B can be used. ASO increases 2-4 weeks following an upper respiratory tract infection, reaches its peak after 3-5 weeks and remains elevated for several months; DNase B increases 2 weeks after infection and reaches its peak in 6-8 weeks[5,11]. Therefore, an increase in ASO titer over time is diagnostic. Although high DNase B levels can also be seen in upper respiratory tract infections, unlike ASO, they are also increased in pyodermal infections[11]. In this paper, we aimed to present the laboratory and clinical findings of patients who applied to Adana City Training and Research Hospital pediatric nephrology clinic in a short period of six months and were followed up with the diagnosis of PIGN.

## MATERIAL AND METHODS

Following the approval of the ethics committee with decision number 3233 at the 149$^{th}$ the approval was received from Adana City Training and Research Hospital Clinical Research Ethics Committee (decision no: E-60116787-020-476142, date: 15.01.2024). The medical records of 30 patients who were followed up in with the diagnosis of PIGN were evaluated retrospectively. The clinical, laboratory and treatment data of the patients were recorded. Patients who applied to the pediatric nephrology outpatient clinic in a six-month period (between October 2022 and March 2023) and had a minimum follow-up period of 3 months were included in the study. PIGN was diagnosed in the presence of proteinuria accompanying hematuria, low C3 that resolved within 2 months, a history of previous infection or evidence of streptococcal infection (ASO, anti-DNAse B elevation and/or throat culture positivity). ASO >200 IU/mL and anti-DNAse B >200 U/mL were defined as high ASO and anti-DNAse B levels, respectively. C3<0.79 g/L was defined as low C3 level. Non-nephrotic proteinuria was defined as protein/creatinine ratio 0f 0.2-2 mg creatinine in spot urine, and nephrotic range proteinuria as protein/creatinine ratio of >2 mg creatinine in spot urine. Serum creatinine above normal limits for age was defined as azotemia.

### Statistical Analysis

All statistical analyses were performed by SPSS version 21 software package. Normal distribution of numeric variables was tested with the Kolmogorov-Smirnov test. Continuous data were defined by means of mean ± SD under the parametric conditions and median under the non-parametric conditions. Independent sample t-test was used for the comparison of normally distributed numeric variables. P values less than 0.05 were considered to be statistically significant.

## RESULTS

Of the patients, 20 were male and 10 were female. The mean age of the patients was 6.8±1.8 years. The mean ages of boy and girl patients were statistically similar (p>0.05). All of the patients mentioned a previous medical history of infection, including upper respiratory tract infection in 25 patients, skin infection (scabies) in 4 patients, and acute gastroenteritis in 1 patient (Table 1). Acute nephritic syndrome was the most common clinical presentation with 22 patients (73.3%). Four (13.3%) of the patients presented with nephrotic syndrome, two (6.7%) with rapidly progressive glomerulonephritis, and two (6.7%) with hypertensive encephalopathy Table 2. The patients who applied with the diagnosis of hypertensive encephalopathy were those whose applications to the health

institution were delayed due to the earthquake disaster in our country. They had a history of macroscopic hematuria.

Macroscopic hematuria was the most common presenting symptom in 25 of the patients. At admission, 19 patients had edema, 14 patients had hypertension, six patients had oliguria, two patients had respiratory distress, and two patients had convulsion (Table 2). Twenty-one of the patients were hospitalized and nine were treated as outpatients. The mean length of stay for the patients was 8.1±3.6 days. Fluid-sodium restriction and loop diuretics (furosemide) were used for the treatment of hypervolemia. Amlodipine was added as antihypertensive treatment in seven (23.3%) patients.

The median urea and creatinine levels of the patients at the time of diagnosis were 38 mg/dL and 0.52 mg/dL, respectively. The laboratory parameters of the patients at the time of admission are shown in Table 3. Kidney biopsy was performed in three patients, two with rapidly progressive glomerulonephritis (RPGN), and one with persistent hypocomplementemia beyond 8 weeks. The pathological diagnosis of these patients was reported as diffuse proliferative glomerulonephritis. These patients were commenced on corticosteroid immunosuppressive therapy. The C3 levels of the patients returned to normal in a mean of 35±32 days. Macroscopic hematuria resolved in a mean of 7±5 days, and microscopic hematuria resolved in mean of 68±34 days. At the end of the study, 12 patients still had microscopic hematuria. Follow-up data of the patients are shown in Table 4.

**Table 1. Previous infections before PSGN**

| | n (%) |
|---|---|
| Upper respiratory tract infection | 25 (83.3) |
| Skin infection | 4 (13.3) |
| Gastroenteritis | 1 (3.33) |

PSGN: Post-streptococcal glomerulonephritis

**Table 2. The clinical and laboratory features of the patients at admission**

| **Clinical presentation** | **n (%)** |
|---|---|
| Acute nephritic syndrome | 22 (73.3) |
| Nephrotic syndrome | 4 (13.3) |
| Rapidly progressive glomerulonephritis | 2 (6.7) |
| Hypertensive encephalopathy | 2 (6.7) |
| **Clinical and laboratory features** | **n (%)** |
| Macroscopic hematuria | 24 (83.3) |
| Edema | 19 (63.3) |
| Hypertension | 14 (46.7) |
| Oliguria | 7 (23.3) |
| Respiratory distress | 2 (6.7) |
| Convulsion | 2 (6.7) |
| Azotemia | 7 (23.3) |
| Proteinuria<br>Nephrotic range<br>Non-nephrotic range | 28 (93.4)<br>17 (56.7)<br>11 (36.7) |
| Hypoalbuminemia (<3.5 g/L)<br><2.5 g/L<br>≥2.5 g/L | 18 (60.0)<br>3 (10.0)<br>15 (50.0) |
| Hypocomplementemia (low C3 level) | 30 (100.0) |
| Increased ASO (>200 IU/mL) | 25 (83.3) |
| Increased anti-DNAse B (>200 U/mL) | 8 (26.7) |

ASO: Anti-streptolysin O, C3: Complement 3

## DISCUSSION

Acute glomerulonephritis is the pathological process characterized by inflammation and/or cellular proliferation of the glomeruli, in which the kidneys are not directly infected but are damaged as a result of autoimmune inflammation[11].

Acute post-streptococcal glomerulonephritis (APSGN) is seen between the ages of 4 and 14 years, and is extremely rare under the age of two years. The male to female ratio has been reported as 2:1[1,12]. In our patient population, male gender was predominant with a percent of 67%, similar to the literature, and the mean age of the patients was 7 years.

**Table 3. The laboratory parameters of the patients at the time of admission**

| | Median (IQR) |
|---|---|
| Urea (mg/dL) | 38 (22) |
| Creatinine (mg/dL) | 0.52 (0.20) |
| Sodium (mmol/L) | 139 (12) |
| White blood count (×10³/µL) | 10.3 (4.3) |
| Platelet (×10³/µL) | 375 (166) |
| | **Mean ± SD** |
| Uric acid (mg/dL) | 5.3±1.7 |
| Hemoglobin (g/dL) | 10.8±1.0 |
| Potassium (mmol/L) | 4.6±0.5 |
| Calcium (mg/dL) | 9.0±0.6 |
| Phosphate (mg/dL) | 4.9±0.7 |
| Total protein (g/dL) | 6.5±0.7 |
| Albumin (g/dL) | 3.3±0.6 |

**Table 4. Follow-up data of the patients**

| Follow-up data | Mean ± SD (days) |
|---|---|
| Follow-up time | 165±51 |
| Macroscopic hematuria resolving time | 7±5 |
| Hypoalbuminemia resolving time | 17±9 |
| Hypocomplementemia (C3) resolving time | 35±32 |
| Proteinuria resolving time | 48±32 |
| Microscopic hematuria resolving time | 68±34 |

C3: Complement 3

Nephritis often occurs after skin and upper respiratory tract infections, but it is also seen after infections affecting different organ systems[1,3]. In a study conducted in Armenia on 474 patients with acute post-infections glomerulonephritis over a 5-year period, 51% of the patients had a previous history of upper respiratory tract infection, 23% had a history of scarlet fever, 13% had impetigo, and 5% had a history of cervical adenitis[5]. Atmış et al.[3] reported that 84.6% of the patients had a history of upper respiratory tract infection and 15.4% had gastroenteritis. In our study, the majority of our patients, 83.3%, had a history of upper respiratory tract infection, 13.3% had a history of skin infection, and 3.3% had a history of acute gastroenteritis. The fact that the majority of our patient population had a history of upper respiratory tract infection and the low frequency of acute gastroenteritis was thought to be related to the seasonal characteristics of the period in which the study was conducted.

The clinical spectrum of PSGN is quite broad. Patients classically present as acute nephritic syndrome with hematuria, proteinuria, and volume overload. However, it can also occur as a disease characterized by nephrotic syndrome (severe proteinuria, hypoalbuminemia and edema) or particularly rapidly progressive glomerulonephritis[5,11]. In our study, consistent with the literature, the majority of patients (73.3%) presented with acute nephritic syndrome.

In the acute phase of the disease, congestive heart failure and pulmonary edema may occur as a complication of hypervolemia, as well as severe encephalopathy due to hypertension[13]. Hypertension-related seizures with documented posterior reversible encephalopathy syndrome may occur in children with PSGN. In the early stages of the patients with PSGN, it is particularly important to continue to have blood pressure assessed at regular intervals, even in children who are normotensive at presentation and are being monitored as outpatients[5]. In our study, although patients presenting with hypertensive encephalopathy and convulsion had a history of macroscopic hematuria, it was observed that their admission to the hospital was delayed due to the earthquake disaster in our country. This shows that patients who are not followed-up and treated appropriately and whose diagnosis is delayed may present with worse clinical presentations.

Considering the presenting findings of the patients, macroscopic hematuria is reported with a frequency of 17-93%, edema with a frequency of 59-72%, hypertension with a frequency of 64-82% and oliguria with a frequency of 18-51%[5,7,14-18]. The frequencies of macroscopic hematuria, edema and oliguria in our study were similar to those in the literature. Although the frequency of hypertension with 47% is lower than previously reported, we think that it can be explained by our low oliguria frequency of 23% in this study. Patients with PSGN often present with non-nephrotic proteinuria, and nephrotic proteinuria is relatively rare and has been reported with a frequency of up to 35%. In fact, there are publications reporting nephrotic range proteinuria with a frequency as low as 1%[5,7,14-18]. In this study, nephrotic level proteinuria was present in 56.7% of our patients, which was significantly higher than the rate in the literature. Although we think that the re-encounter of patients with viral and bacterial infections, from which they are largely protected due to quarantine, after the COVID-19 pandemic, causes a stronger immunological stimulation, we do not have sufficient supporting evidence on this issue. However, the limited number of our patients may be one of the factors affecting these results. Multicenter and prospective immunological studies involving more patients are needed in this field.

The classical pathway of complement activation is partially blocked by immunoglobulin binding proteins on the streptococcal surface, and the alternative complement pathway is often activated. Therefore, C3 is usually low in blood tests, but classical pathway activation and decreased C1 and C4 can be seen in 15-30% of patients. However, despite significant complement activation, normal complement levels may also be observed in 10% of cases[13].

Therefore, the most useful confirmatory test for PSGN is usually a low C3 level, which classically returns to normal levels within 6-8 weeks. Although there are some reports that it takes longer for C3 levels to return to normal, if recovery exceeds 3 months, an alternative diagnosis such as membranoproliferative glomerulonephritis should be considered. Evidence of a previously diagnosed streptococcal infection (such as ASO and DNase B) may facilitate diagnosis in patients with PSGN[5].

Most children with a clear diagnosis of APSGN do not require an initial diagnostic kidney biopsy. In atypical cases or progressive loss of kidney function, biopsy helps make a definitive diagnosis and can rule out another cause that may require special treatment for recovery[5]. C3 levels were initially low in all of our patients; two presented with RPGN, and one with the low C3 level that lasted for more than two months and required kidney biopsy. Biopsy results were evaluated as diffuse proliferative glomerulonephritis.

Edematous or hypertensive patients should also be recommended a sodium-restricted diet and fluid restriction may be required. Since the presumed cause for hypertension is volume excess resulting from sodium and water retention, diuretics or the combination of a diuretic and a vasodilator such as a calcium channel blocker are often sufficient. Angiotensin-converting enzyme inhibitors or angiotensin receptor blockers should be avoided in the acute phase as they may exacerbate any reduction in glomerular perfusion resulting from glomerulonephritis itself[5]. In our study, all of our patients were placed on a liquid and sodium restricted diet; amlodipine was added to the treatment of hypertensive patients and blood pressure was controlled in all patients.

Most clinical signs and symptoms resolve spontaneously within a few weeks, but microscopic hematuria may persist for up to 2 years[5]. During an average follow-up period of 165 days, the macroscopic hematuria of our patients resolved in an average of 7 days. In 40% of the patients, microscopic hematuria continued during this period.

Finally, while Atmış et al.[3] evaluated 13 patients in nine months, Demircioglu Kılıc et al.[13] evaluated 75 patients in two years, and in our study, we evaluated 30 patients in six months. Being the only pediatric reference center in the region was effective in evaluating so many patients in short time. In addition, the earthquake disaster we experienced caused the hospital applications of patients to be delayed and caused patients to come with more serious clinics.

### Study Limitations

The most important limitation of our study is its retrospective design. Although the small number of patients is another limitation, the fact that these patients presented within a short period of 6 months is one of our reasons for reporting this article.

## CONCLUSION

PIGN usually occurs as benign nephritis, but it should be kept in mind that patients who are not treated appropriately and whose diagnosis is delayed may present with poor clinical pictures, like hypertensive encephalopathy. In addition, the higher frequency of serious clinical conditions such as nephrotic proteinuria in this study is a striking difference compared to previous studies. Multicenter and prospective immunological studies involving more patients are needed to understand the reason for this difference and stronger immunological stimulation.

### Ethics

**Ethics Committee Approval:** The approval was received from Adana City Training and Research Hospital Clinical Research Ethics Committee. (decision no: E-60116787-020-476142, date: 15.01.2024).

**Informed Consent:** Retrospective study.

### Authorship Contributions

Surgical and Medical Practices: G.A.Y., S.T., Concept: G.A.Y., S.T., Design: G.A.Y., S.T., Data Collection or Processing: G.A.Y., S.T., Analysis or Interpretation: G.A.Y., S.T., Literature Search: G.A.Y., S.T., Writing: G.A.Y., S.T.

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# The Role of Faecal Calprotectin in the Evaluation of Disease Activity in Spondyloarthritis Patients: A Cross-sectional Study

## Spondiloartrit Hastalarında Hastalık Aktivitesinin Değerlendirilmesinde Fekal Kalprotektinin Yeri: Bir Kesitsel Çalışma

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### ABSTRACT

**Aim:** Spondyloarthritis (SpA) is a group of chronic, inflammatory diseases characterized by involvement of the axial and peripheral joints, as well as extra-articular manifestations. It is important to assess disease activity (DA) for treatment and follow-up. Although there are commonly used scoring systems, they may lack sensitivity and specificity in determining DA. One potential biomarker for DA is calprotectin, a calcium-binding protein released from monocytes and macrophages during inflammation. Faecal calprotectin (fCal) is frequently used in the diagnosis and follow-up of inflammatory bowel diseases. Significant intestinal inflammation has also been shown in active SpA. The aim of this study was to test the utility of fCal as a marker of DA.

**Materials and Methods:** The study included patients with ankylosing spondylitis and psoriatic arthritis, admitted to our hospital between October 2019 and February 2020. Patients with gastrointestinal symptoms were excluded. Demographic data, DA scores, and laboratory test results were obtained from patient records. The correlation between fCal levels and DA parameters was analysed.

**Results:** fCal levels were correlated with the erythrocyte sedimentation rate (ESR) but not with C-reactive protein levels. Among the DA scores, only the AS Disease Activity Score (ASDAS)-ESR was found to be correlated. Non-steroid users and cigarette smokers exhibited lower levels of fCal.

**Conclusion:** fCal levels were found to be associated with ESR but not with C-reactive protein levels. fCal is only correlated with ASDAS-ESR, and lower fCal levels were observed in those using non-steroidal drugs and smokers.

**Keywords:** Ankylosing spondylitis, biomarker, fecal calprotectin, inflammatory bowel disease, spondyloarthritis

### ÖZ

**Amaç:** Spondiloartritler (SpA), aksiyal ve periferik eklem tutulumu ve ekstra-artiküler bulgularla seyreden kronik, enflamatuvar bir hastalık grubudur. Hastalık aktivitesinin (HA) değerlendirilmesi tedavi ve izlem açısından önemlidir. Güncel pratikte HA'yı değerlendirmede kullanılan çeşitli klinik ölçekler ve laboratuvar testleri mevcuttur. Ancak bu laboratuvar belirteçlerinin HA'yı belirlemedeki duyarlılık ve özgüllükleri istenilen seviyede değildir. Kalprotektin, enflamasyon halinde monosit, makrofajlardan salınan kalsiyum bağlayıcı bir proteindir. Enflamatuvar bağırsak hastalıklarının tanı ve takibinde sıkça kullanılmaya başlanan bir biyobelirtecidir. SpA'da de ciddi oranda bağırsak enflamasyonu gösterilmiştir. Bu çalışmada fekal kalprotektinin (FK) HA belirteci olarak SpA'de kullanılabilirliğinin test edilmesi amaçlamıştır.

**Gereç ve Yöntem:** Ekim 2019-Şubat 2020 tarihi arasında Ege Üniversitesi Tıp Fakültesi Hastanesi Romatoloji polikliniğine başvuran ankilozan spondilit (AS) ve psoriatik artritli (PsA) hastalar dahil edildi. Enflamatuvar bağırsak hastalığı olan ya da gastrointestinal semptomları olanlar dahil edilmedi. Demografik veriler, hastalığa ilişkin bilgiler, HA skorları ve laboratuvar tetkikleri hasta kayıtlarından elde olundu. Tüm olgularda FK testi çalışıldı. Ardından FK düzeyleri ile hastalık aktivite parametreleri arasındaki korelasyon incelendi.

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**Bulgular:** FK seviyesi, eritrosit sedimantasyon hızı (ESH) ile ilişkiliyken C-reaktif protein düzeyleri ile ilişkili bulunmadı. HA ölçeklerindense yalnızca ankilozan spondilit hastalık aktivite skoru (ASDAS)-ESH ile ilişkili bulundu. Non-steroid ve sigara kullananlarda FK düzeyi daha düşük saptandı.

**Sonuç:** FK seviyesi, ESH ile ilişkili bulunurken C-reaktif protein düzeyleri ile ilişki göstermemiştir. FK, sadece ASDAS-ESH ile ilişkilidir ve non-steroid ilaçlar ve sigara kullananlarda FK düzeyinin daha düşük olduğu gözlemlenmiştir.

**Anahtar Kelimeler:** Ankilozan spondilit, biyobelirteç, fekal kalprotektin, enflamatuvar bağırsak hastalığı, spondiloartrit

## INTRODUCTION

The definition of spondyloarthritis (SpA) refers to a group of chronic inflammatory rheumatologic diseases with common clinical features and genetic risk factors, all of which may involve axial or peripheral joints characterized by new bone formation[1]. The diseases considered as forms of SpA include ankylosing spondylitis (AS), non-radiographic axial SpA (nr-axSpA), peripheral SpA, psoriatic arthritis (PsA), arthritis associated with inflammatory bowel disease (IBD), reactive arthritis (formerly known as Reiter syndrome) and childhood-onset SpA[2,3]. Although AS and IBD are distinct diseases, there is clinical and genetic evidence supporting an overlapping pathogenic relationship[4]. There are studies showing that 5-10% of AS patients have concomitant IBD and that 25-50% of asymptomatic AS patients may have macroscopic and 50-60% may have microscopic intestinal inflammation[5,6]. PsA is also associated with extra-articular symptoms including inflammatory bowel disease, but these symptoms are rarer than in AS[7]. The prevalence of IBD associated with PsA is reported to be 3.3%[8]. Compared to patients with psoriasis and irritable bowel disease, patients with PsA have been shown to have more lymphocytic cell infiltration in the duodenal epithelium and villi[9].

