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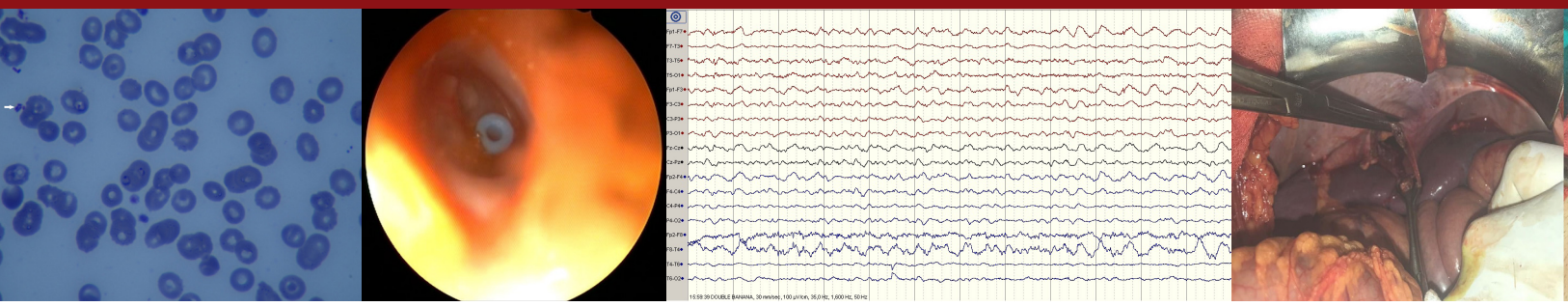

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## Contents

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# CYPRUS JOURNAL OF MEDICAL SCIENCES

CYPRUS JOURNAL OF MEDICAL SCIENCES

Original Article

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# The Efficacy of Endoscopic Ventilation Tube Insertion in Pediatric Populations

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**BACKGROUND/AIMS**
The aim of the present study was to evaluate the effectiveness of endoscopic ventilation tube insertion procedure among pediatric populations.

**MATERIAL and METHODS**
This was an intervention study with a ventilation tube inserted in patients between May 2016 and August 2018. All patients aged <18 years were included except those with syndromic diseases and chronic suppurative otitis media. Ventilation tube insertion was performed with endoscopic and microscopic techniques.

**RESULTS**
A total of 790 ventilation tube insertions were performed in 395 patients. Of the 395 patients, 200 were treated with microscopic interventions, and 195 had endoscopic surgery. The mean operation times were 5.31±2.11 min for endoscopic intervention and 9.05±3.53 min for microscopic intervention. The complication rate was 12 (6.15%) out of 195 patients at the endoscopic group (8 of them had a perforation of the tympanic membrane and 4 of them displayed granulation tissue). In the microscopic groups, 15 (7.5%) complications were seen from 200 patients (all 15 patients had tympanic membrane perforation).

**CONCLUSION**
The endoscopic ear ventilation tube insertion procedure reduces operation times. In addition, the complication rate of this technique appears to not significantly differ from the microscopic technique.

**Keywords:** Endoscopic, microscopic, ventilation tube insertion

## INTRODUCTION

The existence of serous or mucous fluid in the middle ear cavity that persists >3 months is defined as chronic serous otitis media (CSOM) (1). A minimum 3-month follow-up period is recommended to prevent unnecessary surgical processes (2). The insertion of the ventilation tube is one of the most frequent ongoing surgical applications at otorhinolaryngology clinics to diagnose CSOM (3).

Two distinct techniques were identified for this surgical intervention. The microscopic technique was first described by Armstrong in the early 1950's (4). Then, in the early 2000's, endoscopic technique studies started to appear (5, 6). The aim of the present study was to show the difference in operation time and complication rates of these two different techniques when compared against each other.

## MATERIAL and METHODS

A total of 395 patients who underwent ventilation tube insertion at the University of Gaziantep Otorhinolaryngology Department between May 2016 and August 2018 were included in the study. Only patients who were aged <18 years with a diagnosis of CSOM were included. All the patients underwent a complete ear, nose, and throat examination and tympanogram tests in at least three separate occasions in the 3-month follow-up period. Only patients who displayed type B tympanogram and any signs of serous fluid during their ear examination during the 3-month follow-up period were diagnosed with CSOM, and the indication of an operation was accepted. Patients with chronic suppurative otitis media

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or any syndromic diseases (Down syndrome and Treacher Collins syndrome) that could cause hearing loss and craniofacial anomaly that result in the deformity of the external ear canal or non-recurrent acute otitis media were excluded from the study.

**Operational Procedure**

Of the 395 patients, 200 were treated with microscopic interventions, and 195 had endoscopic surgery. All operations were performed under general anesthesia. All patients' heads were positioned with an anesthesiologist on both sides with attention toward the endotracheal tube. The sterilization of the surgical side was completed with an antiseptic solution that clears the auricula and surgical drapes. The patients were separated into two groups according to the types of surgical intervention. The operation time was calculated, in both groups, from the start of the myringotomy to the completion of the ventilation tube insertion.

*Endoscopic group*: A 3 mm, 0°, 18 cm rigid endoscope (Storze, Berlin, Germany) was connected to a camera and display screen. Then, it was introduced into the external ear canal, avoiding any skin in the ear canal. An anti-fog solution (liquid soap) was used to maintain clear vision. An anteroinferior or anterosuperior portion of the tympanic membrane was incised by a myringotomy knife. Thereafter, an adequate size suction probe was introduced toward the incision, and the glue material in the middle ear was suctioned. Finally, a ventilation tube was inserted on the tympanic membrane with an aid of needle or alligator forceps. The same procedure was applied to the side of the other tympanic membrane (Figure 1).

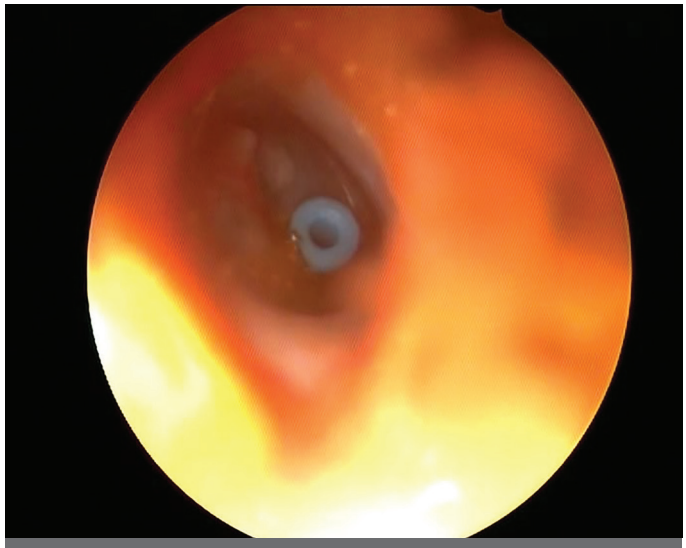

**FIGURE 1.** Endoscopic image of ventilation tube

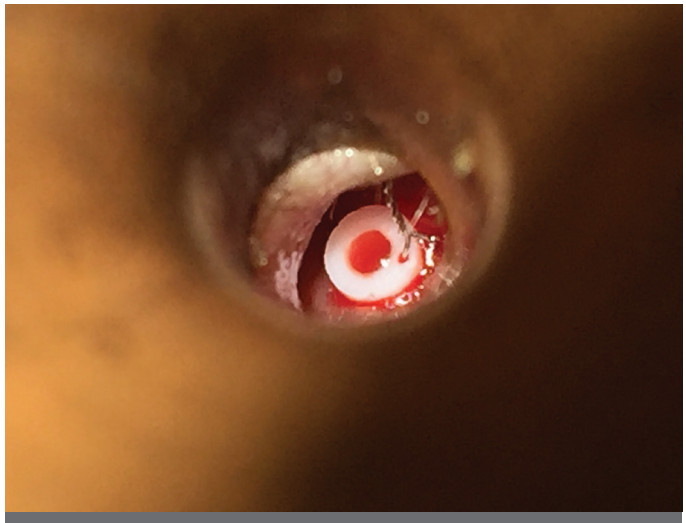

**FIGURE 2.** Microscopic image of ventilation tube

*Microscopic group*: A traditional microscope (Leica M525; Zurich, Switzerland) was used to visualize the tympanic membrane with the aid of an ear speculum. A meticulous approach was needed to ensure that the external ear skin was not touched otherwise this could result in a hemorrhage that obscures vision. Additionally, the head must be tilted with the aid of anesthesia for the adequate surgical vision of the anteroinferior or anterosuperior portion of the tympanic membrane. After these preparations, the surgical steps of microscopic ventilation tube insertion were applied in both tympanic membranes with the same methods that were described in the endoscopic group (Figure 2).

All patients in both groups were evaluated a week after the surgery and then monthly until tube extrusion. The study was approved by the ethics committee of the University of Gaziantep. Informed consents were obtained from all participants in the study. The SPSS 21 (IBM, USA) software program was used for statistical analysis. Student's t-test was used to identify the statistical difference between surgical procedure times. An odd ratio was obtained to determine if there is any increased risk for complications between endoscopic and microscopic surgical techniques.

**RESULTS**

A total of 395 patients had ventilation tube insertions applied in both ears (790 ears) with 195 patients undergoing the endoscopic method (390 ears) and 200 undergoing the microscopic method (400 ears). Of the 395 patients, 165 (41.8%) were female, and 230 (58.2%) were male. The total mean age of the patients was 5.85±3.51 (min 1–max 17) years.

The patients were divided into two groups according to the type of surgical intervention. The average times of the surgery were 5.31±2.11 (min 3–max 12) min in the endoscopic group and 9.05±3.53 (min 5–max 20) min in the microscopic group. There was a statistically significant difference between these two methods of ventilation tube insertion ($p<0.001$) as shown in Table 1.

The complication rate was 12 (6.15%) out of 195 patients (8 of them had perforation of the tympanic membrane and 4 of them displayed granulation tissue) in the endoscopic method. There were 15 (7.5%) complications from a total of 200 patients. All 15 patients had tympanic membrane perforation in the microscopic method. The odds ratio was found to be 0.809 between these two different techniques, and overall it was 1.015. There was no additional significant risk between techniques that will lead to a complication.

These 395 patients were then divided into different groups as categorized by age: <5 years and >5 years. Descriptive findings in both groups are displayed in Table 2.

**TABLE I.** Values of ventilation tube insertion according to different procedures

| Operation time | Endoscopic technique | Microscopic technique | p |
|---|---|---|---|
| Min-Max | 3.0-12.0 | 5.0-20.0 | 0.001 |
| Mean± Std. Deviation | 5.31 ±2.11 | 9.05±3.53 | |
| Median | 5.0 | 8.0 | |

**TABLE 2.** Comparison of endoscopic and microscopic techniques of ventilation tube insertion according to operation times at different age groups

| | Operation time (<5 years) | Operation time (>5 years) | p |
|---|---|---|---|
| Endoscopic technique | 5.99±2.23 | 4.27±1.37 | 0.002 |
| Microscopic technique | 9.64±3.99 | 8.36±2.76 | 0.001 |
| Total (both techniques) | 7.73±3.67 | 6.5±3.02 | 0.001 |

The complication rates were 9.5% for the aged <5 years group and 4% for the aged >5 years group. To determine the age as a risk of complication, the odds ratio was found to be 0.48.

## DISCUSSION

The visualization ability of endoscopes in otologic surgery provides better access for the middle ear and even further location pathologies (7). There are several reports about the insertion of the ventilation tube with the endoscopic technique (8-11). The present study compared the operation time and complication rates of these two different techniques among different pediatric groups with a larger number of participants.

Many of the previous reports concluded that the operation time of the endoscopic technique was shorter than that of the microscopic method (9, 11, 12). Many variables may impact the duration of these procedures. Age is one of these factors that affect the course of the surgery (12). The human auricula is approximately 80% of its adult size at aged approximately 4–5 years, and it reaches full adult size at aged approximately 9 years (13). The bone formation of the external ear canal is nearly complete at aged approximately 3 years (13). This growth pattern of the human auricula and external ear canal might explain the difference of operation times that differs before and after the age of 5 years.

Lee et al. (14) suggested that referring patients who are over the age of 6 years to an endoscopic approach could result in effective surgical outcomes including the reduction of complication rates. According to the current study's complication results between different ages and with respect to the odd ratio, it appears that age may not have a strong relationship with complication rates, which was also consistent with the study by Nassif et al. (10).

Different diameters of endoscopes are also available that include 2.7–3 and 4 mm. In the present study, a 3 mm endoscope was used that could obtain a clear vision that was also emphasized in an earlier study (15). In addition, the usage of a 2.7 mm endoscope was suggested by some authors for better vision (5, 8, 10). According to this research, the use of a 3 mm diameter endoscope did not lead to the conversion of any endoscopic procedure to a microscopic approach.

One of the major obstacles to performing a standard endoscopic technique is bleeding (9, 16, 17). To prevent bleeding, which results in a blurred vision of operation field, meticulous attention must be performed to ensure that the external ear canal is not touched. A careless endoscope maneuver can lead to the tearing of the external ear canal that results in hemorrhaging. Placing a piece of sponge soaked with epinephrine 1/1000 or pontocaine 2% (or any vasoconstricting agent) to the external ear canal and waiting for 2 min are enough to allow adequate homeostasis (10).

When looking into another perspective of these two techniques, endoscopic ear surgery was found to be superior to microscopic ear surgery when considering time and cost (18, 19, 20). According to Patel et al. (18), the endoscopic application was found to be AUS$ 2978.79 cheaper and 56 min quicker than the microscopic method. Tseng et al. (19) also reached similar cost-effective findings regarding endoscopic ear surgery at a different center. It is the opinion of the researchers that health caregivers must keep in mind their ability to utilize their resources efficiently and fairly (21).

One of the limitations of the present study is that surgery was not performed for patients who had external ear deformity caused by a syndromic disease. Another limitation of the present study was the inability to use different diameters of endoscopes to evaluate different variables for surgical intervention.

In conclusion, the endoscopic ear ventilation tube insertion procedure reduces operation times. In addition, the complication rate of this technique appears to not significantly differ from the microscopic technique.

**Ethics Committee Approval:** Ethics committee approval was received for this study from University of Gaziantep Ethical Committee (Approval Date: 10.10.2018, Approval Number: 2018/233).

**Informed Consent:** Informed consent was obtained from the patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** The author have no conflicts of interest to declare.

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# Outcomes of Chronic Hepatitis B Virus Infection in Children: A 20-Year Follow-up

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**BACKGROUND/AIMS**
The aim of the present study was to evaluate seroconversion rates in children with chronic hepatitis B (CHB) infection and determine the factors influencing the natural course of liver diseases.

**MATERIAL and METHODS**
A total of 458 hepatitis B surface antigen (HBsAg)-positive patients aged 0.75–17 years were tested for hepatitis markers, liver function tests, and hepatitis B virus (HBV) DNA levels at baseline and periodically at every 3 months following recruitment. Patients with CHB (n=321) were divided into two groups: treated and untreated patients. The seroconversion rates between the two groups were compared, and their relationship with age, sex, vaccination status, coinfections, aminotransferases, HBV DNA levels, and cirrhosis was determined.

**RESULTS**
Hepatitis B e antigen (HBeAg) seroconversion rates were 30 in 97 patients 30/97 (30.9%) in untreated patients and 67/147 (45.5%) in treated patients (p=0.023). HBsAg seroconversion rates were 10/174 (5.7%) in untreated patients and 16/147 (10.8%) in treated patients (p=0.10). No significant difference was observed in HBeAg and HBsAg seroconversion times between the two groups (p>0.05).

**CONCLUSION**
In our study, although the HBeAg seroconversion rate was significantly higher in treated patients than in untreated patients, the HBsAg seroconversion rate was not different between the groups.

**Keywords:** Children, chronic hepatitis B, cirrhosis, HBsAg, HBeAg, outcome

## INTRODUCTION

Chronic hepatitis B virus (HBV) infection is a major cause of liver diseases associated with the development of cirrhosis and hepatocellular carcinoma (HCC). The natural course of HBV infection is greatly influenced by the age of the individuals at infection, host immune response to the virus, and the level of HBV replication (1). It The natural course of HBV infection comprises four phases: immune tolerance, immune clearance [hepatitis B e antigen (HBeAg)-positive chronic hepatitis B (CHB)], low or non-replication (inactive carrier state), and reactivation (HBeAg-negative CHB) (1-3).

The risk factors associated with the loss of hepatitis B surface antigen (HBsAg) and/or HBeAg that can occur spontaneously or following treatment and the development of progressive liver inflammation, fibrosis, and cirrhosis remain unclear. Given that it is not certain that treatment with interferon (IFN) increases seroconversion rates and improves prognosis, the benefits of treatment (IFN, lamivudine) have not been established (4-6). The risk for cirrhosis and HCC is low; therefore, treatment is not recommended because of the development of viral resistance in carriers who are in the immune tolerance phase (6). However, children with CHB infection would require regular screening for the progression of infection to cirrhosis and HCC because some of them will subsequently have flares of hepatitis and develop HBeAg-positive immune active hepatitis or HBeAg-negative active hepatitis (5, 6).

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The present study aimed to evaluate seroconversion rates in children with CHB infection and determine the factors influencing the natural course of liver diseases.

## MATERIAL and METHODS

A total of 458 HBsAg-positive children who were admitted to the Division of Pediatric Gastroenterology of Sisli Hamidiye Etfal Training and Research Hospital (Istanbul, Turkey) between 1998 and 2018 were retrospectively evaluated. Data on vaccination status, duration of HBsAg positivity, type of onset (acute or chronic), mode of transmission, concomitant hepatitis D virus (HDV) and/or hepatitis C virus (HCV) positivity, and physical examination signs (hepatomegaly, splenomegaly, and ascites) were obtained.

Complete blood count, biochemical tests, coagulation tests, HBsAg, antibody to hepatitis B surface antigen (anti-HBs), total antibody to hepatitis B core antigen (anti-HBc), antibody to hepatitis B e antigen (anti-HBeAg), anti-HCV, anti-HDV, and antibody to human immunodeficiency virus were examined in all patients using commercially available enzyme-linked immunoassays (Cobas Core, Roche Diagnostics, Pleasanton, CA, USA). HBV DNA and HCV RNA levels were measured using quantitative real-time polymerase chain reaction (COBAS TaqMan 48; Roche Diagnostics, Pleasanton, CA, USA). Liver biopsy was performed according to the Menghini technique. The stage and grade of liver involvement were scored according to Knodell's hepatic activity index (HAI) (7) in all treated patients. Children were followed up by testing HBeAg, HBsAg, HBV DNA, and liver function every 3 months for the first year and then annually. The study was approved by the Ethics Committee of the institution. Authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki ' Ethical Principles for Medical Research Involving Human Subjects' (amended in October 2013).Informed consents were obtained from all the parents of the children before biopsy and other procedures.

Patients whose alanine aminotransferase (ALT) and aspartate aminotransferase levels were three times the upper normal limits at admission were regarded as acute onset patients. Patients whose HBsAg continued to be positive for >6 months, indicating chronic HBV infection, were included in the study. A total of 44 (9%) patients who developed seroconversion during the first 6 months were regarded as acute hepatitis B infection and those with fulminant hepatitis were excluded from the study. Of the 414 HBeAg-positive children, 93 were excluded because of incomplete patient data or were lost to follow-up.

Inactive carriers were defined as HBeAg negativity, anti-HBe, undetectable or low levels of HBV DNA (<2000 IU/mL or $10^4$ copies/mL), persistent normal levels of ALT, and inactive liver histology (2, 4). HBeAg-negative chronic hepatitis (mutant HBV infection/precore or core promoter mutant) is defined as HBeAg negativity with anti-HBe positivity, detectable serum HBV DNA levels (2000–20 million IU/mL or $10^4$–$10^8$ copies/mL), increased ALT, and moderate or severe necroinflammation with variable amounts of fibrosis on liver biopsy (2).

HBeAg seroconversion was defined as loss of HBeAg and gain of anti-HBe antibody occurring either spontaneously or following treatment. HBsAg seroconversion was defined as loss of HBsAg and gain of anti-HBsAg antibody. Reactivation was defined as an increase in ALT (more than twice the upper normal limit) with the reappearance of HBV DNA, with or without reversion to HBeAg.

During follow-up, patients whose HBsAg, HBeAg, and HBV DNA positivity persisted for >6 months, aminotransferase levels were twice the upper normal limit, who had liver biopsy, and in whom the other causes of liver diseases were excluded were started on treatment. Patients were treated with IFN alpha-2a or IFN alpha-2b (at a dose of 3–5 MU/$m^2$ three times a week for 6 months, subcutaneously) alone or in combination with lamivudine (4 mg/kg/day once daily for 12 months, orally). Response to treatment was defined as HBV DNA undetectability, loss of HBeAg with seroconversion to anti-HBe, and ALT normalization (ALT ≤1× upper normal limit).

### Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 11.5 (SPSS Inc.; Chicago, IL, USA) software. All results are expressed as mean±SD. Statistical comparisons were made using the unpaired Student's t-test and Pearson correlation test. Qualitative variables were analyzed using Fisher's exact and chi-square tests. A p value of <0.05 was considered statistically significant.

## RESULTS

The age of 321 patients with CHB of the 458 HBsAg-positive patients ranged from 0.75 to 17 (8.99±3.75) years, and the male-to-female ratio was 3.1:2. The mean duration of follow-up was 15±4.7 years. The mode of transmission was mostly (60%) vertical (from mother to child), and 6% of the patients were vaccinated against hepatitis B. Although HBV immunoglobulin prophylaxis is used in Turkey to prevent the vertical transmission of CHB, the patients recruited in the present study were those whose mothers have not been prenatally screened for HBV; therefore, such patients have not been given HBV immunoglobulin prophylaxis.

Overall, 75% of the patients were HBeAg positive. All the 4 patients with concurrent HCV infection had been infected with HCV via blood transfusion. None of the patients had a family history of HCC. The clinical characteristics and laboratory results of the patients have been summarized in Table I.

Of the 270 patients, 12.2% (n=33) were HBV DNA negative and 87.8% (n=237) were positive at admission. HBV DNA levels could not be analyzed in 51 patients because the parents could not charge the cost of the tests. Of the 237 HBV DNA positive patients, 11% (n=26) were HBeAg negative, and these patients were regarded as mutant HBV infection (HBeAg-negative chronic hepatitis).

A mild correlation was observed between cirrhosis and HBsAg titer (r=0.298, p<0.006). A significant correlation was observed between HDV (n=4) and HCV (n=4) coinfections and cirrhosis (r=0.666, p<0.000 and r=0.213, p<0.000, respectively). No correlation was established among HBV DNA, HAI scores, and cirrhosis (r=0.130, p<0.112 and r=–0.046, p<0.447, respectively). Similarly, no correlation was observed between HBV DNA and cirrhosis (r=–0.046, p<0.447). The seroconversion rates between treat-

ed and untreated patients and their relationship with age; sex; vaccination status; mode of transmission; physical examination signs; initial laboratory results; and HBsAg, HBeAg, and HBV DNA titers are summarized in Table 2.

A total of 147 patients were treated with IFN alpha-2a (n=54), IFN alpha-2b (n=48), IFN alpha-2b and lamivudine (n=34), and IFN alpha-2a and lamivudine (n=11) during 6 months. The correlations among treatment modalities, and seroconversion rates are shown in Table 3.

**TABLE I.** Clinical characteristics of the patients at admission

| | |
|---|---|
| Age (mean±SD, range years) | 8.99±3.75 (0.75–17 years) |
| Sex (M/F) | 197/124 (3.1:2) |
| Mode of transmission | |
| Mother | 194 (60%) |
| Father | 47 (14%) |
| Siblings | 35 (11%) |
| Blood transfusion | 32 (10%) |
| Surgical operation | 7 (2%) |
| Other (tooth extraction, cousin vs) | 6 (1.86%) |
| Hepatitis B vaccination | |
| Yes | 302 (94%) |
| No | 19 (6%) |
| HBeAg positivity | 241 (75%) |
| Anti-HBe positivity | 80 (25%) |
| Coinfection | |
| HDV | 4 (1.24%) |
| HCV | 4 (1.24%) |
| Splenomegaly | 10 (3.1%) |
| Hepatomegaly | 15 (4.6%) |
| Ascites | 4 (1.2%) |
| Cirrhosis | 5 (1.5%) |
| Biopsy | |
| Yes | 147 |
| No | 174 |
| HAI score | |
| Minimal | 23 (16%) |
| Mild | 68 (46%) |
| Moderate | 35 (24%) |
| Severe | 21 (14%) |
| Fibrosis stage | |
| None | 13 (9%) |
| Minimal | 91 (62%) |
| Mild | 31 (21%) |
| Moderate | 11 (7%) |
| Severe | 1 (0.6%) |

HBeAg: Hepatitis B e antigen; Anti-HBe: Hepatitis B e antibody; HDV: Hepatitis Delta virus; HCV: Hepatitis C virus

Only 97 of the 174 untreated patients were HBeAg positive. HBeAg seroconversion rates were 30/97 (30.9%) in untreated patients (spontaneous seroconversion) during 20.8±21.9 months and 67/147 (45.5%) in treated patients during 27.8±17.7 months (p=0.023) (Figure 1). HBsAg seroconversion rates were 10/174 (5.7%) in untreated patients (spontaneous seroconversion) during 13.9±12 months and 16/147 (10.8%) in treated patients during 26.5±11.9 months (p=0.10). No significant difference was observed in HBeAg and HBsAg seroconversion times between the two groups (p>0.05). No difference was also observed with respect to HBeAg and HBsAg seroconversion rates between treatment with IFN alpha-2a or IFN alpha-2b alone and in combined treatment with lamivudine (p=0.07 and p=0.64, respectively).

Although the HBeAg seroconversion rates were significantly higher in patients with high pretreatment HBV DNA and serum ALT levels (p=0.005), no significant difference was observed in HBsAg seroconversion rates in these patients. In addition, spontaneous seroconversion rates were not significantly different in patients with high serum ALT and HBV DNA levels (p>0.05).

HBeAg reversion was observed only in 3 of the 5 male patients [mean age 6±3.3 (range 3–11) years] with cirrhosis, whereas HBsAg seroconversion was observed in none of them. One of the two patients in whom seroconversion was not observed had concurrent HCV infection. None of these children with cirrhosis developed HCC. One of the patients with cirrhosis died of liver-related causes, and one of them underwent liver transplantation during follow-up.

## DISCUSSION

The development of chronic HBV infection is higher in individuals infected perinatally (90%) or during childhood (20%–30%), which is thought to be due to immaturity of the immune system, than in adults (<1%) (1, 3). The most frequent mode of transmission is vertical transmission (from mother to child) in countries where moderate and high rates of HBsAg carriage are observed (1, 2). In our study, perinatally acquired HBV infection was observed in 60% of the patients. When compared with other transmission methods, no significant difference was observed in HBsAg and HBeAg seroconversion rates among patients who had vertical transmission of HBV.

Turkey is a moderate endemic area for HBV infection, and vaccination against hepatitis B is recommended for infants, children, and adolescents since 1998 in routine vaccination programs; therefore, 94% of our patients were unvaccinated against HBV.

Factors associated with an increased risk of developing liver diseases and progression to cirrhosis include older age (>40 years), male sex, presence of HBeAg, HBV genotype, mutations in the precore and core promoter regions of the viral genome, recurrent ALT flares, severity of fibrosis stage at presentation, HBV genotypes C>B and D>A, and concurrent infections (HBV/HCV and/or HBV/HDV) (1). In the present study, a correlation was established between cirrhosis and initial HBsAg titer, fibrosis score, and coinfections with HDV and HCV. Male sex has been identified as an independent risk factor of cirrhosis (8). Molecular mechanisms between sex and fibrosis are unknown, but the antifibrogenic effect of estrogen has been proposed (9).

**TABLE 2.** The association between seroconversion rates and clinical features and laboratory parameters

| | HBeAg (+) patients (n=241) | | HBeAg (–) patients (n=80) | | |
|---|---|---|---|---|---|
| | Treated (n=141) | Untreated (n=100) | Treated (n=3) | Untreated (n=77) | P |
| Age (mean±SD) | 8.2±3.9 | 8.9±4.1 | 5.5±4.27 | 10±3.8 | 0.81 |
| Gender (M/F) | 90/51 (1.7:1) | 60/40 (1.5:1) | 2/1 (2:1) | 45/32 (1.4:1) | 0.61 |
| Vaccination status | | | | | |
| Yes | 130 (92%) | 94 (94%) | 3 (100%) | 75 (97.4%) | 0.68 |
| No | 11 (7.8%) | 6 (6%) | 0 | 2 (2.5%) | 0.34 |
| Hepatomegaly | 10 | 3 | 2 | 0 | 1.00 |
| Splenomegaly | 8 | 1 | 0 | 1 | 1.00 |
| Ascites | 3 | 0 | 1 | 0 | 1.00 |
| Cirrhosis | 5 | 0 | 0 | 0 | 1.00 |
| Total bilirubin | 0.8±0.76 | 0.7±0.61 | 0.9±0.62 | 0.8±0.35 | 0.91 |
| AFP | 591±504.9 | 540±470.2 | 536±450.3 | 520±460.1 | 1.00 |
| Serum albumin | 4.0±0.6 | 4.0±0.5 | 4.1±0.6 | 4.0±0.5 | 1.00 |
| AST | 104±158.6 | 72.7±144.7 | 38.6±16.2 | 113.9±269.2 | 0.82 |
| ALT | 114.6±152 | 89.2±121.1 | 54.3±23.1 | 126.8±306 | 0.56 |
| HBV DNA | 9455±12323 | 11224±14018 | 4589±2635 | 3973±8911 | 1.00 |
| HAİ | | | | | |
| Minimal | 22 (15.2%) | - | 1 (33.3%) | - | 1.00 |
| Mild | 68 (47.2%) | - | 0 | - | 1.00 |
| Moderate | 34 (23.6%) | - | 1 (33.3%) | - | 1.00 |
| Severe | 20 (13.8%) | - | 1 (33.3%) | - | 1.00 |
| Fibrosis stage | | | | | |
| None | 12 (8.3%) | - | 1 (33.3%) | - | 1.00 |
| Minimal | 90 (62.5%) | - | 1 (33.3%) | - | 1.00 |
| Mild | 30 (20.8%) | - | 1 (33.3%) | - | 1.00 |
| Moderate | 11 (7.6%) | - | 0 | - | 1.00 |
| Severe | 1 (0.69%) | - | 0 | - | 1.00 |

AFP: alpha-fetoprotein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; HAI: hepatic activity index; HBeAg: hepatitis B e antigen; HBsAg: hepatitis B surface antigen
p<0.05 is statistically significant

In our patients with CHB and all patients with cirrhosis, 61.4% were male patients.

Longitudinal studies have demonstrated that HBeAg seroconversion leads to the inactive HBsAg carrier state in most children (1, 4, 10-12). Spontaneous HBeAg and HBsAg seroconversion rates occur at an average rate of approximately 10%–16% and 0.6%–1% per year, respectively (1, 4, 10-13). Spontaneous HBeAg seroconversion rates have also been reported to be <2% per year among children aged ≤3 years and 4%–5% per year in older children (>3 years) (13). In the present study, the spontaneous HBeAg seroconversion rate was 30.9% and spontaneous HBsAg seroconversion rate was 5.7%, and these seroconversion rates did not change according to age.

Long-term follow-up studies of adult inactive HBsAg carriers have shown that infection in these patients rarely progresses to cirrhosis or HCC (14). Patients who clear HBeAg with sustained reduction of HBV DNA, ALT normalization, and eventually HBsAg loss have a very low risk of developing HCC and have increased survival compared with patients with cirrhosis and persistent high levels of HBV replication. Cirrhosis is infrequent, its incidence is estimated to be 3%–4% in cohort studies (11, 15), and it has been reported to be an early complication. Among the 5 patients with cirrhosis, 3 were aged <4 years.

Hepatitis B virus DNA and ALT levels persistently or intermittently increase in some patients who undergo HBeAg seroconversion. These patients have a naturally occurring mutant form of HBV that abolishes or down-regulates the HBeAg promoter region (4) and are redetermined as having HBeAg-negative CHB. Progression to HBeAg-negative chronic hepatitis due to HBV variants not expressing HBeAg occurs at a rate of 1–3 per 100 person-years following HBeAg seroconversion (2). These patients have the ability to significantly replicate HBV

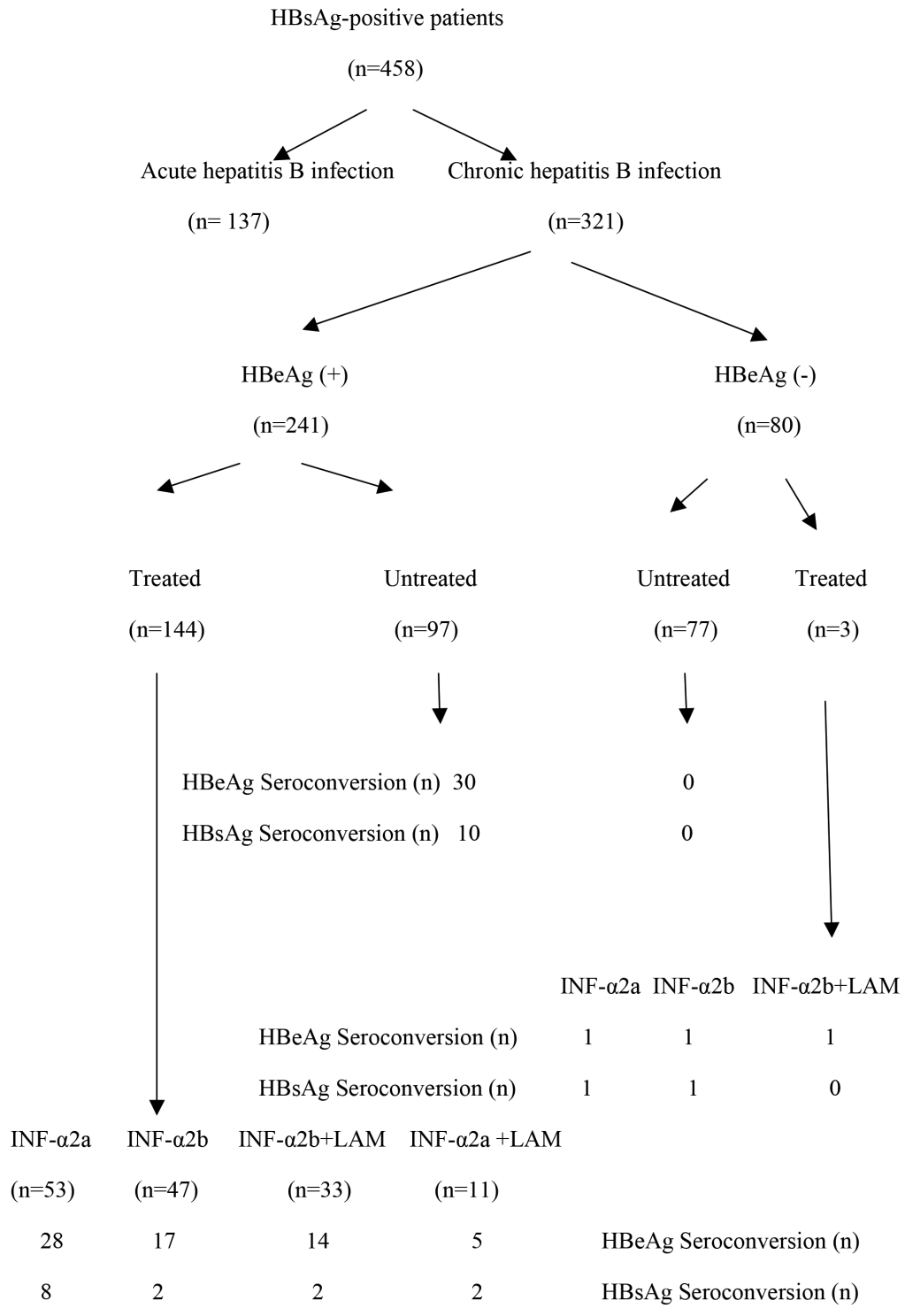


**FIGURE I.** Distribution of HBsAg-positive patients

in the presence of anti-HBe, even when ALT levels are normal. HBeAg-negative CHB is not typically acquired as a de novo infection, although there is reportof transmission of precore mutant HBV (16). Sustained spontaneous remission is uncommon in patients with HBeAg-negative CHB (6%–15%), and the long-term prognosis is poorer in HBeAg-negative patients than in HBeAg-positive patients (16). In our study, 32.5% of HBeAg-negative patients had HBV DNA positivity.

It has been reported that ALT is a poor predictor of outcomes and that IFN alone is not an appropriate indication for therapy (3, 17). The best predictors of adverse outcomes and treatment responses are HBV DNA levels in hepatitis B carriers (1, 17). ALT activity may be independently related to body mass index, sex, geographic origin, genotype, and abnormal lipid and carbohydrate metabolism, and ALT increases also occur during spontaneous HBeAg loss, in association with other viruses (18).

**TABLE 3.** The correlation between treatment modalities and seroconversion rates

| Treated patients (n=147) | HBeAg seroconversion | HBsAg seroconversion | p |
|---|---|---|---|
| Interferon alfa-2a (n=54) | 29 (53.7%) | 9 (16.6%) | 0.0001 |
| Interferon alfa-2b (n=48) | 18 (37.5%) | 2 (4%) | 0.0001 |
| Interferon alfa 2b+lamivudine (n=34) | 15 (44%) | 3 (8.8%) | 0.002 |
| Interferon alfa-2a+lamivudine (n=11) | 5 (45%) | 2 (1.8%) | 0.36 |
| p<0.05 is statistically significant | | | |

Keeffe et al. (18) reported that higher seroconversion rates are observed in patients who have high pretreatment serum ALT and HBV DNA levels than the patients who have normal serum ALT and HBV DNA levels. In our study, higher HBeAg seroconversion rates were also observed in patients with high pretreatment serum ALT and HBV DNA levels than in other patients, but spontaneous seroconversion rates were not different in these patients than other patients.

The primary aim of antiviral therapy is the elimination and durable suppression of serum HBV DNA to the lowest levels possible (maximally $<10^4$ and preferably $<10^3$) and prevention of the progression of liver diseases to cirrhosis (17). It has also been reported that, compared with no antiviral therapy, antivirals improve the HBV DNA suppression and frequency of ALT normalization and HBeAg seroconversion in children with CHB (18). IFN lacks resistance, is expensive, has to be administered via injections, and has many side effects such as flu-like symptoms, nausea, vomiting, anemia, autoimmune diseases, mood disorders, stroke and increased infections . Lamivudine is well tolerated, safe, and efficient, but it is also associated with high rates of resistance; therefore, it is not recommended as a first-line therapy in HBeAg-positive patients (19-23).

Fattovich et al. (15) and Iorio et al. (24) reported that antiviral treatment does not significantly influence HBeAg clearance, whereas Komatsu et al. (25) reported that antiviral treatment can accelerate the achievement of HBeAg seroconversion in children. HBeAg seroconversion rates have been reported to be 76%–86% in treated patients and 37%–75% in untreated patients (5, 19). Although some trials have suggested that combination therapy with IFN alpha-2a and lamivudine results in higher rates of HBeAg seroconversion, HBV DNA undetectability, and ALT normalization than those obtained by treatment with lamivudine alone (26-28), some trials have suggested no benefit and advantages of combination therapy over treatment with IFN alone with respect to HBeAg seroconversion in accordance with our study (29, 30).

HBeAg seroconversion rates have been reported to be 16%–40% with lamivudine treatment (31-32). Keeffe et al. (19) compared seroconversion rates according to the given treatment (IFN and lamivudine) and found that HBeAg seroconversion rates are 18% with INF and 16%–18% with lamivudine and that HBsAg seroconversion rate is 11%–25% with IFN. When compared, our seroconversion rates were higher at 46% with IFN alone and 44% with combined therapy with respect to HBeAg seroconversion. HBsAg seroconversion rates were similar at 10.7% with IFN alone and 11.1% with combined therapy. In our study, although the highest seroconversion rate was obtained with IFN alpha-2a treatment alone (n=29, 53.7%, p=0.0001), it is thought that it would not be used as a single agent in the treatment of CHB because of antiviral drug resistance. There was no difference in HBsAg seroconversion rates and seroconversion times between treated and untreated patients in our study.

It has been reported that the progression of liver diseases, perinatal transmission, and response to antiviral drugs may be influenced by genotypes (3). Keeffe et al. (19) recommended that patients should be routinely genotyped to help identify patients who may be at a greater risk for disease progression, particularly those who are the most appropriate candidates for treatment with IFN. The limitation of our study was that viral genotyping and sequence analysis were unavailable at our hospital.

In conclusion, a total of 30.9% patients underwent spontaneous HBeAg seroconversion and the spontaneous HBsAg seroconversion rate was 5.7%. No significant difference was observed between treated and untreated patients with respect to seroconversion rates and seroconversion times. Further well-designed prospective studies are needed to clarify the natural course of CHB and predictors of disease progression, such as HBV genotypes, mutants, and viral load, for improving the management of children with CHB.

**Ethics Committee Approval:** Authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013).

**Informed Consent:** Written informed consent was obtained from the parents of the patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept - N.U.; Design - N.U., D.K.; Supervision - N.U.;Resource - N.U.; Materials - N.U., D.K.; Data Collection and/or Processing - N.U., D.K.; Analysis and/or Interpretation - N.U., D.K.; Literature Search - D.K.; Writing - D.K., N.U.; Critical Reviews - N.U.

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# Cardiopulmonary Functions and Aerobic Capacity in Patients with Systemic Sclerosis

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**BACKGROUND/AIMS**
Systemic sclerosis (SSc) is a chronic inflammatory multisystem disorder characterized by microvascular damage and extensive fibrosis. Cardiopulmonary involvement is strongly associated with the severity of the disease itself and the mortality and morbidity of SSc. The aim of this study was to evaluate cardiopulmonary functions and aerobic capacity of SSc patients through cardiopulmonary exercise testing and compare them to healthy individuals.

**MATERIAL and METHODS**
A total of 27 patients (25 females, 2 males; mean age 43.96±13.01 years; mean body mass index 26.34±5.33 kg/m$^2$) who were diagnosed with SSc according to the American Rheumatism Association criteria and a control group of 23 healthy age-matched individuals (18 females, 5 males; mean age 42.04±12.28 years; mean body mass index 26.89±3.99 kg/m$^2$) were included in the study. All subjects underwent a treadmill cardiopulmonary exercise test. A computerized gas analysis system collected and analyzed expired gases during the exercise.

**RESULTS**
The mean peak oxygen consumptions were significantly decreased in the patient group (14.09±6.24 mL/kg/minute) as compared to the control group (19.65±5.97 mL/kg/minute) (p=0.002). In addition, the patient group had significantly lower peak minute ventilation (p=0.011), $O_2$ pulse (p=0.003), and exercise time (p=0.027), while having higher VD/VT rest and VD/VT peak in comparison with the control group.

**CONCLUSION**
The results of this study showed that patients with SSc had a lower aerobic capacity as compared to healthy individuals. The cardiopulmonary exercise test is a useful tool to detect exercise intolerance and provide additional information on the mechanism of exercise limitation in SSc.

**Keywords:** Systemic sclerosis, cardiopulmonary function, exercise test, exercise capacity

## INTRODUCTION

Systemic sclerosis (SSc) is a chronic inflammatory multisystem disorder characterized by microvascular damage and extensive fibrosis. The disease not only affects the skin but also damages multiple internal organs such as the lungs, kidneys, heart, and gastrointestinal tract. Organ involvement most often occurs early in the course of SSc, especially in the first 5 years (1). Cardiopulmonary involvement comprises interstitial pneumonia, pulmonary hypertension, conduction system defects, pericardial effusion and myocardial ischemia, hypertrophy, or failure of the cardiovascular system (2). Cardiopulmonary involvement was found to be strongly associated with the severity of the disease itself and the mortality and morbidity of SSc (3).

Exercise capacity is an independent, long-term predictor of mortality from cardiovascular disease in healthy individuals (4). The cardiopulmonary exercise test (CPET) is a useful tool in the evaluation of undiagnosed exercise intolerance of patients with respiratory and/or cardiovascular disease, functional work capacity, response to treatment following surgery, rehabilitation or pharmacological treatment, and in the detection of gas exchange abnormalities and determination of potential exercise-limiting factors (5).

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Of the reasons responsible for low exercise capacity and dyspnea in patients with SSc, cardiopulmonary impairment is more important than cutaneous lesions, chronic inflammation, and deconditioning (6, 7). The determination of exercise capacity is crucial to detect early cardiopulmonary impairment in patients with SSc. The aim of this study was to evaluate cardiopulmonary functions and aerobic capacity through CPET in patients with SSc compared to healthy individuals.

## MATERIALS and METHODS

### Patients

A total of 27 patients (25 females, 2 males; mean age 43.96±13.01 years; mean body mass index (BMI) 26.34±5.33 kg/m$^2$) diagnosed as having SSc according to the American College of Rheumatology (ACR) criteria (8) and a control group of 23 healthy age-matched individuals (18 females, 5 males; mean age 42.04±12.28 years; mean BMI 26.89±3.99 kg/m$^2$) were included in the study. All patients were recruited from the Ankara University School of Medicine, Department of Rheumatology. Patients with either diffuse systemic sclerosis (n=8, 29.6%) or limited scleroderma (n=19, 70.4%) were included in the study. The consenting subjects were interviewed and put through a complete medical assessment, which included a detailed medical history and routine laboratory measurements in addition to a physical examination, before participating in the study. The inclusion criteria were: (1) age of ≥18 years, (2) SSc as defined by the ACR classification criteria, (3) being ambulatory and able to perform walking treadmill exercise, and (4) no previous history of any regular exercise training or sporting activity. Patients were excluded if they had: (1) unstable angina, (2) severe arterial hypertension at rest (>200/120 mmHg), (3) uncontrolled heart failure, (4) uncontrolled arrhythmia, (5) severe mental impairment, (6) high degree AV block, and (7) significant pulmonary hypertension or orthopedic impairment. The study protocol was approved by the Local Research Ethics Committee of Ankara Physical Medicine and Rehabilitation Training and Research Hospital. The study adhered to the guidelines of the Declaration of Helsinki and informed consent was obtained from all participants.

### Cardiopulmonary Exercise Testing (CPET)

All subjects performed a treadmill CPET after a resting spirometric measurement. A computerized gas analysis system collected and analyzed expired gases during exercise (Sensormedix, CA, USA). A standard open-circuit method was used to collect expired gases. It was calibrated with known gas concentrations and volumes prior to each test. The system consisted of a mask, a two-way breathing valve, a rolling seal spirometer, an oxygen analyzer, and a carbon dioxide analyzer. The breathing apparatus was attached to the mask after placing it on the subject's face. Heart rate and electrocardiogram were displayed throughout the CPET. Capillary oxygen tension was measured on an oxygen photometer attached to the earlobe. A modified Bruce protocol was used (9), which protocol had fixed increments in speed and inclines every 3 minutes. The initial 3-minute stage occurred at a speed of 2.74 km/hour and a 0% gradient. The second and third stages had the same speed and duration but the gradients were increased by 5% and 10%, respectively. Each subsequent stage had an increment of 1.29 km/hour in speed and 2% in gradient. The criteria for termination of CPET included chest pain suggestive of angina, ischemic electrocardiogram changes, complex ectopy or 2$^{nd}$ or 3$^{rd}$ degree atrioventricular block, a >20 mmHg drop in systolic blood pressure from the highest value during the test, hypertension (systolic blood pressure >250 mmHg; diastolic blood pressure >120 mmHg), severe desaturation, oxygen saturation ($SaO_2$) ≤80% with accompanying symptoms and signs of hypoxemia, loss of coordination, and mental confusion (10).

Oxygen consumption ($VO_2$), carbon dioxide exhaled ($VCO_2$), minute ventilation (VE), respiratory rate (RR), respiratory exchange ratio (RER), the ratio of physiological dead space to tidal volume (VD/VT), the ventilatory equivalent for $VCO_2$ ($VE/VCO_2$), and $SaO_2$ were recorded for every breath during CPET. The anaerobic threshold was determined with a computerized V-slope method of gas exchange data.

### Overview of Procedures

Baseline characteristics including age, gender, body mass index (BMI), smoking habit, duration of disease, type of SSc, and comorbidities were recorded. The patients were tested with an echocardiogram and an electrocardiogram. Pulmonary hypertension was assessed by recording pulmonary artery systolic pressure (PASP) and a value of to 25 mmHg at rest was accepted as normal (11). The findings of chest x-ray and high-resolution computerized tomography (HRCT) were also noted from medical records.

### Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences for Mac, Version 20.0 software (IBM Corp.; Armonk, NY, USA). The data were stated as mean ± standard deviation for continuous variables and as proportions for categorical variables. The Chi-square test was applied for comparison of proportions. The Student's *t*-test was used to compare the mean values of continuous variables between the groups. If the distribution of the continuous variables was not normal, the Mann–Whitney test was used for comparison. A value of $p < 0.05$ was accepted as statistically significant.

## RESULTS

The baseline characteristics of the patients and the healthy individuals are presented in Table 1. There were no differences between the groups in terms of age, gender, and BMI. The mean duration of the disease was 4.87±4.69 years. A total of 19 (70.4%) patients had limited cutaneous SSc and the rest (eight patients, 29.6%) had diffuse SSc. Cardiac abnormalities were detected in the echocardiograms of 11 (40.9%) patients (Table 2). Five (18.6%) of the patients had a PASP value of over 25 mmHg, while six patients (22.2%) had significant interstitial lung disease based on HRCT and chest x-ray findings.

Cardiopulmonary values obtained at maximum exercise are presented in Table 3. CPET was terminated due to generalized muscle fatigue in 14 patients, dyspnea in five patients, desaturation in two patients, and blood pressure criteria in two patients. In the control group, the test was terminated due to generalized muscle fatigue in 19 control subjects, dyspnea in two subjects, and blood pressure criteria in two subjects. There was no significant difference in the blood pressure measurements between the two groups at baseline and at peak exercise ($p>0.05$). Sig-

**TABLE I.** Baseline characteristics of both groups

| | Patient Group (n=27) | Control Group (n=23) | p |
|---|---|---|---|
| Age (years)* | 43.96±13.01 | 42.04±12.28 | 0.596 |
| Gender (female/male) | 25/2 (92.6/7.4) | 18/5 (78.3/21.7) | 0.145 |
| BMI (kg/m²)* | 26.34±5.33 | 26.89±3.99 | 0.686 |
| Smoking habit | | | 0.182 |
| *Non-smoker* | 16 (59.3%) | 19 (82.8%) | |
| *Current smoker* | 7 (25.9) | 2 (8.6%) | |
| *Ex-smoker* | 4 (14.8) | 2 (8.6%) | |
| Duration of disease (years) | 4.87±4.69 | | |
| Type of SSc | | | |
| *Limited cutaneous (lcSSc)* | 19 (70.4%) | | |
| *Diffuse cutaneous (dcSSc)* | 8 (29.6%) | | |
| Comorbidities | | | |
| *Arterial hypertension* | 5 (18.5%) | | |
| *Diabetes mellitus* | 3 (11.1%) | | |
| *Cardiovascular Disease* | 2 (7.4%) | | |
| *Hypothyroidism* | 3 (11.1%) | | |
| *Other* | 5 (18.5) | | |

*Mean ± standard deviation
BMI: Body Mass Index; SSc: Systemic Sclerosis

**TABLE 2.** Cardiac findings of the patients with SSc (n=27)

| **Echocardiogram** | |
|---|---|
| Normal | 16 (59.1%) |
| TR | 7 (25.9%) |
| MR | 2 (7.4%) |
| MS | 1 (3.7%) |
| *Pericardial effusion* | 1 (3.7%) |
| Mean PASP (mmHg)* | 24.9±5.3 |
| *PASP >25 mmHg* | 5 (18.6%) |
| *PASP ≤25 mmHg* | 22 (81.4%) |
| Electrocardiogram | |
| *Normal* | 22 (81.5%) |
| *Tachycardia* | 3 (11.1%) |
| *Bradycardia* | 2 (7.4%) |

TR: tricuspid regurgitation; MR: mitral regurgitation; MS: mitral stenosis; PASP: pulmonary arterial systolic pressure

nificant electrocardiographic ST-segment changes indicating a positive test were obtained in four patients during the CPET. $SaO_2$ was <88% in two patients at rest and dropped by >4%, i.e., it dropped to <88% and desaturation developed in 11 patients during the CPET. At the peak exercise, the values of $VO_2$, VE peak, $O_2$ pulse, and exercise time were significantly lower and VD/VT rest and peak were significantly higher in the patient group than in the control group. Normal anaerobic threshold responses were determined in two patients (at a level ≥40% of the predicted $VO_{2\,m}$ax). In 25 of 27 patients, no anaerobic threshold was identified.

**TABLE 3.** Cardiopulmonary values obtained at peak exercise in the patient group and the control group

| | Patient Group (n=27) | Control Group (n=23) | p |
|---|---|---|---|
| $VO_2$ peak (mL/kg/min) | 14.09±6.24 | 19.65±5.97 | 0.002 |
| $VO_2$ peak (L/min) | 0.94±0.38 | 1.35±0.43 | 0.001 |
| $O_2$ pulse (mL/beat) | 5.94±2.38 | 8.16±2.62 | 0.003 |
| HR peak (bpm) | 158.33±26.07 | 167.57±22.05 | 0.187 |
| VE peak (L/min) | 55.57±21.43 | 71.26±20.20 | 0.011 |
| RR (bpm) | 41.29±10.43 | 38.82±7.49 | 0.349 |
| HRR | 13.41±28.16 | 4.39±21.75 | 0.218 |
| BR (VE max/MVV%) | 72.07±27.06 | 72.26±18.61 | 0.977 |
| VD/VT rest | 0.56±0.06 | 0.47±0.053 | <0.001 |
| VD/VT peak | 0.31±0.08 | 0.23±0.06 | <0.001 |
| RER | 1.41±0.17 | 1.47±0.17 | 0.233 |
| $SaO_2$ (%) | 92.41±2.91 | 92.74±2.97 | 0.693 |
| $SaO_2$ end (%) | 87.0±4.48 | 87.74±4.01 | 0.542 |
| Exercise time (min) | 11.46±4.22 | 13.81±3.19 | 0.027 |
| SBP/DBP (mm Hg) rest | 112.5 ±18.93/77.7±10.5 | 117.8±13.1/78.2±10.2 | 0.207 |
| SBP/DBP (mm Hg) peak | 155.9±39.1/87.0±11.7 | 159.5±24.7/90.0±11.2 | 0.702 |

*Mean ± standard deviation.
$VO_2$: oxygen consumption; HR; heart rate; VE; minute ventilation; RR; respiratory rate; bpm; beat per minute; HRR; heart rate reserve; VD/VT; the ratio of physiological dead space to tidal volume; RER; respiratory exchange ratio; SaO2; oxygen saturation; SBP; systolic blood pressure; DBP; diastolic blood pressure

## DISCUSSION

The present study evaluated cardiopulmonary functions and aerobic capacity using CPET in SSc. The results showed that the values of $VO_2$ peak, VE peak, $O_2$ pulse, and exercise time were significantly lower and VD/VT values at rest and peak were significantly higher in patients with SSc as compared to healthy individuals. Poor cardiopulmonary findings were revealed in patients with SSc.

The identification of patients with cardiopulmonary complications is challenging even with the use of specific investigations in SSc patients (12). Resting pulmonary and cardiac function testing cannot exactly reflect exercise performance and functional capacity. The 6-minute walk test (6MWT) is a practical simple test but it does not provide peak oxygen uptake, diagnose the cause of dyspnea on exertion, or evaluate the causes or mechanisms of exercise limitation (13). However, CPET allows the detection of organ involvement in asymptomatic patients without cardiopulmonary involvement. In a study by Alkotob (14), it was shown that early pulmonary vascular pathology could be determined using CPET in asymptomatic SSc patients without cardiopulmonary involvement. CPET may also be used in SSc patients with multisystem involvement to globally assess the exercise response in the pulmonary, cardiovascular, hematopoietic, neuropsychological, and skeletal muscle systems.

Oxygen consumption peak has been traditionally identified as the major indicator of aerobic capacity and fitness. This is

calculated from the difference between the volume of $O_2$ in the inhaled and exhaled air during exercise per unit of time (15). A reduced $VO_2$ peak represents reduced exercise capacity. Normal values are ≥85% predicted (16). Similar to previous studies, the present study result showed exercise capacity impairment in the patients with SSc, evidenced by a significantly decreased $VO_2$ peak value (17-19). This value was significantly lower in the patient group as compared to the control group. Although cardiopulmonary involvement is the main culprit for reduced exercise limitation in SSc, a sedentary lifestyle, fatigue, and articular and skin deformities may also contribute to exercise intolerance. Oliveira et al. (19) found a mean $VO_2$ peak value of 19.8 ml/kg/minute in patients with SSc, while the mean $VO_2$ peak was 14.9 mL/kg/minute in the current study. This value was reported by Plazak et al. (20) as 16.5 mL/kg/minute. The difference could be attributed to the fact that Oliveira et al. (19) investigated exercise capacity especially in SSc patients without pulmonary involvement, which indicates that pulmonary involvement is a major contributing factor for lower exercise capacity. Also, the shorter exercise time is an indicator of exercise capacity impairment. In the present study, the patient group had a significantly shorter exercise time as compared to the control group. CPET can be used to comprehensively evaluate exercise capacity and cardiopulmonary involvement in patients with SSc and aerobic exercise programs can be considered in the treatment of patients who have reduced exercise capacity.

Minute ventilation is the volume of air exhaled from the lungs in 1 minute. $VE_{max}$ is the maximal ventilation achieved during exercise and it represents ventilator demand (15). Abnormality in $VE_{max}$ can reflect respiratory and neuromuscular limitation to exercise. There are few data related to $VE_{max}$ achieved in patients with SSc. In a study by Rosato et al. (21), it was reported that patients with SSc had a lower $VE_{max}$ level as compared to the healthy individuals, which was similar to the results of the present study. This finding might be interpreted as a respiratory limitation to exercise.

$O_2$ pulse ($VO_2$/HR) is the amount of oxygen consumed by the tissue per heartbeat. It is calculated by dividing $VO_2$ by HR and is expressed as mL/beat. Reduced $O_2$ pulse can indicate decondition, cardiopulmonary disease, and early exercise limitation due to ventilator restriction (16). Normality is defined as >80%. The current study findings showed that the patients had a lower $O_2$ pulse level compared to the control group. Sudduth et al. (18) and Plazak et al. (20) investigated exercise capacity in patients with SSc using CPET and both those studies also found a lower level $O_2$ pulse in the patient groups than in the control groups.

Respiratory exchange ratio is obtained from the ratio of $VCO_2$/$VO_2$ and corresponds to the gas exchange ratio. RER is the best non-invasive indicator for the level of exercise during the performed CPET. A peak value of ≥1.1 is widely accepted as a marker of maximal exercise effort (22). If the patient does not reach this value, other limiting factors apart from cardiac dysfunction should be considered. In the current study, only two patients did not reach the peak value of RER, with the mean RER value determined as 1.41±0.17. Moreover, in the comparison of the two groups, no difference was determined with respect to the RER peak value. Similarly, Rosato et al. (21) found no difference in the RER peak value of SSc patients when compared with a healthy control group.

Ratio of physiological dead space to tidal volume is one of the indicators of the adequacy or efficiency of gas exchange. An elevated VD/VT or absence of a reduction in VD/VT with exercise suggests the presence of a pulmonary vascular disease such as pulmonary hypertension (15). In a study by Schwaiblmair et al. (23), 78 patients with SSc had increased VD/VT during exercise, which was comparable with the current study. These findings suggested that gas exchange abnormality in SSc patients is a common problem.