Calprotectin is a proinflammatory protein produced mainly by neutrophils, monocytes and macrophages in a calcium-dependent manner in response to damage at the site of inflammation[10]. Calprotectin can be detected in various tissues and body fluids such as blood, mucosal epithelium, synovium and feces[11,12]. Faecal calprotectin (fCal) levels have been shown to increase in IBD patients as a direct result of increased neutrophil migration across the inflamed mucosa in the intestinal lumen[12]. Moreover, in individuals with IBD, fCal levels correlate with the endoscopic and histologic grade of intestinal inflammation and are used in the diagnosis and monitoring of the disease[13]. Due to its proinflammatory properties, calprotectin levels have also been studied in rheumatologic diseases[14]. Serum calprotectin levels have been shown to predict relapse in rheumatoid arthritis, PsA and anti-neutrophil cytoplasmic antibody-associated vasculitis[14,15]. In SpA, there are many publications supporting that both serum and fCal are significantly increased and correlated with disease activity[14,16,17].

This study aimed to test the usability of fCal levels as a marker of disease activity in SpA. With this aim, fCal levels were measured in SpA patients and the correlation of these levels with disease activity scales in use was examined.

## MATERIAL AND METHODS

### Patient Selection

Our single-center, cross-sectional study was conducted in consecutive patients admitted to the Internal Medicine-Rheumatology outpatient clinics of Ege University Faculty of Medicine Hospital between October 2019 and February 2020. Patients who met the diagnosis of AS[18] according to the criteria of the Assessment of Spondyloarthritis International Society and PsA[19] according to the Classification of Psoriatic Arthritis Study Group, were ≥18 years old and gave written informed consent to participate were included in our study. Patients with gastrointestinal symptoms (diarrhea, abdominal pain, bloody-mucous stools), IBD in themselves or their families, malignancy or active infection were excluded. The approval for the study was received from the Ege University Faculty of Medicine Clinical Research Ethics Committee (decision no: 04.09.2019_19-9T/60, date: 04.09.2019). The study was performed in accordance with the principles of the Declaration of Helsinki.

### Characteristics Related to the Disease

Bath ankylosing spondylitis disease activity index (BASDAI) was used as a tool to assess disease activity in patients with AS[20]. In addition, the ankylosing spondylitis disease activity score (ASDAS), which was thought to reflect inflammatory processes better than the BASDAI and which was created by integrating [C-reactive protein (CRP)] or [erythrocyte sedimentation rate (ESR)] into some parameters of the BASDAI (questions two, three and six of the scale), was also used[21,22]. According to the BASDI scale, those with BASDI<4 were classified as inactive and those with BASDI≥4 as active disease. According to ASDAS, ASDAS<1.3=inactive disease; 1.3≤ASDAS<2.1=low activity; 2.1≤ASDAS<3.5=high activity; ASDAS≥3.5=very high activity disease[23]. (ESR, mm/hour) and (CRP, mg/L) levels, which are commonly used to measure disease activity in clinical practice, were obtained from the records of the patient file, from the notes of the visit of the period when the stool sample was taken.

Bath ankylosing spondylitis disease functional index was used as a chronicity score indicating the level of functional limitation[24]. Demographic data (age, gender, disease duration, current treatment, smoking) were also obtained from outpatient follow-up files.

### Laboratory Methods

### Fecal Calprotectin Measurement

Participants were asked to give their stool samples during their hospital visits and to bring them to the laboratory without waiting. fCal in stool samples was analyzed by lateral flow method in Ege University Medical Faculty Clinical Biochemistry Laboratory (CalFast® XT Eurospital Diagnostic, Italy). The measurement range was 50-1005 mg/kg and a cut-off value of 70 mg/kg was used. Values <70 mg/kg were considered negative and values above 70 mg/kg were considered positive (for statistical evaluation, results given as <50 and ≥1005 mg/kg in laboratory results were recorded as 49 and 1006 mg/kg, respectively).

### Statistical Analysis

In summarizing the data obtained from the study, mean ± standard deviation or median, minimum and maximum values were presented in tables depending on the distribution for continuous (numerical) variables. Categorical variables were summarized as number and percentage. Normality of numerical variables was evaluated by the Shapiro-Wilk, Kolmogorov-Smirnov and Anderson-Darling tests. To compare the differences of categorical variables between groups, the Pearson's chi-square test was used in 2x2 tables with an expected number of observations of five or more, and the Fisher's exact test was used when the expected number of observations was less than five. In comparing numerical variables between two independent groups, the Mann-Whitney U test was applied when numerical variables did not show normal distribution. The correlation of fCal, disease activity scores and acute phase reactants was evaluated by the Spearman's correlation analysis. IBM® SPSS® version 25.0 software was used for statistical analysis. In the analyses, the limit of statistical significance was accepted as $p<0.05$.

## RESULTS

A total of 100 patients, including 88 (88%) diagnosed with AS and 12 (12%) with PsA, were included in the study. 62% of the patients were male. The mean age was 44±11.06 (19-67) years and the median disease duration was 9 (10-35) years. Extra-articular involvement was anterior uveitis in 13 cases and 39 patients were active smokers. The distribution of disease characteristics laboratory and disease activity scale scores according to all groups and diagnoses is summarized in Table 1. The median fCal level in the entire group was 72.5 mg/kg (49-1006) and its positivity (>70mh/kg) was 51%.

While 79 (79%) of the patients included in the study were using non-steroidal anti-inflammatory drugs (NSAIDs), 73 (73%) patients were using conventional synthetic disease modifying anti-rheumatic drugs (sulfasalazine 32 patients, methotrexate 32 patients, leflunomide 9 patients) and 38 (38%) patients were using anti-TNF agents. Corticosteroid use was present in only 3 (3%) patients.

When we examine the distribution of the patients according to activity scores, 28% of the patients were active according to BASDAI, 56% according to ASDAS-CRP, and 44% had high and very high disease activity according to ASDAS-ESR. The distribution of patients according to disease activity scales is summarized in Figure 1.

**Table 1. Distribution of age, disease duration, disease assessment scores, acute phase responses and fecal calprotectin levels in all groups and diagnostic subtypes**

| | All group | AS | PsA |
|---|---|---|---|
| [§]Age (year) | 44 (11.06) | 44 (10.84) | 44 (13.09) |
| [†]Disease duration (year) | 9 (10-35) | 8 (0.1-35) | 11.5 (1-26) |
| [†]fCal level (mg/kg) | 72.5 (49-1006) | 76.5 (49-1006) | 49 (49-470) |
| fCal positivity (%) | 51 | 52.2 | 41.6 |
| [†]BASDAI | 2.29 (0-8.9) | 2.25 (0-7.2) | 2.36 (0-8.9) |
| BASFI | 1.75 (0-8) | 1.75 (0-8) | 1.61 (0-7.29) |
| [§]ASDAS-CRP | 2.24 (0.96) | 2.22 (0.91) | 2.40 (1.31) |
| [§]ASDAS-ESR | 2.11 (0.98) | 2.07 (0.94) | 2.38 (1.25) |
| [†]CRP (mg/L) | 5.06 (0.3-50) | 5.78 (0.3-46.30) | 4.54 (1.51-50) |
| [†]ESR (mm/h) | 13 (1-91) | 13 (1-91) | 11.5 (3-89) |

[§]Mean (SD), [†]median (min-max), BASDAI: Bath ankylosing spondylitis disease activity index, fCal: Faecal calprotectin, BASFI: Bath ankylosing spondylitis functional index, ASDAS: Ankylosing spondylitis disease activity score, CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate, AS: Ankylosing spondylitis, PsA: Psoriatic arthritis

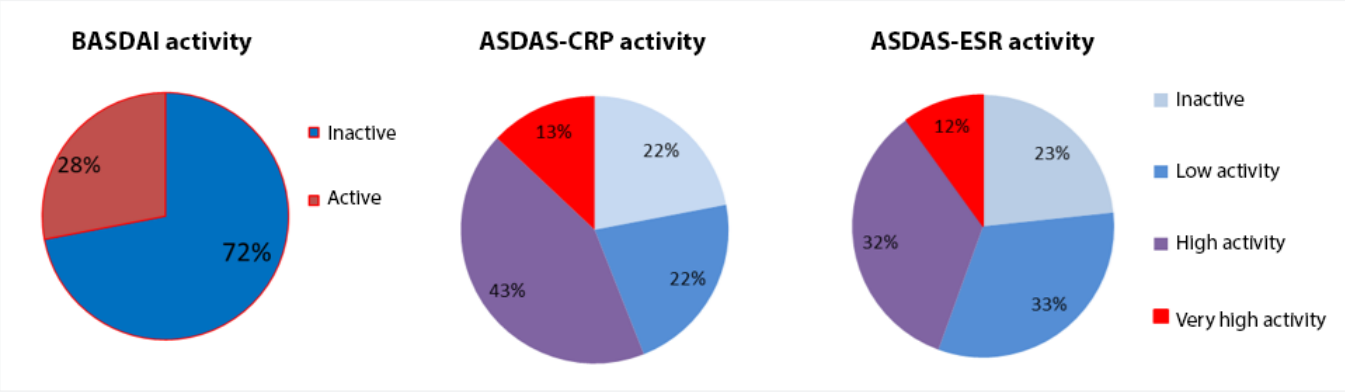


**Figure 1.** Distribution of disease activity according to BASDAI, ASDAS-ESR and ASDAS-CRP

*BASDAI: Bath ankylosing spondylitis disease activity index, ASDAS: Ankylosing spondylitis disease activity score, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate*

### Correlation of Fecal Calprotectin with Disease Activity

While fCal levels showed a significant correlation with ESR, which is one of the laboratory parameters (r=0.289, p=0.003), it did not show a significant correlation with CRP levels (p=0.403). Among the composite disease activity indices, only ASDAS-ESR was found to have a significant relationship with fCal levels (r=0.218, p=0.029). No statistically significant relationship was found between fCal levels and BASDAI and ASDAS-CRP scores (p=0.420, p=0.361, respectively). It could not be evaluated whether the fCal and ESR, fCal and ASDAS-ESR relationships found in the whole group continued in the subgroups (AS and PsA) because the distribution of patients in the subgroups was unbalanced (88AS, 12 PsA) and the number was not sufficient, especially in the PsA group.

In addition, the effects of medications and smoking on fCal levels were also reviewed as possible confounding factors. fCal positivity was detected at a significantly lower rate in patients using NSAIDs than in those not using NSAIDs (45.57% vs. 71.42%, respectively; p=0.035). However, when the relationship between NSAID use alone and fCal positivity was evaluated, no statistically significant relationship was detected (p=0.310). No significant relationship was detected when the use of drugs in the other treatment group [sulfasalazine, methotrexate, leflunomide, anti tumor necrosis factor (TNF)] and fCal were tested. fCal positivity was found to be significantly lower in active smokers than in non-smokers (38.46% vs. 59.02%, respectively; p=0.045).

## DISCUSSION

In this study, the relationship between fCal levels and disease activity in SpA patients was examined. According to the data obtained, the median fCal level was 72.5 mg/kg (49-1006) and 51% of the results were positive. In the literature, fCal positivity in SpA patients is reported in a wide range, between 38% and 72% (B16,25-27). As expected, this may be due to differences in the methods, kits and limit values used to measure fCal levels, racial characteristics that vary depending on the geographical region where the study was conducted, or differences in the patients' nutritional habits and intestinal microbiota. In this regard, since the study conducted by Ercalik et al.[28] was conducted in our country, it can be assumed that it was conducted in patient populations with similar genetic characteristics and even similar environmental and nutritional habits in terms of region. However, in this study, fCal positivity in AS patients is given as 11.3%, which seems to be quite low compared to our results. Considering that the patient age (44±11.06 in our study versus 43.90±13.42 in the other study) and the use of biological agents (38% in our study versus 40% in the other study) were similar in both studies, it may be possible to suggest that the main reason for the difference is the use of NSAIDs. Likewise, 79% of the patients in our study used NSAIDs, while this rate was 33% in the study by Ercalik et al.[28] NSAIDs are the first and most frequently used drug group in the treatment of SpA. NSAIDs have been shown to increase intestinal permeability and inflammation in healthy individuals[30]. However, the results regarding the relationship between NSAIDs and fCal positivity are contradictory[25-27,31,32]. It has been reported that fCal levels are higher in patients who regularly use NSAIDs[26] and that a 3-week drug interruption causes a decrease in fCal levels[31]. On the contrary, there are also studies suggesting that NSAID use does not affect the level of fCal[25,27,32]. In our study, we found that fCal positivity was more frequent in NSAID users (fCal was positive in 45.6% of NSAID users and 71.4% of non-NSAID users, p=0.035). Of course, these different results in the literature may be due to

differences in other drugs used concomitantly with NSAIDs and differences in patient cohorts depending on the inclusion/exclusion criteria. In this context, we think that more detailed and prospective studies are needed to examine the relationship between NSAID use and fCal levels.

Another drug group frequently used in the treatment of SpA is biological agents, especially TNF alpha inhibitors[29]. There are some studies showing that fCal levels may decrease with the use of TNFα inhibitors[26,33], but there are also studies in which this was not observed[25,27]. Although we could not show a significant relationship between the use of TNFα inhibitors and the level of fCal in our study, it is not possible to comment on this issue since the number of patients was not sufficient.

In the previous literature, it has been shown that there is a correlation between fCal level and CRP and/or ESR, and therefore, it has been suggested that fCal may be an auxiliary laboratory parameter for disease activity[26,34,35]. In our study, a significant correlation was observed between fCal levels and ESR (r=0.289, p=0.003), supporting that fCal may be related to inflammatory processes. However, in contrast to ESR, we did not detect a significant correlation with CRP in our study (p=0.403). This suggests that fCal may be more strongly associated with certain inflammatory markers, but may not be equally associated with all biomarkers. Unlike ESR, CRP is a protein produced by the liver and is not affected by age, gender, erythrocyte count and serum protein levels[36]. Therefore, the fact that the level of fCal is correlated with ESR while it is not correlated with CRP may be due to the fact that the level of fCal increases as a result of chronic subclinical intestinal inflammation rather than disease activity. The fact that CRP increases more rapidly in acute inflammation and decreases more rapidly when inflammation subsides is also supportive of this.

Similar to acute phase responses, the results of studies examining the correlation of fCal with composite disease activity scales also contain contradictions. Contrary to studies showing that fCal level and BASDAI were correlated[25,34], we did not find a correlation between fCal and BASDAI. Similarly, we did not find a correlation between fCal and ASDAS-CRP, another composite disease activity scale. Similar to our data, Simioni J et al.[27] also failed to show a significant relationship between fCal level and CRP and ASDAS-CRP, although many studies support the opposite[16,25,26]. On the other hand, we found a significant association between fCal level and only ASDAS-ESR among the disease activity scores. We think that the reason why fCal was associated with ASDAS-ESR while it was not associated with ASDAS-CRP was the indirect effect of ESR and CRP, which we used to calculate these scores.

Finally, when the relationship between smoking and inflammation is considered, it is known that smoking may have both proinflammatory and anti-inflammatory activity. Previous studies have demonstrated that disease activity increases with smoking in autoimmune diseases such as systemic lupus, Crohn's disease and Graves' disease[37-39]. In contrast, active smoking has been shown to reduce oral aphthae in Behçet's disease (BD) and to be inversely associated with disease activity in ulcerative colitis (UC)[40,41]. In this study, similar to patients with BD and UC, we found a negative correlation between active smoking and fCal positivity (p=0.045). It is known that intestinal BD and inflammatory bowel diseases show similar characteristics in terms of genetic background, pathogenesis, clinical features and response to anti-TNF therapies[42]. Therefore, our finding of a relationship between smoking and fCal level may be due to the fact that intestinal inflammation in SpA shows similar pathogenetic features with inflammatory bowel diseases and thus with BD.

### Study Limitations

The most important limitation of our study is that its cross-sectional nature limits the evaluation of the effects of fCal levels on long-term disease progression. In addition, the fact that it was conducted in a single center covering a specific geographical region limits the generalizability of the findings to different populations and ethnic groups. Other limitations include the lack of a control group, the lack of scoring systems such as PsARC, which is more specific for PsA activity, and the lack of restrictions on the use of NSAIDs before stool sampling. These limitations should be taken into consideration when interpreting the results of our study and guiding future research. On the other hand, the inclusion of PsA patients, the exclusion of patients with The gastrointestina symptoms and concomitant IBD, and the comparison of different disease activity indices can be considered as strengths of the study. Our findings may serve as a basis for deepening and expanding research in this field.

## CONCLUSION

The results of this study support the use of fCal levels in SpA patients as a potential biomarker for a more sensitive and specific assessment of disease activity as an indicator of inflammation. This may contribute to more effective management of treatment plans, especially given its significant correlation with ESR. However, the finding that fCal was not correlated with CRP suggests that this biomarker may not be a universal indicator for all patients and should be evaluated on a patient-by-patient basis. Larger and longer-term studies are needed to confirm the findings and integrate fCal into clinical practice. In particular, studies examining how fCal levels change under different treatment strategies and how they affect disease progression would be useful.

## Ethics

**Ethics Committee Approval:** The approval for the study was received from the Ege University Faculty of Medicine Clinical Research Ethics Committee (decision no: 04.09.2019_19-9T/60, date: 04.09.2019). The study was performed in accordance with the principles of the Declaration of Helsinki.

**Informed Consent:** Written informed consent to participate were included in our study.

## Authorship Contributions

Concept: E.S., B.B., F.Y.Z., Design: E.S., B.B., F.Y.Z., Data Collection or Processing: E.S., N.G.U., F.Y.Z., Analysis or Interpretation: E.S., N.G.U., F.Y.Z., Literature Search: E.S., B.B., N.G.U., Writing: E.S., F.Y.Z.

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# The Relation of Disease Characteristics with Type D Personality and Illness Perception in Patients with Gout

## Gut Hastalarında Hastalık Özelliklerinin D Tipi Kişilik ve Hastalık Algısı ile İlişkisi

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### ABSTRACT

**Aim:** The aim of this study was to evaluate type D personality and its relationship with illness perception and other disease parameters in patients with gout.

**Materials and Methods:** The study included 59 patients with gout who met the eligibility criteria and a control group of an equal number of relatively healthy individuals. Type D personality and psychological evaluation of all participants were assessed with the Type D Personality Scale (DS14) and Beck Depression Inventory (BDI), respectively. The Brief Illness Perception Questionnaire (B-IPQ) was used to evaluate patients' illness perception.

**Results:** The median age of the patients was 48 years (20); 79.7% were male. The frequency of type D personality was 30.5% in patients with gout, and this rate was higher than that in healthy controls (13.5%) (p=0.026). Patients with type D personality had higher numeric rating scale-pain (NRS-pain), C-reactive protein, erythrocyte sedimentation rate, Gout Activity Score (GAS), BDI, and B-IPQ scores than those without type D personality. A significant positive moderate-strong correlation (Spearman's rho ranged from 0.525 to 0.753) was found between the B-IPQ score and the tender joint count, NRS-pain, GAS, BDI, DS14 total score, social inhibition, and negative affectivity subscale scores.

**Conclusion:** The results of our study showed that type D personality was associated with negative illness perception, psychological health, perceived pain severity, and disease activity in individuals with gout. In this context, it supports the possibility that assessing personality traits, interventional planning, and education that change disease perception may be an effective strategy to improve health outcomes in patients with gout.

**Keywords:** Gout, type D personality, negative affectivity, illness perception, disease activity

### ÖZ

**Amaç:** Bu çalışmanın amacı gut hastalarında D tipi kişiliği ve bu kişilik tipinin hastalık algısı ve diğer hastalık parametreleri ile ilişkisini değerlendirmektir.

**Gereç ve Yöntem:** Çalışmaya uygunluk kriterlerini karşılayan 59 gut hastası ve aynı sayıda görece sağlıklı bireyden oluşan bir kontrol grubu dahil edildi. Tüm katılımcılarda D tipi kişilik varlığı ve psikolojik değerlendirmeler sırasıyla D Tipi Kişilik Ölçeği (D14) ve Beck Depresyon Ölçeği (BDÖ) ile değerlendirildi. Hastaların hastalık algılarını değerlendirmek için Kısa Hastalık Algısı Ölçeği (KHAÖ) kullanıldı.