Desaturation is regarded as any decrease in oxygen saturation measured by standard pulse oximetry ($SpO_2$) of 4% or more, or to a nadir of 88% or less, regardless of the baseline $SpO_2$ (24). Desaturation is common in patients with interstitial lung disease, pulmonary hypertension, and chronic obstructive disease (22). Exercise-induced oxygen desaturation is one of the predominant factors contributing to exercise limitation in patients with SSc. Desaturation during the exercise test is also associated with high mortality risk and increased severity of disease (25, 26). In the present study, $SaO_2$ was below 88% in two patients at rest and it dropped by >%4, following which it dropped below 88% and desaturation developed in 11 patients during the CPET. Thus, desaturation developed in a total of 13 patients. Similar to these findings, in a study by Ciurzyński et al. (27) that evaluated left and right ventricular diastolic function in patients with systemic sclerosis, a higher rate of desaturation was seen in the patient group as compared to the control group. This desaturation seen during the exercise test provides prognostic information about the patients with SSc.

Respiratory rate is the number of breathing cycles per minute. It reflects abnormalities in the mechanics of breathing, control of breathing, and/or hypoxemia or psychological disorders. The normal value of RR as <60 breaths per minute is during peak exercise time. A normal value of RR of <60 breaths per minute is 34 during peak exercise time. In the current study, three patients had a value of >60 breaths/minute. Rapid and shallow breathing resulting in a high RR may be evidence of inefficient ventilation in systemic sclerosis. To the best of our knowledge, there has been no study in literature that has evaluated the value of RR in SSc to date.

Ventilatory equivalent for $VCO_2$ is a good non-invasive estimator of inefficient ventilation (28). The normal value is <34 and higher values reflect increased dead space ventilation or hyperventilation during exercise. In addition, $VE/VCO_2$ has been shown to be a predictor of mortality in patients with pulmonary arterial hypertension and chronic heart failure (29). Sudduth et al. (18) revealed that higher $VE/VCO_2$ is indirectly related with early pulmonary vasculopathy. The current study results showed one patient with a higher value. The study conducted by Rosato et al. (21) showed that $VE/VCO_2$ correlated with vascular involvement and SSc severity in patients without cardiopulmonary involvement. Therefore, $VE/VCO_2$ is an important value which should be considered when evaluate cardiopulmonary involvement in SSc patients.

The present study achieved the goals that it aimed to substantiate. However, there were some limitations. First, healthy individuals were not assessed with HRCT, echocardiogram, and chest x-ray. Unnecessary radiation exposure was avoided be-

cause doing so might have caused an ethical violation as these individuals did not have any pulmonary symptom. The second limitation is the cross-sectional design of the study. Further prospective researches that assess CPET over a longer period of time will better define the development of cardiopulmonary impairment in patients with SSc.

A comprehensive evaluation of all CPET variables can provide clinicians with more information on the mechanism of exercise limitation, prognosis, and mortality risk. Cardiopulmonary involvement most often occurs in the course of SSc, especially in the first 5 years. CPET can be applied for the detection of cardiopulmonary abnormalities not revealed by routine testing. In addition, the ability to detect cardiopulmonary involvement using CPET combined with advanced treatment options may prevent mortality and organ-based complications of SSc. If abnormalities are established, ventilatory exercise and aerobic training programs can be considered to improve the aerobic capacity of SSC patients.

**Ethics Committee Approval:** Ethics committee approval was received for this study from Ankara Physical Medicine and Rehabilitation Training and Research Hospital. (Approval Date: 07.01.2013, Approval Number: 4.06.23.34-902/111).

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Original Article

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# Rare Complication of Stereotactic Guide-Wire Localization of Nonpalpable Breast Lesions: Breakdown of the Wire-The First Series From Turkey Involving 20 Case Analyses

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**BACKGROUND/AIMS**
In cases wherein percutaneous biopsy cannot be performed via imaging, a wire-guided breast biopsy is used for the diagnosis of nonpalpable breast cancer. Although known as a safe procedure, complications may develop rarely. In this study, we analyzed 20 cases of instances of guide-wire breakdown that occurred when the procedure was performed.

**MATERIAL and METHODS**
We retrospectively analyzed 818 patients with guide wire-localized breast lesions between January 2015 and June 2017 from electronic files at our hospital.

**RESULTS**
Wire breakdown occurred in 20 patients. Although the guide wire broke down in the breast specimen of 15 patients (75%), it broke apart from the specimen in 5 patients (25%); 3 of these 5 wires were noticed intraoperatively in the remaining tissue and were removed by re-excision. The remaining 2 wires were noticed using imaging methods during the postoperative period and removed by inserting a second wire. The guide-wire indications in these 20 patients were as follows: microcalcification in 14 (70%); structural distortion in 3 (15%); and focal, asymmetrical area in 3 (15%) patients. Pathology results revealed that the breast specimens were benign in 13 (65%) patients and malignant in 7 (35%) patients.

**CONCLUSION**
Although guide-wire breakdown was rarely reported in wire-marked breast biopsies, the frequency is not fully known. Because the wire may breakdown inside the specimen, it can also be found in the remaining breast tissue. Residual wire should be removed by coordinating with the radiologist to prevent possible complications.

**Keywords:** The wire-guided localization, wire breakdown, nonpalpable breast cancer

## INTRODUCTION

Nonpalpable breast cancer became a common finding on mammograms taken for screening asymptomatic women (1). Approximately 25%–35% breast cancers consist of nonpalpable breast lesions (2). These lesions are observed to have a linear configuration, asymmetrical density, and structural distortion, and more frequently, they are seen as microcalcification and are classified as BIRADS 4-5 (2). For this reason, the lesions have to be excised and diagnosed. The wire-guided localization (WGL) technique was first described by Dodd et al. (3) in 1965. In this technique, the lesion in the breast is marked with a thin wire under the guidance of ultrasound or mammography, and the marked area is excised surgically. The excised tissue is sent to the radiology department for confirmation that the lesion has been removed, and the surgical procedure is terminated after this is confirmed. By this method, breast cancer can be diagnosed early, the size of the excised specimen for biopsy can be reduced, and simultaneously, bad cosmetic results can

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be prevented (4). As any other interventional processes, this process can also lead to some complications. Vasovagal reflex may develop during wire insertion, pneumothorax can occur, the location may change after insertion, and although rarely, the wire can breakdown (5).

If the wire breaks and stays in the breast long enough, the metal induces carcinogenesis, and nickel complexes cause chromosomal damage, activating signaling pathways and altering the cell genetics, which can cause cancer. Conversely, if the patient sees the wire broken during control mammography, it can lead to medicolegal and psychological problems. We were sued by one of our patients for this reason; therefore, caution is important.

In this study, we analyzed 20 cases in which the wire broke down during the procedure.

## MATERIAL and METHODS

We analyzed 818 patients with guide wire-localized breast lesions between January 2015 and June 2017, and this was approved by the Ethics Committee of our hospital. The surgical notes, imaging methods, pathology reports, and medical history were analyzed by a retrospective search of patients records. Data on complications during the procedure were obtained via patient observation forms.

The IBM Statistical Package for Social Sciences version 20.0 for Windows (IBM Corp.; Armonk, NY, USA) was used to record the data.

An approval was received for this study from the local Ethics Committee of Dr. Abdrurrahman Yurtaslan Oncology Training and Research Hospital (AOH 11/3/2017). Written informed consent was obtained from patients who participated in this study.

## RESULTS

Overall, there were 20 instances of wire breakdown, proven by imaging methods, which were detected from 818 cases in an approximately 30-month period. A guide wire was introduced under mammographic guidance in all cases. The mean age of the patients was calculated as 51.3 (range, 41–71). Although the guide wire broke down in the breast specimen of 15 patients (75%), it broke apart from the specimen in 5 patients (25%) (Table 1). The wires that broke down in the specimens were detected by radiological examination of tissue samples obtained during surgery (Figure 1); 3 of the 5 wires that broke down out of the specimen were noticed intraoperatively by manual examination of lumpectomy cavity and were removed by re-excision (Figure 2). The remaining 2 wires were noticed via imaging methods in the postoperative period (1 year later) and removed by inserting a second wire (Figure 3). Guide-wire indications in the 20 patients were as follows: microcalcification in 14 (70%); structural distortion in 3 (15%); and focal, asymmetrical area in 3 (15%) patients. Pathology results revealed that breast specimens were benign in 13 (65%) patients and malignant in 7 (35%) patients. Although 5 patients with malignant results underwent surgery, 1 was administered prophylactic chemotherapy and 1 was lost to follow-up.

**TABLE 1.** Localization of broken wires

| Patient no. | Age | Wire indication | Pathology | The site of breakdown |
|---|---|---|---|---|
| 1 | 48 | Microcalcifications | DCIS, Grade 3 | In specimen |
| 2 | 51 | Structural distortion | Fibrocystic change | In specimen |
| 3 | 47 | Microcalcifications | Atypical ductal hyperplasia | In specimen |
| 4 | 47 | Microcalcifications | DCIS, Grade 3 | In specimen |
| 5 | 55 | Structural distortion | Sclerosing adenosis | In specimen |
| 6 | 47 | Focal asymmetric area | Fat necrosis | In specimen |
| 7 | 55 | Microcalcifications | Fibrocystic change | In specimen |
| 8 | 60 | Microcalcifications | DCIS, Grade 2 | In specimen |
| 9 | 53 | Microcalcifications | Adenosis | In specimen |
| 10 | 42 | Microcalcifications | Apocrine cyst | In specimen |
| 11 | 66 | Microcalcifications | DCIS, Grade 3 | In specimen |
| 12 | 46 | Microcalcifications | Invasive ductal carcinoma | In specimen |
| 13 | 49 | Microcalcifications | Fibrocystic change | In specimen |
| 14 | 71 | Microcalcifications | Fibrocystic change | In specimen |
| 15 | 41 | Microcalcifications | Adenosis | In specimen |
| 16 | 44 | Microcalcifications | Ductal ectasia | Out of specimen |
| 17* | 45 | Focal asymmetric area | Adenosis | Out of specimen |
| 18 | 46 | Focal asymmetric area | LCIS | Out of specimen |
| 19 | 57 | Microcalcifications | Micropapillary cancer, Grade 2 | Out of specimen |
| 20* | 57 | Structural distortion | Fat necrosis | Out of specimen |

DCIS: ductal carcinoma in situ; LCIS: lobular carcinoma in situ
*Removed with a second wire during the second procedure (1 year later)

## DISCUSSION

Because palpable breast cancer is usually symptomatic and patients report to the hospital frequently, it is more easily detected. However, nonpalpable breast cancers are usually asymptomatic, and as long as there is no nipple discharge, pain, or erythema, they are usually diagnosed using radiological screening methods. The incidence of nonpalpable breast lesions has increased with the widespread employment of mammography screening programs. Approximately 15%–20% of these lesions are malignant; therefore, those that are radiologically suspicious must be excised (6). The most commonly used methods for the excision of nonpalpable lesions are percutaneous biopsy, radio-guided occult lesion localization (ROLL), and WGL; percutaneous biopsy is the gold standard. ROLL was reported as a new method in 1998, in which a radiopharmaceutical drug is injected into the tumor, and the lesion is removed via excisional biopsy using a gamma probe (7). In the WGL method, the region of the lesion is surgically excised using a guide wire positioned under the guidance of ultrasound or mammography (8). Certain criteria must be adhered to when a wire is placed by a radiologist. Although Abrahamson suggested that the wire should be placed within 5 mm of the lesion to increase the success rate, Sagutti suggested that the wire should be advanced through the lesion to a depth of <1 cm (8, 9). General or local anesthesia may be preferred during the surgery. General anesthesia is preferred for patients with deep-seated lesions and those with large breasts (4). In our series, 18 patients were operated under local and 2 under general anesthesia. Two different wire types are used in WGL: stainless steel and nitinol (nickel and titanium). Results of *in vitro* study has revealed that metal induces carcinogenesis, and nickel complexes cause chromosomal damage, activate signaling pathways, and alter cell genetics (10). We used stainless steel wires (Anbao, USA, 20G/10cm) for all our patients. WGL has some preoperative and postoperative disadvantages. It is difficult to place the guide wire in dense breasts (11); 16 (80%) of our patients had dense breasts.

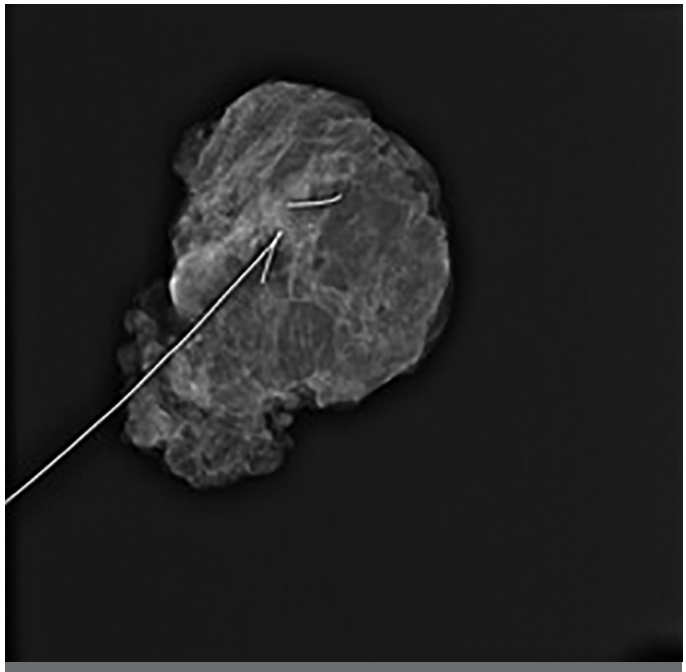

**FIGURE 1.** Break-down of a wire in the specimen

Furthermore, the wire may dislocate during surgery. As the surgeon removes the healthy tissue to find the lesion, more tissue than necessary might be removed. In addition, the wire may cause pneumothorax by migration or by the racquet effect (the wire can be pulled by the pectoral muscle to cause pneumothorax). The guide wire can be broken down during surgery (5, 12). The broken part of the wire should be removed when it is noticed. Forgotten wires can be observed later during control mammography. In literature, guide-wire breakdown was reported at a rate of 0%–3% (12). In our series, 20 (2.5%) instances of guide-wire breakdown among 818 cases in the last 2.5 years were noted, and this ratio is compatible with the one in the literature. Handa et al. (13) reported low-grade adenosquamous carcinoma in the tissues around the broken-down wire in a pa-

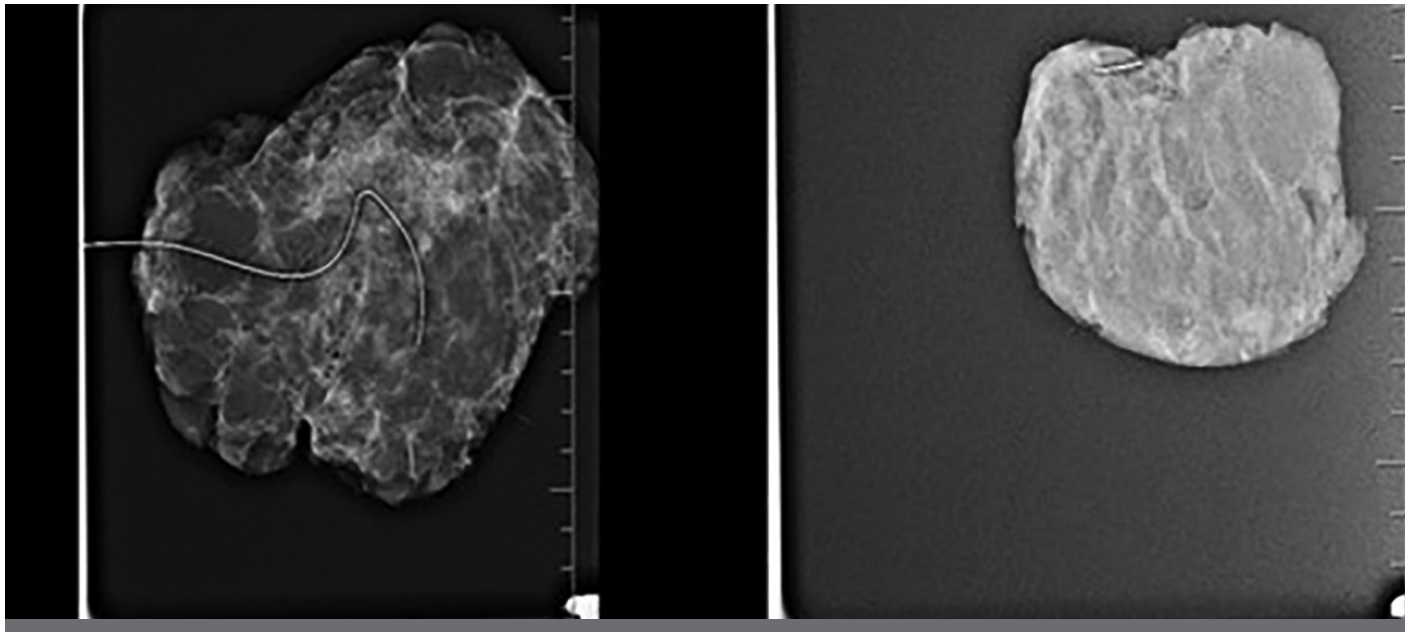

**FIGURE 2.** Wire break-down out of specimen, removed tissues at the same procedure

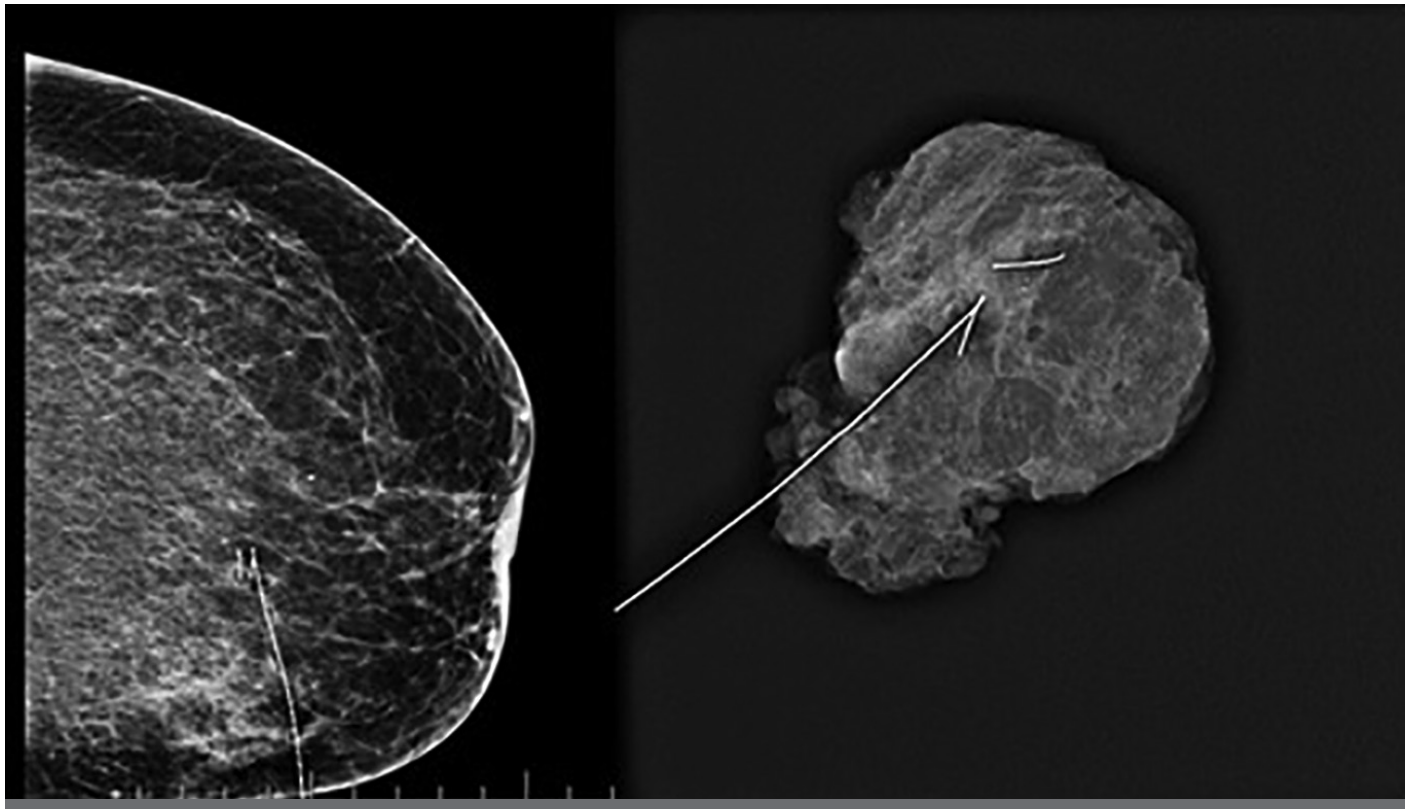

**FIGURE 3.** Wire break-down out of specimen, wire removed with surgery by inserting a second wire

tient who underwent WGL 10 years ago. The pathology report was evaluated as fat necrosis in two cases that we removed 1 year later, malignancy was not detected. In a study by Monterey et al. (12), 8 broken-down wires were reported on 32,473 mammograms. The length of these wires ranged from 0.7 to 4.2 cm. The remaining wires are usually asymptomatic and can cause pain by movement. A second wire localization may be necessary to remove the residual broken wire (13). In our series, a second wire localization was necessary in 2 patients (Table 1, patients 17 and 20). Results of primary and secondary biopsies revealed that the specimens of these patients were benign. The remaining wires may stay in the same region or migrate to the infraclavicular fossae, subcutaneous tissue, cervical muscles, and even to the contralateral axillae (14, 15). Homer suggested periodic mammography owing to the possibility of wire migration in patients who do not agree to undergo surgery (16). As long as the residual wires do not penetrate the pectoral muscle, they usually do not create a medical problem. However, the patient may be in trouble because she does not know the kind of problems it may cause in the breast; therefore, medicolegal problems may occur (13). Consequently, the radiology and surgical team should work in coordination to improve quality standards while performing WGL, especially in reference centers, such as our hospital, where breast surgery is often performed. The direction and distance of the wire inserted by the radiologist during the preoperative period should be presented in a 3D format. In cases of doubt, intraoperative re-excision should be performed and broken wire should not be left behind. The patient should be referred to the surgical team for the wires seen in the post-operative mammograms. If necessary, surgery must be performed under general anesthesia to increase the comfort of the patient and surgeon.

At the end of the procedure, surgeons should control the cavity. When a wire fracture is noticed during the postoperative period, the patient should be informed to avoid medicolegal problems, and the wire should be removed if possible. If not, the patient should be followed up by mammography to prevent wire migration.

**Ethics Committee Approval:** Ethics committee approval was received for this study from Dr.Abdurrahman Yurtaslan Oncology Training and Resarch Hospital (Approval Date: 03.11.2017, Approval Number: 2017118).

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

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Original Article

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# The Effect of Staging Laparoscopy Prior to Neoadjuvant Chemotherapy on Treatment Management in Locally Advanced Gastric Cancer

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**BACKGROUND/AIMS**
The prevalence of gastric adenocarcinoma is 951.000 in the world and cases in Western countries are diagnosed in a more advanced stage. In this study, the efficacy of neoadjuvant chemotherapy in gastric cancer was demonstrated and neoadjuvant chemotherapy was recommended for patients with ≥T2 resectable gastric tumors. The key point for administering neoadjuvant chemotherapy is the distant location of a solid organ and the status of peritoneal metastasis at the beginning of the treatment. We aimed to investigate the effect of the peritoneal status, which is assessed using staging laparoscopy, on the treatment protocol of gastric cancer patients.

**MATERIAL and METHODS**
This retrospective study included 60 neoadjuvant chemotherapy patients, who were divided into 2 groups according to staging methods.

**RESULTS**
Out of a total of 60 patients, 30 were staged by radiological methods and staging laparoscopy prior to neoadjuvant chemotherapy. The remaining 30 patients were staged only by radiological methods. In the laparoscopic staged group, peritoneal metastases were detected in 43% of radiologically non-metastatic patients by staging laparoscopy. In the non-laparoscopic staged group, metastatic disease was identified in 7 patients, right before or during the gastrectomy operation.

**CONCLUSION**
Peritoneal metastasis is not uncommon in gastric cancer. If the evaluation of peritoneal status is made only by radiological examinations, the treatment may be started with an incorrect low-grade diagnosis. The use of staging laparoscopy prior to neoadjuvant chemotherapy makes a significant contribution to the multidisciplinary treatment of gastric cancer.

**Keywords:** Cancer staging, gastric cancer, Laparoscopy, neoadjuvant therapy, peritoneal carcinomatosis

## INTRODUCTION

The prevalence of gastric adenocarcinoma is 951.000 in the world and the prevalence in underdeveloped countries is estimated to be 677.000 (1). The cases in Western countries are diagnosed in a more advanced stage of the disease, unlike the countries in the Far East (2). The incidence of gastric cancer in our country's region is found to be more similar to Far Eastern countries than to European and North American countries (1). Effective treatment methods should be discussed on priority for patients with advanced stage gastric cancer, especially in our region.

The efficacy of neoadjuvant chemotherapy in advanced gastric cancer patients was demonstrated in Western origin studies (3). Neoadjuvant chemotherapy is recommended for patients who have T2 and above resectable gastric tumors and no distant metastasis or peritoneal spread findings (4).

We aimed to investigate the effect of the peritoneal status, which is assessed using staging laparoscopy, on the treatment protocol of gastric cancer patients.

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## MATERIAL and METHODS

### Patients

Between August 2015 and September 2018, 1283 presentations registered to Dokuz Eylül University, Faculty of Medicine Upper Gastrointestinal (GI) were examined retrospectively at a case discussion meeting. Gastric cancer patients who had undergone a neoadjuvant chemotherapy plan were identified. Twenty-six patients were excluded from the study due to the lack of data.

### Staging, Chemotherapy, and Surgery

The radiologic staging routinely was performed with computed tomography (CT) scanning. In both groups, patients with T2 or higher tumors and/or metastatic lymph nodes were considered to meet the criteria for neoadjuvant chemotherapy. All patients underwent the same neoadjuvant chemotherapy regimen. The second radiologic staging was performed in the third or fourth week after the previous neoadjuvant chemotherapy session. Surgery was performed at least 4 weeks later, following the last chemotherapy session.

The 60 consecutive patients with complete data were divided into 2 equal groups according to the staging methods. In the first group, patients were evaluated without using staging laparoscopy. The second group consisted of patients who underwent staging laparoscopy procedure prior to neoadjuvant chemotherapy.

### Staging Laparoscopy Technique

Under general anesthesia, an infra-umbilical camera port and a right upper quadrant five-millimeter working port were placed in the supine position. The anterior abdominal wall, bilateral diaphragmatic surfaces, pelvic peritoneal, rectovesical/rectouterine region, omentum, and gastric wall were inspected. The location of the tumor, its mobilization, and its relation to the serosa and other organs were examined. The liver, spleen, jejunum, ileum, cecum, colon, and their mesenteric structures were examined. If an implant was detected, it was excised and then fixed with formaldehyde solution. If acid was found, it was aspirated and examined by cytological methods. In each case, the abdominal cavity was washed with 1000 ml physiologic normal saline. By changing the position of the operating table, it was ensured that the fluid would come into contact with the entire abdominal wall, small intestines, omentum, diaphragmatic surfaces, pelvis, and abdominal organs. Then, the washing liquid was aspirated. This aspirated liquid was centrifuged and the precipitate was obtained. Two smears were prepared for pathological examination; one of them was stained with hematoxylin eosin and the other was stained with Papanicolaou stain.

This research was conducted according to the principles of the World Medical Association's Declaration of Helsinki, "Ethical Principles for Medical Research Involving Human Subjects" (amended in October 2013). Ethics committee approval was received for this study from Dokuz Eylül University (Approval Date: 23.01.2019, Approval Number: 2019/02-05). Oral informed consent was obtained from all the patients.

### Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences program Statistical Package for the Social Sciences version 22.0 (IBM Corp., Armonk, NY, USA). Data were expressed as mean±SD or median (minimum-maximum). Frequencies and percentages were used to depict categorical variables. A p-value of <0.05 was considered statistically significant.