**Bulgular:** Hastaların ortanca yaş değerleri 48 (20) olup %79,7'si erkek idi. Gut tanılı hastalarda D tipi kişilik görülme oranı %30,5 olup bu oran sağlıklı kontrollere (%13,5) göre yüksekti (p=0,026). D tipi kişiliğe sahip gut tanılı hastaların numerik ağrı derecelendirme ölçeği (NRS-ağrı), C-reaktif protein, eritrosit sedimentasyon hızı, Gut Aktivite Skoru (GAS), BDÖ ve KHAÖ skorları, D tipi kişiliğe sahip olmayan hastalara göre istatistiksel olarak anlamlı yüksek saptandı. KHAÖ skoru ile hassas eklem sayısı, NRS-ağrı, GAS, BDÖ, D14 total skor, sosyal içe dönüklük ve negatif duygulanım alt ölçek skorları arasında orta-güçlü pozitif korelasyon bulundu (Spearman's rho 0,525 ile 0,753 arasında değişmekte).

**Sonuç:** Çalışmamızın sonuçları gut tanılı bireylerde D tipi kişiliğin negatif hastalık algısı, psikolojik sağlık, algılanan ağrı şiddeti ve hastalık aktivitesi ile ilişkili olduğunu göstermiştir. Bu bağlamda gut hastalarında sağlık sonuçlarını iyileştirmek için kişilik özelliklerinin değerlendirilmesinin, hastalık algısını değiştiren müdahalelerin ve eğitim planlanmasının etkili bir strateji olma olasılığını desteklemektedir.

**Anahtar Kelimeler:** Gut, D tipi kişilik, negatif duygulanım, hastalık algısı, hastalık aktivitesi

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## INTRODUCTION

Gout disease is one of the common causes of inflammatory arthritis, which affects 0.68-3.9% of adults in the world. It is characterized by an increase in monosodium urate in blood and deposition of monosodium urate crystals in joints, tendons and tissues. It is associated with hypertension, chronic kidney disease, obesity, diabetes and cardiovascular disease. The initial exacerbation is typically characterized by asymptomatic hyperuricemia followed by acute arthritis involving the foot/ankle joint. The acute attack is self-limiting within 1-2 weeks and the signs and symptoms of inflammation completely resolve during the so-called intercritical period. If hyperuricemia persists, polyarticular exacerbations may develop, which become increasingly frequent and may affect many joints, including the joints of the upper extremities. If left untreated, chronic gouty arthritis characterized by tofus accumulation and joint erosion which occurs as a result of recurrent arthritis attacks[1]. Only 22% of patients with gout successfully reach the target serum urate level. This contributes to recurrent gout flares, reduced productivity and health-related quality of life[2].

The need to consider approaches to pessimistic disease perceptions in order to increase treatment compliance and improve health outcomes in patients diagnosed with gout is emphasized in the literature[3,4]. Disease perceptions are belief and opinion patterns that an individual develops in response to a perceived health threat. It is related to how patients evaluate living with a disease[5]. According to the results of a recent study examining the relationship between disease perceptions of patients with gout and health outcomes and covering a 12-month follow-up period, it was reported that pessimistic disease perception was associated with poorer medication compliance and health-related quality of life. Accordingly, interventions that change disease perception in patients diagnosed with gout can be seen as an effective way to improve health outcomes[3].

Type D personality is characterized by a personality pattern consisting of two personality traits: negative affectivity and social inhibition[6]. Individuals with type D personality tend to experience negative emotions such as distress, dissatisfaction, irritability, anxiety, and depression[7]. The prevalence of type D personality in the general population varies between 13% and 34%[6-8]. This personality type has been shown to play an important role in the clinical progression of certain diseases, such as coronary artery disease, hypertension, heart failure, and brain dysfunction. Many studies have reported the negative impact of type D personality on various health-related factors (such as quality of life and functionality)[9-12].

There are a limited number of studies in the literature that define the personality types or characteristics of patients diagnosed with gout, but no studies have been found that examine type D personality and the relationship of this personality type with disease perception and other disease parameters in this patient group[13-15]. The aim of this study is to evaluate the relationship between disease characteristics and type D personality and disease perception in patients diagnosed with gout.

## MATERIALS AND METHODS

This cross-sectional study included patients aged 18 years and over, under 65 years old, diagnosed with gout for at least 6 months according to the 2015 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) Criteria, who applied to the Physical Medicine and Rehabilitation outpatient clinic, and the same number of relatively healthy individuals who applied to our outpatient clinic, matched in terms of age and gender, as the control group[16]. The addition of the term "relatively" to healthy controls is because some of these individuals had various non-specific musculoskeletal pain. None of the individuals included in the control group had the study exclusion criteria. Exclusion criteria from the study were determined as the presence of concomitant autoimmune inflammatory disease, having had an acute gout attack (flare) within the last 4 weeks, serious psychological disorder (e.g. psychotic disorders), concomitant neurological disease, presence of uncontrolled systemic disease and history of malignancy and alcohol/substance addiction.

Written and verbal informed consent was obtained from the patients included in the study regarding the use of their medical information, and the study was conducted in accordance with the principles of the Declaration of Helsinki. The approval obtained from the Çukurova University Faculty of Medicine Non-invasive Clinical Research Ethics Committee (decision no: 138/23, date: 3.11.2023).

Age, gender, marital status, education level, employment and annual income status, current smoking and alcohol use, and body mass index (BMI) of all participants were determined and recorded. The patients' gout-related characteristics such as diagnosis period (months), number of attacks in the last year, tender joint count (TJC), disease activity, laboratory parameters [C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and serum urate level] and pain level were evaluated. The pain felt in the last month was determined by the numerical pain rating scale (NRS-pain).

The Gout Activity Score (GAS) was used to evaluate disease activity. This scoring is calculated with a special formula using the serum urate level, number of tophi, disease severity level reported by the patient (according to the visual pain scale) and the number of attacks in the last year. Accordingly, high scores indicate high disease activity[17,18].

The presence of type D personality in the participants was assessed using the Type D Personality Scale (DS14). In addition to assessing type D personality (14 items), the scale also provides information about the presence of negative affect (seven items) and social inhibition (seven items). It consists of a total of 14 questions, and each item statement is scored according to five ordered categories from 0 (wrong) to 4 (correct). The negative affect and social inhibition subscales can be used as continuous parameters (0-28) to assess these personality traits separately. Scores of ≥10 from the subscales indicate the presence of type D personality[6,19,20].

The psychological status of participants was evaluated using the Beck Depression Scale (BDS). The BDS is a 21-item questionnaire in which each item is scored from 0 to 3. Minimum and maximum scores range from 0 to 63, with higher scores representing more severe depressive mood[21,22].

The Brief Illness Perception Questionnaire (B-IPQ) was used to assess patients' perceptions of illness. This scale consists of 9 questions that assess the cognitive and emotional representations of the illness. The total score obtained from the scale varies between 0 and 80, and an increase in the score indicates that the person is affected by the illness, perceives the illness as worrying, and has an increased negative effect[23,24].

### Statistical Analysis

Sample size was determined using G*Power Software (Heinrich-Heine-Universität Düsseldorf, Germany). The system used "t-test means: difference between two independent means (two groups)". In the study conducted by Gokcen et al.[25], when DS14 was taken as the study variable, the effect size was determined as 0.61. The sample size was estimated as at least 58 for gout patients and 58 for controls, with a statistical power of 0.90 and an alpha level of 0.05. Considering the possible data loss, the sample size was increased to 59 for each group.

The obtained data were evaluated in a computer environment using the IBM SPSS Statistics for Windows, Version 26.0 package program (IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test was used to evaluate the distribution of continuous variables. Categorical variables were presented as numbers and frequencies. Continuous variables were expressed as mean with standard deviation and median with interquartile range. The chi-square test was used to compare the differences between the groups (comparison of the patient and control groups and the patients with gout and without type D personality) in terms of categorical variables, and the Mann-Whitney U test or Student's t-test was used for continuous variables, depending on the distribution of the data. The possible correlation of DS14 and B-IPQ scores with clinical variables was tested with Spearman correlation analysis. The results were considered statistically significant if the p values were less than 0.05.

## RESULTS

59 patients with a median age of 48 (20) and 59 relatively healthy individuals as a control group were included in the study. 20.3% of the patients were female and 79.7% were male. There was no statistically significant difference between the patient and control groups in terms of age, gender, marital status, education level, employment status, income level, smoking and BMI (p>0.05). The frequency of alcohol use was significantly higher in the patient group than in the controls (p=0.003). The mean DS14 scores of the patient and control groups were 18.6±10.9 and 13.2±7.5, respectively, and the DS14 scores were significantly higher in the patient group (p=0.002). In addition, the negative affect scores of the patient group were significantly higher than the controls (p<0.001), while no significant difference was observed between the groups in terms of social withdrawal scores (Table 1).

The disease-related data of patients diagnosed with gout are shown in detail in Table 2. Accordingly, the diagnosis period of

**Table 1. Comparative analysis of socio-demographic and clinical data of patients with gout and control group**

| Variables | Patient n=59 | Control n=59 | p value |
|---|---|---|---|
| **Age**[a] | 48 (20) | 48 (16) | 0.823† |
| **Gender**[b] | | | |
| Female | 12 (20.3) | 8 (13.6) | 0.326ǂ |
| Male | 47 (79.7) | 51 (86.4) | |
| **Marital status**[b] | | | |
| Married | 48 (81.4) | 52 (88.1) | |
| Single | 9 (15.3) | 6 (10.2) | 0.579† |
| Divorced | 2 (3.4) | 1 (1.7) | |
| **Level of education (years)**[a] | 11 (10) | 11 (7) | 0.529† |
| **Occupational status**[b] | | | |
| Workers | 40 (67.8) | 51 (86.4) | 0.016ǂ |
| Non-workers | 19 (32.2) | 8 (13.6) | |
| **Annual income level (TL)**[a] | 102000 (102000) | 168000 (228000) | 0.014† |
| **Current smoking status**[b] | 21 (35.6) | 24 (40.7) | 0.570ǂ |
| **Current alcohol consumption status**[b] | 18 (30.5) | 5 (8.3) | 0.003ǂ |
| **BMI(kg/m²)**[a] | 28.9 (5.5) | 27.8 (5.1) | 0.066† |
| **BDS**[a] | 10 (5) | 4 (7) | <0.001† |
| **DS14** | 18.6±10.9 | 13.2±7.5 | 0.002* |
| **Type D personality +**[b] | 18 (30.5) | 8 (13.6) | 0.026ǂ |
| **Social inhibition**[a] | 8 (7) | 7 (6) | 0.521† |
| **Negative affect**[a] | 11 (12) | 4 (9) | <0.001† |

Values [a] represent mean ± SD except for median (IQR), [b]n (%).

†: Mann-Whitney U test, ǂ: chi-square test, *: Student's t-test

TL: Turkish lira, BMI: Body mass index, BDS: Beck depression scale, DS14: Type D Personality scale

the patients was 36 (77) months. 69.5% of the patients had 1-3 attacks per year. The median NRS-pain values were 3 (4) and the mean GAS values were 4.3±1.0. The most common comorbidities were hypertension in 33.9% of the patients and hyperlipidemia in 15.3%. The mean B-IPQ scores were determined to be 36.1±13.3 (Table 2).

The comparative analysis of sociodemographic and clinical data of gout patients according to the presence of type D personality is given in detail in Table 3. Accordingly, NRS-pain, CRP, ESR, GAS, BDS and B-IPQ scores of patients diagnosed with gout with type D personality were found to be statistically significantly higher than those of patients without type D personality (p values 0.003, 0.014, 0.016, 0.012, respectively, and p<0.001 for BDS and B-IPQ).

The potential relationship between clinical variables was examined using Spearman correlation analysis and the results are presented in Tables 4 and 5. Accordingly, a moderate to strong positive correlation was found between the B-IPQ score and TJC, NRS-pain, GAS, BDS, DS14 total score, social withdrawal and negative affect subscale scores (Spearman's rho ranged between 0.525 and 0.753, p<0.001 for all). In addition, a weak negative correlation was found between the B-IPQ score and education and income level (Spearman's rho ranged between -0.331 and -0.292, respectively) (Table 4). A weak to moderate positive correlation was found between the DS14 total score and TJC, NRS-pain, GAS, BDS and B-IPQ scores (Spearman's rho ranged between 0.354 and 0.642) (Table 5).

**Table 2. Disease-related data of patients with gout**

| Clinical variables | Patient (n=59) |
|---|---|
| **Diagnosis time (months)**[a] | 36 (77) |
| **TJC**[a] | 1 (0) |
| **Number of episodes (years)**[b] | |
| 0 | 13 (22) |
| 1-3 | 41 (69.5) |
| >3 | 5 (8) |
| **NRS-pain**[a] | 3 (4) |
| **Medications**[b] | |
| NSAID | 9 |
| Colchicine allopurinol | 12 |
| Allopurinol | 12 |
| Febuxostat | 5 |
| Allopurinol+colchicine | 17 |
| Allopurinol+ NSAID | 4 |
| **Associated comorbidities**[b] | |
| Hypertension | 20 (33.9) |
| Coronary artery disease | 7 (11.9) |
| Hyperlipidemia | 9 (15.3) |
| Chronic kidney disease | 6 (10.2) |
| Diabetes | 4 (6.8) |
| Nephrolithiasis | 6 (8.3) |
| **GAS** | 4.3±1.0 |
| **Laboratory** | |
| CRP[a] | 4.2 (5) |
| ESH[a] | 13 (15) |
| Serum urate | 8.2±1.8 |
| **B-IPQ** | 36.1±13.3 |

Values represent mean ± SD except for [a] median (IQR), [b]n (%).

TJC: Tender joint count, NRS: Numerical rating scale, NSAID: Non-Steroidal Anti-Inflammatory Drugs, GAS: Gut activity score, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, B-IPQ: Brief illness perception questionnaire

## DISCUSSION

In our study evaluating the relationship between disease characteristics and type D personality and disease perception in patients with gout, the rate of type D personality was 30.5%, which was considerably higher than healthy controls (13.5%). Patients with gout had higher type D personality, negative affect and depression scores than healthy controls. In addition, this study showed that patients with type D personality had more pessimistic disease perception, increased pain intensity, higher GAS and more mental involvement. The disease perception and type D personality scores of the patient group were related both to each other and to the number of affected joints, pain level, GAS and depression scores.

Personality traits have been extensively studied to date in various musculoskeletal disorders, especially rheumatic diseases, and their effects on clinical outcomes have been reported[9,25-27]. In the study by Donisan et al.[27] investigating the relationship between personality types and quality of life and GAS in patients with ankylosing spondylitis and rheumatoid arthritis, it was confirmed that type A personality was associated with better quality of life and reduced disease activity. However, types C and D personality were associated with impaired quality of life and higher GAS in patients with rheumatoid arthritis and ankylosing spondylitis. Gokcen et al.[25] reported that fibromyalgia patients with type D personality had lower self-esteem, worse general health status, and higher anxiety and depression levels. However, studies investigating the effects of personality traits on clinical parameters in patients with gout are quite limited, and there is no study investigating the presence and potential effect of type D personality in this patient population[13-15]. Pazcoguin et al.[13] showed that there is a significant relationship between aggression, which is one of the personality trait types in individuals diagnosed with gout, and disease characteristics such as pain level, disease duration and serum urate level of the patients. The results of our current study showed that inflammatory markers (CRP and ESR) were higher in patients with type D personality. This situation can be attributed to the activation of the inflammatory process by negative affect. In support of this interpretation, GAS was found to be high in patients

**Table 3. Comparative analysis of socio-demographic and clinical data of patients with gout according to the presence of type D personality.**

| Variables | Type D personality (+) n=18 | Type D personality (-) n=41 | p value |
|---|---|---|---|
| **Age**[a] | 48 (22) | 47 (19) | 0.889[†] |
| **Gender**[b] | | | |
| Female | 6 (33.3) | 6 (14.6) | 0.100[ǂ] |
| Male | 12 (66.7) | 35 (85.4) | |
| **Level of education (years)**[a] | 9.5 (10) | 11 (10) | 0.478[†] |
| **Annual income level (TL)**[a] | 99000 (78000) | 120000 (108000) | 0.447[†] |
| **Current smoking status**[b] | 8 (44.4) | 13 (31.7) | 0.347[ǂ] |
| **Current alcohol consumption status**[b] | 5 (27.8) | 13 (31.7) | 0.763[ǂ] |
| **BMI (kg/m²)**[a] | 30.2 (7.5) | 28.7 (5.7) | 0.256[†] |
| **Diagnosis time (months)**[a] | 20.5 (50) | 51 (105.5) | 0.174[†] |
| **Number of gout attacks (in the last 12 months)**[b] | | | |
| 0 | 2 (11.1) | 11 (26.8) | 0.175[ǂ] |
| 1-3 | 13 (72.2) | 28 (68.3) | |
| >3 | 3 (16.7) | 2 (4.9) | |
| **TJC**[a] | 1 (0.3) | 1 (1) | 0.105[†] |
| **NRS-pain**[a] | 5 (3) | 2 (3) | 0.003[†] |
| **CRP**[a] | 7.8 (6) | 4 (3.2) | 0.014[†] |
| **ESR**[a] | 19 (13) | 10 (13) | 0.016[†] |
| **Serum urate** | 8.1±2.1 | 8.2±1.7 | 0.953[*] |
| **GAS** | 4.7±1.1 | 4.04±0.8 | 0.012[*] |
| **BDS**[a] | 13.5 (6.5) | 8 (5.5) | <0.001[†] |
| **B-IPQ** | 46.2±10.4 | 31.7±12.0 | <0.001[*] |

Values[a] represent mean ± SD except for median (IQR), [b]n (%).

[†]: Mann-Whitney U test, [ǂ]: Chi-square test, *: Student's t-test

BMI: Body mass index, TJC: Tender joint count, NRS: Numerical rating scale, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, GAS:Gut activity score, BDS: Beck depression scale, B-IPQ: Brief illness perception questionnaire

**Table 4. Correlation of B-IPQ score with clinical variables**

| | B-IPQ | |
|---|---|---|
| | Spearman's rho | p value |
| **Age** | 0.089 | 0.505 |
| **Diagnosis time (months)** | -0.164 | 0.216 |
| **Level of education (years)** | -0.331 | 0.010 |
| **Income level** | -0.292 | 0.025 |
| **BMI (kg/m²)** | 0.118 | 0.373 |
| **TJC** | 0.553 | <0.001 |
| **NRS-pain** | 0.753 | <0.001 |
| **CRP** | 0.048 | 0.717 |
| **ESR** | 0.281 | 0.031 |
| **Serum urate** | 0.228 | 0.082 |
| **GAS** | 0.731 | <0.001 |
| **BDS** | 0.750 | <0.001 |
| **DS14** | 0.611 | <0.001 |
| **Social inhibition** | 0.525 | <0.001 |
| **Negative affect** | 0.582 | <0.001 |

BMI: Body mass index, TJC: Tender joint count, NRS: Numerical rating scale, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, GAS: Gut activity score, BDS: Beck depression scale, B-IPQ: Brief illness perception questionnaire, DS14: Type D personality scale

**Table 5. Correlation of DS14 total score with clinical variables**

| | DS14 | |
|---|---|---|
| | Spearman's rho | p value |
| **Age** | 0.004 | 0.967 |
| **Diagnosis time (months)** | -0.101 | 0.446 |
| **Level of education (years)** | -0.176 | 0.056 |
| **Income level** | -0.256 | 0.005 |
| **BMI (kg/m²)** | 0.154 | 0.097 |
| **TJC** | 0.354 | 0.006 |
| **NRS-pain** | 0.469 | <0.001 |
| **CRP** | 0.219 | 0.096 |
| **ESR** | 0.175 | 0.185 |
| **Serum urate** | -0.036 | 0.789 |
| **GAS** | 0.417 | 0.001 |
| **BDS** | 0.642 | <0.001 |
| **B-IPQ** | 0.611 | <0.001 |

BMI: Body mass index, TJC: Tender joint count, NRS: Numerical rating scale, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, GAS: Gut activity score, BDS: Beck depression scale, B-IPQ: Brief illness perception questionnaire, DS14: Type D personality scale

with type D personality in our study and negative affect was higher in the patient group compared to the control group. It has also been shown that type D personality is associated with disease perception, depression, GAS and pain scores. Patients may evaluate self-reported measurements such as disease activity, functional impairment and pain perception more exaggeratedly than they actually are regarding their personality characteristics. This suggests that the differences between patients with similar clinical presentation may be partly related to personality characteristics[28]. Therefore, it is valuable for clinicians to consider personality characteristics in the clinical evaluation and management of these patients.