## RESULTS

The median age of the patients was 62 (37–84) years. Fifteen patients (25%) were female and 45 (75%) were male. Thirty patients underwent neoadjuvant chemotherapy without staging laparoscopy (non-laparoscopic staged group, GROUP 1), and 30 patients underwent staging laparoscopy prior to neoadjuvant chemotherapy (laparoscopic staged group, GROUP 2). No gender-based significance was detected between the groups (Group 1: 25 males & 5 females; Group 2: 20 males & 10 females; p=0.136). No age-related significance was detected between the 2 groups (Group 1: 63.93 years; Group 2: 59.67 years; p=0.107)

In group 1, radiological imaging after neoadjuvant therapy showed progression under treatment in 3 patients. The remain-

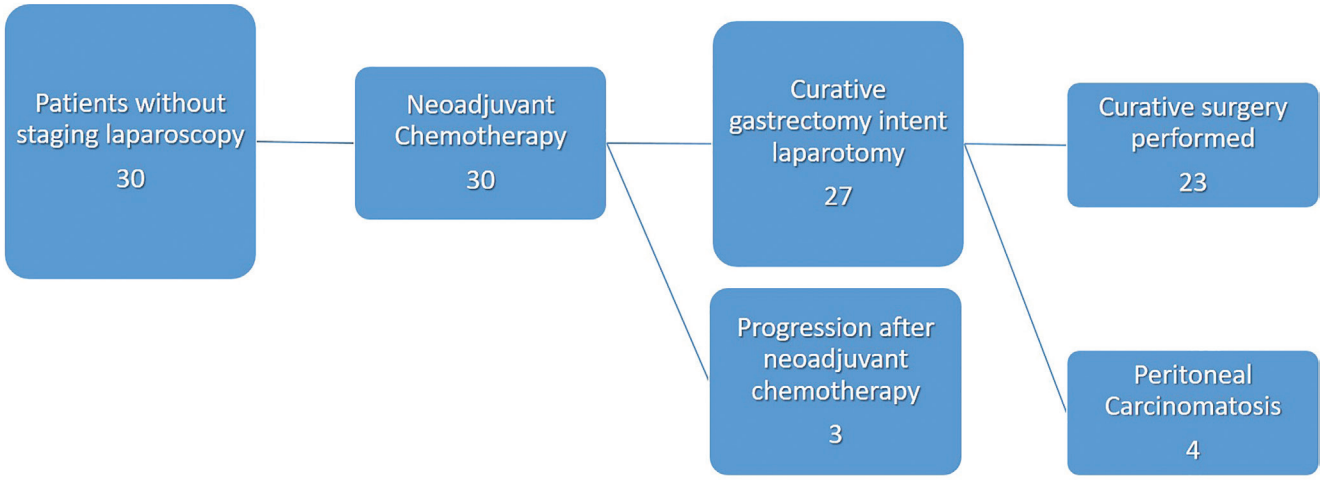


**FIGURE 1.** Patients without staging laparoscopy

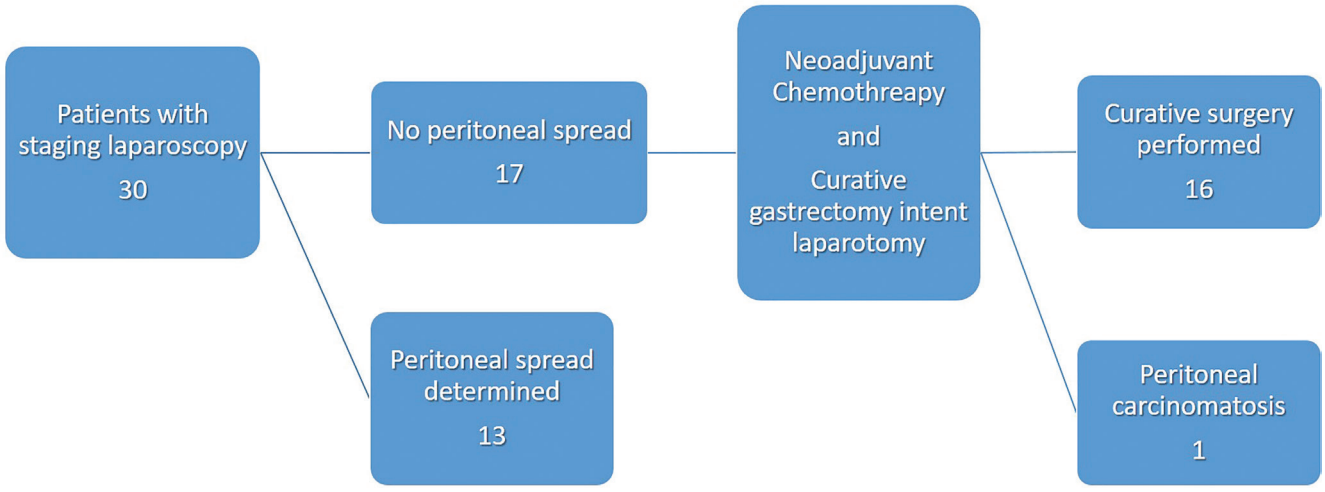


**FIGURE 2.** Patients with staging laparoscopy

ing 27 patients in this group were operated on and peritoneal carcinomatosis was detected in 4 patients (Figure I).

Thirteen of 30 patients in group 2 showed peritoneal spread of cancer. Ten of these I3 patients had peritoneal implants and 3 had malignant cells in the peritoneal lavage precipitate. Seventeen patients who had no peritoneal spread on laparoscopy were operated on after neoadjuvant chemotherapy. The peritoneal spread was determined during gastrectomy operation in I patient (Figure 2).

## DISCUSSION

Chemotherapy and radiotherapy regimens are added to radical gastric and lymph node resection procedures to provide an adjuvant effect in gastric cancer treatment protocols (5, 6). In the treatment protocol, adjuvant therapies are added only if patients have poor prognostic factors such as advanced tumor invasion, lymph node metastasis, and lymphovascular invasion (7).

Neoadjuvant and adjuvant (perioperative) chemotherapy is a relatively new concept in the treatment of gastric cancer. This option is offered for ≥T2 tumors without distant organ or peritoneal metastasis as per the guidelines (8). It has been proposed that neoadjuvant therapy has more advantages than adjuvant therapy in our study. Adjuvant chemotherapy is delayed due to feeding problems and a prolonged recovery period, and treatment is often not completed in the postoperative period. Preoperative neoadjuvant chemotherapy is tolerated better than postoperative adjuvant chemotherapy. Although there are no difficulties and problems of tolerance in postoperative chemotherapy, there are other troubles with respect to the effectiveness of the treatment, such as surgical dissection causing deterioration of vascularity in the remaining tissues after resection. This effect causes a lower distribution of adjuvant chemotherapeutics in these tissues.

Neoadjuvant chemotherapy seems to be more successful in this context (3, 4). Neoadjuvant chemotherapy contributes to the achievement of the R0 resection goal by decreasing the tumor and lymph node staging. Further, according to the response of the tumor to the treatment, information about the efficacy of the chemotherapeutic can be obtained, with which the transition to effective chemotherapy regimens are easily achieved if the treatment response is not enough.

Stomach cancer can metastasize to a wide variety of organs and/or structures (9). The key point for neoadjuvant chemotherapy is the distant location of a solid organ and the status of peritoneal metastasis at the beginning of treatment. Although preoperative radiological and/or nuclear medicine methods are very reliable for distant solid organ metastases, it is difficult to detect small peritoneal implants by these methods.

The sensitivity of contrast-enhanced multi-slice spiral CT and Positron Emission Tomography are reported in the literature as 25%–I00% and 58%–I00% respectively, for detecting peritoneal metastasis. Unfortunately, these values are decreased in the presence of small (<5 mm) peritoneal metastases (I0, II).

In this study, staging laparoscopy determined peritoneal metastasis in 43% of radiologically negative patients. The clinical stage and treatment protocol changed in these I3 patients by staging laparoscopy. This rate of change was reported as 48% by Shelat et al. (II)3 RI resections and I R2 resection and as 37.8% by Muntean et al. (I2).

In group I, 3 metastatic patients were detected after neoadjuvant chemotherapy by preoperative CT, and 4 patients were detected with peritoneal metastasis after neoadjuvant chemotherapy during the operation. In these 4 patients, radiological images could not determine the peritoneal spreads due to their small dimensions (in millimeters) just before the operation. No clear comment was made on their peritoneal status prior to initiating neoadjuvant therapy for I0 patients.

In group 2, the progression despite administration of chemotherapy was determined in I patient. We concluded that this chemotherapy regimen does not have sufficient anti-tumoral activity. With this information, missed effective drugs were identified and the chemotherapy protocol was reorganized.

When the results of this study were interpreted, 3 choices regarding the treatment suitable for 7 patients without curative gastrectomy in group 2 were put forward; A. Minimal response to treatment, B. Stable disease under treatment, C. Progression under treatment. It was decided that this question could not be answered with absolute accuracy with the present data. The effects of the applied chemotherapy regimens on the tumors were unknown. Unfortunately, the treatment protocols were revised without this information for these 7 patients.

The present study had several potential limitations. The major limitations were the retrospective design and small sample size. Another limitation was the lack of polymerase chain reaction testing, which is a more sensitive method for the evaluation of abdominal washing fluids.

Peritoneal metastasis is not uncommon in gastric cancer. When clinical staging is performed at the beginning of the treatment and if the evaluation of peritoneal status is made only by radiological examinations, the treatment may be started with an incorrect low-grade diagnosis. With the information in the previous literature and the results obtained from our study, we can conclude that the probability of error in the clinical staging is about 40%. Staging laparoscopy prior to neoadjuvant chemotherapy can make a significant contribution in the multidisciplinary treatment of gastric cancer.

**Ethics Committee Approval:** Ethics committee approval was received for this study from Dokuz Eylul University Ethical Committee. (Approval Date: 23.01.2019, Approval Number: 2019/02-05).

**Informed Consent:** Informed consent was obtained from the patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept - S.D., K.A.; Design - S.D., C.A.; Supervision - K.A., M.Ü.; Resource - M.Ü., K.A.; Materials - C.A., M.Ü.; Data Collection and/or Processing - S.D.; Analysis and/or Interpretation - S.D., C.A., K.A., M.Ü.; Literature Search - S.D.; Writing - S.D.; Critical Reviews - K.A., M.Ü., C.A.

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**Conflict of Interest:** The authors have no conflicts of interest to declare.

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

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Original Article

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# Evaluation of Thyrotropin and Thyroxine Levels in The First Month of Life

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**BACKGROUND/AIMS**
Thyrotropin (TSH) and thyroxine (FT4) levels in neonates are not similar to those in older children and adults. The aim of this study is to determine the TSH and FT4 hormone levels in newborns aged >37 gestational weeks.

**MATERIAL and METHODS**
The blood samples for TSH and FT4 obtained from newborns aged >37 gestational weeks were analyzed at postnatal 4-7, 8-14, 15-21, and 22-30 days and established as reference intervals for the first week of life, in addition to the mean and median levels for other observation points.

**RESULTS**
There was no significant difference according to gender, gestational age, and mode of delivery (p>0.05); therefore, a pooled analysis was performed. The lower and upper limits of the TSH and FT4 levels from the 4$^{th}$ to 7$^{th}$ day of life were 1.20-10.70 mIU/mL and 0.87-2.20 ng/dL, respectively.

**CONCLUSION**
We demonstrated that the TSH and FT4 levels change during the neonatal period without a significant sex difference.

**Keywords:** Neonate, thyrotropin, thyroxine, congenital hypothyroidism

## INTRODUCTION

Congenital hypothyroidism (CH) is one of the most common etiologies of preventable mental retardation all over the world (1) and the signs and symptoms are not very apparent at birth. Therefore, neonatal screening has been performed in many countries, including Turkey, under a nationwide neonatal thyroid screening program to diagnose and treat CH on time (2). Thyrotropin (TSH) is measured using a filter paper collection method at discharge, and elevated levels are reevaluated for confirmation as soon as possible using both serum TSH and thyroxine (FT4) values in Turkey. The American Academy of Pediatrics' (AAP) recommendations for screening are filter paper collection at 2-4 days of age or at discharge for term infants delivered in the hospital, within 7 days for term neonates staying in the neonatal intensive care unit (NICU), and within 7 days for preterm neonates and for infants delivered at home. Screen cord blood is suggested for neonates whose mothers are on thyroid medication and/or have a family history of CH. A low FT4 level and TSH concentrations >40 mIU/L are indicative of CH (3). The European Society for Pediatric Endocrinology Consensus Guidelines on Screening, Diagnosis and Management of CH recommendations suggest starting treatment if capillary TSH concentration obtained for neonatal screening is ≥40 mU/L, which is similar to AAP suggestions, and/or if venous TSH concentration is >20 mU/L even if the FT4 concentration is normal.

During the neonatal period, reference ranges of thyroid hormones and TSH are not similar to older children and adults (4). Therefore, neonatal reference values for TSH and FT4 are required for accurate assessment. The aim of this study was to determine the TSH and FT4 reference ranges in hospitalized neonates with gestation age ≥37 weeks.

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## MATERIAL and METHODS

In this retrospective study, we included 615 neonates with gestational age ≥37 weeks and hospitalized in the NICU between May 2015 and October 2017.

The gestational age was calculated by using the last menstrual date of the mother; if this date was not known, Ballard score or fetal USG measurements were used. Inclusion criteria were an Apgar score >7 at the 1st and 5th min of life, ≥37 weeks' gestation, mothers having no history of thyroid disease, and appropriate height, weight, and head circumference for appropriate gestational age (AGA). Neonates who had congenital malformations, chromosomal anomaly, metabolic disease, received any medication that might interfere with thyroid or pituitary function (e.g., corticosteroids, dopamine, or propranolol), and whose mothers had any thyroid disease were excluded.

The results of the FT4 and TSH serums were analyzed at postnatal 4-7, 8-14, 15-21, and 22-30 days in AGA neonates from the existing laboratory data. Further, the thyroid function results of neonates on L-thyroxine treatment due to congenital hypothyroidism were collected, but separately evaluated.

The analyses of the FT4 and TSH levels from the serum samples were measured by a Beckman-Coulter DxI device (Minnesota, USA) using the electrochemiluminescence immunoassay method.

Gaziosmanpasa Taksim Research and Training Hospital Ethics Committee approved the study protocol (approval number 90) and all the procedures were conducted in accordance with the Declaration of Helsinki. The study was retrospective, so informed parental consent was not obtained.

### Statistical Analysis

Normal distribution was obtained by using Kolmogorov-Smirnov tests and histogram, Q-Q plot, and box plot graphics. The data were expressed as mean, standard deviation, median, minimum, maximum, IQR, frequency, and percentage. Logarithmic transformation was used for non-normally distributed variables to provide normality. The first-week TSH and FT4 levels, which were normally distributed between two categorical variables, were analyzed with independent samples t test and the other results that were non-normally distributed were analyzed by the Mann-Whitney U test. Variables with three or more categories were compared with the Kruskal–Wallis test. Multiple comparisons were not performed as there was no significant difference. Correlation between the measurable variables was performed by Spearman's correlation test. Normality was not provided for the determination of a reference interval; therefore, the nonparametric percentile method (C28-A3, CLSI guideline) was used. The controls of the outliers were obtained by Rosner's test for multiple outliers. The statistical analysis was carried out using NCSS 10 statistical software (2015, Kaysville, Utah, USA): p values with significance of less than 5% were considered to be statistically significant.

## RESULTS

We studied 615 healthy newborns with mean gestational age of 38.61±1.89 weeks, mean birth weight was 3500±540 g, mean head circumference was 33.5±1.3 cm, and mean birth length was 49.83±1.8 cm. Here, 271 females (44.1%) and 344 males (55.9%) were included. Further, 44% neonates (n: 275) were delivered by caesarean section. Furthermore, 15% neonates (n: 93) exhibited prolonged jaundice. The FT4 and TSH levels during the 4-7th day were higher among neonates with prolonged jaundice, but none of them were diagnosed and treated for CH. There was no significant difference related to the FT4 and TSH levels for other observation points with or without prolonged jaundice. There was no significant difference according to gender, gestational age, and mode of delivery (p>0.05); therefore, a pooled analysis

**TABLE 1.** TSH (mIU/mL) and FT4 (ng/dL) levels according to postnatal day

| | 4-7th DOL | 8-14th DOL | 15-22th DOL | 23-30th DOL |
|---|---|---|---|---|
| TSH (median) | 3.67 (0.85-15.63) | 3.65 (0.93-12.07) | 3.61 (0.76-17.10) | 3.20 (1.28-13.50) |
| FT4 (mean±SD) | 1.45±0.33 | 1.3±0.20 | 1.16±0.22 | 1.11±0.30 |

TSH: thyrotropin; FT4: free thyroxine; DOL: day of life

**TABLE 2.** Reference ranges for TSH (mIU/mL) and FT4 (ng/dL) during postnatal 4-7th day

| | 95% Reference range lower limit (90%CI) | 95% Reference range upper limit (90%CI) | Median (IQR) |
|---|---|---|---|
| TSH | 1.20 (1.13-1.28) | 10.70 (10.04-11.36) | 3.67 (2.35-5.85) |
| FT4 | 0.87 (0.80-0.96) | 2.20 (2.16-2.27) | 1.45 (1.24-1.66) |

TSH: thyrotropin; FT4: free thyroxine; DOL: day of life

**TABLE 3.** Demographic variables and TSH and FT4 levels belonging to patients diagnosed with hypothyroidism

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 |
|---|---|---|---|---|---|---|
| HC (cm) | 33 | 32 | 32,5 | 33 | 34 | 35 |
| BW (g) | 3310 | 2300 | 2580 | 2500 | 3170 | 3800 |
| BL (cm) | 49 | 46 | 47 | 47 | 49 | 50 |
| GA (week) | 39 | 36 | 36 | 36 | 383/7 | 382/7 |
| Gender | Male | Female | Male | Male | Male | Female |
| TSH (mIU/mL) | DOL 3>48 | DOL 3: 30.6 | DOL 3: 21 | DOL 3>100 | DOL 3:32.99 | DOL11>50 |
| | DOL 6>48 | DOL 7: 32.25 | DOL 6: 45 | DOL 5>100 | DOL6>46.8 | DOL16>50 |
| FT4 (ng/dL) | DOL 3: 0.35 | DOL 3: 1.53 | DOL 3: 1.36 | DOL 3: 0.36 | DOL 3: 1.24 | DOL11: 0.35 |
| | DOL 6:1.40 | DOL 7: 1.02 | DOL 6: 0.9 | DOL 5: 0.24 | DOL 6: 0.81 | DOL6: 0.23 |

TSH: thyrotropin; FT4: free thyroxine; DOL: day of life; HC: head circumference; BW: birth weight; BL: birth length; GA: gestataional age

was performed. The median TSH and mean FT4 levels during the 4-7th day (n: 482), 8-14th day (n: 131), 15-22nd day (n: 57), and 23-30th day (n: 87) are listed in Table 1. We tried to determine the 95% reference range of TSH and FT4 level during the 4-7th day by using a nonparametric percentile method (C28-A3, CLSI). The reference intervals for TSH and FT4 of the neonates during the 4-7th day of life are listed in Table 2. The median TSH level of neonates with prolonged jaundice during the 4-7th day (n: 55), 8-15th day (n: 43), 16-22nd day (n: 29), and 23-30th day (n: 30) were 4.3 (01.36-15.6), 3.66 (1.01-12.06), 3.69 (1.3-17), and 2.95 (1.3-13.5), respectively. The mean FT4 levels of neonates with prolonged jaundice during the 4-7th day, 8-15th day, 16-22nd day, and 23-30th day were 1.33±0.30, 1.30±0.30, 1.14±0.20, and 1.02±0.20, respectively. The TSH and FT4 levels measured during the 4-7th day of life were statistically different among neonates with and without prolonged jaundice (p: 0.038, p: 0.002), but the hormone values of these newborns were not as high as the values required for treatment for CH mentioned in the literature; in other words, none of these newborns were treated for CH.

In this study, we discuss the period in which we diagnosed and started the treatment for 6 neonates for CH along with pediatric endocrinology. Information regarding these neonates is listed in Table 3. The TSH level of patient 4 was the highest; an enlarged thyroid gland was distinctly visible from the outside.

**DISCUSSION**

Primary hypothyroidism is diagnosed when there is a high concentration of serum TSH and/or low concentration of serum FT4. Therefore, the determination of the reference ranges for these hormones during the neonatal period is very essential. The FT4 and TSH levels of newborns increase dramatically just after birth because of cold stress. These hormones reach their peak levels in the first 24-36 h, and these high levels continue for 2 or 3 days and slowly decrease to their adult levels after 4-5 weeks (4). Mutlu et al. (5) demonstrated that the TSH and thyroid hormones peak in the first 24 h of life and then slowly decline during the neonatal period. According to their study, both TSH and thyroid hormone levels were significantly higher during the neonatal period as compared to those in adults (5). However, Kawahara et al. (6) found that the TSH level reached adult levels after 5 or 7 days of life. They also reported that FT4 was lower at 72 h and thereafter, comparable to the first 48 h of life, but still higher than adult levels. In our unit, we did not evaluate the TSH and FT4 levels as early as these studies because of the rise in these hormone levels following birth to prevent recurrent measurements regarding high levels. We generally measured these hormones after 72 h in our NICU. Neonates whose TSH and FT4 levels were measured on the 4th day of life and thereafter were included in the present study. We investigated several factors that may affect thyroid function: gestational age, gender, and mode of birth. There was no significant difference between these hormone levels and gestational age, gender, and mode of delivery at any observation point; therefore, we decided to combine all these values. Kawahara et al. (6) and Mutlu et al. (5) also demonstrated that there was no significant difference between the hormone levels and the mode of delivery or gender. Imamoglu et al. (7) reported that the serum TSH levels at the postnatal 1 week and 1 month were not correlated with the gestational age, but the FT4 levels were correlated with gestational age at both postnatal 1 week and 1 month. Kapelari et al. (8) found no gender difference except for free T3. Kratzch reported no gender difference involving the thyroid function tests in newborns aged 1 day to 1 month (9).

Kapelari et al. (8) reported the FT4 and TSH reference intervals (2.5, 50, and 97.5%) of neonates from 1 day to 1 month as 0.66/1.56/2.36 ng/dL and 0.70/3.50/18.10 mIU/L, respectively. Elmliger et al. (10) also studied these reference intervals of TSH for neonates aged 1 to 7 days and 8 to 15 days, which were 1.79/4.63/9.69 and 1.80/3.71/7.97 mIU/L, respectively. In another study performed by Hubner et al. (11), the lower limit of TSH for neonates aged 1 to 3 days and 4 to 30 days was lower as compared to the abovementioned studies; however, the upper limit was similar to the results of Elmliger et al. (10), but significantly lower than the results of Kapelari et al. (8). Imamoglu et al. (7) obtained the serum TSH and FT4 levels at the postnatal 1 week and 1 month and determined the reference ranges for TSH and FT4. The reference ranges for TSH (mIU/L) irrespective of the gestational age at the postnatal 1 week and 1 month were 3.71 (0.57-13.11) and 3.30 (1.0-8.37), respectively. Verburg et al. (12) studied the reference ranges for thyroid function in children. They evaluated 139 newborns and determined the reference intervals (2.5, 50, and 97.5%) of TSH and FT4 at the 7th, 14th, 21st, and 28th day of life. The values of TSH at the 7th, 14th, 21st, and 28th day of life were 3.11 (0.32-2.27), 3.01 (0.34-11.44), 2.89 (0.35-10.43), and 2.80 (0.36-9.75) mU/L, respectively. The values of FT4 at the 7th, 14th, 21st, and 28th day of life were 18 (8.9-33.6), 17.9 (8.9-32.9), 17.8 (9-32.3), and 17.7 (9-31.8) pmol/L. The design of the study by Sheikhbahaei et al. (13) was similar to ours. They evaluated the TSH, total, and FT4 hormone levels during 5-7, 8-14, 15-21, and 22-30 days of life and established the reference ranges. The reference ranges (2.5, 50, and 97.5%) of TSH and FT4 during 5-7 days were 0.25/4.7/21.25 mIU/mL and 0.75/1.40/2.12 ng/dL, respectively. The FT4 levels are similar to our results, but the upper TSH level is higher and the lower TSH level is less than those in our results. The TSH level during 22-30 days of life is similar to that obtained in our study, but the FT4 level was higher when compared to that obtained in our study.

We observed that the TSH and FT4 levels decreased with postnatal age. Mutlu et al. (5) found an inverse relationship between the TSH and FT4 levels and age after the 3rd day of life. Najam et al. (14) demonstrated that the decline in the TSH and T4 levels was more apparent in the first week.

Mutlu et al. (5) found that if the TSH level is >20 mIU/L on the 3rd day, >16 mIU/L during the 5-7 days, and >5 mIU/L on the 28th day, these patients should be carefully followed-up with regard to CH. Lott et al. (15) suggested a cutoff value of TSH ≥20 mIU/L for newborns older than 72 h for CH diagnosis. In our unit, 6 patients were diagnosed for CH during the study period. The TSH serum level of our patients on the 3rd day and during the 5-7th days were >20 mIU/L and >30 mIU/L, respectively. The TSH level of 1 out of the 6 patients diagnosed for CH was higher than 100 mIU/mL on the 3rd and 5th days of life with a diffuse goiter visible from the outside.

Prolonged jaundice is defined as jaundice persisting beyond 14 days of life in term neonates and beyond 21 days in preterm neonates (16). One of the pathological causes associated with prolonged jaundice is congenital hypothyroidism (16-19).

Agrawal V et al. (19), Najati N et al. (20), Sabzehei MK et al. (17), Boskabadi H et al. (21), and Cetinkaya et al. (22) studied the etiology of prolonged jaundice in newborns and demonstrated that the incidence of hypothyroidism was 4-8%. Further, 13% (n: 93) of our cases diagnosed as having prolonged jaundice were investigated for the underlying disease. Surprisingly, none of the infants had hypothyroidism. The FT4 and TSH levels measured during the 4-7$^{th}$ days of life are higher, but not as high as levels that necessitate treatment. The FT4 and TSH levels at other observation points were in the normal range.

The main limitation of our study is that it comprises a hospital-based population. Ideally, reference intervals should be determined using blood samples, obtained from a large cohort of healthy subjects. However, due to ethical and practical considerations, reference interval determination is usually performed on the basis of the hospital database by applying appropriate selection criteria. We addressed this concern by excluding all the subjects with diagnoses and concomitant medications that might affect the thyroid function. The other limitations are that the study is retrospective and the number of subjects is small.

In conclusion, it is known that neonatal reference intervals for thyroid function tests are different from older children and adults and should not be used interchangeably. It is important to diagnose CH in a timely manner due to long-term sequelaes. Therefore, we decided to evaluate retrospective data of our unit to determine the upper and lower limits for TSH and FT4 levels during the neonatal period at different observation points. We demonstrated that the TSH and FT4 levels change during the neonatal period without a significant sex difference. Both TSH and FT4 levels were prone to decrease with postnatal age; however, higher than adult levels have been reported in most studies.

**Ethics Committee Approval:** Ethics committee approval was received for this study from Gaziosmanpasa Taksim Research and Training Hospital Ethics Committee (Approval Date: 15.11.2017, Approval Number: 90).

**Informed Consent:** Informed consent is not necessary due to the retrospective nature of this study.

**Peer-review:** Externally peer-reviewed.

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Original Article

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# A Comparison of The Effects of Lidocaine and Saline Injection on Pain, Disability, and Shear-Wave Elastography Findings in Patients with Myofascial Trigger Points

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**BACKGROUND/AIMS**
To compare the effects of lidocaine injection (LI) and saline injection (SI) on the myofascial trigger points (MTrPs) in the trapezius muscle on pain, disability, and shear-wave elastography (SWE) in patients with myofascial pain syndrome (MPS). The secondary aim was to evaluate the correlations between SWE and pain with disability scores.

**MATERIAL and METHODS**
This prospective study included 45 patients with MTrPs due to MPS. The patients were evaluated using the visual analog scale (VAS), Neck Disability Index (NDI), and SWE immediately before and 15 days after the injections. The patients were randomly assigned to an LI (n=20, 30 MTrPs) or an SI (n=25, 32 MTrPs) group. The LI group was treated with lidocaine, and the SI group was treated with SI.

**RESULTS**
Visual analog scale and NDI scores improved significantly in both groups after injection ($p \leq 0.05$). In addition, 16 MTrPs in the LI group and 3 MTrPs in the SI group were completely resolved. Maximum shear-wave velocity (V(s)max) and mean shear-wave velocity significantly decreased in the SI group after injection ($p=0.025$). The size of MTrPs decreased in the LI group ($p=0.02$). Pre-injection V(s)max and resting VAS were weakly correlated ($r=0.309$). No significant correlation was found on other SWE measurements with VAS and NDI scores ($r<0.3$).

**CONCLUSION**
Lidocaine injection and SI effectively improved the disability and pain in patients with MPS. LI is more effective than SI in reducing the size of the trigger points and resolving MTrPs. SWE findings may not completely reflect the severity of pain and correlate with disability.

**Keywords:** Disability, pain, lidocaine injection, myofascial trigger points, shear-wave elastography

## INTRODUCTION

Myofascial pain syndrome (MPS) is a syndrome characterized by pain and accompanying muscle spasm, referring pain patterns, stiffness, restricted range of motion caused by myofascial trigger points (MTrPs) on constricted fibers of muscles, and/or fasciae (1). The prevalence of MPS varies from 21% to 30% (2). Detecting tenderness, taut bands, and MTrPs depends on the examiner's clinical skills (3). MPS treatment targets breaking down the chain reaction of "spasm–pain–spasm" and resolving MTrPs. Currently, different modalities, such as MTrPs injections, spray and stretch technique, or physiotherapy, can be used for treatment. These modalities inactivate the MTrPs with various effects (1). Lidocaine hydrochloride is a local anesthetic commonly used for treating pain due to MTrPs. It is a reversible blocker of conduction along the small nerve fibers carrying pain and autonomic impulses (4, 5).

Objective characterization and quantitative measurement of the properties of MTrPs can improve their localization, diagnosis, and treatment. Sonographic techniques can play a role in objectively identifying active MTrPs and detecting

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the improvement in outcome measurements after therapeutic interventions (6). Ultrasound (US) elastography is a new, low-cost, fast, and non-invasive technique assessing the stiffness of the lesions in real time. Several elastography techniques, including strain elastography and shear-wave elastography (SWE), are available (7-9). SWE is an objective method and uses shear waves produced by the interaction of the conventional US waves within the tissue (10). It allows direct assessment of elasticity and quantification of the soft tissue stiffness in real time. However, a few studies in the literature have evaluated MTrPs by SWE. This novel study compared lidocaine injection (LI) and saline injection (SI) treatment modalities by SWE.

The primary objective of the present study was to compare the improvement in pain, disability, and SWE measurements with LI and SI into the trapezius muscle with MTrPs. The secondary objective was to evaluate the correlations of SWE measurements with pain and disability scores.

## MATERIAL and METHODS

This prospective, randomized, controlled study was approved by the Baskent University Institutional Review Board and Ethics Committee (Project No.: KA16/179) and supported by the Baskent University Research Fund. Written informed consent was obtained from all of the patients.

Power analysis during the biostatistical preliminary assessment indicated a study population of 40 patients (20 patients for each group) with 95% confidence level and 80%power. Power analysis was performed using mean values of pain intensity according to the study by Ballyns et al. (6). All patients had active MTrPs on the upper part of the trapezius muscle on physical examination. The diagnostic criteria by Simons and Travell were used for diagnosing MPS (11). Simons and Travell's criteria take into account the existence of MTrPs during muscle palpation and restriction in compromised muscles.

Exclusion criteria were as follows: mental retardation, local anesthetic allergy, bleeding diathesis, cervical and/or thoracic disk herniation, radiculopathy, and receiving any drug-targeting pain (e.g., nonsteroidal anti-inflammatory drugs (NSAIDs), paracetamol, and myorelaxant drugs). Patients with injection treatment, manual therapy, massage, or any interventions into the MTrPs within the last 2 months were also excluded from the study.