Illness perceptions are opinions and beliefs formed in response to a health threat that may affect a person's self-management behaviors and chronic disease outcomes[5]. Research supports the idea that illness perception is associated with mortality and many health outcomes in individuals with gout[3,29,30]. Selvadurai et al.[3] reported that pessimistic or negative illness perception in patients with gout is associated with poor medication adherence, impaired quality of life, and decreased work productivity. They also reported that patients' pessimistic illness perceptions are associated with more severe illness characteristics. On the other hand, a similar relationship could not be demonstrated with serum urate levels. The results of our study, consistent with existing evidence, showed that negative illness perception is associated with higher disease activity, increased pain levels, and the number of joints affected. However, no relationship was observed between serum urate levels and CRP and illness perception.

Another important finding of our study is the positive relationship between the disease perception of patients with gout and the presence of type D personality, negative affectivity and social inhibition. Literature overview revealed, the relationship between personality traits and disease perception in various diseases has been investigated[31-35]. However, no study investigating this relationship in patients with gout has been found. A study conducted on patients with myocardial infarction reported that the disease perception profile of patients with type D personality showed a statistically significant difference compared to those without type D personality[31]. Another study conducted on peritoneal dialysis patients reported a strong relationship between type D personality, disease perception, social support and quality of life[32]. These results support the possibility that evaluating personality traits, planning interventions that change disease perception and education are effective strategies to improve health outcomes in patients with gout.

### Study Limitations

This study has some limitations and strengths. One of the most important strengths of this study is the comprehensive evaluation of the relationship between type D personality and its subdomains and many health-related outcomes including disease perception and GAS in patients with gout. Its cross-sectional design is one of its limitations as it cannot establish any cause-effect relationship. Fluctuations in the relevant parameters and definite relationships can be revealed with longitudinal studies. In addition, other personality types were not evaluated in the study. In addition, no comparisons were made with other inflammatory rheumatic diseases. Another limitation is that most of the selected questionnaires were subjective and possess a risk of bias.

## CONCLUSION

This study showed that approximately one third of individuals diagnosed with gout have type D personality, and type D personality is associated with many variables such as negative illness perception, psychological health, perceived pain intensity and disease activity. These findings suggest that illness perception may have a potential role in explaining the negative effects of type D personality on various health parameters in patients with gout. In this context, it would be appropriate to consider these close relationships when treating these individuals. Patient education, coping strategies and psychological interventions should be considered as an integral part of the treatment algorithm. Further studies with longer follow-up period should be conducted to fully elucidate these relationships in a more comprehensive and objective manner.

### Ethic

**Ethics Committee Approval:** The approval obtained from the Çukurova University Faculty of Medicine Non-invasive Clinical Research Ethics Committee. (decision no: 138/23, date: 3.11.2023). The study was conducted in accordance with the principles of the Declaration of Helsinki.

**Informed Consent:** Written informed consent to participate were included in our study.

### Authorship Contributions

Surgical and Medical Practices: A.S., İ.C.B., K.T., A.Y., E.K., Concept: A.S., İ.C.B., K.T., E.K., Design: A.S., İ.C.B., K.T., A.Y., E.K., Data Collection or Processing: A.S., İ.C.B., K.T., A.Y., E.K., Analysis or Interpretation: A.S., İ.C.B., Literature Search: A.S., İ.C.B., E.K., Writing: A.S., İ.C.B.

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# Prognostic Factors and Treatment Outcomes in Renal Cell Carcinoma: A Comprehensive Analysis

## Renal Hücreli Karsinomda Prognostik Faktörler ve Tedavi Sonuçları: Kapsamlı Bir Analiz

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**ABSTRACT**

**Aim:** We aimed to investigate the prognostic factors, factors affecting survival and the prognostic value of the Memorial Sloan-Kettering Cancer Centre (MSKCC) risk score in renal cell carcinoma (RCC) patients. In addition, we assessed the survival and potential adverse effects of sunitinib and pazopanib tyrosine kinase inhibitors.

**Materials and Methods:** The study included patients diagnosed with RCC aged ≥18 years, who were followed up in our clinic between 2006 and 2020. The clinicopathological characteristics were recorded in the hospital's electronic data system. In the entire patient population, survival and prognostic factors were investigated. Furthermore, prognostic factors in terms of treatment (pazopanib vs. sunitinib) for advanced stage patients were evaluated as well.

**Results:** Two hundred and two patients were included in this study. Fifty-five of the patients were female, 147 patients were male. Most common histological type was clear cell carcinoma (59%). At the time of presentation, 57% of the patients were in the early stage (stage 1,2,3). The median overall survival (mOS) was 16.8 months in stage 4 patients and 82.5 months in early stage patients. mOS was 69.1 months in the favorable MSKCC risk group while it was 6.8 months in the poor risk group. In the sunitinib arm, the median progression-free survival (mPFS) was 11.1 months, and mOS was 18.1 months. In the pazopanib arm, mPFS was 12.2 months, and mOS was 17.4 months. There was no significant difference in response rate, mPFS, and mOS between the two drugs.

**Conclusion:** In this study, we have shown that risk and performance scorings with some laboratory and clinical evaluations, which are still cheap and easily accessible, are valuable and usable in showing prognosis in RCC patients. Disease stage, MSKCC risk score, Eastern Cooperative Oncology Group, and Karnofsky performance scores showed prognostic characteristics in RCC. There was no survival difference between histological subtypes. The efficacy of sunitinib and pazopanib in metastatic first-line treatment was similar, but pazopanib was superior in terms of any grade adverse events.

**Keywords:** Renal cell carcinoma, RCC, MSKCC score, sunitinib, pazopanib

**ÖZ**

**Amaç:** Renal hücreli karsinom (RCC) hastalarında prognostik faktörleri, sağkalımı etkileyen faktörleri ve Memorial Sloan-Kettering Kanser Merkezi (MSKCC) risk skorunun prognostik değerini araştırmayı amaçladık. Ayrıca, tirozin kinaz inhibitörleri olarak sunitinib ve pazopanibin sağkalım sonuçlarını ve yan etkilerini değerlendirdik.

**Gereç ve Yöntem:** Çalışmaya 2006-2020 yılları arasında kliniğimizde takip edilen ≥18 yaş RCC tanılı hastalar dahil edilmiş olup, klinikopatolojik özellikler hastanenin elektronik veri sistemine kaydedilmiştir. Tüm hasta popülasyonunda sağkalım ve prognostik faktörler araştırılmış, ayrıca ileri evre hastalar için tedavi (pazopanib vs. sunitinib) açısından prognostik faktörler de değerlendirilmiştir.

**Bulgular:** Bu çalışmaya 202 hasta dahil edildi. Hastaların 55'i kadın 147'si erkekti. En sık görülen histolojik tip berrak hücreli karsinomdu (%59). Başvuru sırasında hastaların %57'si erken evredeydi (evre 1,2,3). Dördüncü evre hastalarda ortanca genel sağkalım (mOS) 16,8 ay iken, erken evre hastalarda mOS 82,5 aydı. mOS, MSKCC iyi risk grubunda 69,1 ay iken, kötü risk grubunda 6,8 aydı. Sunitinib kolunda medyan progresyonsuz sağkalım (mPFS) 11,1 ay ve mOS 18,1 ay, pazopanib kolunda ise mPFS 12,2 ay ve mOS 17,4 aydı. İki ilaç arasında yanıt oranı, mPFS ve mOS açısından anlamlı bir fark bulunamadı.

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**Sonuç:** Bu çalışmada, halen ucuz ve kolay erişilebilir olan bazı laboratuvar ile klinik değerlendirmelerle birlikte yapılan risk ve performans skorlamalarının RCC hastalarında prognozu göstermede değerli ve kullanılabilir olduğunu gösterdik. Hastalık evresi, MSKCC risk skoru, Eastern Cooperative Oncology Group ve Karnofsky performans skorları RCC'de prognostik özellik gösterdi. Berrak hücreli ve diğer histolojik alt tipleri arasında sağkalım farkı bulunmadı. Metastatik hastalıkta birinci basamak tedavide sunitinib ve pazopanib tiroin kinaz inhibitörlerinin etkinliği benzer bulundu, ancak pazopanib yan etki açısından daha üstündü.

**Anahtar Kelimeler:** Renal hücreli karsinom, RCC, MSKCC skoru, sunitinib, pazopanib

## INTRODUCTION

Renal cell carcinoma (RCC) is the most common kidney cancer in adults and is a renal parenchymal cancer of the adenocarcinoma cell type. It accounts for approximately 4% of adult malignancies and more than 90% of neoplasms arising from the kidneys[1]. According to globocan 2022 data, approximately 435,000 new cases and 156,000 deaths were reported worldwide annually[1]. Although the incidence is increasing, mortality is decreasing due to new treatments[2] .

RCC is characterized by several subtypes with distinct genetic and molecular profiles. Clear cell (~70%), papillary (~15%), chromophobe (~5%), oncocytic (~5%), and collecting duct (Bellini) origin (<1%) subtypes exist[3]. Sarcomatoid differentiation is not a separate histological subtype and may be observed together with other RCC subtypes[4]. These tumors have a more aggressive prognosis, and approximately 75% of the patients were metastatic at the time of diagnosis[5]. Many RCCs are diagnosed when local invasion or metastasis occurs. In addition, recurrence may develop in patients who are initially resectable and undergo surgery, and systemic treatment (targeted agents, immunotherapy, radiotherapy) may be required. Systemic treatment is initiated immediately in metastatic or locally advanced disease. In localized disease, surgical resection is the most effective and curative treatment method. RCC is resistant to most chemotherapeutic agents due to the expression of the multidrug resistance protein P-glycoprotein, which originates from the proximal tubule. In recent years, targeted therapies have come to the forefront in RCC with a better understanding of molecular mechanisms[6].

Overall, the management of RCC has evolved significantly with the introduction of targeted therapies and immunotherapies. Ongoing research continues to unravel the complex biology of RCC, aiming to improve patient outcomes through personalized and combination therapies [7-9].

In this study, we aimed to investigate the predictive factors for recurrence and survival in patients with RCC and to evaluate the efficacy and adverse effects of tyrosine kinase agents.

## MATERIALS AND METHODS

For our study, permission was obtained from the Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee with decision number 25 dated 05.03.2020. Between the years of 2006 and 2020, 202 patients over the age of 18 years, who were diagnosed with RCC and followed up in the Medical Oncology Clinic of Dicle University Faculty of Medicine, were included in the study. Tumors of urothelial epithelial origin were not included. Their files, demographic and clinical characteristics were analyzed. Prognostic factors related to the case and treatment were investigated, and survival analyses were performed. The histopathological type and stage of the tumor, type of surgery performed, time and sites of recurrence and metastasis were examined. Patients were grouped according to the Memorial Sloan-Kettering Cancer Centre (MSKCC) risk scores. The Karnofsky performance status (KPS) and Eastern Cooperative Oncology Group Performance Status Scale (ECOG PS) were used to evaluate the performance status of the patients. In addition, treatment effects, side effects and survival analyses were evaluated in patients receiving sunitinib and pazopanib, two tyrosine kinase inhibitors (TKI).

Overall survival (OS) was defined as the time from the date of disease diagnosis until death or the date of the last follow-up and disease-free survival (DFS) as the time from cure in early stage (stage 1, 2, 3) patients until relapse. Progression-free survival (PFS) was defined as the time from the start of treatment until disease progression or the date when it was decided that the response obtained with treatment was inadequate and the treatment approach should be changed. Treatment response was evaluated as complete response (CR), partial response (PR), srogressive disease (PD), and stable disease (SD) according to the new response evaluation criteria in solid tumors (RECIST 1.1).

### Statistical Analysis

Statistical analyses of the results obtained in the study were performed using the IBM SPSS Statistics version 27 statistical software package. Descriptive statistics for continuous variables were expressed as median value, minimum and maximum value, 95% confidence interval, mean ± standard deviation, while categorical variables were expressed as number and percentage. The chi-square test was used to analyze categorical variables. Overall survival, intragroup survival, DFS and PFS were analyzed by the Kaplan-Meier test. In these tests, a p value less than 0.05 was considered statistically significant.

## RESULTS

In our study, we analyzed the data from a total of 202 patients diagnosed with RCC, including 55 females (27.2%) and 147 males (72.8%). The median age of the patients was 57 years. The most common histological subtype was clear cell carcinoma, with 119 patients (59%). Forty-eight patients (23.8%) were stage 1, 33 patients (16.3%) were stage 2, 34 patients (16.8%) were stage 3, and 86 patients (43%) were stage 4. Seventy-nine patients (39%) were metastatic, and the most common sites of metastasis were the lymph nodes (62.2%) and lungs (58.7%). According to the Karnofsky score, 157 patients (78.1%) had a score greater than 80%. There were 80 patients (39.6%) with ECOG 0. According to MSKCC criteria, 66 patients (32.8%) were in the good risk group, 93 patients (46.3%) were in the intermediate risk group, and 42 patients (20.9%) were in the poor risk group. Nephrectomy was performed in 155 patients (77.1%). Clinicopathological characteristics of the patients are presented in Table 1.

In the survival analysis of the patients, we divided the early-stage patients and stage 4 patients into two separate groups for evaluation.

### Results of Early Stage Patients

In this group of 115 patients, 37 were female and 78 were male. Survival was analyzed for 114 patients. Forty-seven patients (41.2%) were stage 1, 32 patients (28.1%) were stage 2, and

**Table 1. Patient characteristics**

| **Age (years), median (range)** | **57 (21-83)** | | |
|---|---|---|---|
| **Subgroup** | **n (%)** | **Subgroup** | **n (%)** |
| **Sex** | | **MSKCC criteria** | |
| Female | 55 (27.2) | Favorable | 66 (32.8) |
| Male | 147 (72.8) | Intermediate | 93 (46.3) |
| | | Poor | 42 (20.9) |
| **Histologic type** | | **ECOG PS** | |
| Clear cell | 119 (59) | 0 | 80 (39.6) |
| Papillary | 39 (19.3) | 1 | 81 (40.1) |
| Kromofob | 15 (0.4) | 2 | 21 (10.4) |
| Sarcomatoid | 8 (3.9) | 3 | 20 (9.9) |
| Other | 21 (10.4) | | |
| **Stage** | | **Nephrectomy** | |
| 1 | 48 (23.8) | Yes | 155 (76.7) |
| 2 | 33 (16.3) | No | 47 (23.3) |
| 3 | 34 (16.8) | | |
| 4 | 86 (43.1) | | |
| **T grade** | | **Nephrectomy type** | |
| T1 | 56 (28) | Partial | 18 (%11.6) |
| T2 | 48 (24) | Total | 137 (88.4) |
| T3 | 56 (28) | | |
| T4 | 40 (20) | | |
| **N category** | | **Primary metastatic disease** | |
| N0 | 129 (64) | No | 123 (61) |
| N1 | 70 (35) | Yes | 79 (39) |
| Nx | 3 (1) | | |
| | | **Metastasis region** | |
| | | Lymph node | 89 (62.2) |
| | | Lung | 84 (58.7) |
| **Karnofsky score** | | Bone | 62 (43.4) |
| <80% | 45 (22) | Adrenal | 26 (18.2) |
| ≥80% | 157 (78) | Liver | 25 (17.5) |
| | | CNS | 13 (9.1) |
| | | Local | 11 (7.7) |
| | | Thyroid | 1 (0.7) |

MSKCC: Memorial Sloan-Kettering Cancer Centre risk scores, ECOG PS: Eastern Cooperative Oncology Group Performance Status Scale, CNS: Central nervous system

35 patients (30.7%) were stage 3. The number of patients with recurrence/metastasis was 56 (49.1%). There was no gender difference in the recurrence rate. DFS and OS analyses were performed. The median DFS (mDFS) was 47.5 months and the median OS (mOS) was 82.5 months according to the time to recurrence and/or metastasis. There was no significant difference between genders in terms of DFS and OS. In stage 1 patients, mDFS was 79.2 months and mOS was 131.3 months, while in stage 3 patients, mDFS was 29.6 months and mOS was 50.3 months. mDFS and mOS durations were found to be significantly shorter as the stage progressed (mDFS p=0.001, mOS p=0.002). In patients with ECOG 0, mDFS was 72.8 months and mOS was 105 months, while in patients with ECOG 2, mDFS was 13.3 months and mOS was 78.7 months. mDFS and mOS were significantly shorter as ECOG performance score increased (mDFS p=0.001, mOS p=0.020). In the clear cell subtype, mDFS was 47.5 months and mOS was 68.3 months. In patients with non-clear cell subtype, mDFS was 59.1 months and mOS was 135.7 months. In patients with clear cells, mDFS and mOS were shorter, which was not statistically significant (mDFS p=0.327, mOS p=0.147). Survival analyses of early stage patients are shown in Table 2.

## Results of Stage 4 Patients

The data of 86 patients with stage 4 RCC, including 17 female and 69 males, were analyzed. The mOS was 16.8 months in stage 4 patients. This duration was 17.7 months in men and 9.9 months in women, which was shorter in women and statistically significant (p=0.049). The duration of mOS was 17.4 months in the clear cell subtype and 16.8 months in the non-clear cell subtype, with no statistical difference (p=0.564) (Figure 1).

When survival was analysed according to the MSKCC risk assessment score, the mOS was 69.1 months in the favorable risk group, 11.1 months in the intermediate risk group, and 6.8 months in the poor risk group. According to the MSKCC risk score, survival was shorter and statistically significant as the risk status worsened (p<0.001) (Figure 2).

mOS was 32.9 months in patients with a Karnofsky score >80% and 6.8 months in patients with a Karnofsky score <80%. It was significantly shorter (p<0.001), as shown in Figure 3.

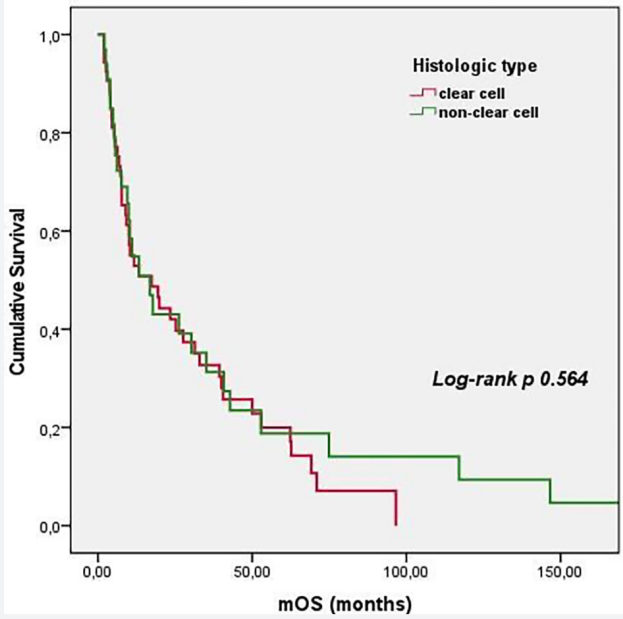


**Figure 1.** Kaplan-Meier curves for median overall survival (mOS) by histologic type

**Table 2. Survival analysis of early stage patients**

| | mDFS (months) (95% CI) | p value | mOS (months) (95% CI) | p value |
|---|---|---|---|---|
| **Overall** | 47.5 (22.6-72.3) | | 82.5 (55.4-109.5) | |
| **Sex** | | | **(Mean)** | |
| Female | 66.8 (40.8-92.9) | 0.069 | 191.8±31 | **0.029** |
| Male | 32.6 (22.3-42.9) | | 82.3±6 | |
| **Stage** | | | | |
| 1 | 79.2 (55.6-102.7) | **0.001** | 131.3 (116.2-146.4) | **0.002** |
| 2 | 47.9 (22-73.9) | | 69 months (36.9-101.1) | |
| 3 | 29.6 (6.6-52.5) | | 50.3 months (34.2-66.5) | |
| **ECOG PS** | | | **(Mean)** | |
| 0 | 72.8 (56.9-88.8) | **0.001** | 105±6.9 | **0.020** |
| 1 | 22.3 (15.5-29.1) | | 100.3±20 | |
| 2 | 13.3 (0-64.8) | | 78.7±16.3 | |
| **Histologic type** | | | | |
| Clear cell | 47.5 (26-69) | 0.327 | 68.3 (51.7-84.9) | 0.147 |
| Non-clear cell | 59.1 (17.5-100.8) | | 135.7 (118-153.4) | |

mDFS: Median disease-free survival, mOS: Median overall survival, CI: Confidence interval, ECOG PS: Eastern Cooperative Oncology Group Performance Status Scale

mOS was 50 months in patients with ECOG 0 and decreased to 1.2 months in patients with ECOG 3. Survival significantly shortened as the ECOG performance score worsened (p<0.001), as shown in Figure 4.