A total of 66 patients with MPS who were admitted to the physical medicine and rehabilitation outpatient clinic with neck and/or back pain were enrolled in the study. A physiatrist diagnosed the patients enrolled in the study (researcher 1). A flowchart of the study population is shown in Figure 1. Eight patients were excluded from the present study for some reasons. The remaining 58 patients were allocated to two groups as LI group and SI group. The patients in the LI group were treated with 0.5 mL lidocaine without epinephrine (5 mg/mL), and the patients in the SI group were treated with 0.5 mL saline (0.9% NaCl) injection. A radiologist blinded to the nature of the study selected the patients by drawing lots and randomly assigned 29 patients to each group (researcher 2). Four patients in the SI group and nine patients in the LI group were excluded from the study for reasons including receiving steroid injections into the MTrPs, could not be reached for control evaluation, performing massage or manual therapy into the MTrPs, or using NSAIDs.

Finally, 20 patients (30 MTrPs) in the LI group and 25 patients (32 MTrPs) in the SI group were included in the study. The radiologist performing SWE and measuring outcomes (visual analog scale (VAS) and Neck Disability Index (NDI)) was unaware of the groups (researcher 2). In addition, data collection was performed by another radiologist blind to the groups (researcher 3).

### Outcomes

#### Visual analog scale

The severity of pain during rest, night, and physical activity was assessed by VAS. VAS is a scale evaluating subjective pain intensity from 0 to 10. Studies have shown that VAS is a reliable and valuable method for evaluating MTrPs (12, 13).

#### Neck disability index

Neck Disability Index was designed for assessing neck pain and disability. It contains 10 self-reported items, including pain intensity, personal care, lifting, reading, headache, concentration, work, driving, sleeping, and recreation. Each item is scored on a 6-point scale from 0 (no disability) to 5 (full disability). The sum score of all 10 items is calculated using a percentage of the maximal score, with higher values representing greater disability (14). The total score ranges from 0 (no disability) to 50 (total disability) or, in percentage terms, between 0 and 100. The modified Turkish version of NDI was used in the present study. Kesiktas et al. (12) demonstrated that this version is a reliable and valid test suitable for daily practice.

#### Shear-wave elastography

Shear-wave elastography was performed by a blind radiologist experienced in musculoskeletal imaging. The US elastography evaluations were performed using a US system (Acuson S2000; Siemens, Erlangen, Germany) with a linear transducer that enabled scanning with a frequency ranging from 4 to 9 MHz. Elastography images were obtained using a freehand technique at the same time as US. US elastography was performed when the patient was in a prone position with arms resting alongside their trunk. The transducer was held in the transverse plane over the trapezius muscle. Sikdar et al. (13) defined MTrPs as focal hypoechoic areas with heterogeneous echotexture on B-mode US. Their criteria were used, but both latent and active MTrPs might have the same imaging characteristics. Thus, the lesion was gently palpated to confirm tenderness and referring pain. B-mode US and SWE were performed for each lesion. The localization of the active MTrPs was marked on the skin before injection. The marked areas were photographed for assessing the same areas 15 days after injection. Two to four regions of interest (ROIs) were calculated due to the size of the lesions (Figure 2). In addition, 1.5×1.5 $mm^2$ box-shaped standard ROIs automatically provided by the US system were used. Application of high pressure was avoided. The qualities of the images were assessed by color-coded quality maps provided by the US system. The color-coded green areas in the maps were considered reliable, whereas yellow and red color-coded areas were considered low-quality scans. All scans were repeated at least three times in MTrPs, and the best representative image with the highest quality on the quality map was selected. Qualitative

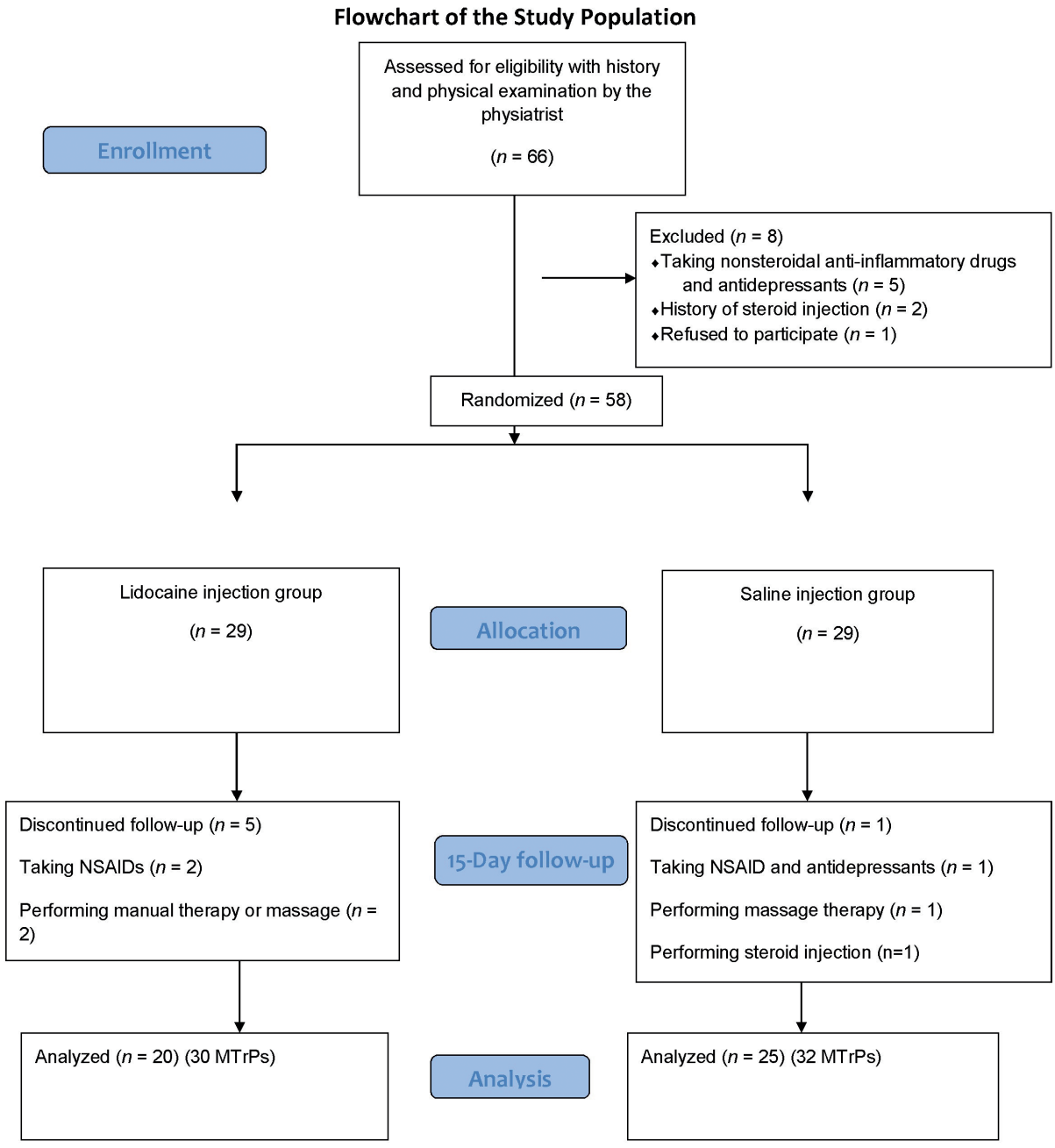


**FIGURE I.** Flowchart of the study population

color-coded elastography images and quantitative maps measuring shear-wave velocity were obtained. Mean and maximum shear-wave velocities (*V(s)mean* and *V(s)max*) were assessed. All of the images were evaluated at a workstation (Synapse version 4.0; Fujifilm Medical Systems Inc., CT, USA).

**Injections**

Baseline SWE was performed before the injection by a radiologist who was blind to the groups. The MTrPs were marked on the skin, and the depth of the lesion was noted by the performing radiologist. US guidance was used for injection into the trigger point. In the LI group, 0.5 mL of 0.5% lidocaine hydrochloride without epinephrine (5 mg/mL) was injected into the MTrPs by a single physiatrist. Antisepsis was performed using 10% polyvinylpyrrolidone iodine (Batticonol, DERMOSEPT), and a sterile needle with a thickness of 26 gauge ×1/2 (0.45×13 $mm^2$) was inserted into the MTrPs at an angle of 30° with respect to the skin. The needle was aspirated before injecting a small amount of injection solution to ensure that the needle was not in a blood vessel. Then, the needle was withdrawn to the subcutaneous tissue into the MTrPs. In the SI group, 0.5 mL saline (0.9%NaCl) was injected to the MTrPs in the same way. The same physiatrist (researcher I) prepared the injection materials and injected them into the MTrPs of the patients. The patients in both the groups were blind to the SI or LI materials.

The patients were instructed not to use any drugs or interventions targeting pain or preventing other symptoms during the study. All of the patients were re-evaluated 15 days after the intervention.

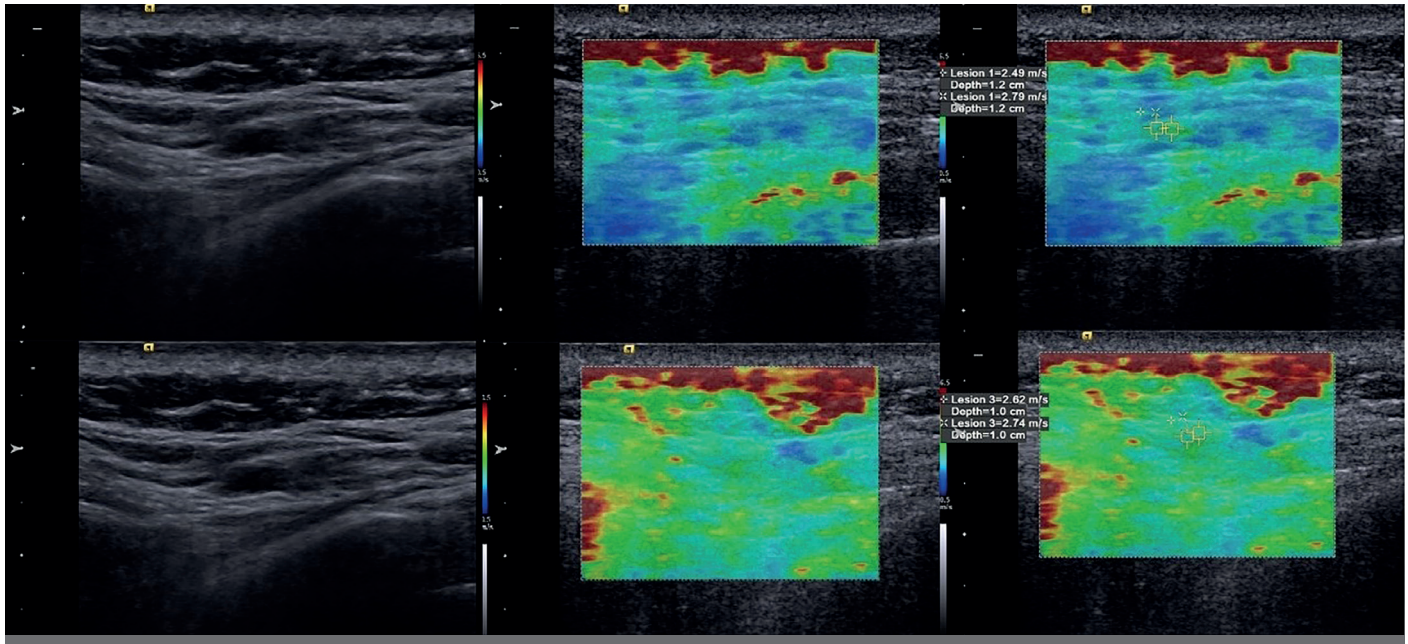


**FIGURE 2.** A 35-year-old man with active MTrP within the trapezius muscle. Upper row demonstrates pretreatment US and SWE images. Lower row shows US and SWE images after saline injection treatment

**Data and Statistical Analysis**

Statistical analysis was performed using the Statistical Package for the Social Sciences software version 17.0 (SPSS Inc., Chicago, IL, USA). If continuous variables were normal, they were described as mean±standard deviation (p>0.05 in Kolmogorov–Smirnov or Shapira–Wilk test (n<30)); and if they were non-normal, they were described as median. The Student's *t* test was used for comparisons between the groups with normally distributed data, and the Mann–Whitney *U* test was used for comparisons between the groups with non-normally distributed data. The Wilcoxon rank-sum test was used to analyze pre- and post-intervention measures. Pearson's chi-square and Fisher's exact tests were used for comparison of categorical variables. A p value ≤0.05 was considered statistically significant.

Correlations were tested using the Pearson's correlation test, and the correlation coefficients were interpreted as either excellent (r≥0.91), good (0.90≥r≥0.71), fair (0.70≥r≥0.51), weak (0.50≥r≥0.31), or little or none (r≤0.3). A p value ≤0.05 was considered statistically significant. Type I and 2 error risks were kept at the minimum level by applying the appropriate power analysis during the biostatistical preliminary assessment stage.

## RESULTS

The LI and SI groups included 30 and 32 MTrPs, respectively. The mean age, gender, and affected dominant extremities did not show statistically significant difference between the groups (p>0.05) (Table I). When we compared the patients who attended and who did not attend the treatment in each group, the result is not statistically significant (p>0.05).

Pre-intervention values of the VAS and NDI and the SWE measurements were similar for both the groups. The only exception was pretreatment size of the MTrPs. It was found to be higher in the LI group (p=0.02). VAS and NDI scores were significantly improved with both LI and SI injections (p≤0.05).

**TABLE I.** Clinical characteristics of the study population

| Characteristics | Group I (n: 20) | Group 2 (n: 25) | p |
|---|---|---|---|
| Age (years) (mean±SD) | 45.6±13.22 | 42.4±11.16 | 0.783 |
| Gender (Females/Males) (%) | 13/7 (65/35) | 19/6 (76/24) | 0.604 |
| Dominant extremity (right/left) (%) | 18/2 (90/10) | 24/1 (96/4) | 0.415 |
| Affected extremity (right/left/bilateral) (%) | 7/7/6 (35/35/30) | 8/14/3 (32/56/12) | 0.237 |

SD: standard deviation; Group I: lidocaine injection+dry needling; Group 2: saline injection+dry needling

A comparison of the pre- and post-injection SWE showed that *V(s)max* and *V(s)mean* significantly decreased in the SI group (p=0.022 and p=0.025, respectively), but they did not change significantly in the LI group (p≥0.05). In addition, 14 of MTrPs in the LI group and 3 of the MTrPs in the SI group were completely resolved after injection. One of the MTrPs was very superficial and did not provide the distance to place the box of ROI after treatment in the SI group.

A comparison of the post-intervention outcome parameters revealed that VAS, NDI, and all of the SWE measurements of the post-treatment period were similar for the groups (p>0.05). Pre- and post-treatment VAS and NDI results are shown in Table 2. B-mode US and SWE results are summarized in Table 3. In addition, *V(s)max* and resting VAS were found to be weakly correlated on evaluating the correlation of VAS and NDI with *V(s)max* and *V(s)mean* (r=0.309). No significant correlation of other SWE measurements with VAS and NDI scores was found (r<0.3, p>0.05). There were no serious side effects following injections including prolonged bleeding and signs of an allergic reaction, such as difficulty breathing or facial swelling. Two patients had moderate pain at the injection side, and one patient had redness, swelling, or warmth at the injection site without swelling or pain.

**TABLE 2.** Pain and disability outcome measurements of the study population (mean±standard deviation)

| Characteristics | Group I | Group 2 | p |
|---|---|---|---|
| Neck Disability Index (%) | | | |
| Before treatment | 28.3±11.9 | 27.36±17.97 | 0.842 |
| After treatment | 16.3±16.26 | 21.5±18.93 | 0.339 |
| p* | 0.001 | 0.022 | |
| VAS/rest | | | |
| Before treatment | 6.3±2.81 | 6.12±2.11 | 0.807 |
| After treatment | 4.5±3.41 | 4.44±2.97 | 0.950 |
| p* | 0.043 | 0.004 | |
| VAS/night | | | |
| Before treatment | 6.65±3.32 | 6.84±3.11 | 0.845 |
| After treatment | 4.4±3.79 | 4.88±4.05 | 0.687 |
| p* | 0.013 | 0.009 | |
| VAS/physical activity | | | |
| Before treatment | 6.4±3.31 | 6.32±3.65 | 0.940 |
| After treatment | 4.5±3.12 | 4.48±3.51 | 0.984 |
| p* | 0.013 | 0.010 | |

p*: Wilcoxon signed-rank test
VAS: Visual Analog Scale; Group I: lidocaine injection+dry needling; Group 2: saline injection+dry needling

**TABLE 3.** B-mode ultrasound and shear-wave elastography measurements of the study

| Characteristics | Group I | Group 2 | p |
|---|---|---|---|
| SWV mean (m/s) | | | |
| Before treatment | 3.17±0.81 | 3.66±1.56 | 0.131 |
| After treatment | 3.22±1.33 | 2.91±0.84 | 0.338 |
| p* | 0.981 | 0.022 | |
| SWVmax (m/s) | | | |
| Before treatment | 3.29±0.92 | 3.82±1.64 | 0.123 |
| After treatment | 3.27±1.32 | 3.07±0.95 | 0.548 |
| p* | 0.913 | 0.025 | |
| Size of MTrPs (mm) | | | |
| Before treatment | 7.79±2.78 | 5.65±2.44 | 0.002 |
| After treatment | 5.61±2.26 | 5.11±3.49 | 0.59 |
| p* | 0.002 | 0.13 | |

p*: Wilcoxon signed-rank test
SWV: shear-wave velocity; SWVmax: maximum shear-wave velocity; Group I: lidocaine injection+dry needling; Group 2: saline injection+dry needling

## DISCUSSION

The aim of the present study was to compare the effects of LI and SI injections into the MTrPs in the trapezius muscle on pain, disability, and SWE findings in patients with MPS. The secondary aim was to evaluate the correlations between SWE results, pain, and disability scores. Pain and disability due to MTrPs were improved with both SI and LI treatments. However, LI did not appear to be more effective than SI in improving pain and disability. Based on these data, it was thought that patients with MTrPs could be treated with lidocaine for improving pain and disability. Lugo et al. (2) investigated LI and physiotherapy, alone or in combination, in patients with MTrPs. They found no difference in pain and quality of life between these treatments.

Although the exact etiology of MTrPs is unknown, one theory is that chronic muscle overuse leads to inflammation (15). Moreover, histological changes within the muscle in response to pain may correspond to changes in mechanical properties. Ballyns et al. (16) showed that *V(s)* is significantly higher in active MTrPs and the surrounding tissue than in the normal tissue. Clinical application of elastography may provide an objective assessment for identifying MTrPs and detecting their changes (15-18). US elastography is a useful tool for monitoring response to injections into trigger points (19). The present study found that pretreatment sizes of the MTrPs in the LI group were significantly higher, but they significantly reduced after treatment. However, the size of MTrPs did not reduce significantly in the SI group after treatment. After injection, more MTrPs in the LI group than those in the SI group were completely resolved. Based on these data, it is proposed that LI injection may be a good option in the local treatment of MTrPs. On the contrary, *V(s)* significantly improved only in the SI group in the present study.

Despite the application of SWE to healthy human skeletal muscles, its use in assessing the treatment of MTrPs with injections has not been investigated adequately. This novel study compared the effects of LI and SI treatment modalities using SWE. Maher et al. (14) evaluated the effects of dry needling and posture on MTrPs using SWE. Seven women with palpable MTrPs in the upper trapezius muscle were investigated in the study. They showed a reduction in the shear modulus after dry needling and a significant difference between prone and upright positions while sitting. They proposed that SWE detected the changes in MTrPs (14). The present study found that some SWE measurements (presence of MTrPs, size of MTrPs, *V(s)mean*, and *V(s)max*) changed with injections. Thus, the results agreed with the findings of Maher et al. (14) on the feasibility of SWE for detecting the changes in MTrPs.

Müller et al. (20) designed a double-blind, randomized, controlled, pilot study for evaluating the effects of acupuncture (AC) and electroacupuncture (EA) in women with painful MTrPs in the upper trapezius muscle using two-dimensional US and US elastography. They found that EA treatment decreased general and local pain intensity, whereas only general pain was decreased in the AC group (20). The post-treatment strain ratios did not change between the groups.

Moreover, a weak correlation was found between pretreatment *V(s)max* and resting VAS. Other outcome measurements were not correlated with each other, although the size of MTrPs is a very good classifier of the site type in the upper trapezius muscle and also quick and simple to implement using clinical sonography. Ballyns et al. (16) did not find a correlation between trigger point size and pain pressure threshold score. They suggested that other mechanisms could be contributing independently to the trigger point size and pain sensitivity (16). No correlation was found between MTrPs size and VAS scores, consistent with the present results. In addition, it was thought that SWE measurements (except *V(s)max*) could be affected by more complicated mechanisms beyond functional outcomes.

Pretreatment VAS, NDI, and SWE measurements were used for correlation analysis because injection treatments could affect these outcomes. Some patients had multiple MTrPs in the present study. When the VAS and NDI are used once in patients with multiple MTrPs, it is difficult to differentiate which trigger point is being reflected. This limitation might have affected the present correlation analysis. In addition, there is another technical difficulty that must be mentioned. MTrP in one patient was very superficial and did not provide the distance to place the box of ROI to measure *V(s)*. The use of gel pads would be better in such conditions (21). SWE provides a quantitative measure of the lesion stiffness obtained by either in a small fixed ROI (single measurement) or pixel-by-pixel in a field-of-view box giving a color map (22). Tissue stiffness measurements in an ROI can be displayed in speed (m/s) or in pressure/elasticity (kPa) depending on the commercially available different US units. As the velocity increases, it suggests increased tissue stiffness.

The first limitation of the present study was that pain was scored by VAS, which is a patient-dependent subjective method. Compared with VAS, algometers are more objective tools for evaluating pain intensity. Algometric evaluation methods are designed to measure deep pressure pain thresholds or tenderness resistance. Hence, algometers can be a more useful option for assessing the treatment effects in these cases (23). The outpatient clinic involved in the present study did not have an algometer. Therefore, the only option was to assess the pain intensity by VAS. Another limitation of the study is that all the elastographic measurements were performed by a single radiologist. It would be better to assess the intrarater reliability of the elastography. Other limitations of the present study included the short duration of the follow-up period and the limited number of patients. Further studies with larger sample sizes and longer follow-up periods are required to independently confirm the present findings. However, the study included patients with multiple MTrPs. Therefore, VAS and NDI results were not completely correlated with SWE measurements. SWE directly measures each MTrP separately; however, VAS and NDI generally evaluate pain regardless of the number of MTrPs. When VAS and NDI are used once in patients with multiple MTrPs, it is difficult to differentiate which trigger point is being reflected. Algometers can be more specific tools in patients with multiple MTrPs and can specifically evaluate each MTrPs. Thus, algometer and SWE measurement correlations can be good evaluation methods for correlation analysis in further studies. Evaluation of patients with single MTrPs can be a second option. In addition, adding another group with just dry needling should be better to compare the effectiveness of these two treatment modalities.

In conclusion, LI is a useful option for short-term treatment of patients with MPS with neck and/or back pain due to the MTrPs in the trapezius muscle. It can be used to reduce pain, disability, and trigger point size and to resolve trigger points. LI and SI effectively improve pain and disability in MPS. SWE can be used for assessing the size of these painful trigger points. However, SWE measurements may not completely reflect the severity of pain and disability in patients with MTrPs due to MPS.

**Ethics Committee Approval:** Ethics committee approval was received for this study from Baskent University Institutional Review Board and Ethics Committee (Approval Date: 16.06.2016, Approval Number: 16/66).

**Informed Consent:** Informed consent was obtained from the patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept - S.T.U., P.D.A., H.A.; Design - S.T.U., P.D.A.; Supervision - S.T.U.; Resource - P.D.A., H.A.; Materials - S.T.U., P.D.A., H.A.; Data Collection and/or Processing - S.T.U., H.A.; Analysis and/or Interpretation - P.D.A., H.A.; Literature Search - P.D.A., H.A.; Writing - S.T.U., P.D.A., H.A.; Critical Reviews - H.A., S.T.U.

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CYPRUS JOURNAL OF MEDICAL SCIENCES

Original Article

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# Glasgow Prognostic Score is a Useful Predictive Factor for Palliative Surgery Outcomes in Advanced-stage Gastric Cancer

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**BACKGROUND/AIMS**
More than one million new gastric cancer cases have been reported in 2018. In many countries other than those in the Far East, gastric cancer could not be diagnosed at an early stage. Surgical options are limited in advanced-stage gastric cancer, and physicians have to make the right decision for the prolongation of patient survival. Surgery can prolong the survival even in advanced disease if the appropriate patient population is selected. The Glasgow prognostic score (GPS) was validated as a predictor of the prognosis in several cancer types. In this study, we aimed to test the hypothesis that GPS is useful to select the most suitable patients for surgical intervention in advanced-stage gastric cancer.

**MATERIAL and METHODS**
Data of 632 gastric cancer patients operated in our institute were investigated in this retrospective study. Eighty-four patients with gastric cancer who underwent palliative surgery and had complete clinical and follow-up data were included in this study.

**RESULTS**
Albumin levels were low in 46 patients. Forty-eight patients had high C-reactive protein (CRP) levels. Palliative gastrectomy was performed in 43 of 84 patients. Patients with a GPS of 2 survived a median 3 (95% confidence interval [CI]: 0.89-5.11) months, GPS of 1 for a median of 7 (95% CI: 4.50-9.50) months, and GPS of 0 for a median of 8 (95% CI: 3.31-12.70) months (p=0.047). Patients with modified GPS scores of 2 survived for a median of 3 (95% CI: 0.89-5.11) months, mGPS of 1 for a median of 3 (95% CI: 0.55-7.45) months, and mGPS of 0 for a median 8 (95% CI: 5.65-10.35) months (p=0.012). The mGPS values of patients with palliative gastric resection were compared; patients with mGPS of 0 had significantly longer survival times than those with mGPS 1 and 2.

**CONCLUSION**
The GPS and mGPS can be calculated prior to surgery using non-invasive and easily available laboratory tests. It has been shown in this study and other previous studies that mGPS can be particularly used easily to predict the prognosis in advanced-stage gastric cancer.

**Keywords:** Gastric cancer, prognosis, palliative surgery, glasgow prognostic score

## INTRODUCTION

Gastric cancer is the third most common cause of cancer-related deaths. More than a million new gastric cancer patients were diagnosed in 2018 worldwide (1). In countries where routine screening is not performed, cases are unfortunately diagnosed in the late stage (2).

Curative surgery cannot be performed in patients with non-resectable tumor or peritoneal spread and/or multiple solid organ metastases. Patients often undergo palliative surgical intervention followed by chemotherapy. Although all patients in this group have advanced-stage disease, the survival time varies.

Recent studies have demonstrated that increased systemic inflammation in patients with advanced solid cancer is related to weight loss, decreased performance, and reduced survival (3-5).

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Abnormalities in C-reactive protein (CRP) and albumin levels are considered indicators of systemic inflammation. In 2005, the Glasgow Prognostic Score (GPS), which is calculated using (CRP) and albumin values, was introduced (6). GPS was validated in prognosis studies of advanced-stage solid organ cancers (7, 8). The purpose of the present study was to test the hypothesis that GPS is useful for predicting prognosis in gastric cancer patients undergoing palliative surgery.

## PATIENTS and METHODS

Ethical approval was obtained from the ethical committee of Dokuz Eylul University medical study with the date and number of 2019/13-39. Oral informed consent was obtained from all the patients.

### Patients

In total, data of 632 patients with gastric cancer who underwent curative surgical resection or palliative surgery at the Department of Surgery, Dokuz Eylul University Hospital, from September 2006 to December 2018 were retrospectively evaluated. Patients who died within 30 days of surgery, those with infection other malignancies, and those who received neoadjuvant chemotherapy that might have affected the CRP levels were excluded from this study. We excluded 53 of included 137 palliative surgery patients from the study due to incomplete clinical data. The remaining 84 patients included in the study had adequate clinical information and follow-up data.

### Preoperative Radiological Evaluation

The patients were evaluated through a computerized tomography (CT) examination of the thorax and whole abdomen in the preoperative period. Investigations were performed using 16-section or 64section multidetector CT devices (Brilliance 16 or Brilliance 64, Philips Medical Systems, Eindhoven, Holland). In contrast-enhanced CT examinations, iodinated contrast agents were applied at a rate of 2 mL/kg (at least 100 mL). Arterial and portal phase images were obtained.

The computed tomography images were used for systemic evaluation of the following parameters: localization and extension of the primary tumor, possible tumor invasion, perigastric or retroperitoneal lymph node mapping, intra-abdominal suspicious implant, and distant lymph node or organ/structure metastases.

### Surgical Procedure

Palliative gastrectomy was defined as the resection of the primary gastric lesion with or without regional lymph node dissection in patients with metastatic disease.

In this procedure, the resection of lesions in peritoneal implants and residual metastases of nonresectable lymph nodes, liver, or distant organs, was not performed. Gastrojejunostomy (GJ) for oral intake or placement of a feeding jejunostomy tube for enteral feeding was performed in nonresectable locally advanced gastric cancer.

### Statistical Analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences version 22.0 software (IBM Corp.; Armonk, NY, USA). Age, gender, surgical method, preoperative disease stage, albumin, and CRP values, survival data were obtained. GPS and modified GPS (mGPS) were calculated (Table 1, 2). Kaplan–Meier survival analysis was applied to determine the factors affecting survival. Also, GPS and mGPS for predicting the prognosis were validated using the Kaplan–Meier survival analysis. Receiver Operating Characteristic (ROC) analysis was performed for GPS and mGPS.