Survival analyses of advanced-stage patients are provided in Table 3.

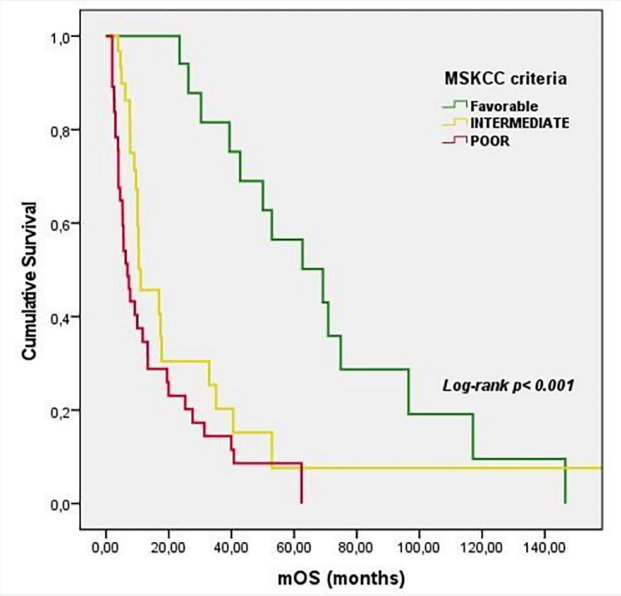


**Figure 2.** Kaplan-Meier curves for median overall survival (mOS) by MSKCC criteria

*MSKCC: Memorial Sloan-Kettering Cancer Centre*

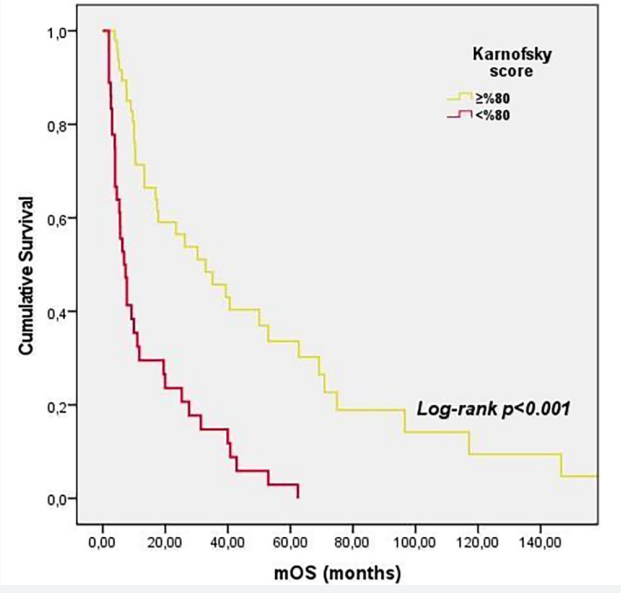


**Figure 3.** Kaplan-Meier curves for median overall survival (mOS) by Karnosfsky score

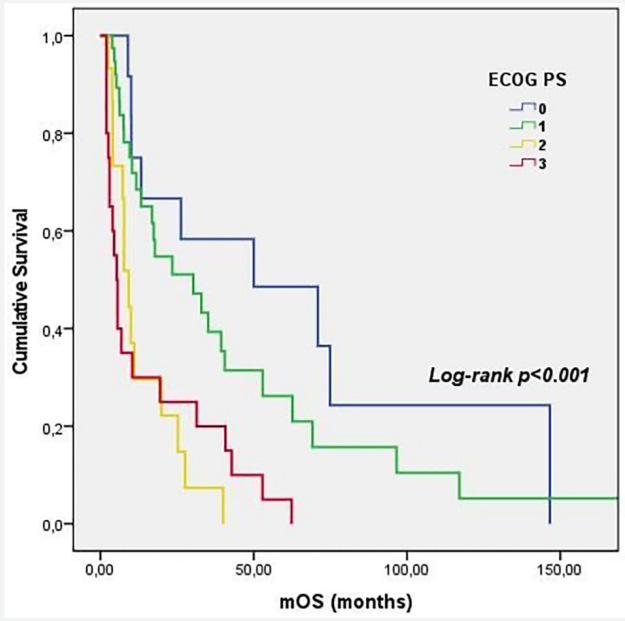


**Figure 4.** Kaplan-Meier curves for median overall survival (mOS) by ECOG PS

*ECOG PS: Eastern Cooperative Oncology Group Performance Status Scale*

**Table 3. Survival analysis of stage 4 patients**

| | mOS (months) (95% CI) | p value |
|---|---|---|
| **Overall** | 16.8 (8.4-25.3) | |
| **Sex** | | |
| Female | 9.9 (1-24.4) | **0.049** |
| Male | 17.7 (1.8-33.6) | |
| **Histologic type** | | |
| Clear cell | 17.4 (5.1-29.6) | 0.564 |
| Non-clear cell | 16.8 (8-25.6) | |
| **Karnofsky score** | | |
| ≥80% | 32.9 (14.7-51) | **<0.001** |
| <80% | 6.8 (4.4-9.2) | |
| **MSKCC criteria** | | |
| Favorable | 69.1 (39.3-99.1) | **<0.001** |
| Intermediate | 11.1 (3.9-18.2) | |
| Poor | 6.8 (4.3-9.4) | |
| **ECOG PS** | | |
| 0 | 50 (1-109) | |
| 1 | 30.0 (11.8-48.8) | **<0.001** |
| 2 | 9.2 (6.9-11.4) | |
| 3 | 5.3 (2.8-7.7) | |
| **Nephrectomy** | | |
| Yes | 35.1 (6.7-63.5) | **0.001** |
| No | 9.5 (4-15) | |

mOS: Median overall survival, CI: Confidence interval, MSKCC: Memorial Sloan-Kettering Cancer Centre risk scores, ECOG PS: Eastern Cooperative Oncology Group Performance Status Scale

### Results of Patients Treated with Sunitinib and Pazopanib

The general characteristics, median PFS (mPFS), and mOS durations of 114 patients who received TKI (sunitinib, pazopanib) were analyzed. Eighty patients received sunitinib and 34 patients received pazopanib. A CR was observed in 2 patients (2.8%), a PR in 24 patients (33.8%), SD in 15 patients (21.1%), and PD in 30 patients (42.3%). Among the patients receiving pazopanib, a PR was observed in 8 patients (27.6%), SD in 8 patients (27.6%), and PD in 10 patients (44.8%). Dose changes were made in 23 patients (30.3%) receiving sunitinib and in 3 patients (8.8%) receiving pazopanib due to adverse effects and the inability to tolerate the drug. The number of patients having adverse events at any grade was significantly higher in the sunitinib arm. The most common adverse events in the sunitinib arm were fatigue, hand-foot skin reaction, and gastrointestinal side effects. In the group receiving pazopanib, the most common adverse events were gastrointestinal adverse events, hematological adverse events, and high arterial blood pressure. Hypothyroidism occurred in 26 (32.5%) patients receiving sunitinib and 5 (14.7%) patients receiving pazopanib. The characteristics, treatment response, and adverse event status of TKI patients are shown in Table 4. In patients who received sunitinib, mPFS was 11.1 months and mOS was 18.1 months. In patients given pazopanib, mPFS was 12.2 months and mOS was 17.4 months. There was no statistically significant difference between the mPFS and mOS durations of patients receiving sunitinib and pazopanib (mPFS p=0.278, mOS p=0.403).

## DISCUSSION

In our study, DFS, OS, and prognostic factors of 202 patients diagnosed with RCC were analyzed retrospectively. Additionally, the efficacy and adverse effects of both drugs were analyzed in patients treated with sunitinib and pazopanib in the first-line treatment for metastasis. The study included both early-stage (stage 1,2,3) and stage 4 patients, and analyses were performed separately for both groups. Of the 115 early-stage patients, 56 (49%) developed recurrence/metastasis during follow-up, with a mDFS of 47.5 months and a mOS of 82.5 months. As the stage progressed and ECOG performance score worsened, mDFS and mOS significantly decreased. Sex and histological subtypes were not associated with survival in early-stage patients. In the 86 patients with stage 4, mOS was 16.8 months, and in this group, the duration of mOS was significantly shorter in females, patients with poor Karnofsky and ECOG performance scores, patients with high MSKCC risk scores, and patients who could not undergo nephrectomy.

In our study, the objective response rates were similar in patients receiving sunitinib and pazopanib. In patients receiving sunitinib, the mPFS was 11.1 months and the mOS was 18.1 months, while in patients receiving pazopanib, the mPFS was 12.2 months and the mOS was 17.4 months. No statistically significant difference was observed between the two groups in survival. Many studies investigating the efficacy of pazopanib and sunitinib treatments have found that both drugs have similar efficacy in terms of PFS and OS durations in patients with metastatic RCC. Pazopanib shows non-inferiority compared to sunitinib[10-13]. Although their efficacy is similar, large-scale studies have reported that pazopanib is associated with less fatigue and a better general health-related quality of life than sunitinib[14,15]. Motzer et al.[13] reported that pazopanib treatment was associated with a lower incidence of some adverse effects such as fatigue and hand-foot skin reaction compared to sunitinib. In our study, while 30% of patients receiving sunitinib had a dose change, this rate was 8% in patients receiving pazopanib and was statistically significantly lower. When the adverse effect profiles were examined, gastrointestinal and hematological side effects and hypertension were similar for both agents. Fatigue, hand-foot skin reaction, and hypothyroidism were significantly more common in the sunitinib arm.

**Table 4. Characteristics of patients given sunitinib and pazopanib, their treatment response and adverse events status**

| | Sunitinib n (%) | Pazopanib n (%) | p value |
|---|---|---|---|
| **Sex** | | | |
| Female | 14 (17.5) | 7 (20.6) | **0.69** |
| Male | 66 (82.5) | 27 (79.4) | |
| **Stage at diagnosis** | | | |
| 1 | 12 (15) | 4 (11.8) | |
| 2 | 15 (18.8) | 2 (5.9) | **0.13** |
| 3 | 12 (15) | 3 (8.8) | |
| 4 | 41 (51.3) | 25 (73.5) | |
| **Histologic type** | | | |
| Clear cell | 49 (61.2) | 25 (73.5) | 0.14 |
| Non-clear cell | 31 (38.8) | 9 (26.5) | |
| **Response** | | | |
| CR | 2 (2.8) | 0 | |
| PR | 24 (33.8) | 8 (27.6) | 0.689 |
| SD | 15 (21.1) | 8 (27.6) | |
| PD | 30 (42.5) | 13 (44.8) | |
| **Adverse events (any grade)** | | | |
| Fatigue | 32 (40) | 2 (6) | |
| Hand-foot skin reaction | 23 (28.7) | 3 (9) | |
| Hypertension | 11 (13.7) | 4 (11.7) | **0.019** |
| Gastrointestinal | 14 (17.5) | 6 (17.6) | |
| Hematologic | 11 (13.7) | 4 (11.7) | |
| Endocrinologic | 12 (15) | 3 (9) | |
| Hypothyroidism | 26 (32.5) | 5 (14.7) | |

CR: Complete response, PR: Partial response, SD: Stable disease, PD: Progressive disease

Although various scoring systems have been used to predict prognosis and decide on treatment in patients diagnosed with RCC, the MSKCC score is one of the most commonly used classifications to classify RCC patients into prognostic groups. After the MSKCC was defined, its effectiveness in predicting prognosis has been confirmed by various studies[16]. In addition, there are studies reporting that the MSKCC score is also prognostic in non-clear cell and sarcomatoid tumors[17,18]. In our study, the analysis models of patients who were metastatic at diagnosis and patients who developed metastasis during follow-up with the MSKCC score revealed prognostic features and survival times were statistically significantly shorter as the score worsened.

The effect of clear cell and non-clear cell histological subtypes on prognosis in RCC is controversial. de Velasco et al.[19] reported that the prognosis of patients with non-clear cell was worse in a study of more than four thousand patients, but the rate of sarcomatoid differentiation was 1.2% in the clear cell group and 26% in the non-clear cell group. Other studies show that the prognosis is better in the clear cell subtype[20,21]. Survival has been shown to be better in chromophobe subtype non-clear cell carcinomas[21]. In our study, mDFS was 47.5 months and mOS was 68.3 months in early-stage clear cell carcinoma, mDFS was 59.1 months and mOS was 135.7 months in the non-clear cell group; although OS was numerically longer in the non-clear cell group, it did not reach statistical significance. In our stage 4 patients, there was no OS difference between each clear cell and non-clear cell arms. We think that this difference in the literature may be due to heterogeneous patient groups in the non-clear cell group, especially tumors with sarcomatoid differentiation and tumors of collecting duct origin.

The KPS and the ECOG PS are established methods used to assess the functional status of cancer patients and are important in predicting patient outcomes. These scales are used to determine the eligibility of patients for clinical trials and to provide prognostic information. Karnofsky and ECOG PS scores have a strong correlation, and there is a high degree of agreement between the two scales, suggesting that they can be used interchangeably to a certain extent[22]. Intra-and inter-observer variability for both KPS and ECOG PS is very low, suggesting that assessments made by clinical oncologists using these scales are reliable in selecting patients for clinical trials[23]. In our study, we demonstrated the prognostic value of both performance scores in both early-stage and metastatic patients, and survival times were significantly shorter as the performance score worsened. We observed that both scores are correlated in showing performance status.

## CONCLUSION

In our study, we investigated prognostic factors in patients diagnosed with RCC and performed survival analysis. We found that the disease stage and MSKCC risk score are prognostic. Additionally, we demonstrated the prognostic value of ECOG and KPS, which evaluate the performance status of patients. There was no survival difference between the histological subtypes. We also showed that sunitinib and pazopanib TKI treatments in metastatic first-line therapy were similar in terms of mPFS and mOS, but pazopanib was superior in terms of any grade of adverse events. This study shows that some inexpensive and easily accessible laboratory and clinical evaluations, as well as risk and performance scoring, are valuable and usable in determining prognosis in RCC patients.

### Ethics

**Ethics Committee Approval:** Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (decision number: 25 date: 05.03.2020).

**Informed Consent:** Retrospective study.

### Authorship Contributions

Concept: Ö.F.E., Design: Ö.F.E., Data Collection or Processing: Ö.F.E., Analysis or Interpretation: Ö.F.E., M.K., Literature Search: Ö.F.E., Writing: Ö.F.E.

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

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# Dysfunctional Personality Disorder Beliefs, Treatment Adherence and Lifetime Suicide Attempts of Bipolar Disorder Patients Type-1

## Tip-1 Bipolar Bozukluk Hastalarında İşlevsel Olmayan Kişilik Bozukluğu İnançları, Tedavi Uyumu ve Yaşam Boyu İntihar Girişimleri

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### ABSTRACT

**Aim:** This study aims to determine the effects of personality-specific dysfunctional beliefs on psychiatric treatment adherence of patients with bipolar disorder (BD) type-1 in relation to their suicide attempt history. Additionally, the clinical features of the patients related to that may influence medication adherence.

**Materials and Methods:** Patients were screened (n=79) using the Morisky Medication Adherence Scale, and the Personality Belief Questionnaire-Short Form to assess psychiatric treatment adherence and personality-specific dysfunctional beliefs, respectively.

**Results:** The results of the study show that the psychiatric medication treatment adherent group consisted of 26 (32.9%) patients, while the psychiatric medication treatment non-adherent group consisted of 53 (67.1%) patients. Participants with a history of suicide attempt had high dysfunctional beliefs specific to avoidant, dependent, histrionic, paranoid, and borderline personalities both in the psychiatric medication treatment adherent and psychiatric medication non-adherent group ($p<0.05$). The number of patients who stopped their medication due to its side effects was significant higher in the psychiatric medication treatment non-adherent group than the psychiatric medication treatment adherent group ($p<0.05$). The age of onset of BD was significantly higher in the psychiatric medication treatment adherent group than in the psychiatric medication treatment non-adherent group ($p<0.05$).

**Conclusion:** This study showed that during the pharmacological management of BD type-1 patients with history of suicide attempt, dysfunctional beliefs specific to avoidant, dependent, histrionic, paranoid, and borderline personalities may be considered. This study highlights the importance of dysfunctional personality beliefs in type-1 BD patients.

**Keywords:** Bipolar disorder, suicide attempted, medication adherence, personality disorders, age of onset

### ÖZ

**Amaç:** Bu çalışma, kişiliğe özel işlevsel olmayan inançların, bipolar bozukluk (BB) tip-1 hastalarında intihar girişimi öyküsü ile ilişkili olarak psikiyatrik tedavi uyumuna etkisini belirlemeyi amaçlamaktadır. Ayrıca hastaların ilaç uyumlarına ilişkin klinik özellikleri de değerlendirilmiştir.

**Gereç ve Yöntem:** Hastalar, psikiyatrik tedavi uyumunu ve kişiliğe özel işlevsel olmayan inançları değerlendirmek için sırasıyla Morisky İlaç Uyum Ölçeği ve Kişilik İnanç Anketi-Kısa Formu kullanılarak tarandı (n=79).

**Bulgular:** Araştırmanın sonuçları, psikiyatrik ilaç tedavisine uyum sağlayan grubun 26 (%32,9) hastadan oluştuğunu, tedaviye uyumsuz grubun ise 53 (%67,1) hastadan oluştuğunu göstermektedir. İntihar girişimi öyküsü olan katılımcıların hem psikiyatrik ilaç tedavisine uyumlu hem de psikiyatrik ilaç tedavisine uyumlu olmayan grupta yüksek düzeyde kaçınmacı, bağımlı, histrionik, paranoid ve borderline kişiliğe özel işlevsel olmayan inançları vardı ($p<0,05$). İlaç tedavisini yan etki nedeniyle bırakan hasta sayısı, psikiyatrik ilaç tedavisine uyumsuz grupta, ilaç tedavisine uyumlu gruba göre

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anlamlı olarak daha yüksekti (p<0,05). BB'nin başlangıç yaşı, psikiyatrik ilaç tedavisine uyumlu grupta, psikiyatrik ilaç tedavisine uyumsuz gruba göre anlamlı olarak daha yüksekti (p<0,05). Birinci derece akrabalarda psikiyatrik bozukluk öyküsü, psikiyatrik ilaç tedavisine uyumsuz grupta, psikiyatrik ilaç tedavisine uyumlu gruba göre istatistiksel olarak daha yüksekti (p<0,05).

**Sonuç:** Bu çalışma, intihar girişimi öyküsü olan BB tip-1 hastalarının farmakolojik tedavisinde kaçınmacı, bağımlı, histriyonik, paranoid ve borderline kişiliklere özel işlevsel olmayan inançların dikkate alınabileceğini göstermiştir. Bu çalışma tip-1 BB hastalarındaki işlevsel olmayan kişilik inançlarının önemini vurgulamaktadır.

**Anahtar Kelimeler:** Bipolar bozukluk, intihar girişimi, ilaç uyumu, kişilik bozuklukları, başlangıç yaşı

## INTRODUCTION

Although bipolar disorder (BD) is an economically costly health service, effective treatment thereof remains to be one of the greatest problems of daily psychiatric practice. Success of medication is affected by factors such as patient's tolerance, suitableness of the regimen, and above all, adherence to the medication[1]. The World Health Organization (2014) defines medication non-adherence as "a case in which a person's behavior in taking medication does not correspond with agreed recommendations from health personnel". In a recent meta-analysis, medication non-adherence for schizophrenia, major depressive disorders, and BD were detected at 56%, 50%, and 44%, respectively[2]. Medication non-adherence was significantly related to more hospitalization, and suicide attempts in patients with BD in a prospective observational study[3]. Moreover, as shown in the same study, costs incurred by non-adherent patients were higher than those of adherent patients[3]. Medication non-adherence previously was found to be related to some factors such as experiencing side effects, insight problems, reluctance to use psychiatric medication, substance use history, and number of hospitalizations in patients with BD[4,5].

Suicide is a crucial public health problem. Every year, almost 800,000 people die by suicide worldwide[6]. Death by suicide has an enormous effect on individuals' mental health[7]. Suicide affects not only the family of the person who died via suicide but also a considerable group of people who have connections with the person who died via suicide. It was shown that each suicide affected 135 people who knew the person died via suicide[8]. Additionally, association between suicide and psychopathology is widely accepted[9].

BDs are usually comorbid with other psychiatric disorders, making BD management difficult[10]. Personality disorders are highly comorbid in patients with BD[11]. In a systematic review of euthymic BD patients, 41.2% had at least one comorbid personality disorder, and cluster B personality disorders were the most common cluster of personality disorders. Moreover, borderline personality disorder (10.1%) was detected as the most common subtype of personality disorder[12]. Personality disorders adversely influence suicide attempts, treatment outcomes, and psychosocial functioning in BD patients[13-17]. The association of personality disorders and suicide has been implicated previously. High mortality rates in personality disorders may be associated with suicide[18,19]. A recent meta-analysis has shown that the lifetime prevalence of suicide attempt is 33.9% in BD[20].

Personality disorders' effect is one of the substantial factors considering medication non-adherence. This effect may be one of the causes of the adverse clinical course in patients with BD[21]. Medication adherence problems in BD were previously associated with personality disorder comorbidity[22]. Additionally, borderline personality disorder was found to be a predictor of antipsychotic treatment non-adherence in BD in a follow-up study[23]. Besides this, the limited clinical research on this topic calls for further investigations to be done[24].

As stated by cognitive theory, a personality disorder is distinguished in the dysfunctional beliefs that characterize and sustain it[25]. Dysfunctional personality disorder beliefs supply explanations for dysfunctional attitudes to past and current experiences[26]. Exploring and modifying dysfunctional personality disorder beliefs are among the main goals of cognitive-behavioral psychotherapy of personality disorders. In the current study, dysfunctional personality disorder beliefs are considered a proxy for personality disorders, as the beliefs themselves are often what cause the difficulties of personality[27].

The present study primarily aimed to evaluate the effect of personality disorder beliefs on medication adherence of patients with BD type-1 (BD-1) in relation to their suicide attempt history. Additionally, to assess the clinical characteristics of the participants in relation to their medication adherence was aimed. To the authors' knowledge, the present study is the first study exploring the effects of personality disorder beliefs on medication adherence of patients with BD-1 in relation to their suicide attempt history.

## MATERIALS AND METHODS

The present cross-sectional study was performed with patients followed in Ataturk University Research and Training Hospital Clinic of Psychiatry Outpatient between June 2017 and February 2019. The sample included 79 patients with BD-1,

who met the following criteria: age of 18-65 years, a diagnosis of BD-1 established with SCID-I[28,29], and being euthymic in BD as defined by the Turkish version of the Hamilton rating scale for depression (HRSD) ≤7[30] and Young Mania rating scale ≤ 5[31] for at least eight weeks. Exclusion criteria were the presence of mental retardation, comorbid axis I, and a medical illness affecting patients' general health condition.

The necessary approval was obtained from the Atatürk University Faculty of Medicine Clinical Research Ethical Committee (decision no: 21, date: 09.06.2017). The study procedures were conducted in line with the Helsinki Declaration. Informed consent was procured from all participants before their participation in the study. A form was developed to obtain necessary socio-demographic and clinical data regarding the study's aims.

### Morisky Medication Adherence Scale

Participants' adherence to medication was evaluated using the MMAS, which was created by Morisky et al.[32-34]. The validity and reliability of the Turkish version of the scale have been evaluated[35]. The scale includes four items about patients' adherence to medication. Medication adherence is considered high if the patient answers "no" to all items. Medication adherence is moderate if the patient answers "yes" to one or two items. Medication adherence is poor if the patient answers "yes" to three or four items. In the present study, poor and moderate medication adherence patients have been grouped as medication non-adherent, and patients with high medication adherence as medication adherent[36].

### Personality Belief Questionnaire-Short Form

The PBQ-SF, developed by Butler et al.[37], is a self-rated tool created to evaluate dysfunctional beliefs associated with personality disorder. The PBQ-SF evaluates beliefs associated with ten personality disorders: paranoid, schizoid, antisocial, borderline, histrionic, narcissistic, avoidant, dependent, obsessive-compulsive, and passive-aggressive. In PBQ-SF, the respondents are asked to rate 65 statements on a 5-point scale ranging from 0= I do not believe it at all to 4= I believe it totally. Total scores of all the personality disorder beliefs included in the scale extend between 0 and 28. A high score indicates high levels of dysfunction. The PBQ-SF has a reliability and validity study in Turkey[38].

### Statistical Analysis

**Statistical Package for Social Sciences:** The Statistical Package for Social Sciences (SPSS) Statistics 20.0 Software was used for statistical analysis. The comparison of the medication adherent and medication non-adherent groups in terms of socio-demographic characteristics and clinical features was done through the Pearson chi-squared test and independent-sample t-test. The Pearson chi-squared test used nominal variables, while the independent sample t-test used continuous variables. Using the Kolmogorov-Smirnov test, the skewness and kurtosis values were examined to evaluate the normal distribution of data. Moreover, the patients' socio-demographic characteristics and clinical features were presented as mean ± standard deviation and percentages (%). In the present study, p values below 0.05 were determined to be statistically significant.

**Two-way Multivariate Analysis of Variance Test:** The MANOVA was used to determine whether the dysfunctional personality beliefs of patients with BD-1 differ according to medication adherence and history of suicide attempts. The test was conducted with the medication adherence and suicide attempt as independent variables, and the passive-aggressive, dependent, obsessive-compulsive, anti-social, narcissistic, histrionic, schizoid, paranoid, borderline, and avoidant personality beliefs scores as dependent variables. MANOVA is a parametric test and requires that some assumptions be met before analysis of the data. In this direction, all assumptions were checked before the test and were met.

## RESULTS

The mean age of patients was 34.49±12.37 years. Among the patients, 34 (43.0%) were female and 45 (57.0%) were male. Their mean number of years of education was 11.56 ± 3.77. Among the patients, 41(51.9%) had psychotic features and 13 (16.5%) had at least one suicide attempt in their medical history. The mean age of BD onset was 24.62±7.78 years.

Medication adherent and medication non-adherent groups were created based on the MMAS. The medication adherent group consisted of 26 (32.9%) patients, while the medication non-adherent group consisted of 53 (67.1%) patients. Table 1 displays a comparison of these two groups with respect to socio-demographic and clinical features and course of illness. In terms of years of education, the medication non-adherent group had significantly higher years of education than the medication adherent group (t=-2.26, p=0.02). In the medication adherent group, the age of BD onset was significantly higher than the medication non-adherent group (t= 2.13, p=0.04). No significant differences were detected regarding the other parameters in Table 1.

While 46.2% (n=12) of patients reported that they experienced drug side effects in the medication adherent group, 67.9 % (n=36) of patients in the non-adherent group reported the same. Statistically, no significant difference was detected between the two groups ($X^2$: 3.46, p=0.06). In terms of stopping medication due to side effects, the number of patients (n=10, 12.7%) was significantly higher in the medication non-

adherent group than the adherent group (n=0, 0.0%) ($X^2$: 5.61, p=0.02).

History of BD in the first-degree relatives was not statistically different between the two groups (n=1, 3.8% in the medication adherent group; n=9, 17.3% in the medication non-adherent group) ($X^2$: 2.81, p=0.15). However, history of psychiatric disorder in the first-degree relatives was statistically higher in the medication non-adherent group (n=25, 48.1%) than the adherent group (n=6, 23.1 %) ($X^2$: 4.52, p=0.03).

In all of the patients, the mean avoidant personality belief scores (14.20±5.93) were the highest, and the mean histrionic personality belief scores (6.96±5.61) were the lowest among all personality disorder belief scores (Figure 1). In both groups, the mean avoidant personality belief scores were higher in the adherent group (14.57±5.31) than in the non-adherent group (14.01±6.25), and the mean histrionic personality belief scores were lower in the adherent group (6.84±5.78) than in the non-adherent group (7.01±5.58) (Figure 2). In the group with history of suicide attempt, the mean avoidant personality belief scores (16.76±6.12) were the highest, and the narcissistic personality beliefs scores (9.07±7.15) were the lowest. In the group without history of suicide attempt, the mean histrionic personality disorder beliefs were the lowest (6.27±5.21), and the mean avoidant personality belief scores (13.69±5.81) were the highest (Figure 3). The means and standard deviation values of dysfunctional personality belief scores of the medication-adherent and medication non-adherent patients according to history of suicide attempt are presented in Table 2.

For the assessment of patients' dysfunctional personality disorder beliefs according to medication adherence and history of suicide attempt, MANOVA was performed. The MANOVA showed a significant multivariate main effect for medication adherence [$F_{(10.66)}$= 3.500, p=0.001, Wilks' Λ = 0.653]. However, there is no significant difference between the personality disorder beliefs in terms of medication adherence at the univariate level in MANOVA (Table 3). Additionally, there was a significant multivariate main effect for history of suicide attempt [$F_{(10.66)}$= 4,290, p= 0.000, Wilks' Λ = 0,606).

**Table 1. Socio-demographic and clinical features of the patients**

| Variable | Medication adherent (n=26) | Medication non-adherent (n=53) | Test | p value |
|---|---|---|---|---|
| Age, mean ± SD | 37.61±14.32 | 32.96±11.13 | t=4.05 | 0.15[b] |
| **Gender, n (%)** | | | | |
| Female | 10 (61.5) | 29 (54.7) | $X^2$: 2.1 | 0.56[a] |
| Male | 16 (38.5) | 24 (45.3) | | |
| **Marital status, n (%)** | | | | |
| Single | 11 (42.3) | 25 (47.2) | $X^2$: 0.17 | 0.91[a] |
| Married | 14 (53.8) | 26 (49.1) | | |
| Divorced | 1 (3.8) | 2 (3.8) | | |
| Years of education, mean ± SD | 10.23±3.72 | 12.22±3.65 | t=-2.26 | **0.02[b]** |
| Age at first onset, mean ± SD | 27.69±10.11 | 23.11±5.89 | t=2.13 | **0.04[b]** |
| Age at first hospitalization, mean ± SD | 28.33±9.56 | 24.16±5.48 | t=1.96 | 0.058[b] |
| Total episode number, mean ± SD | 2.96±1.88 | 7.22±19.39 | t=-1.11 | 0.26[b] |
| **Psychotic feature history, n (%)** | | | | |
| None | 10 (38.5) | 28 (52.8) | $X^2$: 1.44 | 0.23[a] |
| Yes | 16 (61.5) | 25 (47.2) | | |
| Number of hospitalization, mean ± SD | 2.11±1.42 | 1.71±1.68 | t=1.04 | 0.29[b] |
| Hamilton rating scale for depression-17 scores, mean ± SD | 2.15±1.51 | 2.18±2.07 | t=-0.07 | 0.94[b] |
| Young Mania rating scale scores, mean ± SD | 0.30±0.78 | 0.47±1.08 | t=-0.68 | 0.49[b] |
| **Suicide attempt, n (%)** | | | | |
| None | 22 (84.6) | 44 (83) | $X^2$: 0.03 | 1.00[a] |
| Yes | 4 (15.4) | 9 (17) | | |
| Number of suicide attempt, mean ± SD | 0.19±0.49 | 0.39±1.06 | t=-0.92 | 0.35[b] |
| History of psychiatric disorder in first degree relatives, n (%) | 25 (48.1) | 6 (23.1) | $X^2$: 4.52 | 0.03 |

Significant outcomes are reported in bold. [a]Pearson chi squared test, [b]Independent samples t-test. SD: Standard deviation.

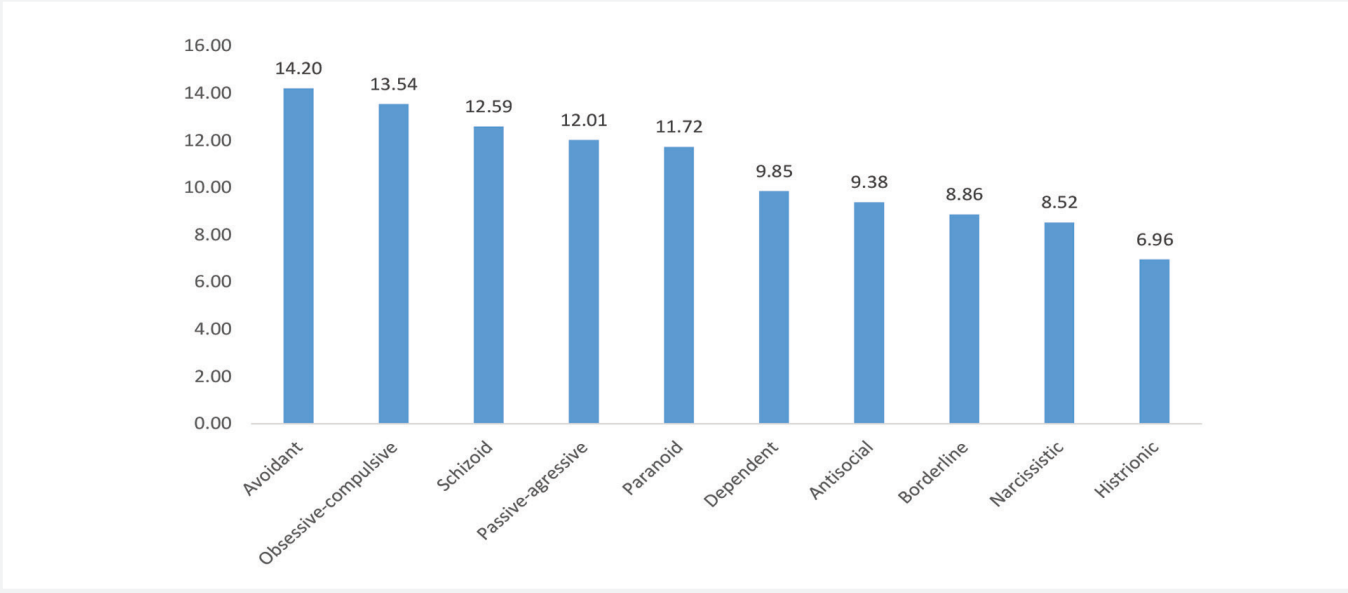


**Figure 1.** Mean scores of the all patients in subscales of the personality belief questionnaire-short form

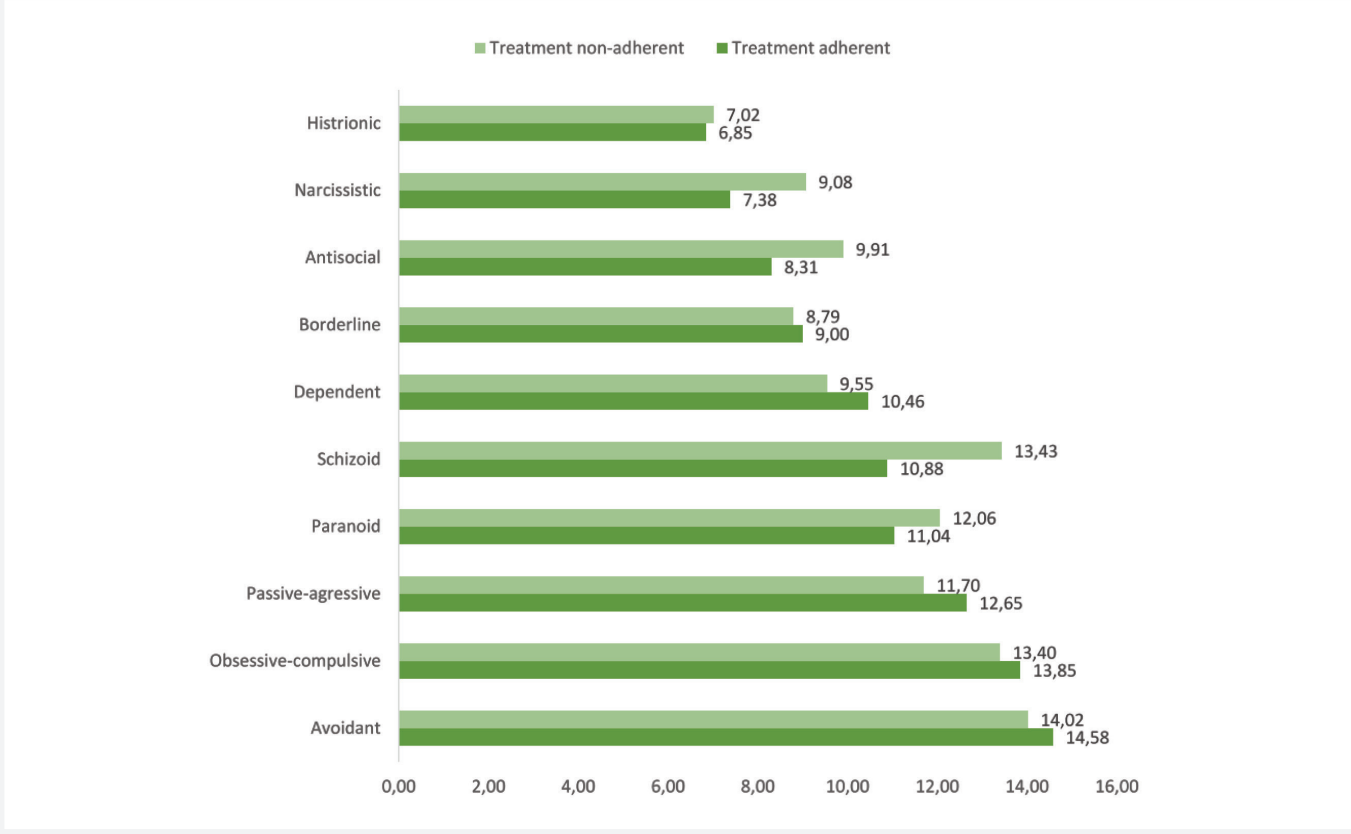


**Figure 2.** Mean scores of the patients according to medication adherence in sub scales of the personality belief questionnaire-short form