## RESULTS

The median age of the 84 patients was 63.5 years. The male/female ratio was 3/2. Palliative gastrectomy was performed

**TABLE 1.** GPS

| | Score |
|---|---|
| CRP≤10 mg/L and albumin ≥3.5 g/dL | 0 |
| CRP≤10 mg/L and albumin <3.5 g/dL | 1 |
| CRP>10 mg/L and albumin ≥3.5 g/dL | 1 |
| CRP>10 mg/L and albumin <3.5 g/dL | 2 |

GPS: Glasgow Prognostic Score; CRP: C-reactive protein

**TABLE 2.** Modified GPS

| | Score |
|---|---|
| CRP≤10 mg/L and albumin ≥3.5 g/dL | 0 |
| CRP≤10 mg/L and albumin <3.5 g/dL | 0 |
| CRP>10 mg/L and albumin ≥3.5 g/dL | 1 |
| CRP>10 mg/L and albumin <3.5 g/dL | 2 |

GPS: Glasgow Prognostic Score; CRP: C-reactive protein

**TABLE 3.** Patient characteristics and survival: Univariate analysis

| | Patients (100%) | Survival, months (median, 95% CI) | p |
|---|---|---|---|
| Age, years | | | 0.860 |
| <65 | 46 (54) | 7 (4.64-9.36) | |
| >65 | 38 (46) | 5 (3.30-6.71) | |
| Gender | | | 0.717 |
| Female | 34 (40) | 4 (1.15-6.85) | |
| Male | 50 (60) | 7 (4.66-9.44) | |
| Albumin | | | 0.204 |
| ≥3.5 g/dL | 38 (46) | 8 (4.68-11.32) | |
| <3.5 g/dL | 46 (54) | 5 (3.14-6.86) | |
| CRP | | | *0.003* |
| ≤10 mg/L | 36 (42) | 8 (5.65-10.35) | |
| >10 mg/L | 48 (58) | 3 (1.18-4.82) | |
| GPS | | | *0.031* |
| 0 | 23 (27) | 8 (3.31-12.70) | |
| 1 | 28 (34) | 7 (4.50-9.50) | |
| 2 | 33 (39) | 3 (0.89-5.11) | |

GPS: Glasgow Prognostic Score; CRP: C-reactive protein; CI: confidence interval

in 43 patients. The remaining patients underwent GJ bypass and/or feeding tube placement surgeries. Albumin levels were low in 46 patients (<3.5 g/dL). Forty-eight patients had high CRP (CRP>10 mg/L) levels. In the univariate analysis, GPS values were found to be effective in predicting prognosis (Table 3).

**TABLE 4.** Modified GPS and survival: Univariate Analysis

| | Patients (100%) | Survival, months (median, 95% CI) | p |
|---|---|---|---|
| mGPS | | | *0.011* |
| 0 | 36 (42) | 8 (5.65-10.35) | |
| 1 | 15 (19) | 3 (0.55-7.45) | |
| 2 | 33 (39) | 3 (0.89-5.11) | |

mGPS: modified Glasgow Prognostic Score; CI: confidence interval

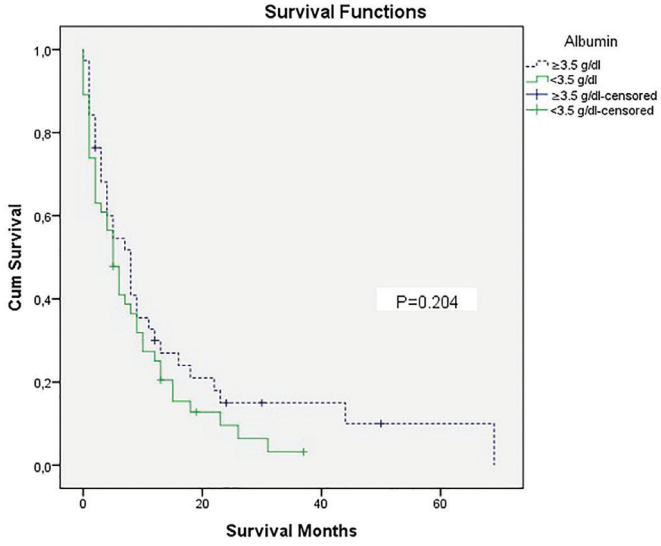



**FIGURE 1.** Kaplan–Meier survival curves of patients with gastric cancer who underwent palliative surgery according to albumin levels (p=0.204). The p value was calculated using the log-rank test

When the survival rates were examined, 9 patients were known to be alive. The median survival was 21.5 (6-50) months for the alive patients. The median survival was 5 (1-69) months for the other patients.

With regard to albumin values, patients with albumin levels ≥3.5 g/dL survived for a median of 8 (95% confidence interval [CI]: 4.68-11.32) months, and those with albumin <3.5 g/dL patients survived for a median 5 (95% CI: 3.14-6.86) months in the postoperative period (Figure 1). With regard to CRP values, patients with CRP≤10 mg/L survived for a median 8 (95% CI: 5.65-10.35) months, and those with CRP>10 mg/L survived for a median 3 (95% CI: 1.18-4.82) months in the postoperative period (Figure 2).

The GPS scores were compared, and patients with GPS 2 survived for a median 3 (95% CI: 0.89-5.11) months, GPS 1 for a median of 7 (95% CI: 4.50-9.50) months, and GPS 0 for a median of 8 (95% CI: 3.31-12.70) months (p=0.047; Figure 3). The area under the curve (AUC) in the ROC analysis was 0.613 for GPS (Figure 4).

Patients with mGPS 2 survived for a median of 3 (95% CI: 0.89-5.11) months, those with mGPS 1 for a median of 3 (95% CI: 0.55-7.45) months, and those with mGPS 0 for a median of 8 (95% CI: 5.65-10.35) months (p=0.012; Table 4; Figure 5). The AUC in the ROC analysis was 0.628 for the mGPS (Figure 6).

The mGPS values of patients with palliative gastric resection were examined, and those with mGPS=0 had significantly longer survival times compared to those with mGPS 1 and 2 (Figure 7).

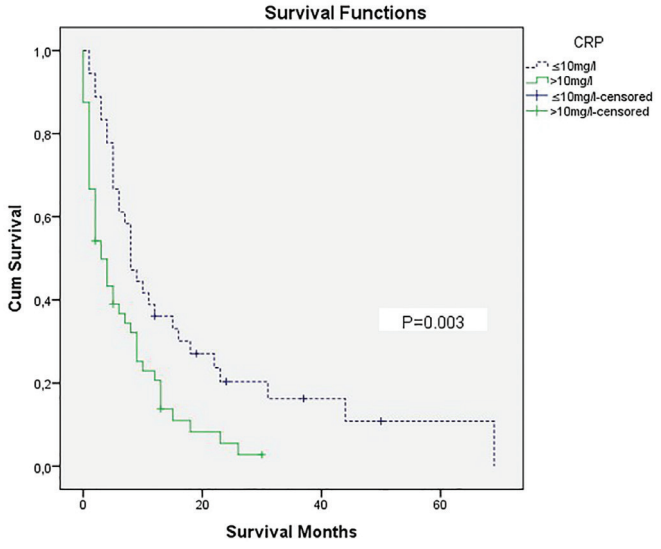



**FIGURE 2.** Kaplan–Meier survival curves of patients with gastric cancer who underwent palliative surgery according to the CRP levels (p=0.003). The p value was calculated using the log-rank test. CRP, C reactive protein

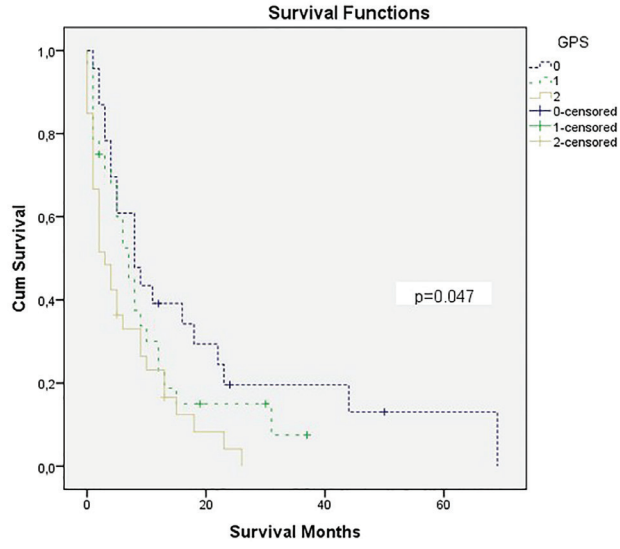



**FIGURE 3.** Kaplan–Meier survival curves of patients with gastric cancer who underwent palliative surgery according to GPS (p=0.047). The p value was calculated using the log-rank test. GPS, Glasgow Prognostic Score

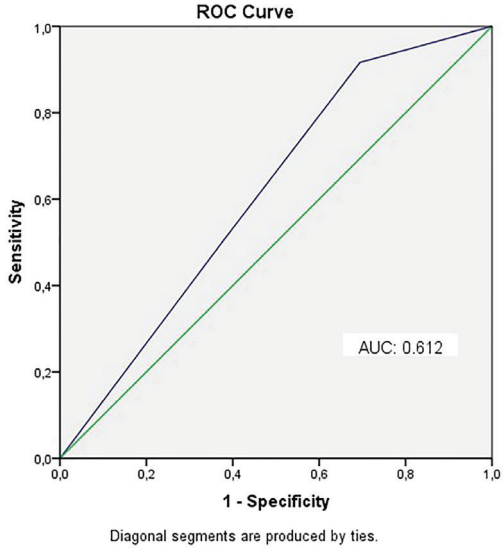



**FIGURE 4.** ROC curves of GPS of patients with gastric cancer with palliative surgery. The AUC of GPS was 0.612. GPS, Glasgow Prognostic Score; ROC, receiver operating characteristics; AUC, area under the curve

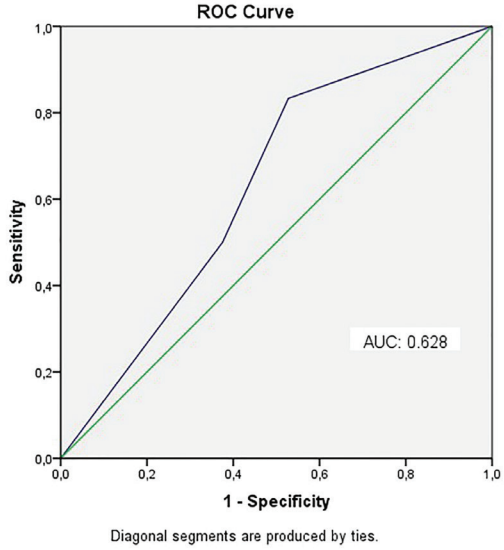



**FIGURE 6.** ROC curves of mGPS of patients with gastric cancer who underwent palliative surgery. The AUC of mGPS was 0.628. mGPS, modified Glasgow Prognostic Score; ROC, receiver operating characteristics; AUC, area under the curve

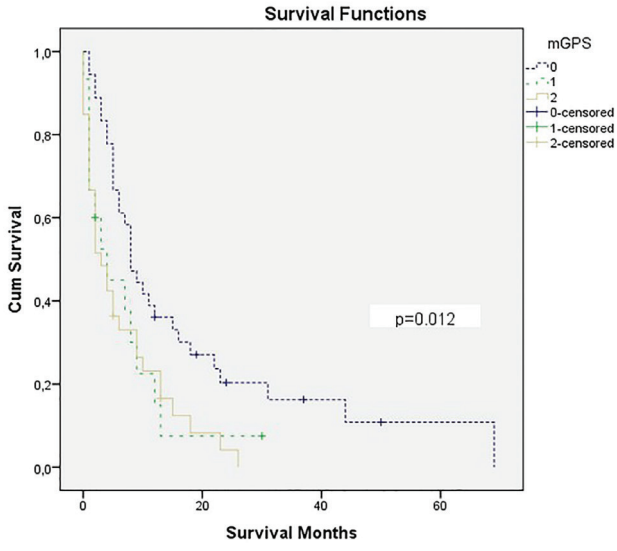
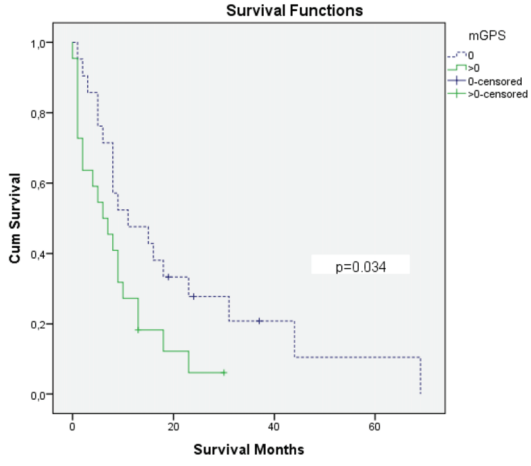



**FIGURE 5.** Kaplan–Meier survival curves of patients with gastric cancer who underwent palliative surgery according to mGPS (p=0.012). The p value was calculated using the log-rank test. mGPS, modified Glasgow Prognostic Score




**Means and Medians for Survival Time**

| | Mean[a] | | | | Median | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 95% Confidence Interval | | | | 95% Confidence Interval | |
| VAR00002 | Estimate | Std. Error | Lower Bound | Upper Bound | Estimate | Std. Error | Lower Bound | Upper Bound |
| 0 | 20.678 | 4.960 | 10.957 | 30.399 | 11.000 | 5.340 | 0.533 | 21.467 |
| >0 | 8.530 | 1.802 | 4.998 | 12.062 | 6.000 | 2.345 | 1.403 | 10.597 |
| Overall | 15.145 | 3.034 | 9.199 | 21.092 | 8.000 | 0.937 | 6.164 | 9.836 |

[a]Estimation is limited to the largest survival time if it is censored

**FIGURE 7.** Kaplan–Meier survival curves of patients with gastric cancer who underwent palliative gastrectomy according to mGPS (mGPS=0 and mGPS>0; p=0.034). The p value was calculated using the log-rank test. mGPS, modified Glasgow Prognostic Score

## DISCUSSION

Palliative surgical interventions in stage IV gastric cancer are performed if obstruction, bleeding, mass compression, intense acid, and perforation are observed. Although there is no consensus yet, there are reports that a combination of palliative resection and chemotherapy prolongs the survival in stage IV gastric cancer (9). However, some criteria should be considered to provide the expected benefit in these applications where patient selection is important. Parameters, such as the presence of comorbid diseases and general performance scores, prior to surgical intervention will guide in this regard. In contrast, cancer biology directly affects prognosis. Inflammation, which is considered the response (the host) to cancer and is frequently the focus of several studies in recent years, has been defined as a parameter of prognostic significance, particularly for advanced-stage cancer (10). GPS using the elevation in the CRP value and a decrease in albumin value, which are considered to be indicators of increased inflammation, were reported by Forrest et al. This scoring system was first described to predict the prognosis of inoperable non-small cell lung cancers. GPS used for estimating the prognosis was examined in various cancers and found beneficial (7, 8, 11).

When the results of the current study were evaluated, it was observed that the decrease in albumin and the increase in CRP values were found to be associated with shorter survival times (Table 3; Figure 1, 2). GPS using these parameters was found to be effective in predicting the prognosis in advanced gastric cancer (Figure 3). These results were consistent with those of Elahi and Mimatsu studies.

In our study, albumin decrease without CRP elevation was associated with shortened survival, but no statistically significant result was found. It was believed that the low levels of albumin alone could be caused by loss of appetite and relative malnutrition in the preoperative period rather than being the marker of the inflammatory process. The AUC in the ROC analysis for the GPS and the mGPS were 0.613 and 0.628, respectively (Figure 4-6). These results were similar to those of other validation studies for predicting prognosis in cancer patients using GPS and mGPS (8, 12, 13).

When the mGPS scores of the patients who underwent palliative gastric resection were examined in terms of survival, patients with an mGPS of 0 had a mean 20.68 (±4.96) months of survival and those with mGPS>0 had a mean of 8.53 (±1.80) months of survival (p=0.034). In other words, as mGPS scores increased, the prognosis was shorter (Figure 5). When the results of this study were evaluated together with the arguments of the researchers who suggested that palliative gastric resection for patients with stage IV gastric carcinoma would contribute positively to the prognosis, it was thought that a significant increase in the survival rate could be achieved by selecting appropriate patients with an mGPS of 0 and having other positive prognostic markers.

The number of patients and retrospective design are the most important limitations fort his study. Although the single-center design of the study benefits in terms of the homogeneity of the data, it can be considered a limitation due to a lower number of patients.

The mGPS can be calculated prior to surgery using non-invasive and easily available laboratory tests. Particularly, in advanced-stage gastric cancer, it can be easily used for predicting the prognosis of patients and choosing the treatment to be applied with other known prognostic markers.

**Ethics Committee Approval:** Ethics committee approval was received for this study from Dokuz Eylul University Ethical Committee. (Approval Date: 22.05.2019, Approval Number: 2019/13-39).

**Informed Consent:** Informed consent was obtained from the patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept - S.D., T.E., K.A.; Design - S.D., T.E., A.Ç., I.B., S.Ü.; Supervision - K.A., Ö.S.; Resource - K.A., Ö.S., I.B., S.Ü., S.D.; Materials - S.D., I.B., S.Ü., Ö.S.; Data Collection and/or Processing - S.D., A.Ç., T.E., I.B.; Analysis and/or Interpretation - S.D., A.Ç., T.E., I.B., S.Ü.; Literature Search - S.D., I.B., T.E., A.Ç., S.Ü.; Writing - S.D., I.B., T.E., A.Ç.; Critical Reviews - K.A., Ö.S.

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Original Article

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# Knowledge of Nursing Students about The Pre-Analytical Phase in Laboratory Analyses

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**BACKGROUND/AIMS**
The aim of the present study was to determine the knowledge of the final year students of the nursing department about the pre-analytical phase in the laboratory analysis.

**MATERIAL and METHODS**
This was a descriptive study conducted during the spring semester of 2017–2018 academic year. A total of 142 volunteer final year nursing department students were included in the study. A questionnaire was prepared by the researcher as a data collection tool.

**RESULTS**
Of the 142 final year nursing students, 93.7% responded correctly to the optimal blood-letting time for biochemical analysis. It was determined that 92.9% of the students correctly know blood-letting positions affect blood values or not, and 94.7% know the reason why blood-letting is performed in sitting or lying positions. Of the 142 students, 90.1% correctly identified the medium urine samples. It was determined that 96.4% of the students know within how many minutes at maximum blood samples should be sent to the laboratory after drawing.

**CONCLUSION**
The study has provided evidence that the study course of "clinical biochemistry" included into the syllabus in the academic year of 2017–2018 for the first time increased the knowledge of the students about the collection, storage, and transfer of biochemistry laboratory specimens. The study concludes that it is advisable to include the study course of "clinical biochemistry"that predominantly handles the pre-analytical phase into the syllabi designed for nursing schools.

**Keywords:** Pre-analytical phase, biochemical analyses, laboratory evaluation, sample taking, student nurse

## INTRODUCTION

Laboratory tests play an important role in the diagnosis and treatment of diseases. Almost 70% of all the decisions about the diagnosis, treatment, hospitalization, and discharge of patients are obtained based on the outcomes of laboratory tests. The test time consists of three phases in analyses: pre-analytical, analytical, and post-analytical phases. The correctness and reliability of a laboratory outcome depends on the quality of each of these phases. Errors may occur in any phase of an investigation. Pre-analytical phase accounts for 75% of all the errors occurring in laboratory tests; they can occur at any point in the process extending from the preparation of the patient, sample collection, transfer/transport, processing, storage to physiological effects, and/or interventional factors (1-7). Lad Hasit Dalpatbhai et al. (7) categorize the errors that occurred in the pre-analytical phase into four groups: (1) inappropriate form (28.24%), (2) inappropriate specimen (3.52%), (3) inappropriate transfer (22.16%), and (4) inappropriate centrifuge (7.29%). Since collection of blood specimen is the first step, any error made in this step would put the test results in jeopardy regardless of whether or not the tests have been correctly analyzed. Most of the analytical errors occur due to human factors and can be prevented by proper education and training. Healthcare professionals, including nurses, phlebotomists, personnel in laboratories, and transportation services, play an important role with respect to the emergence of analytical errors (6). Lichtinghagen (2016) underlines in his book entitled "Tips and Techniques in Pre-analytical" that the pre-analytical phase is the most import-

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ant factor in laboratory tests with respect to the correctness of tests. He also points out that nurses have great responsibilities in specimen collection (4). Previous research has provided evidence that training programs specifically designed for nurses do substantially reduce pre-analytical errors and have a positive effect on the quality of laboratory specimens. Knowledge is a powerful tool in healthcare, and a well-educated nurse can be the difference between a patient's life and death. Providing nurses the skills and tools they need to positively impact individual's lives is important (8-10).

In our previous study, it was determined that the students did not have information about obtaining, storing, and transferring biochemical laboratory samples (11). Within the concept of the present study, nursing students were provided training for 28 h before they begin their professional life with respect to biochemical analyses, such as appropriate specimen collection, appropriate storage of specimens, and transport to the laboratory, as a part of the responsibilities they would assume. The present study includes the results of the inquiry performed at the end of the training to control the information the students received. The aim of the present study was to see the results of our training.

## MATERIAL and METHODS

This was a descriptive study conducted during the spring semester of the 2017–2018 academic year. A total of 142 volunteer final year nursing faculty students out of 160 were included in the study. As the results obtained in the study with the title "Determination of the nursing students' knowledge regarding the biochemical laboratory specimens," which was performed in the academic year of 2016–2017, to determine the responsibilities of nurses in the pre-analytical phase were insufficient (11), the course program of "Biochemistry" was added to the syllabus of the Faculty of Nursing of Near East University, and a new group of students were informed in a course of 2 h during the week for 14 weeks(February–June2018)regarding the evaluation of the results of the pre-analytical phase and the post-analysis results. To collect data, the question form was used as designed by the researchers for our previous study based on the models available in the literature (11), including the descriptive features of the students and the questions regarding biochemical analyses.

As a data collection tool, a questionnaire, which was prepared by the researcher in the line with the literature (1-12) and included "Descriptive characteristics of the students and information questions about the taking and transferring of the biochemistry laboratory samples," was used.

The questionnaire related to the descriptive characteristics of students was composed of four questions regarding age, gender, school graduated, and hometown. The questionnaire related to the biochemical analyses was composed of four parts. There are a total of 24 questions, including 11 questions about pre-sample taking in section 1, 4 questions about the order of sample taking in section 2, 5 questions about sample taking in section 3, and 4 questions about the evaluation of results in section 4. The questions were prepared as multiple choices, and their answers were evaluated as true and false. The questionnaire was applied to students in the class on June 1, 2018. Students spend approximately 40min to answer the questions.

Statistical Package for the Social Sciences (SPSS) package program, version 21 (IBM Corp.; Armonk, NY, USA) was used for statistical analysis of the responses obtained from the data collection forms. Students' answers were evaluated as percentages as true and false.

Written permission was received from the Near East University Scientific Research and Ethics Committee (26.4.2018/565) to conduct the research. Verbal and written informed consents were obtained from the students before application of the questionnaire.

After completion of the study, the researcher gave a conference on biochemical analyses to complete the missing information, and the students were informed before graduation.

## RESULTS

It was determined that 76.0% of the students were female, 50.6% were graduate of public high schools, 60.8% were Turkish nationals, and the average age was 23.27±1.13 (22–28) years.

The tables describe the students' knowledge about the biochemical analyses.

As shown in Table 1, 3.5% of the students answered the information that should be in the laboratory sample request document, and 93.6% responded correctly to the optimal blood-letting time for biochemical analysis. It was determined that 92.9% of the students correctly know whether the sitting, lying, or on-foot blood-letting positions affect blood values or not, and 94.7% know the reason why blood-letting is performed in the sitting or lying positions. It was observed that 90.1% of the students correctly identified the medium urine sample, and 93.7% correctly understood the 24-hour urine collection practices.

It was reported that 88.0% of the students correctly answered the sample to be first taken from the patient during blood-letting, and 78.2% correctly answered for which analyses the blood sample taken into purple-cover tube is used (Table 2).

As shown in Table 3, it was determined that 96.5% of the students know within how many minutes at maximum blood samples should be sent to the laboratory after drawing, and 2.8% correctly know which values increase in case of delayed delivery of blood samples to the laboratory. They gave correct answers to the questions related to storage conditions of urine container as as 93.7%, 90.1%, 66.9%, respectively.

It was determined that 96.4% of the students know the normal value of fasting blood glucose, 98.6% know the routine blood tests very frequently requested from the emergency patients, and 34.5% know which of the blood proteins increase much in acute infections (Table 4).

## DISCUSSION

Erroneous results do not only lead to incorrect diagnoses but also affect the patients both materially and morally. As is known, the responsibility of collecting and labeling proper specimens for analysis in the pre-analytical phase and delivering them to the laboratory generally depends on the nurses (12, 13).

Nurses need not be specialized in the technical details of laboratory analyses. However, knowledge about pre-analytical variables is important because it has a significant effect on the results of laboratory tests. Of the pre-analytical errors, 60% result from insufficient quantity of specimens and inappropriate specimen quality. Confusion about the process of blood sampling, errors in patient identification and preparation, faults at specimen collection device/container, and errors in processing the specimens ultimately jeopardize the laboratory results. Such errors can seriously affect the reliability of test results, also impacting the diagnosis and treatment process of the disease. As the specimens are collected by nurses, these errors can be rarely detected by laboratories. The "human factor" plays a role in concealing errors in an unrealistic way; however, recognizing and defining probable error fields through adequate trainings provided on a repetitive and continual basis can substantially reduce errors (14-18).

The steps of sample taking, labeling, preservation, and delivery to the laboratory should be followed carefully. The name and surname of the patient must be verified before obtaining any samples. The name, protocol number, department of the patient, and the date and time of sample taking should be labeled on the sample container. It is necessary that the requests are properly coded, and that sufficient samples are delivered to the laboratory.

In the previous study we performed to determine the responsibilities of nurses in the pre-analytical phase, few students (11.0%) had complete knowledge about the information required on the specimen request form (11). The same rate was found to be 3.5% in the present study, leading to the conclusion that they do not learn here attentively enough (Table 1). Special focus should be given to this matter in future courses.

Time of taking is important for blood components that undergo a significant diurnal variation and for controlling drug treatment. The time passing after drug administration affects the drug level (12, 19). In our previous study, the number of students who had correct knowledge about the most appropriate hour of blood sampling for biochemical analyses was the highest (83.9%) as far as knowledge regarding the specimen pre-collection phase is concerned. As for answers about the most appropriate time for blood sampling in the present study, 93.6% of the students gave the correct answer (Table 1). In our previous study, while the rate of correct answers about the appropriate time for blood sampling was 78%, it was calculated to be 88% after the course. Aykal et al. (20) observed that an inadequate sample is found to be important among the rejection reasons of laboratory samples.

The rates of correct answers regarding blood specimens with anticoagulants and blood gases were 51.7% and 68.6%, respectively. In the present study, the rate of correct answers regarding blood specimens with anticoagulants decreased to 40.1% (Table 2), but that regarding blood gases increased to 97.1% (Table 1).

The positioning of the patient is important during blood sampling. The patient should comfortably lie on his/her back or sit for 15 min before blood-letting. Depending on the lying/sitting and on-foot position, there is a transition of body fluid between the cells at significant levels. The blood volume in a standing person is 600–700 mL less than that in a person lying. This situation shows a reduction of approximately 10% in blood volume. As a result of that, blood-letting is performed by lying or sitting positions, as significances are observed in the concentration of all proteins, including enzymes and protein hormones, calcium, bilirubin partially bound to proteins, and drugs bound to protein. Owing to postural effect, plasma proteins may be found to be higher in patients subject to on-foot blood-letting than in patients who sat and waited for 30 min. For this reason, the patients should be required to wait in a sitting position until his/her turn comes for blood-letting, and it should be ensured that blood-letting is certainly performed in the sitting position (19, 21-23).

In our previous study, the rates of correct answers to our questions about patient positioning during the blood sampling process were 52.5% and 45.8%, respectively. The present study performed after the course found a correct answer rate of 92.9% to our question whether blood sampling performed in the sitting, lying, or standing position has an effect on blood values and 94.7% to the question why blood sampling should be undertaken in the sitting or lying position. We are glad to report that our students have quite clearly understood this process with great importance in blood sampling.

Küme et al. (24) found that the majority of mistakes (73%) occur during sample taking. The content of the first drawn blood

**TABLE 1.** Knowledge of the student nurses about pre-sample taking (n=142)

| | Correct answers | |
|---|---|---|
| **Questions related to pre-sample taking** | **n** | **%** |
| Sample used in the blood gases test | 138 | 97.1 |
| The reason why blood-letting is performed in the sitting or lying positions | 134 | 94.7 |
| The optimal blood-letting time for biochemical analysis | 133 | 93.7 |
| Applications related to 24-hour urine collection | 133 | 93.7 |
| Whether the sitting, lying, or on-foot blood-letting positions affect blood values or not | 132 | 92.9 |
| Definition of medium urine sample | 128 | 90.1 |
| Definition of spot urine | 128 | 90.1 |
| Proper blood sample taking | 125 | 88.0 |
| The most widely preferred vein in arterial blood-letting | 109 | 76.8 |
| Finding in serum samples arising as a result of an error in blood-letting timing | 17 | 11.9 |
| Information required in the laboratory sample request document | 5 | 3.5 |

**TABLE 2.** Knowledge of the student nurses about the order of sample taking (n=142)

| | Correct answers | |
|---|---|---|
| **Questions related to sample taking and order of taking** | **n** | **%** |
| Sample to be first taken from the patient during blood-letting | 125 | 88.0 |
| For which analyses the blood sample taken into purple-cover tube is used | 111 | 78.2 |
| Which blood sample includes anticoagulant | 95 | 66.9 |
| What color blood tube has to be shaken by slowly turning upside-down following blood-letting | 57 | 40.1 |

shows best the composition of the circulating blood. Therefore, the first sample obtained should be used for important tests in critical medical decisions, such as calcium. When the tourniquet is applied, the blood that is drawn later is the content in the small veins and capillaries. It significantly shows the effect of venous stasis. The first tube may show a 5% increase, whereas the third tube may show a 10% increase in protein. Concentrations of protein-bound compounds are also affected by stasis (12, 23, 25).