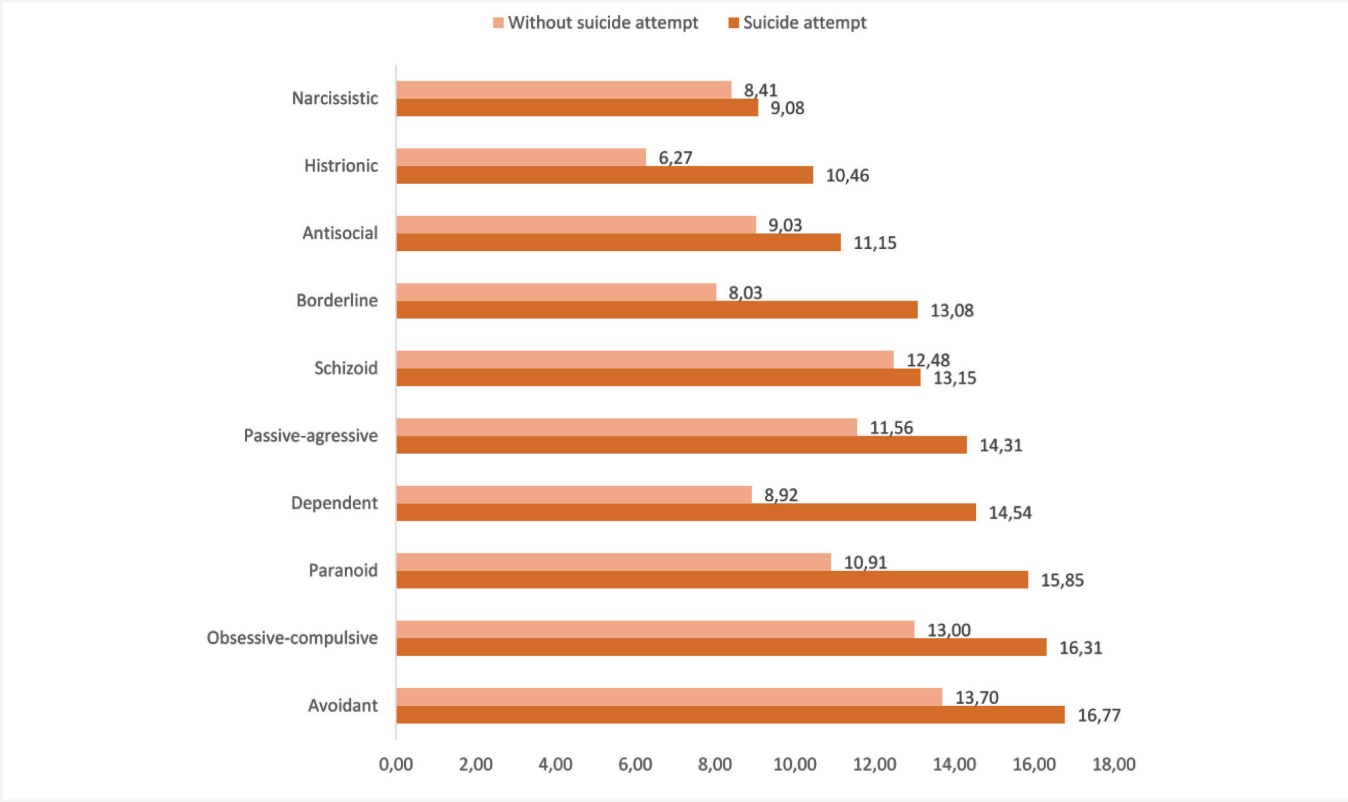


**Figure 3.** Mean scores of the patients according to suicide attempt in sub scales of the personality belief questionnaire-short form

**Table 2. Descriptive statistics of the personality belief questionnaire-short form scores of participants**

| Personality belief questionnaire-short form subscale | History of suicide attempt | Medication adherent | Medication non-adherent |
|---|---|---|---|
| **Avoidant** | Positive | 20.00±1.82 | 15.33±6.89 |
| | Negative | 13.59±5.15 | 13.75±6.17 |
| **Dependent** | Positive | 18.00±4.69 | 13.00±7.44 |
| | Negative | 9.09±5.97 | 8.84±5.76 |
| **Passive aggressive** | Positive | 17.25±3.77 | 13.00±6.76 |
| | Negative | 11.81±5.73 | 11.43±5.14 |
| **Obsessive compulsive** | Positive | 18.50±7.54 | 15.33±9.61 |
| | Negative | 13.00±7.58 | 13.00±6.99 |
| **Antisocial** | Positive | 10.25±8.18 | 11.55±8.15 |
| | Negative | 7.95±5.70 | 9.56±6.69 |
| **Narcissistic** | Positive | 6.00±1.82 | 10.44±8.29 |
| | Negative | 7.63±4.38 | 8.79±5.93 |
| **Histrionic** | Positive | 13.25±4.71 | 9.22±6.97 |
| | Negative | 5.68±5.24 | 6.56±5.23 |
| **Schizoid** | Positive | 10.50±8.10 | 14.33±7.10 |
| | Negative | 10.95±5.13 | 13.25±6.21 |
| **Paranoid** | Positive | 16.00±3.55 | 15.77±7.91 |
| | Negative | 10.13±6.72 | 11.29±6.56 |
| **Borderline** | Positive | 13.75±2.21 | 12.77±6.61 |
| | Negative | 8.13±5.47 | 7.97±5.77 |

Data presented as mean ± standard deviation.

**Table 3. Evaluation of PBQ-SF subscale scores according to medication adherence and history of suicide attempt with two-way multivariate analysis of variance test**

| Effect | PBQ-SF subscale | F | p value |
|---|---|---|---|
| **Main effect: medication adherence** | Avoidant | 1.373 | 0.245 |
| | Dependent | 1.792 | 0.185 |
| | Passive aggressive | 1.679 | 0.199 |
| | Obsessive compulsive | 0.415 | 0.521 |
| | Antisocial | 0.445 | 0.507 |
| | Narcissistic | 2.215 | 0.141 |
| | Histrionic | 0.779 | 0.380 |
| | Schizoid | 2.332 | 0.131 |
| | Paranoid | 0.046 | 0.831 |
| | Borderline | 0.092 | 0.762 |
| **Main effect: suicide attempt history** | Avoidant | 4.316 | **0.041** |
| | Dependent | 11.101 | **0.001** |
| | Passive aggressive | 3.827 | 0.054 |
| | Obsessive compulsive | 2.540 | 0.115 |
| | Antisocial | 0.959 | 0.331 |
| | Narcissistic | 0.000 | 0.997 |
| | Histrionic | 8.253 | **0.005** |
| | Schizoid | 0.025 | 0.876 |
| | Paranoid | 5.593 | **0.021** |
| | Borderline | 7.804 | **0.007** |
| **Main effect: medication adherence history of suicide attempt** | Avoidant | 1.574 | 0.214 |
| | Dependent | 1.467 | 0.230 |
| | Passive aggressive | 1.166 | 0.284 |
| | Obsessive compulsive | 0.415 | 0.521 |
| | Antisocial | 0.005 | 0.944 |
| | Narcissistic | 0.761 | 0.386 |
| | Histrionic | 1.907 | 0.171 |
| | Schizoid | 0.147 | 0.703 |
| | Paranoid | 0.100 | 0.753 |
| | Borderline | 0.048 | 0.828 |

Significant outcomes are reported in bold. PBQ-SF: Personality belief questionnaire-short form

As presented in Table 3, avoidant, dependent, histrionic, paranoid, and borderline personality disorder beliefs were differentiated in terms of history of suicide attempt at the univariate level in MANOVA. In light of these outcomes, patients with history of suicide attempt had significantly higher avoidant, dependent, histrionic, paranoid, and borderline personality disorder beliefs both in the medication adherent and medication non-adherent group ($p<0.05$).

## DISCUSSION

In this cross-sectional study, it was found that the medication non-adherence rate was 67.1, and suicide attempt history was 16.5% in patients with BD-1. Avoidant personality belief scores were the highest, and histrionic personality belief scores were the lowest among the participants in the present study. BD-1 patients with a history of suicide attempts showed higher avoidant, dependent, histrionic, paranoid, and borderline personality disorder beliefs both in the medication adherent and non-adherent groups.

In the present sample, the medication non-adherence rate was 67.1%. Literature shows that medication non-adherence rate in BD is roughly reported at 50%[39]. In a clinical trial involving eight countries, 57 % of patients with BD had problems with adherence to treatment[40]. The differences between the present sample's high non-adherence rate (67.1%) and the previous studies' results may be due to the varied sample characteristics and assessment tools used. Besides this, in a clinical trial carried

out in Turkey using the same evaluation tool for medication adherence as this clinical trial, a similar medication non-adherence rate (67.5%) was found in patients with BD-1[36].

In terms of personality pathology, scores of avoidant personality disorder beliefs were found to the highest among the whole sample in the current study. Previously, cluster B and C traits were detected to be more frequent than cluster A disorders in BD[41,42]. In remitted patients with BD, cluster B and C disorders were similarly more frequent than cluster A, and borderline personality disorder was the most frequent comorbid personality disorder[12]. In another meta-analysis that included studies on both outpatient and inpatients, obsessive-compulsive personality disorder ranked first when comparing the frequency of personality disorders[42].

In this sample, 16.5% of the participants have a history of suicide attempt. A history of suicide attempt was found at 40.8 % in another study with remitted BD-1 patients[43]. In a comprehensive report of 101 cases, it was found that 31.1% of BD patients attempted suicide at least once[44]. Additionally, in the same study, it was stated that small sample size and brief exposure times might overestimate suicidal risk in BD. Additionally, it is assumed that the lifetime prevalence of suicide attempts is associated with geographic region, and geographic differences may have influences on the outcomes[20].

In the current sample, personality disorder beliefs from clusters B and C (avoidant, dependent, histrionic, borderline), as well as paranoid personality disorder beliefs from cluster A, were significantly related to the history of suicide attempt. In a ten-year follow-up trial, borderline and narcissistic personality disorders were detected to be associated with suicide attempts[45]. Cluster B personality disorder comorbidity was related to suicide attempts in remitted BD-1 participants[43]. The relationship between cluster B personality disorders and suicide attempt is widely acknowledged; however, there are some differences in results among studies. Only cluster C personality disorders were significantly related to suicide attempts in a study that investigated both unipolar and BDs[46]. In another study, paranoid, dependent, narcissistic, borderline, and histrionic personality disorder beliefs were reported to be associated with suicide attempts[26]. This study used the same personality belief assessment tool as the present study. Considering the mentioned results, when assessing suicidal risk, the focus should not only be given to cluster B personality disorder traits, but also to cluster C and paranoid personality disorder traits.

High level of education is mostly considered to lead to better medication adherence outcomes in BD[47,48]. Contrarily, in a study with BD-1 patients receiving lithium treatment, no difference was found between medication adherent and non-adherent groups regarding years of education[49]. In the present study, the non-adherent group had higher levels of education than the adherent group. The inconsistency with the previous findings may be due to this study's sample characteristics. Different population characteristics and inadequate psychoeducation may have contributed to the present study's results regarding the association between education level and medication adherence. Nonetheless, the influence of education on medication adherence should be considered in future studies.

Regarding the course of BD, results show that the age of onset was significantly lower in the medication non-adherent group in the current study. Early-onset of BD was shown to be related to medication adherence only in some studies[50-52]. The course of the disorder, early-onset, and medication adherence may influence each other reciprocally. For deeper explanations, further studies are necessary.

Medication-related side effects are considered to be one of the important contributors to medication adherence[2]. On the contrary, previous reports state that clinicians pay less attention on the causality of the side effects on medication adherence than patients with BD[40]. Additionally, it was reported that side effects primarily concerning clinicians (e.g., hypothyroidism, diabetes insipidus, hypercalcemia)and patients with BD (e.g. weight gain, tremors, cognitive impairment, and sedation) are different[53]. In accordance with the previous findings, this study found that the adherent group's history of discontinuing medication due to side effects was significantly lower than the non-adherent group. Studies on medication-related side effects are crucial to understand this better and satisfy patients' needs. Hence, the impacts of side effects on patients with BD necessitate the generation of different perspectives in clinical applications.

A family history of BD was related to compliance with psychoeducation programs[54]. No significant influence of the family history of BD on medication adherence was found in different studies with patients with BD[49,52]. In this study, a significant relationship was detected between medication adherence and family history of psychiatric disorder, but not with a family history of BD. Interestingly, in a systematic review, a family history of BD was identified as a factor affecting medication adherence in pediatric BD patients[55]. Considering the above-mentioned outcomes regarding family history, a family history of BD or psychiatric disorder may have detrimental influences on the adherence of patients with BD.

## Study Limitations

The limitations of this clinical trial need to be emphasized. First, the recruitment of the participants from a tertiary hospital where many complex patients were enrolled may limit the generalizability of the results. Second, the MMAS used in the present trial to evaluate medication adherence is a self-report assessment tool. Third, the relatively small sample

size limits the results. Fourth, the pharmacological agents that the patients used were not assessed. Fifth, including only euthymic patients may limit the generalizability of the present study's results. A further study considering the present study's topics would be performed with BD-1 patients during their episodes. Sixth, only BD-1 patients were enrolled in the present study. The exclusion of other types of BD patients, such as patients with cyclothymia or BD type-2, may have generated limitations. Finally, comorbid psychiatric diagnoses such as substance use disorder, attention deficit hyperactivity disorder, and obsessive-compulsive disorder were excluded, which may have contributed to the generalizability problems of the present study.

## CONCLUSION

As far as we know, this clinical trial is the first to assess the influence of dysfunctional personality disorder beliefs on medication adherence of patients with BD-1 in relation to their previous suicide attempts. This study showed that patients with a history of suicide attempt exhibited higher avoidant, dependent, histrionic, paranoid, and borderline personality disorder beliefs both in the medication adherent and non-adherent groups. This study suggests that assessing avoidant, dependent, histrionic, paranoid, and borderline personality traits is crucial in patients with BD-1 and a history of suicide attempt. Additionally, medication-related side effects, early age of onset of BD-1, and family history of psychiatric disorder may influence the medication adherence of patients with BD-1. Longitudinal studies are necessary in order to comprehend the contributions of dysfunctional personality disorder beliefs on medication adherence in relation to a history of suicide attempt.

### Ethics

**Ethics Committee Approval:** Atatürk University Faculty of Medicine Clinical Research Ethical Committee (decision no: 21, date: 09.06.2017).

**Informed Consent:** Informed consent was procured from all participants before their participation in the study.

### Authorship Contributions

Concept: E.F.A., H.A.C., M.Ş., F.T.O., C.K., Design: E.F.A., H.A.C., M.Ş., F.T.O., C.K., Data Collection or Processing: E.F.A., F.T.O., C.K., Analysis or Interpretation: E.F.A., Literature Search: E.F.A., Writing: E.F.A., H.A.C., M.Ş., F.T.O., C.K.

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

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CASE REPORT

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# Video-Assisted Thoracoscopic Carinal Resection and Reconstruction in A Case of Primary Tracheal Tumor

## Primer Trakeal Tümör Olgusunda Video Yardımlı Torakoskopik Cerrahi ile Karinal Rezeksiyon ve Rekonstrüksiyonu

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**ABSTRACT**

Tracheal tumors located in the carina are quite rare. Resection and reconstruction of carinal tumors are technically more challenging due to their locations. The most commonly preferred method is right posterolateral thoracotomy. In this study, a case of carinal resection and reconstruction performed using video-assisted thoracoscopy is presented. According to our knowledge, this case is the first video thoracoscopic carina resection and reconstruction performed in Turkey.

**Keywords:** Video-assisted thoracoscopic surgery, carinal resection, carinoplasty

**ÖZ**

Karina yerleşimli primer trakeal tümörler oldukça nadir görülür. Yerleşim yeri itibariyle karinal tümörlerin rezeksiyonu ve rekonstrüksiyonu teknik açıdan çok daha zordur. Sıklıkla tercih edilen yaklaşım yöntemi, sağ posterolateral torakotomidir. Çalışmamızda, video yardımlı torakoskopik karina rezeksiyon ve rekonstrüksiyonu yapılan olgu sunulmuştur. Sunulan olgu Türkiye'de gerçekleştirilen ilk başarılı Video yardımlı torakoskopik cerrahi ile karina rezeksiyonu ve rekonstrüksiyonudur.

**Anahtar Kelimeler:** Video yardımlı torakoskopik cerrahi, karinal rezeksiyon, karinoplasti

## Introduction

Carinal resection is usually performed concurrently with lung parenchymal resection when primary lung cancer invades the carina. Isolated carinal resection is defined as the resection of only the tracheal bifurcation without lung parenchymal resection and is indicated in cases of primary tracheal tumors located in the carina. It was first described by Abbott et al. [1] in 1950. Subsequently, Barclay et al.[2] described the technique of end-to-end anastomosis of the right main bronchus to the trachea and end-to-side anastomosis of the left main bronchus to the trachea in carinal resections. In the following years, the Double-barrel[3] and Miyamoto[4] and Yamamoto[5] described techniques in which the left main bronchus was anastomosed end-to-end to the trachea, and the right main bronchus or intermediate bronchus was anastomosed end-to-side to this anastomotic line. This technique has been reported to have advantages such as not requiring a new anastomotic hole that would disrupt tracheal blood flow, leaving neocarina open as a result, distributing tension in three different directions along the anastomotic line to minimize tension[5]. The authors also believe that this method is safer. Therefore, this anastomotic technique was chosen in this study.

Carinal resections are technically challenging operations and are more challenging to perform with video-assisted thoracoscopic surgery (VATS). The authors would like to present this case, which is the first in Turkey to their knowledge. The permission for the study was obtained from the approval

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was obtained from the Non-Interventional Clinical Research Ethics Committee of Ethics Committee of Tekirdağ Dr. İsmail Fehmi Cumalığlu City Hospital (approval number: 97, date: 19.04.2024). The informed consent form was obtained from the patient.

## CASE REPORT

A 66-year-old male patient presented to our clinic with a complaint of cough like hemoptysis that started two months ago. He had a smoking history of 70 pack-years. A 1 cm lesion was observed on the carina in the chest computed tomography scan (Figure 1A). The positron emission tomography assessment reported the lesion's maximum standardized uptake value as 1.8. Fiberoptic bronchoscopy examination revealed a mass on the carina (Figure 1B), and a punch biopsy was performed. Pathological examination of the lesion reported squamous cell carcinoma.

After achieving single-lung ventilation with single lumen intubation by using a bronchial blocker on the right side, in the left lateral decubitus position, a 2 cm camera port incision was made into the thorax at the 7$^{th}$ intercostal space (ICS) along the midaxillary line. Subsequently, a 3 cm utility incision was made from thea 4th ICS along the midaxillary line towards the anterior axillary line, and a wound retractor was inserted. In the case where carinal resection/reconstruction was planned, the azygos vein was initially sacrificed and divided with an endovascular stapler. To ensure proper exposure, azygos vein stumps were secured posteriorly to the chest wall and anteriorly to the mediastinal pleura. After being suspended with tape by turning the trachea and right main bronchus, the left main bronchus was exposed through blunt and sharp dissections (Figure 2A).

The endobronchial blocker was deployed, and the endotracheal tube was pulled back from the trachea to the proximal carina. The right main bronchus was cut with a scalpel distal to the carina. Two 2/0 absorbable sutures were placed for traction purposes at the distal end of the left main bronchus, and it was cut distal to the carina (Figure 2B). At this stage, a no. 5 spiral endotracheal tube was inserted through the utility incision, intubating the left main bronchus (Figure 2C). The endotracheal tube was then connected to the newly established external circuit. Finally, the trachea was cut with a scalpel at the level of the carina. The specimen was removed from the chest.

After the surgical margins were reported as tumor-free with pathological examination of the frozen section, the anastomosis was initiated. At this stage, the patient's head was abducted to reduce tension at the anastomotic site. The left main bronchus was anastomosed to the trachea using a continuous end-to-end technique with 3/0 Prolene sutures (Figure 2D). During the anastomosis, the endotracheal tube in the left main bronchus was intermittently removed, and suturing was completed. Before completing the anastomosis, approximately a 1 cm gap was left for the anastomosis of the right main bronchus. At this stage, the endotracheal tube in the trachea was pushed distally, achieving intubation of the left main bronchus, and the external circuit was secured. Subsequently, to calibrate the gap left for the right main bronchus, a piece of cartilaginous ring was excised from the trachea using a scalpel. The right main bronchus was sutured to the trachea-anastomosis line of the left main bronchus in an end-to-side fashion using continuous technique with 3/0 prolene. The cuff of the endotracheal tube in the left main bronchus was lowered, and leakage control in the suture lines was performed with a small amount of physiological saline spilled into the thoracic cavity. To reduce tension on the anastomotic site, no additional release maneuvers were required, except for the liberation of the inferior ligament.

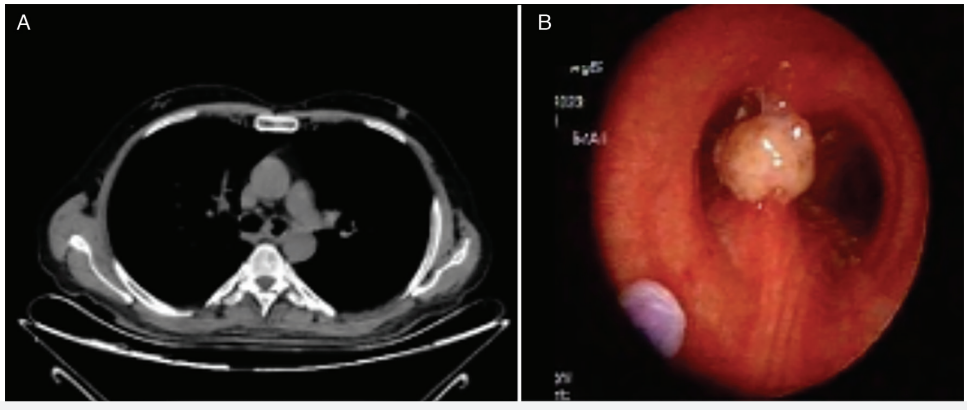


**Figure 1. A)** The tumor was observed on the level of carina in computed tomography. **B)** A mass on the carina was observed with fiberoptic bronchoscopy

Finally, tissue adhesive was applied to the anastomotic line, and the ends of the azygos vein were sutured together to cover the anastomotic area. No additional support tissue was brought to the anastomotic site. Bilateral lung expansion was observed, and there was no leakage at the anastomotic site. Neocarina was visualized with fiberoptic bronchoscopy examination before extubating. After placing the patient in the supine position, the patient's chin was sutured to the manubrium sterni with 2 0/0 silk sutures to prevent hyperextension. The patient was extubated in the operating room. Head flexion was maintained during the first week. Chin sutures were removed at the end of the first week, but neck extension was restricted for one more week. The patient, whose right chest tube was removed on the 2nd day of the operation, was discharged on the 7th day of the operation. The final pathology result reported negative surgical margins for squamous cell carcinoma. The patient is living a healthy life at the postoperative 6th month.

## DISCUSSION

Carinal resections are technically challenging operations. The preferred approach has long been the right posterolateral incision and, less frequently, median sternotomy[6]. The first carinal resection with VATS was performed by Nakanishi in 2013[7]. In the following years, cases with a "larger case series". have been reported[8]. With the development of the uniportal approach, Diego et al. [9] first described uniportal approach in carinal resection in 2016.

Squamous cell carcinoma and adenoid cystic carcinoma are the most common primary tumors of the trachea and are the main indicators of carinal resection. Provided that the disease is not metastatic and resection is completed, surgical treatment is significantly superior to palliative treatment in terms of survival. When preoperative intubation cannot be conducted due to an obstructing lesion at the carina level, the lesion can either be removed with rigid bronchoscopy and an endotracheal tube can be placed, or veno-venous bypass can be initiated. In studies, it is recommended to perform VATS sleeve after 20 sleeve with open surgery and 200 lobectomy with VATS[10].

Centrally located non-small-cell lung cancers encompassing the carina or distal trachea is relatively rare and regardless of tumor size, classified as locally advanced T4 stage disease. Such patients are associated with a poor prognosis. The difficulty of tracheal anastomosis requires high surgical skills; therefore, this method can be performed by experienced thoracic surgeons in

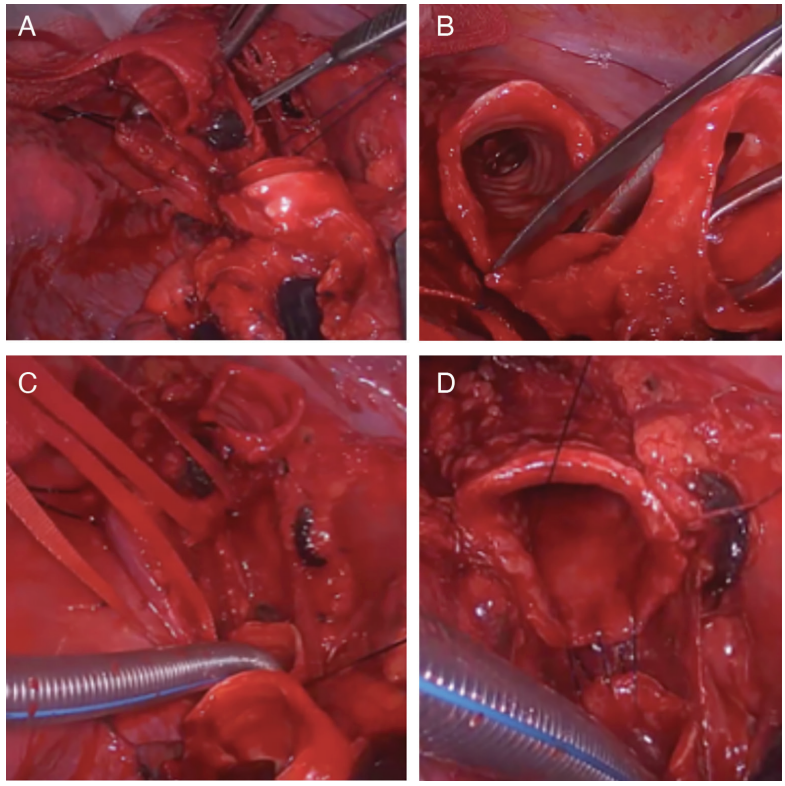


**Figure 2. A)** After the right main bronchus was cut, the left main bronchus was released and cut. **B)** Cutting the trachea above the level of the carina. **C)** Ventilation of the left main bronchus with an endo-tracheal tube. **D)** Starting an anastomosis between the left bronchus and the trachea

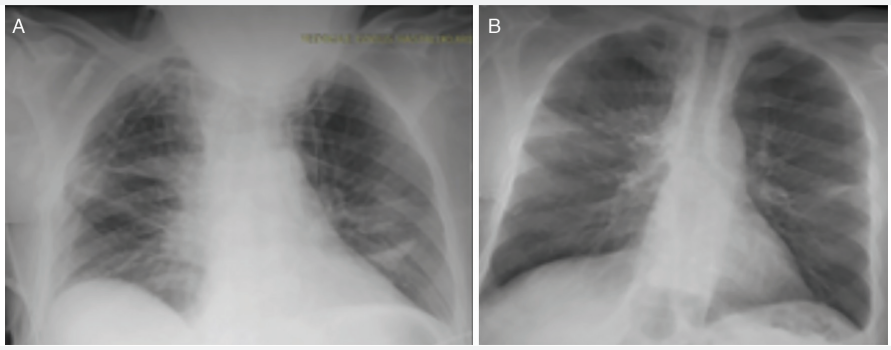


**Figure 3. A)** Chest radiography on the first day after surgery. **B)** Chest radiography on the second week after surgery

a few centers. In the study conducted by Pan et al. in 2023, the mortality rate was 10.81%. Mortality rates ranging from 8.3% to 20% have been reported in the literature[11].

In this case, a biportal approach was chosen. In VATS carinal resections, high-frequency jet ventilation with the patient intubated is a frequently preferred approach to facilitate surgery during anastomosis. However, due to the absence of this equipment in the hospital, during the operation, the end-blocker-assisted intubation technique was used.

## CONCLUSION

As a result of the increasing use of VATS and its adaptation to extended resections, the use of VATS in extended resections has become widespread. It was observed that surgical trauma was less and postoperative recovery was faster with VATS. Considering these reasons, VATS can also be performed in extended surgical procedures in clinics with high thoracoscopy experience.

### Ethics

**Ethics Committee Approval:** The approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Ethics Committee of Tekirdağ Dr. İsmail Fehmi Cumalıoğlu City Hospital (approval number: 97, date: 19.04.2024).

**Informed Consent:** The informed consent form was obtained from the patient.

### Authorship Contributions

Surgical and Medical Practices: M.M., Concept: M.Ü., A.Ç., Design: V.E., M.Ü., D.K., Data Collection or Processing: D.K., Analysis or Interpretation: M.Ü., Literature Search: V.E., Writing: M.Ü.

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

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CASE REPORT

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# Pembrolizumab Associated Autoimmune Diabetes Mellitus: Case Series

## Pembrolizumab İlişkili Otoimmun Diabetes Mellitus: Olgu Serisi

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**ABSTRACT**

The use of immune checkpoint inhibitors is increasing every day. With such frequent use, the side effects and management associated with these treatments are becoming difficult. One of rare side effects is autoimmune diabetes mellitus. In this review, we will discuss two cases of pembrolizumab-associated diabetes mellitus treated in our clinic and those reported in the literature. Most of the reported cases presented with diabetic ketoacidosis and they were on insulin therapy. The relationship between diabetes and immune checkpoint inhibitors needs to be clarified.

**Keywords:** Autoimmune diabetes, intensive insulin therapy, immunotherapy-related adverse events, cancer immunotherapy, pembrolizumab induced diabetes mellitus

**ÖZ**

İmmün kontrol noktası inhibitörlerinin kullanımı gün geçtikçe artmaktadır. Bu sık kullanım göz önüne alındığında bu tedavilere bağlı yan etkiler ve yönetimi zorlaşmaktadır. Nadir görülen bir tanesi otoimmün diyabettir. Bu derlemede kliniğimizde tedavi edilen pembrolizumab ilişkili iki diyabet olgusundan ve literatürde bildirilen olgulardan bahsedeceğiz. Bildirilen olguların çoğu diyabetik ketoasidoz ile başvurmuştur ve insülin tedavisine ihtiyaç göstermektedir. Diyabet ile immün kontrol noktası inhibitörleri arasındaki ilişkinin açıklığa kavuşturulmasına ihtiyaç vardır.

**Anahtar Kelimeler:** Otoimmün diyabet, intensif insülin tedavisi, immünoterapi ilişkili advers olaylar, kanser immünoterapisi, pembrolizumabın indüklediği diyabetes mellitus

## INTRODUCTION

Pembrolizumab and other checkpoint inhibitors are increasingly being used to treat various cancers. Pembrolizumab, a programmed cell death 1 (PD-1) inhibitor, has the ability to overstimulate the immune system, leading to the activation of a large number of hematopoietic cells, macrophages, dendritic cells, and pancreatic cells that express its ligand[1,2]. As such, it may interfere with cell function in other non-cancer tissues during treatment. This results in a collection of immune-related adverse events (irAEs), the most common of which is the hypofunction of the pituitary, thyroid and adrenal glands.

Type 1 diabetes mellitus (T1DM) is an autoimmune disease due to absolute insulin deficiency related to islet β cell death[3-5]. PD-1 inhibitors may indirectly damage islet β cells through excessive activation of the autoimmune system, leading to the development of T1DM.

Among relatively common autoimmune adverse events associated with pembrolizumab, including pneumonia, colitis, hepatitis, hypophysitis, hyperthyroidism, hypothyroidism and nephritis, diabetes was rarely observed in only 0.1-1.4% of patients in clinical trials. In the few case reports of checkpoint inhibitor associated T1DM, no correlation between the number

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of administered cycles and the development of diabetes was outlined.

In this case report, we present two patients with triple-negative breast cancer who developed checkpoint inhibitor associated autoimmune diabetes after receiving (neo) adjuvant pembrolizumab therapy. Written consent was obtained from the patients for the presentation of the cases.

## CASE REPORT

The patient was a 25-year-old premenopausal female with a history of thalassemia minor, who presented with a right breast mass measuring 4 cm on breast MRG. A tru-cut biopsy was performed of the right upper quadrant mass, revealing metaplastic breast carcinoma and ductal carcinoma in situ.

The ki-67 was 50%; estrogen-receptor, progesterone-receptor and Cerbb2 were negative in immunohistochemistry staining. A staging positron emission tomography/computed tomography (PET/CT) scan did not detect any evidence of distant metastases. No evidence of axillary lymph node involvement was noted. Neoadjuvant chemotherapy consisting of 4 cycles of adriamycin plus cyclophosphamide (AC) followed by weekly carboplatin plus paclitaxel for a total of 12 weeks simultaneously with pembrolizumab administered every 21 days was initiated.

Following neoadjuvant chemotherapy, the patient underwent bilateral subcutaneous mastectomy, right sentinel lymph node sampling, and immediate breast reconstruction. The postoperative pathology showed a complete pathological response. The removed 2 sentinel lymph nodes were benign as well. Adjuvant treatment with pembrolizumab was continued for a total of 17 cycles. Due to worsening fatigue at 11 months after surgery, an adrenocorticotropic hormone stress test was performed, which was consistent with adrenal insufficiency. Treatment with hydrocortisone was started with near resolution of symptoms. Over a course of 3 months, hydrocortisone was tapered and eventually discontinued.

Approximately 3 months after the discontinuation of hydrocortisone treatment and 8 months after the end of adjuvant pembrolizumab, the patient presented with nausea. A random plasma glucose was found to be 500 mg/dL (baseline fasting plasma glucose at diagnosis was 84 mg/dL); There was no ketone in the urine and no acidosis in the arterial blood gas. Anti-GAD and anti-insulin antibodies requested to identify the etiology of diabetes were negative. The C-peptide level was found to be low (HbA1c value 11.8% and C-peptide value 0.2 ng/mL). HLA haplotypes, which have been shown to be closely associated with T1DM, requested from the tissue type laboratory were negative. After consultation with the endocrinology clinic, the diagnosis of classical T1DM was ruled out because of the patient's advanced age at onset of diabetes, negative autoantibodies, absence of HLA haplotypes and the reported development of autoimmune diabetes after the end of immunotherapies in the literature. Diagnosis of checkpoint inhibitor related autoimmune T1DM was made and intensive insulin treatment was started. Education on diabetes, diabetic nutrition and lifestyle was given and blood sugar levels were closely monitored. After 2 years of follow-up, the patient still remains on insulin therapy.

The second patient was a 56-year-old postmenopausal female who underwent further evaluation and testing after detecting a 2.8 cm mass in the right breast. Tru-cut biopsy was found to be compatible with triple negative invasive ductal carcinoma. PET/CT revealed no metastases to distant organs. Axillary imaging performed at the time of diagnosis was negative. The patient was treated with pembrolizumab every 21 days in combination with neoadjuvant four cycles of AC followed by weekly carboplatin plus paclitaxel for 12 weeks, per Keynote 522 study. After neoadjuvant treatment, the patient underwent sentinel axillary lymph node biopsy and partial mastectomy. Pathology revealed a 2 mm in situ ductal carcinoma, but no viable invasive tumor was found. There was no evidence of metastasis in the sentinel lymph node specimens.

At the postoperative month 3, while pembrolizumab treatment was ongoing, fasting blood sugar was detected as 330 mg/dL (pre-treatment fasting plasma glucose was 97 mg/dL) and HbA1c was 7.7%. Autoantibody tests performed after referral to the endocrinology clinic revealed that anti-GAD antibodies were 0.70 IU/mL (less than 10 IU/mL) and anti-insulin antibodies were 2.30 U/mL (less than 10 IU/mL). The C-peptide was 0.31 ng/mL (normal 0.9-1.8 ng/mL). The patient was started on intensive insulin therapy and put on a diabetic diet after being diagnosed with checkpoint inhibitor related T1DM. Since blood sugar regulation was not accomplished with intermittent insulin therapy, an insulin pump was installed, which improved blood sugar management. The patient completed 1-year course of pembrolizumab and remains on insulin therapy. She has been disease-free for four years.

## DISCUSSION

T1DM caused by PD-1 inhibitors is extremely rare. Diabetic ketoacidosis (DKA) is the most frequent form of presentation in most cases reported in the literature[6,7]. Although T1DM in general is not as common among irAEs, PD-1 inhibitor-associated T1DM can rapidly worsen to the point that patients may not survive if not identified and treated in a timely manner[8]. Physicians should inform patients receiving checkpoint inhibitors about the possibility of treatment induced diabetes and educate them about the related clinical symptoms. Nivolumab, another immune checkpoint inhibitor, has been

**Table 1. Comparison-of-T1DM-and-IO-related-DM**

| | IO related autoimmune DM | Type 1 DM |
|---|---|---|
| Risk factors | *Anti PD-1/PDL-1 checkpoint inhibitors | *Unclear |
| Presentation | *More frequent DKA | *DKA mostly in childhood |
| Clinical course | *Median 12 weeks after IO treatment,<br>*C-peptide mostly low. | *Honeymooning |
| Autoantibodies | *Generally negative (60%) | *Present in 90% |
| Genetik | *Rare | *Almost 90% haplotypes |
| Exocrine pancreatic involvement | *Amylase lipase simultaneously high | *Reduced pancreatic volumes but enzymes generally normal |

T1DM: Type 1 diabetes mellitus IO: Immunotherapy, PD-1: Programmed cell death 1, PDL-1 programmed cell death ligand, DKA: Diabetic ketoacidosis, DM: Diabetes mellitus

associated with a higher risk of T1DM in studies[9]. The primary pathogenesis-related processes proposed are: t cell activation and proliferation-induced damage to pancreatic beta islet cells; increased autoantibodies to insulin; predisposition due to HLA genotype; and an increase in proinflammatory cytokines.

Studies show that the incidence of autoimmune diabetes increases with combination immunotherapy (anti-PD-1, anti-CTLA-4)[10]. There have been reports of cases of diabetes mellitus (DM) with a fulminant course and DKA presentation, particularly in combination treatment settings[11]. By reviewing previous case reports[12,13], the features of diabetes associated with PD-1 inhibitors can be summarized as follows: late-onset diabetes [Based on the literature reviewed, the time from administration of immunotherapy (IO) to hyperglycemia ranged from 5 days to 23 months], rapid islet cell destruction, and initially low C-peptide levels. In cases reported in the literature, frequent complaints include polydipsia, polyuria, nausea, vomiting, dizziness, fatigue, upset stomach, diarrhea, progression to coma and other diseases of the endocrine glands. Table 1 summarizes the significant clinical differences between patients with IO-related DM and patients with conventional T1DM.

Our results validate previous correlations in case series, showing that 50% of patients will manifest within 12 weeks of starting IO treatment and that IO-associated DM is almost exclusively linked to anti-PD1 and anti-PD-L1 medication. Both of our patients had no family history of DM and had no additional risk factors that might predispose to diabetes mellitus. Although concomitant elevated amylase lipase levels have been reported in the literature, the amylase lipase levels of our patients were found to be within the normal range. With a high incidence of DKA and a rapid decrease in the synthesis of endogenous insulin, the disease not only manifests itself more quickly but also more fulminantly. In contrast, in general, no precipitating factor is identified in classic T1DM, and the disease typically has a much slower onset, with a lower prevalence of DKA and autoantibodies occurring years before the onset of clinical symptoms[14,15]. The exact criteria that put pembrolizumab users at risk for autoimmune diabetes remain unknown in today's setting.

## CONCLUSION

Although IO related T1DM is uncommon, it can have fatal consequences. Therefore, it is important to educate patients and their families about the warning signs and symptoms of DKA and hyperglycemia. Because of the rapid loss of pancreatic β-cell function and the significant risk of DKA, clinical oncologists and endocrinologists should be aware of this condition and offer appropriate treatment. In addition, further studies are needed to determine the precise mechanism of underlying IO-related T1DM. It would also be useful to investigate novel strategies or islet cell and insulin nanoformulations for the treatment of diabetes. To best monitor and manage this rare adverse event, it is necessary to identify possible predisposing variables and update existing guidelines.

### Ethics

**Informed Consent:** Written informed consent was obtained from the patient to present this case.

### Authorship Contributions

Surgical and Medical Practices: O.Ö., A.G., E.G., Concept: O.Ö., A.G., E.G., Design: O.Ö., A.G., E.G., Data Collection or Processing: O.Ö., A.G., E.G., Analysis or Interpretation: O.Ö., A.G., E.G., Literature Search: O.Ö., A.G., E.G., Writing: O.Ö., A.G., E.G.

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

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