Of the students who participated in our previous study, 22.0% gave the correct answer to the question about which specimen is to be obtained right at the beginning during the blood sampling process, and 45.8% to the question about which analyses are to be undertaken with the blood specimen taken into the tube with a violet lid (11). These rates increased to 88.0% and 78.2% after the course, respectively (Table 2). As full blood examination is needed for certain tests, such as hemogram, blood gas, ammonia, and lactate, the blood to be used for such tests is drawn into a tube containing an anticoagulant. Since the anticoagulant used affects certain tests, a tube proper for the parameter to be analyzed must be used. These error sources should be known, defined, and controlled to the extent possible. These points should especially be underlined in the training programs designed for students.

Our previous study found that the students had insufficient knowledge about urine sampling. Of the students, 57.1% correctly defined what clean-catch urine specimen is, and only 14.3% had correct knowledge about the practices regarding 24-hour urine specimen (11). These rates increased to 90.1% and 93.7% after the course, respectively (Table 1).

The type of urine sample to be collected depends on the test to be performed. Generally, urine samples should be collected in a predetermined time period, such as 1, 4, 12, and 24 h. The first urine, which is obtained early in the morning with a clean and hungry stomach, is usually the most concentrated urine, and microscopic examination is preferred for identification of abnormal amounts of contents, such as protein, and for identification of compounds, such as human chorionic gonadotropin. The collection period of timed samples should be long enough to minimize the effect of short-term biological variations. The bladder should be emptied before the collection starts, and this urine should be discharged. Thereafter, all urines should be collected until the end of the time period. Patients should be informed by the nurse with regard to dietary and drug intake before urine collection is started to avoid interference with the compounds taken with the analytical transactions (22, 25, 26).

The results about the correct answers of the students regarding urine sampling and storage conditions in our previous study were also not satisfactory (11). Of the students participating in that study, 50.8% correctly answered to our question about the preservative agents to be added to urine specimens, and 68.6% to our question about the storage conditions of urine container used for 24-hour urine specimens. These rates increased to 66.9% and 93.7% after the course, respectively (Table 3).

Küme et al. (24) found that the errors related to urine analysis among erroneous sample types are 8%. Before starting the urine collection, suitable preservatives (e.g., 6N hydrochloric acid) should be placed in the collection container to prevent degradation of the parameters to be analyzed. When the 24-hour urine is collected, the bladder is emptied by first urinating in the morning, and then the entire urine during the day is poured into the collection container. On the next morning, the first urine is taken into the collection container and delivered to the laboratory as soon as possible. During the urine collection period, the collection container is stored in a cool place (22, 24-26).

Sharaki et al. (27) found that 87.84% of the errors in urine samples are in the pre-analytic stage and are particularly related to sample collection.

Errors can occur during specimen collection at hospitals, and 60% of the errors occurring in the pre-analytical phase are attributed to insufficient specimen quality or quantity. Therefore, nurses should be aware of pre-analytical errors and make efforts to reduce them.

For patient safety, it is necessary to know the sources of errors, to control them, and to increase the knowledge level of health professionals through in-service trainings. Nurses play an important role in collecting and handling blood specimens and providing information to patients prior to tests. For this reason, healthcare professionals should be very conscious of the effects of individual pre-analytical factors and their combinations on test results (28-32).

We asked the nursing student for information about the evaluation of some laboratory test results, such as; the normal value of fasting blood glucose (Table 4). Nurses are the ones who

**TABLE 3.** Knowledge of the student nurses about post-sample taking (n=142)

| | Correct answers | |
|---|---|---|
| **Questions related to post-sample taking** | **n** | **%** |
| Within how many minutes at maximum blood samples should be sent to the laboratory after drawing | 137 | 96.5 |
| Condition of storage of the urine collection container for 24-hour urine collection duration | 133 | 93.7 |
| If the urine sample will not be analyzed promptly, condition of storage after taking | 128 | 90.1 |
| If the urine sample will not be analyzed within 2 h, the chemical protectors that have to be added into the urine collection container | 95 | 66.9 |
| Which values increase in case of delayed delivery of blood samples to the laboratory | 4 | 2.8 |

**TABLE 4.** Knowledge of the student nurses about the evaluation of results (n=142)

| | Correct answers | |
|---|---|---|
| **Questions related to the evaluation of results** | **n** | **%** |
| Routine blood tests very frequently requested from the emergency patients | 140 | 98.6 |
| Normal value of fasting blood glucose | 137 | 96.4 |
| Important indicators of the blood count results for bleeding patient | 72 | 50.7 |
| Which blood proteins significantly increase in acute infections | 49 | 34.5 |

generally see the results of the tests requested from the inpatients. Therefore, it is very important in early intervention that the nurses realize abnormal laboratory results of patients and inform the physician. It is important for nursing students to have knowledge about laboratory results, especially critical values, by having the necessary training. Teaching some critical laboratory values in the education of nursing students will be useful in practice.

In conclusion, the study has provided evidence that the study course of "clinical biochemistry" included into the syllabus in the academic year of 2017–2018 for the first time increased the knowledge of the students about the collection, storage, and transfer of biochemistry laboratory specimens. The study results indicate that a program to be designed for nurses should especially focus on the pre-analytical phase. The information about the post-analytical phase can be learned in a short time beginning with the most important pieces of information. In conclusion, it is advisable to include the study course of "clinical biochemistry" that predominantly handles the pre-analytical phase into the syllabi designed for nursing schools.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ethics Committee of Near East University School of medicine. (Approval Date: 26.09.2018, Approval Number: 565).

**Informed Consent:** Written informed consent was obtained from the patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept - Ü.D.Y., T.Y.; Design - T.Y., Ü.D.Y.; Supervision - T.Y., Ü.D.Y.; Resource - T.Y., Ü.D.Y.; Materials - Ü.D.Y., T.Y.; Data Collection and/or Processing - Ü.D.Y., T.Y.; Analysis and/or Interpretation - Ü.D.Y., T.Y.; Literature Search - T.Y., Ü.D.Y.; Writing - Ü.D.Y., T.Y.; Critical Reviews - Ü.D.Y., T.Y.

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# Effects of Parent Characteristics on the Presence and the Progression of Retinopathy of Prematurity

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**BACKGROUND/AIMS**
To investigate the effects of maternal and paternal risk factors in the development of retinopathy of prematurity (ROP).

**MATERIAL and METHODS**
The medical records of premature infants examined between 2015 and 2018 were included in this retrospective study. Gestational age, birth weight, sex, time spent in the neonatal intensive care unit (NICU), maternal and paternal ages, level of education, employment, and smoking were recorded.

**RESULTS**
Data of 438 infants who were screened for ROP were included in the study. The mean gestational age of the infants was 31.9±2.9 weeks, mean birth weight was 1727.8±543.7 g, and mean time spent in the NICU was 36.5±24.5 days. Lower gestational age, lower birth weight, and more time spent in the NICU significantly increased the presence of any stage and treatment requiring ROP ($p<0.001$). Maternal and paternal ages, smoking, education level, and employment did not have any significant relationship with the presence of any stage or treatment requiring ROP ($p>0.05$).

**CONCLUSION**
The major risk factors for ROP are gestational age, birth weight, and time spent in the NICU where infants receive supplemental oxygen. There was no any relationship between paternal demographics and presence of any stage and treatment requiring ROP.

**Keywords:** Retinopathy of prematurity, risk factors, parent characteristics, parental age, parental smoking

## INTRODUCTION

Retinopathy of prematurity (ROP) is one of the leading causes of preventable blindness in children that primarily targets preterm infants (1, 2). ROP is a multifactorial disease with many risk factors (3). The major risk factors for development of ROP are small gestational age, low birth weight (4), and use of supplemental oxygen (5). Other infantile risk factors include respiratory distress syndrome (6), mechanical ventilation (7), blood transfusion (8), necrotizing enterocolitis (9), sepsis (10), and the time spent in the neonatal intensive care unit (NICU) (11).

Previous studies investigated the maternal risk factors of ROP. There are conflicting results regarding the effects of pre-eclampsia (12, 13), maternal age (14, 15), maternal diabetes (16, 17), and smoking (18, 19) on the development of ROP.

The infantile risk factors for development of ROP have been well established, but there are less and conflicting data regarding the maternal risk factors and very limited data about the paternal risk factors in the literature. The aim of the present study was to investigate the effects of maternal and paternal risk factors in the development of ROP.

## MATERIAL and METHODS

This was a retrospective study was conducted in Gaziantep University Hospital, Turkey. The study was approved by the ethics committee in accordance with the Declaration of Helsinki. The medical records of premature infants examined between 2015 and 2018 in the Ophthalmology Department of Gaziantep University Hospital were included in the study.

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Gestational age, birth weight, sex, time spent in the NICU, maternal and paternal ages, level of education, employment, and smoking during pregnancy were recorded. The presence of ROP, stage and zone of the disease, treatment for ROP, and any adverse event regarding ROP were also recorded. The classification of ROP and the decision for treatment were performed according to the criteria determined by the early treatment for ROP study group (20). Informed consent was not obtained from the patients and parents due to the retrospective nature of the present study.

**Statistical Analysis**

Statistical analysis was performed using Statistical Package for the Social Sciences version 22.0 for Windows (IBM Corp.; Armonk, NY, USA). Chi-square test, independent samples t-test, and Mann–Whitney U test were used to evaluate the results. A p value <0.05 was considered statistically significant.

## RESULTS

Data of 438 infants who were screened for ROP were included in the study. The patient and parent demographics are shown in Table I. Data regarding the presence and severity of ROP are shown in Table 2.

Maternal and paternal ages were not significantly associated with the presence of ROP. Maternal and paternal smoking did not affect the presence of ROP. Lower gestational age, lower birth weight, and more time spent in the NICU significantly increased the presence of ROP (Table 3). The collected data were also evaluated between treated and untreated infants for ROP. Gestational age, birth weight, and time spent in the NICU were significantly different between the treated and untreated ROP groups (Table 4).

## DISCUSSION

Retinopathy of prematurity affects preterm infants (1). Most of the infantile risk factors for development of the disease have been discussed by previous studies. However, there are less data regarding the maternal and paternal risk factors of the disease. Moreover, existing data of maternal and paternal risk factors have conflicting results.

The most important risk factors for the disease are small gestational age, low birth weight, and oxygen requirement. Palmer et al. (4) reported that small gestational age and low birth weight are risk factors for development of ROP. York et al. (5) showed that not only supplemental oxygen requirement but also fluctuations in $PaO_2$ are associated with the presence and progression of ROP. In our study, gestational age and birth weight were significantly lower, and time spent in the NICU where infants are treated with supplemental oxygen was significantly higher in infants with ROP. These significant differences persisted among infants with ROP when we divided them into the treated and untreated groups.

There are conflicting results about the effect of maternal risk factors on the development of ROP. Wu et al. (14) reported that older maternal age is a risk factor for development of ROP. On the other hand, Uchida et al. (15) showed that ROP develops more frequently from younger mothers. Concurrently, Kanungo et al. (21) reported that maternal age does not affect the development of stage 3 or higher ROP. Similarly, in our study, maternal age did not have an influence on the presence of any stage ROP or treatment requiring ROP. Maternal smoking increases the risk of low birth weight and preterm delivery (22). Hirabayashi et al. (23) reported that maternal smoking decreases the incidence of ROP. However, Spiegler et al. (18) showed that maternal smoking increases the incidence of ROP. In our study, we could not find an association between maternal smoking and presence of any stage or treatment requiring ROP. Previous studies showed that lower maternal education is associated with preterm birth

**TABLE I.** Patient and parent demographics

| | n (%) | Mean±SD |
|---|---|---|
| Gestational age (weeks) | 438 (100) | 31.9±2.9 |
| Birth weight (g) | 438 (100) | 1727.8±543.7 |
| Sex | | |
| —Male | 227 (51.8) | |
| —Female | 211 (48.2) | |
| Time spent in the NICU (days) | 438 (100) | 36.5±24.5 |
| Maternal age (years) | 438 (100) | 28.0±6.2 |
| Paternal age (years) | 438 (100) | 32.1±6.9 |
| Maternal smoking (pack-year) | 438 (100) | 0.9±2.8 |
| Paternal smoking (pack-year) | 438 (100) | 6.6±9.3 |
| Maternal education | | |
| —Primary school | 312 (71.2) | |
| —High school | 69 (15.8) | |
| —University | 57 (13) | |
| Paternal education | | |
| —Primary school | 267 (61.0) | |
| —High school | 101 (23.0) | |
| —University | 70 (16.0) | |
| Maternal employment | | |
| —Unemployed | 384 (87.7) | |
| —Civil servant | 32 (7.3) | |
| —Own business | 19 (4.3) | |
| —Worker | 3 (0.7) | |
| Paternal employment | | |
| —Unemployed | 4 (0.9) | |
| —Civil servant | 60 (13.7) | |
| —Own business | 122 (27.9) | |
| —Worker | 252 (57.5) | |

N: number; SD: standard deviation; NICU: neonatal intensive care unit

**TABLE 2.** ROP presence and severity among the screened infants

| | n (%) |
|---|---|
| No ROP | 201 (45.9) |
| Mild ROP | 134 (30.6) |
| Type 2 ROP | 13 (3) |
| Type 1 ROP | 90 (20.5) |

N: number; ROP: retinopathy of prematurity

**TABLE 3.** Factors affecting the presence of ROP

| | No ROP | Any stage ROP | p |
|---|---|---|---|
| Gestational age (weeks) | 33.3±2.5 | 30.7±2.7 | <0.001 |
| Birth weight (g) | 1979.8±551.8 | 1508.5±430.5 | <0.001 |
| Time in the NICU (days) | 27.9±22.4 | 43.9±23.8 | <0.001 |
| Maternal age (years) | 28.6±6.4 | 27.6±6.1 | 0.108 |
| Paternal age (years) | 32.5±6.9 | 31.8±6.8 | 0.272 |
| Maternal smoking (pack-year) | 0.8±2.3 | 1.1±3.2 | 0.320 |
| Paternal smoking (pack-year) | 5.6±7.8 | 7.5±10.4 | 0.077 |
| Sex | | | |
| —Male | 94 | 133 | 0.051 |
| —Female | 107 | 104 | |
| Maternal education | | | |
| —Primary school | 137 | 175 | 0.396 |
| —High school | 34 | 35 | |
| —University | 30 | 27 | |
| Paternal education | | | |
| —Primary school | 115 | 152 | 0.109 |
| —High school | 46 | 55 | |
| —University | 40 | 30 | |
| Maternal employment | | | |
| —Unemployed | 169 | 215 | 0.068 |
| —Civil servant | 17 | 15 | |
| —Own business | 14 | 5 | |
| —Worker | 2 | 1 | |
| Paternal employment | | | |
| —Unemployed | 1 | 3 | 0.172 |
| —Civil servant | 34 | 26 | |
| —Own business | 59 | 63 | |
| —Worker | 107 | 145 | |

ROP: retinopathy of prematurity; NICU: neonatal intensive care unit

**TABLE 4.** Factors affecting the treatment need for ROP

| | Treated ROP | Untreated ROP | p |
|---|---|---|---|
| Gestational age (weeks) | 29.9±2.8 | 31.1±2.6 | 0.001 |
| Birth weight (g) | 1423.0±411.8 | 1553.8±434.6 | 0.026 |
| Time in the NICU (days) | 50.0±26.1 | 40.7±21.9 | 0.007 |
| Maternal age (years) | 27.6±5.7 | 27.6±6.3 | 0.959 |
| Paternal age (years) | 31.4±6.4 | 31.9±7.0 | 0.621 |
| Maternal smoking (pack-year) | 1.1±3.2 | 1.0±3.3 | 0.874 |
| Paternal smoking (pack-year) | 8.1±12.1 | 7.2±9.4 | 0.518 |
| Sex | | | |
| —Male | 52 | 81 | 0.183 |
| —Female | 32 | 72 | |
| Maternal education | | | |
| —Primary school | 63 | 112 | 0.792 |
| —High school | 13 | 22 | |
| —University | 8 | 19 | |
| Paternal education | | | |
| —Primary school | 62 | 90 | 0.070 |
| —High school | 14 | 41 | |
| —University | 8 | 22 | |
| Maternal employment | | | |
| —Unemployed | 78 | 137 | 0.673 |
| —Civil servant | 5 | 10 | |
| —Own business | 1 | 4 | |
| —Worker | 0 | 1 | |
| Paternal employment | | | |
| —Unemployed | 1 | 2 | 0.196 |
| —Civil servant | 5 | 21 | |
| —Own business | 20 | 43 | |
| —Worker | 58 | 87 | |

ROP: retinopathy of prematurity; NICU: neonatal intensive care unit

and poor health of the newborn (24, 25). In our study, there was no any difference between maternal education levels and presence of ROP. Maternal employment has been shown to reduce or does not affect the incidence of preterm delivery (26, 27). Similarly, we could not find a relationship between maternal employment and presence of ROP.

Existing literature has limited information about the paternal risk factors of ROP. There are conflicting studies about the effect of paternal age on preterm birth. Astolfi et al. (28) reported that the risk of preterm delivery increases with advanced paternal age. Conversely, Alio et al. (29) found that preterm delivery risk decreases with paternal age. One might think that if paternal age affects preterm delivery, it can affect the presence of ROP, but that was not the case in our study. We could not find any relationship between paternal age and presence of ROP. Ko et al. (22) showed that paternal smoking does not have an effect on preterm birth of low birth weight, which are major risk factors for development of ROP. Similarly, we could not find an association between paternal smoking and presence of ROP. Shapiro et al. (30) reported that lower paternal education increases the risk for preterm birth. In our study, we did not find a relationship between paternal education and presence of ROP. In addition, there was no any relationship between paternal employment and presence of ROP.

Our study has some limitations. First, the retrospective nature of our study limits the accuracy of our results. Second, our study was conducted in a single center; a multicentric study is required for more detailed results.

In conclusion, the major risk factors for ROP are gestational age, birth weight, and time spent in the NICU where infants receive supplemental oxygen. These risk factors affect the presence of both any stage and treatment requiring ROP. Maternal and paternal ages, smoking, education level, and employment did not have any significant relationship with the presence of any stage or treatment requiring ROP. Further studies are needed because of the conflicting results in the literature.

**Ethics Committee Approval:** Ethics committee approval was received for this study from Gaziantep University Clinical Research and Ethics Committee (Approval Date: 19.06.2017, Approval Number: 247).

**Informed Consent:** Informed consent was not obtained from the patients and parents due to the retrospective nature of the study.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** The author has no conflicts of interest to declare.

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# The Relationship Between Long Non-Coding RNA Expressions and Ponatinib in Breast Cancer

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**BACKGROUND/AIMS**
Breast cancer is the most common type of cancer in women and is among the leading causes of cancer-related deaths. Long non-coding RNAs (lncRNAs) play significant roles in cell proliferation, transcriptional regulation, cell cycle progression, apoptosis, carcinogenesis, and metastasis. Studies have shown that ponatinib has an antiproliferative effect in some types of cancer. The aim of the present study was to evaluate the effect of ponatinib on cytotoxicity and to determine changes in lncRNA expression levels with the use of ponatinib treatment in estrogen receptor (ER)-independent MDA-MB-231 and ER-dependent MCF-7 breast cancer cells.

**MATERIAL and METHODS**
The cytotoxic effects of ponatinib were determined by using the xCELLigence system. Changes in lncRNA expression profiles were determined using quantitative reverse transcription polymerase chain reaction to investigate the antiproliferative roles of ponatinib in breast cancer.

**RESULTS**
In human breast adenocarcinoma cell lines (MCF-7 and MDA-MB-231), the IC50 doses of ponatinib were determined to be 4.59 μM (72 h) and 1.41 μM (48 h), respectively. After ponatinib treatment, we observed changes in lncRNA expression profiles in ER-independent MDA-MB-231 and ER-dependent MCF-7 breast cancer cells compared with the control group.

**CONCLUSION**
The changes in the lncRNA expression profiles and the anti-cancer agent of ponatinib play roles in the definition of therapeutic target for new approach in breast cancer.

**Keywords:** Long non-coding RNAs (lncRNAs), ponatinib, MCF-7, MDA-MB-231, breast cancer

## INTRODUCTION

Breast cancer is common in women and is characterized by high rates of malignancy and metastasis (1). Since breast cancer has a heterogeneous molecular structure, there is no common treatment strategy. Most patients develop resistance during treatment. Therefore, it has been found that alternative medicine sources are used as new options for breast cancer treatment (2).

Ponatinib, a strong tyrosine kinase inhibitor, targets BCR-ABL1 oncoprotein. It has antiangiogenic and antineoplastic activities. It has been applied in the treatment of hematological malignancies, such as Philadelphia chromosome-positive acute lymphoblastic leukemia and chronic myeloid leukemia (3). In addition, the antineoplastic effects of ponatinib on various cancer cells, such as endometrial, bladder, stomach, breast, lung, and colon cancers, have been demonstrated (4). Ponatinib has been shown to induce dose-dependent G2/M arrest in ovarian and MCF-7 breast cancer cells, but does not block the cell cycle in colon and SKBR3 breast cancer cells (5).

Long non-coding RNA (lncRNA) is a kind of non-coding RNA (ncRNA) molecule longer than 200 nucleotides (6, 7). lncRNAs play biological roles in cell proliferation, proliferation, metabolic functions and differentiation, and the development of many diseases. They also play a role in genomic imprinting, gene regulation, alternative splicing, chromatin organization, and genomic packaging. The abnormal regulation of lncRNAs is associated with the formation, development, and progression of different

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types of diseases, particularly cancer. Specific lncRNAs are considered as indicators of diagnostic, prognostic, or predictive therapeutic responses for various diseases (8). Various studies have been conducted on lncRNAs. For example, it was shown that HOXAII-AS inhibits the formation of cell colonies in the breast cancer cell line and arrests the cell cycle in the G0/GI phase (9).

In conclusion, in our study, the antiproliferative effects of ponatinib and lncRNA expression profile were found in breast cancer. Some lncRNA genes have been shown to have anti-cancer effects. We believe that ponatinib can be used as a candidate biomarker for future effective treatment of breast cancer.

## MATERIAL and METHODS

### Cell Culture

MDA-MB-231 and MCF-7 cell lines were obtained from ATCC (Guernsey, Ireland). MCF-7 cells were cultured with RPMI-1640 (Biological Industries, Beit-Haemek, Israel), and MDA-MB-231 cells were cultured with Leibovitz's L-15 (Biological Industries) media containing 1% L-glutamine (EMD Millipore, K0282, Darmstadt, Germany), 10% inactivated fetal bovine serum (Capricorn Scientific, FBS-11B, Ebsdorfergrund, Germany), and 1% penicillin/streptomycin (Biochrom, A2213, Berlin, Germany) in 5% $CO_2$ and 37 °C. Ponatinib (Selleckchem, Munich, Germany) was suspended in dimethyl sulfoxide (Sigma-Aldrich, Taufkirchen, Germany). Our study was conducted according to the Declaration of Helsinki.

### Cytotoxicity Assay

MCF-7 and MDA-MB-231 ($1\times10^4$ cells/well) were seeded in 96-well E-plates in triplicate to investigate the cytotoxic effects of ponatinib. The cells were incubated for 24 h before ponatinib treatment. MCF-7 cells were treated with ponatinib with concentrations of between 100 µM and 3.1 µM, whereas MDA-MB-231 cells were treated with ponatinib doses of 50 µM–1.5 µM. The cells were incubated for 48 h, and impedance was monitored every 15 min throughout the period using the xCELLigence system. Cytotoxicity was evaluated by comparing the viabilities of the ponatinib-treated cells to the untreated control cells using the xCELLigence RTCA software.

### lncRNA Expression Profiling

### Total RNA isolation and cDNA synthesis

For lncRNA expression profiling studies, RNeasy Mini Kit (Qiagen, Kat. No: 74134) was used to extract total RNA (including small RNAs) from the ponatinib-treated and -untreated MDA-MB-231 and MCF-7 cells ($2\times10^6$ cells/mL). The concentration and purity of RNA samples were determined by measuring absorbance at wavelengths of 260/280 nm and 230/260 nm using the NanoDrop instrument (Thermo Scientific, Wilmington, DE, USA). For further analysis, RNA samples with A260/A280 and A230/A260 absorbance ratios >2.0 were used.

### qRT-PCR analysis

For cDNA synthesis, an RNAQuant cDNA Synthesis Kit (System Biosciences, CA, USA) was used according to the manufacturer's instruction. The Disease-Related lncProfiler Array was used to investigate the antiproliferative roles of lncRNAs in breast cancer after ponatinib treatment. Relative quantitation of 83 lncRNAs was measured by using a Maxima SYBR Green qPCR Master Mix (Thermo Scientific) on LightCycler 480 II (Roche Life Science, Indianapolis, IN, USA). In addition to seven human housekeeping genes (ACTB, B2M, PGKI, GAPDH, HPRTI, RPLIA, and RPLI3A) and four small RNA transcript primers (7SL scRNA, 5.8S rRNA, U87 scaRNAU6, and smRNA) for normalization, the quantitative reverse transcription polymerase chain reaction (qRT-PCR) array plate included one genomic DNA control and one negative control. The relative expression of lncRNAs was determined by using the $2^{-\Delta\Delta CT}$ method. Fold changes of lncRNA expression levels after ponatinib treatment were evaluated by comparing with the untreated control groups. Log2 transformation was applied to the $2^{-\Delta\Delta Ct}$ values of the lncRNA expression in the control and ponatinib-treated groups. Fold changes for lncRNA expressions and their significance were calculated by Student's t-test using an online software (https://www.qiagen.com/jp/shop/genes-and-pathways/data-analysis-center-overview-page/) (GeneGlobe Data Analysis Center; Qiagen, Valencia, USA). Changes in lncRNA expression of ±2-fold were compared with the control group. A p value <0.05 was considered significant.

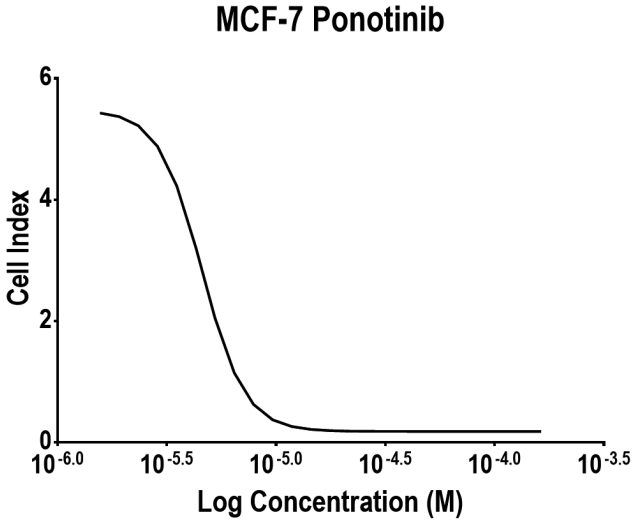


**FIGURE 1.** Cytotoxic effect of ponatinib in MCF-7 cell line

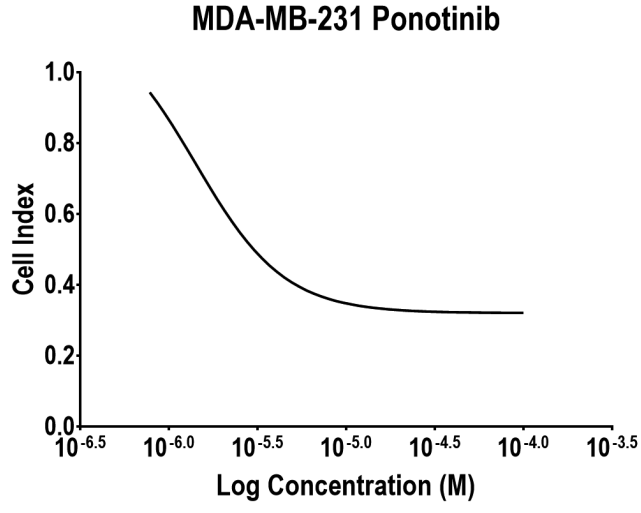


**FIGURE 2.** Cytotoxic effect of ponatinib in MDA-MB-231 cell line

## RESULTS

### Cytotoxic Effect of Ponatinib

MDA-MB-231 and MCF-7 cell lines revealed the cytotoxic effect of ponatinib by MTT analysis. The $IC_{50}$ doses of ponatinib were determined to be 1.41 μM for MDA-MB-231 cell line (48 h) and 4.59 μM for MCF-7 cell line (72 h) at 48 h by using the xCELLigence system (Figure 1, 2). Ponatinib inhibited cell proliferation in a dose- and time-dependent manner compared with the untreated MDA-MB-231 and MCF-7 control cells.

### Expression Profile Changes of lncRNA

Following ponatinib treatment, changes in lncRNA expression were found in MCF-7 and MDA-MB-231 cells compared with the control groups. According to log2 transformation, 17 lncRNAs (AAAI, aHIF, BC200, DISC2, EGO, HOXA11AS, MALAT1, MEG3, NEAT1, NCRMS, PCAT-1, PCAT-14, PCAT-43, SAF, SRA, WTI-AS, and ZEB2NAT) were downregulated, whereas 5 lncRNAs (CMPD, DGCR5, HI9-AS, HARIA, and LIT) were upregulated in MCF-7 cell lines ($p<0.05$) (Table 1).

**TABLE 1.** Exchange of lncRNAs expression in ponatinib treated MCF-7 cell line

| lncRNA Symbols | Fold Change (Log2Transformed) | p |
|---|---|---|
| AAAI | -11.7 | 0.000124 |
| aHIF | -4.79 | 0.000056 |
| BC200 | -2.13 | 0.000081 |
| CMPD | 2.44 | 0.00029 |
| DGCR5 | 2.02 | 0.000234 |
| DISC2 | -3.6 | 0.000097 |
| EGO | -2.79 | 0.00005 |
| HI9-AS | 2.29 | 0.000842 |
| HARIA | 5.38 | 0.000072 |
| HOXA11AS | -6.05 | 0.000086 |
| LIT | 2.04 | 0.000616 |
| MALAT1 | -2.69 | 0.000043 |
| MEG3 | -3.68 | 0.000144 |
| NEAT1 | -2.85 | 0.000019 |
| NCRMS | -3.06 | 0.000063 |
| PCAT-1 | -5.29 | 0.000045 |
| PCAT-14 | -5.02 | 0.000157 |
| PCAT-43 | -2.20 | 0.000114 |
| SAF | -2.37 | 0.000106 |
| SRA | -2.97 | 0.000069 |
| WTI-AS | -7.86 | 0.000072 |
| WTI-AS | -2.54 | 0.000432 |

lncRNAs: Long non-coding RNA; aHIF: Hypoxia Inducible Factor; CMPD: Cancer mutant proteom Database; DGCR5: DiGeorge Syndrome Critical Region Gene 5; DISC2: Disrupted in schizophrenia 2; EGO: Eosinophil Granule Ontogenesis; LIT: Late inhibitor of T4; MALAT1: Metastasis Associated Lung Adenocarcinoma Transcript 1; NCRMS: Non-coding RNA in Rhabdomyosarcoma; SAF: Serum Amyloid A- Activating Factor; SRA: Steroid Receptor Activator

In addition, according to log2 transformation, 22 lncRNAs (AAAI, aHIF, BC200, BCMS, DLG2AS, GAS5, HI9, HOXA11AS, IPW, LIPA16, LIT, MALAT1, MERIIC, NEAT1, PCAT-14, PCGEM1, RMRP, SOX2OT, TELOMERASE RNA, TMEVPG1, TUG1, UCA1) were downregulated, whereas 6 lncRNAs (IGF2AS, MEG3, PCAT-43, ST7OT2, ST7OT3, and WTI-AS) were upregulated in MDA-MB-231 cell lines ($p<0.05$) (Table 2).

## DISCUSSION

In recent years, ncRNAs have been extensively studied in various biological processes and in human diseases, including cancer (10). Currently, a large number of human lncRNAs can act as biomarkers for cancer diagnosis and prognosis (11). Most of the lncRNAs are abnormally expressed in breast cancer; they act as tumor suppressors and oncogenes according to their function and expression patterns (12).

**TABLE 2.** Exchange of lncRNAs expression in ponatinib treated MDA-MB-231 cell line

| lncRNA Symbols | Fold Change (Log2Transformed) | p |
|---|---|---|
| AAAI | -7.73 | 0.000312 |
| aHIF | -2.66 | 0.000187 |
| BC200 | -6.16 | 0.000031 |
| BCMS | -4.80 | 0.000146 |
| DLG2AS | -2.90 | 0.000132 |
| GAS5 | -5.39 | 0.000082 |
| HI9 | -5.15 | 0.000066 |
| HOXA11AS | -7.83 | 0.00012 |
| IPW | -5.15 | 0.00006 |
| IGF2AS | 2.93 | 0.00013 |
| LIPA16 | -2.44 | 0.000147 |
| LIT | -2.99 | 0.000435 |
| MALAT1 | -7.38 | 0.000015 |
| MEG3 | 4.46 | 0.000065 |
| MERIIC | -5.65 | 0.000055 |
| NEAT1 | -4.17 | 0.000016 |
| PCAT-14 | -2.93 | 0.000381 |
| PCAT-43 | 3.54 | 0.00004 |
| RMRP | -7.73 | 0.000007 |
| SOX2OT | -5.28 | 0.000216 |
| ST7OT2 | 3.33 | 0.000204 |
| ST7OT3 | 2.67 | 0.000113 |
| TELOMERASE RNA | -3.46 | 0.000539 |
| TMEVPG1 | -3.10 | 0.000714 |
| TUG1 | -3.48 | 0.000122 |
| UCA1 | -7.97 | 0.000073 |
| WTI-AS | 2.91 | 0.000211 |

lncRNAs: Long non-coding RNA; aHIF: Hypoxia Inducible Factor; BCMS: Gene associated with multiple splicing B-cell neoplasia; IPW: Imprinted Gene in Prader-Willi Sendrom; LIT: Late inhibitor of T4; MALAT1: Metastasis Associated Lung Adenocarcinoma Transcript 1; MEG3: maternally expressed 3 TMEVPG1: Theiler's mouse encephalomyelitis virus resume candidate gene; ITUG1: Taurine RNA arranged upward 1; UCA1: Associated with urethral cancer 1

Long non-coding RNA H19 is known to play an oncogenic role in breast cancer. Studies have shown that H19 plays a critical role in cell survival and proliferation in estrogen receptor (ER)-positive breast cancer cells (13). While silencing of H19 in breast cancer cells reduces proliferation, overexpression of H19 is suggested to accelerate cell cycle progression (14). Inhibition of H19 reduces the metastasis of pancreatic cancer in vivo. Therefore, they showed that H19 is a new candidate for treatment of pancreatic cancer (15). In our study, ponatinib was suppressed in ER-negative breast cancer cells of H19. We can associate the 5.15-fold reduction in H19 expression with the tumorigenesis process.

Chen et al. (16) have shown that IGF2AS is involved as an epigenetic tumor suppressor in human prostate cancer. In our study on breast cancer, it was evaluated that the expression of lncRNA IGF2AS had a 2.93-fold increase, playing a role as a tumor suppressor.

Wang et al. (17) showed that overexpressed PCAT-14 is associated with a poor prognosis in patients with hepatocellular carcinoma (HCC). PCAT-14 has been proposed as a new prognostic factor and therapeutic target because it regulates proliferation and cell cycle in HCC cells (17). Qiao et al. (18) showed that the downregulation of lncRNA PCAT-1 inhibits proliferation, blocks cell cycle passage, and suppresses cyclin and c-myc expression in colorectal cancer cells. In our study, the expression of lncRNA PCAT-14 and PCAT-1 decreased with the effect of ponatinib. This may suggest that they induce apoptosis.

In their study on breast cancer, Iranpour et al. found that SOX-2OT overexpresses tumor tissues compared with non-cancerous tissues. It has been observed that SOX2OT acts as oncogenesis, and that its expression is more negative for patients (19).

Farhangian et al. (20) found that SOX2OT expression shows a significant reduction compared with non-tumor tissues in gastric cancer (GC) samples. They showed that SOX2OT plays a tumor suppressor role with the downregulation of SOX2 in GC. It can also be a good biomarker in the diagnosis of the disease (20). In our study, at the expression level of SOX2OT, a 5.25-fold decrease was found in ER-negative breast cancer cells. The decrease in the expression level of SOX2OT in ponatinib-treated breast cancer cells reveals the antiproliferative effect of ponatinib. We think that studies on this subject can be used as a biomarker in the diagnosis and treatment of the disease.

In the present study, it was observed that the expression of BC200 increased in breast cancer. Among breast cancer tissues, it was observed that BC200 was expressed at a higher level in ER-positive tumors than in ER-negative tumors. BC200 has been shown to play a role as an oncogene in breast cancer. Therefore, BC200 can be shown as a prognostic marker and target to minimize irregular cell proliferation in ER-dependent breast cancer (21). In their study with carboplatin, Wu et al. (22) found that the expression of BC200 increases in ovarian cancer cell lines. It was observed that the cells decreased their sensitivity to the drug by inhibiting BC200 (22). We found that this lncRNA BC200, which is defined as an oncogene in breast cancer, is suppressed with ponatinib in hormone-sensitive and -independent breast cancer cells.

CMPDI showed an antiproliferative effect in GC cell lines MKN-45 and SGC790I. In addition, CMPDI induced time- and dose-dependent apoptosis in MKN-45 cell lines (23). In our study, we showed that ponatinib-treated MCF-7 cells regulated the expression of the tumor suppressor lncRNA CMPDI. As with GC, CMPDI may also contribute to the antiproliferative effect of ponatinib in breast cancer cells.

Fung et al. (24) found that DGCR5 expression is significantly lower in bladder cancer tissues than in healthy tissues. The increased expression of DGCR5 showed a high survival rate. In addition, it was found that the overexpression of DGCR5 inhibited proliferation, colony formation, and cell cycle progression (24). According to these data, after ponatinib treatment, the expression of DGCR5 is increased in ER-positive breast cancer cells. We investigated that ponatinib may suppress metastasis and induce apoptosis in tumorigenesis progression.

It has been observed that the decreased expression of PVTI and the increased expression of HARIA in patients with diffuse glioma increased the survival rate of patients receiving chemotherapy and radiotherapy. PVTI and HARIA can be used as biomarkers in the diagnosis and treatment of diffuse gliomas (25). In our study, we observed that the expression level of HARIA was increased in ER-dependent breast cancer cell lines. As a result, ponatinib may have an apoptotic effect on MCF-7 cell lines in cytotoxic and tumorigenesis.

TUGI was found to be abnormally expressed in cancer. lncRNA TUGI expression decreases in glioma and non-small cell lung cancer. However, it can act as a potential tumor suppressor by inhibiting cell proliferation and promoting apoptosis (26, 27). Fan et al. (28) found that TUGI expression decreases in various cancer tissues and cell lines. They observed that TUGI induces apoptosis and promotes cell cycle arrest in breast cancer cells (28). We have observed that TUGI is downregulated in ER-negative breast cancer cells after ponatinib treatment. We can suggest that TUGI can suppress proliferation, cell migration, and invasion.

Li et al. (29) found that the downregulation of UCAI reduces cell proliferation, cell migration, and invasion in esophageal squamous cell carcinoma (ESCC). We observed that UCAI decreased expression, such as ESCC cells, in MDA-MB-23I cell lines. As a result, in breast cancer cells treated with ponatinib, ponatinib can suppress metastasis and proliferation.

The downregulation of MALAT-I by SiRNA was found to inhibit prostate cancer cell growth, invasion, and migration in the G0/GI phases and inhibited the castration-resistant prostate cancer cell cycle (30). We observed a decrease in expression levels of MalatI in breast cancer cells treated with ponatinib. We suggest that the downregulation of Malat-I can play a role as a prognostic factor in breast cancer.

Li et al. (31) found that the expression levels of ZEBI ASI are higher in HCC tissues than in healthy neighboring tissues. They observed that ZEBI ASI expression increases with HCC metastasis. It was determined that survival time was shortened, and that recurrence rates were higher in patients with HCC who had high ZEBI ASI expression. ZEBI ASI supports the prolifer-

ation and metastasis of HCC; therefore, they asserted that they played an oncogene role in HCC (32). According to our results, lncRNA ZEB2NAT has a 2.54-fold decrease in the level of the hormone-sensitive breast cancer cells seen as oncogene.

Sun et al. (33) found that the expression of MEG3 is lower in breast cancer tissues than in adjacent tissues. The heterogeneous expression of MEG3 has been observed to induce proliferation by inducing the G0/GI proliferation phase and decreases cells in the S (mitotic) phase under in vitro conditions (33). In our study, MEG3 expression was decreased in ER-positive breast cancer cells. Therefore, we believe that apoptosis may be induced.

NEATI acts as an oncogene and tumor suppressor. While the expression of NEATI increases in most cancer tissues, there is a decrease in leukemia and multiple myeloma expression. Ghaforui-Fard et al. (34) reported a decrease in NEATI expression in various types of breast cancer, esophageal carcinomas, and gliomas. According to our data, the decrease in the expression of lncRNA NEATI determined as an oncogene was found to be significant. As a result, we think that target therapies can be considered as prognostic markers. In our study, the proliferation of the cells should be checked periodically.

We believe that ponatinib can be used as a candidate biomarker for future treatment of breast cancer. Such studies may be promising for a variety of diseases, allowing new lncRNA-based therapeutic strategies to be developed and the identification of new markers to make a diagnosis.

**Ethics Committee Approval:** Authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" (amended in October 2013).

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Review

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# Inflammasomes in Pediatric Autoinflammatory Diseases with Recurrent Fever

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Recurrent fever is common in young, especially preschool, children, and is associated with repeated episodes of fever that, in some cases, may last for weeks. Most often, these episodes are due to repeated infections; however, when there is a periodic recurrence, they can also be because of conditions such as autoinflammatory diseases (AIDs). Since their discovery nearly two decades ago, AIDs received a growing interest in research studies that not only increased our understanding of the AID pathologies, but also enriched the literature on innate immune responses, including inflammasome-mediated pathways. Inflammasomes are protein complexes formed in response to the activation of a group of intracellular pathogen-recognition receptors by a variety of inducers originating from the infection or cellular stress. They are involved in the generation of caspase-1, which is required for the generation of active interleukin (IL)-1β and IL-18, and were also shown to induce the generation of a wide variety of other cytokines, including tumor necrosis factor (TNF)-α, IL-6, and interferons (IFNs). This review aims not only to raise awareness about pediatric AIDs with periodic fever, but also to present the current literature about the molecular basis of inflammasome-mediated pathways involved in the disease pathogenesis. Future studies that would further enlighten the relationship between inflammasome-mediated pathways and AIDs would contribute to the development of more effective treatment strategies aiming to improve the patient life quality and help to avoid long-term complications.
**Keywords:** Autoinflammatory diseases, inflammasomes, periodic fever

## INTRODUCTION

The term *autoinflammatory disease* was first used in a study by Galon in 1999, which also identified the gene responsible for tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS). Autoinflammatory diseases (AIDs) were initially distinguished from autoimmune diseases by their characteristic feature of apparently unprovoked inflammation episodes without any auto-reactive T lymphocyte or auto-antibody induction (1). However, today it became apparent that both innate and adaptive immune responses can be activated during AIDs, and therefore, a new continuum model was suggested by McDermott and McGonagle in which immunological diseases are regarded as a continuum, with "pure monogenic autoinflammatory diseases" and "pure monogenic autoimmune diseases" being located at opposite ends (2).

Nevertheless, AIDs are considered to be mainly innately immune driven and are associated with neutrophil, macrophage, or monocyte-mediated inflammation with dysregulated cytokine production. They are also characterized by systemic inflammation, influencing tissues such as the skin, joints, conjunctiva, and serosal tissues (1). Over the last 20 years, the studies conducted on AIDs not only helped to improve our understanding of the AID pathogenesis, but also of the innate immune mechanisms, namely inflammasomes, which have also been linked to autoinflammatory, as well as autoimmune diseases.

### Inflammasomes

Innate immune responses are triggered as a result of the interaction between pattern recognition receptors (PRRs) and pathogen-associated molecular patterns (PAMPs), or damage-associated molecular patterns (DAMPs). Depending on their localization, PRRs can be divided into two broad families: (1) transmembrane receptors (e.g., Toll-like receptors [TLRs]) that are found in the plasma membrane and in endosomes, and (2) PRRs, which are expressed in intracellular compartments (e.g, nucleotide-binding domain and leucine-rich repeat-containing proteins [NLRP]).

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In response to a variety of stimuli originating from infection or cellular stress, while some intracellular PRRs, activate nuclear factor-κB (NF-κB), activator protein I, and interferon regulatory factors, some stimulate the assembly of protein complexes called inflammasomes. A typical inflammasome is composed of a sensor protein, which is an intracellular PRR; apoptosis-associated speck-like (ASC) protein that acts as an adaptor protein and possesses a caspase-recruitment domain (CARD); and pro-caspase-1. Some inflammasomes were also reported to include chaperone proteins (e.g., heat shock protein), which are thought to play an important role in stabilizing the protein–protein interactions (3). Up until today, there have been at least seven types of inflammasomes identified, and each is named according to the intracellular PRR associated: NLRP1, NLRP3, NLRP6, NLRP12, NLR Family CARD Domain Containing 4 (NLRC4), retinoic acid-inducible gene (RIG)-I, and absent in melanoma (AIM)-2. Among those, the NLRP3-inflammasome is regarded as the most important one as it is responsive to a wide range of PAMPs and DAMPs (4).

Upon detection of an activating signal, inflammasome sensors bind pro-caspase-1 either directly via CARD or indirectly via the pyrin domain (PYD), which interacts with ASC that has both the PYD and CARD domains. Pro-caspase then undergoes oligomerization in inflammasomes, as well as the autoproteolytic cleavage that yields caspase-1 (4). Active caspase-1 is a cysteine-dependent protease that is involved in the generation of interleukin (IL)-1β and IL-18 from pro-IL-1β and pro-IL-18, respectively. While both cytokines are involved in inflammatory diseases, infection, and cancer, IL-1β is regarded as one of the major mediators of fever in humans and is regarded as a "prototypic alarm cytokine" because of its ability to induce the NF-κB activation and inflammatory cytokine release. In addition to the IL-1 family member cytokines, inflammasomes were also associated with the induction of other wide variety of cytokines such as TNF-α, IL-6 IL-11, IL-17A, interferon (IFN)-α, and IFN-β (5).

Caspase-1 activation can also lead to pyroptosis, which is an inflammatory form of cell death associated with the release of proinflammatory cellular contents due to rupture of the plasma membrane. Its activation results in the pore formation on the plasma membrane, which disrupts the cellular ionic gradient causing increased osmotic pressure, swelling, and consequently, lysis. For this reason, caspase-1 is suggested to help host immune defense by both mediating the cytokine release and destruction of infected or damaged host cells (6).

***Inflammasomes and Pediadtric AIDs with Fever***

Recurrent fever is a common clinical presentation observed in preschool children, and is characterized by repeated episodes of fever that last for days or weeks with symptom-free intervals for weeks or months. While it is mostly caused by repeated infections, in cases of continued recurrence, it is suspected to be due to other conditions such as primary immunodeficiencies, anatomic and metabolic abnormalities, malignancies, and AIDs (5).

Fever is common among AIDs; however, some cases are associated with other prominent features, including pyogenic and granulomatous lesions, and are classified accordingly. On the other hand, AIDs associated with repeated fever episodes are classified differently and include monogenic forms of autoinflammation, as well as genetically complex autoinflammatory diseases such as the periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome (7).

**Hereditary Periodic Fever Syndromes**

***Familial mediterranean fever (FMF)***

Familial mediterranean fever is the most common monogenic inflammatory disease and is characterized by the episodes of fever, polyserositis abdominanal pain, and long-term complication of amyloidosis. The affected gene, known as the Mediterranean fever gene (MEFV), encodes the pyrin protein, which is involved in the inflammasome formation, regulation of apoptosis, inflammation, and IL-1β processing (8). The protein was also suggested to facilitate the autophagic degradation of inflammasome components including NLRP1, NLRP3, and pro-caspase I (9).

Up until today, there had been over 310 different mutations associated with FMF, most of which were positioned in the C-terminal B30.2 (PYR) domain involved in the protein–protein interactions (10). The most common FMF-associated mutations are M694V, M694I, V726A, M680I, and E148Q, which account for 70% of cases in the Mediterranean population (11). Among those, the M694V and E148Q mutations are responsible for severe and milder forms of the disease, respectively (8). FMF is known to primarily affect the Mediterranean populations, and our recent study demonstrated a high MEFV gene mutation carrier rate of 12.5% in the Turkish Cypriot population (12).

Albeit FMF was initially associated with the loss-of-function autosomal recessive mutations, previous studies reported that 30% of the FMF patients possessed only a single demonstrable mutation, and mice inserted with FMF-associated human B30.2 domains displayed phenotypes similar to those observed in patients with the FMF phenotype, while deletion of mouse pyrin did not result in any inflammatory phenotype (13, 14). Additionally, monocytes isolated from patients with FMF were demonstrated to release higher LPS-induced levels of IL-1β than those from healthy donors (15).

While these studies suggest that the gain-of-function mutations are responsible for the disease, Jamilloux et al. (16) showed that the disease is associated with hypermorphic mutations that result in reduction of the activation threshold of the pyrin inflammasome. Moreover, the B30.2 domain mutations were shown to decrease the activation threshold by inhibiting pyrin phosphorylation by pyrin kinases, and thereby reducing the 14–3–3 binding, which is involved in blocking of the inflammasome activity (17). On the other hand, the ASC-dependent NLRP3-independent inflammasomes were also suggested to facilitate the FMF development in mouse models (14).

***Cryopyrin-associated periodic syndromes (CAPS)***

Cryopyrin-associated periodic syndromes is a broad term used to cover a group of autosomal-dominant inherited diseases, including familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome (MWS), and neonatal onset multisystem inflammatory disease (NOMID), among which FCAS and NOMID are the least and most severe forms, respectively. The symptoms include fever, rash, headache, and conjunctivitis. Unique fea-

tures include cold-induced fever in FCAS; hearing loss in MWS and NOMID; overgrowth of knees; and symptoms associated with the central nervous system inflammation (CNS) of patients with NOMID. If left untreated, amyloidosis leading to end-stage renal disease can also be observed in patients with MWS (3).

While they were initially thought to be caused by distinct mechanisms, later studies reported that CAPS were all associated with the NLRP3 inflammasome (18). Up to date, over 175 different NLRP3 gene sequence variants and over 90 heterozygous NLRP3 mutations were associated with CAPS disease (19). It is a rare disease (prevalence of approximately 1–3 per 1 million), predominantly affecting the Northern European population (20).

An *in vitro* study reported increased levels of lipopolysaccharide (LPS)-induced IL-1β and IL-18 production in the caspase-1-dependent manner in CAPS patients, when compared with that from control subjects (21). Additionally, in contrast to monocytes from control patients, the FCAS patient monocytes were able to release IL-1β following incubation at 32°C which is the skin temperature of subjects exposed to cold (22). In the same study, the generation of several cytokines was IL-1β dependent, as reported by reduction in the levels following treatment with an IL-1 receptor antagonist (22). In another study, a fast IL-1β production by CAPS patients was associated with an altered redox state, as demonstrated by enhanced reactive oxygen species (ROS) and antioxidant levels in the CAPS monocytes that were left unstimulated (23). Moreover, macrophages from mice expressing CAPS mutations also displayed enhanced levels of the IL-1β and IL-18 production in response to TLR ligands in the absence of ATP. This was thought to be due to a reduced inflammasome activation threshold, which could be as a result of conformation changes introduced by the associated mutation (24).

***Mevalonate kinase deficiency (MKD)***

Mevalonate kinase deficiency is a rare disease with two phenotypes that include hyperimmunoglobulinemia D syndrome (HIDS), which is less severe but more common; and mevalonic aciduria (MVA), which is a more severe but a less common form (25). MKD presents with recurrent inflammatory attacks, with a sudden onset of high fever that may last up to 6 days, which is usually accompanied by other features including lymphadenopathy and gastrointestinal, mucocutaneous, musculoskeletal, and neurological symptoms (7). In a severe MKD form, children may also suffer from growth retardation, congenital defects, psychomotor retardation, progressive cerebellar ataxia, and hypotonia (25).

It is inherited in an autosomal recessive manner and is caused by a mutation of the mevalonate kinase (MVK) gene. While the MVK activity is usually below the detection levels in cultured fibroblast cells isolated from MVA, it is approximately 1%–7% of the control value in fibroblasts and leukocytes from patients with HIDS (26).

The MVK protein is important for the mevalonate pathway of cholesterol and isoprene biosynthesis, and its mutation in MKD leads to reduced mevalonate-derived intermediates, including geranylgeranyl pyrophosphate (GGPP). GGPP is the geranylgeranylation substrate required for the membrane targeting of ras homolog gene family member A (RhoA), which is involved in the regulation of the pyrin inflammasome activity via kinases that block pyrin effects by phosphorylation (7, 17, 27). Accordingly, pyrin inflammasomes were shown to be activated in response to Rho inactivation, and the inhibition of mevalonate pathway was reported to elevate the NALP3 expression, which suggests its involvement in MKD (7, 27, 28).

While its epidemiology is still unclear, and its incidence rate seems to vary depending on the region, the Netherlands is reported to have the highest prevalence with an incident rate of 1:200,000 (29). MKD is a very rare disease, and there are approximately 300 MKD patients known today. However, this is more likely an underestimated number due to lack of genetic screening programs for MKD, as well as undocumented and undiagnosed patients that stay unreported in literature (25).

***Tumor necrosis factor receptor-associated periodic fever syndrome (TRAPS)***

TRAPS, which was initially known as Hibernian fever, was first described in 1982 in a boy of Irish–Scottish origin (30). It is regarded as the most common autosomal-dominant AID with symptoms including fever, abdominal, chest and testicular pain, conjunctivitis, arthralgia, and myalgia, which may last up to 3 weeks or even longer. In addition to those, some patients may also suffer from periorbital edema and painful migratory erythematous rash (20). It is caused by the missense mutation of the TNF receptor (TNFR)-1 extracellular domain that influences the folding and trafficking of the protein (31).

Even though the gene responsible for TRAPS is not a part of inflammasomes, inflammasome-mediated pathways are still thought to be involved in the pathogenesis since ROS, which acts as a driver in the inflammation responsible for TRAPS, is known to contribute to the NLRP3 inflammasome activation (32, 33). In another study, mutant TNRF-1 was demonstrated to be trapped within the cell and increase the LPS-induced MAPK activation, as well as the proinflammatory cytokine secretion levels (31). Moreover, TNF-α was also reported to induce the inflammasome activation in the absence of any bacterial infection and was shown to act as an important transcriptional regulator of the NLRP3 inflammasome components (34, 35). On the other hand, while literature on the prevalence of TRAPS is still scarce, its incidence rate among the pediatric population in Germany was estimated at approximately 5.6 in $10^7$ persons (36).

***Familial cold autoinflammatory syndrome 2 (FCAS 2)***

Familial cold autoinflammatory syndrome 2 is a very rare genetic disease associated with NLRI2 mutations that is transmitted with autosomal-dominant inheritance. Current literature on the effect of this mutation on the immune response is not clear: Jeru et al. (37) reported a deleterious effect on the NF-κB signaling; however, Borghini et al. (38) did not observe such an inhibitory effect. On the other hand, its possible effect on the noncanonical NF-κB/p100 processing pathway is still unclear (38). The clinical symptoms resemble those reported for FCAS and include fever that may last up to 10 days, together with headache, joint symptoms, and skin rash triggered by the exposure to cold. In some cases, urticaria, abdominal pain and lymphadenopathy, and hearing loss were also observed (39).

### PFAPA

PFAPA, which is named according to its symptoms that include fever flares associated with pharyngitis, adenitis, and/or aphthous stomatitis in the absence of any infection, is the most common pediatric AID in regions without a high FMF prevalence and is known as the most common cause of recurrent fever among the pediatric population in Europe (40, 41). It is considered as a self-limited condition as it generally fades away before adulthood (7).

Today, even though the cause of the disease is not clear, it is thought to be associated with dysregulated innate immunity as the patients exhibit increased serum levels of proinflammatory cytokines, neutrophilia, and are responsive to corticosteroids and IL-1 blockade (2). Accordingly, in our study, tonsil samples isolated from patients with PFAPA displayed similar antimicrobial peptide expression levels when compared with those isolated from the group of patients with A beta-hemolytic streptococcal recurrent tonsillitis (42). Tonsils seem to play a crucial role in PFAPA since tonsillectomy was shown to be an effective treatment strategy for the PFAPA disease, and tonsillar microbiome was shown to differ between the control subjects and patients with PFAPA (43-45).

In contrast to most of the periodic fever syndromes, which were shown to be hereditary monogenic disorders, the genetic basis for PFAPA is not yet clear. Recent studies that examined siblings and related mothers suggested a familial susceptibility and demonstrated an association between PFAPA and some candidate genes. However, lack of a clear monogenic trait suggests heterogenous, polygenic, or complex inheritance of PFAPA syndrome (46).

Among those are included inflammasome-related genes, mostly NLRP3 and MEFV variants, which implies inflammasome-mediated pathways in the PFAPA development (47). In support of its role, PFAPA was also reported to be associated with the CARD8 mutation that disrupts its binding to the NLRP3 inflammasome and thereby reducing its negative regulatory activity (40, 48). This correlates with previous reports showing increased IL-1β expression during PFAPA febrile attacks (49, 50).

## CONCLUSION

Since the discovery of AID, the literature on the disease pathogenesis and innate immunity including inflammasome-mediated pathways have proceeded hand in hand, such that due to its important role in inflammation, IL-1 blocking strategy is being used to treat AID with periodic fever (5). Today clinical application of this strategy involves using medications that either target the IL-1 molecule directly (e.g., canakinumab, rilonacept, gevokizumab) or indirectly via antagonistic effects on the IL-1 receptor (e.g., anakinra) (5). Nevertheless, as the inflammasome activity is also needed for host response to microbial pathogens, a greater understanding of the balance between beneficial and detrimental inflammasome activation would contribute to the discovery of new and better inflammasome-focused treatment strategies.

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# Dog Bites and Their Treatment in Federation of Bosnia and Herzegovina

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## ABSTRACT

Cohabitation of humans and dogs often results in dog bites that may lead to severe health risks due to viral, bacterial, and parasitic zoonoses. Dog bites result in wounds and the dysfunction of damaged tissues, as well as possible infection, alongside the risk of rabies and tetanus, if appropriate and timely treatment is not administered. Pediatric and geriatric patients, as well as pregnant women, are the most vulnerable categories, being the most susceptible to psychological trauma. Research results suggest that in the Federation of Bosnia and Herzegovina (FB&H) during the 1996–2005 period, there were 6.9% more bites inflicted by dogs of known owners compared to the number of bites inflicted by stray dogs, but during the 2006–2010 period, this the percentage increased in favor of stray dogs. Dog bites are a serious social problem and pose a potential health risk due to viral, bacterial, parasitic, and fungal zoonoses. Timely and adequate treatment of bite wounds and the implementation of rabies-postexposure prophylaxis can significantly reduce health risks in patients who have suffered dog bites.

**Keywords:** Stray dogs, dogs of known owners, dog bite wounds, microorganisms in the canine oral cavity

## INTRODUCTION

It is estimated that approximately 2 million people worldwide visit a doctor every year due to bite wounds caused by dogs and cats. Although there are no accurate statistical data, the importance and consequences of these injuries should not be underestimated (1). Under certain circumstances, dogs attack indiscriminately.

In the United States, more than ten deaths per year occur due to dog bites. Most of the victims are children (2-4). Children are a particularly vulnerable population group (5, 6). It is interesting to note that 78% of dog bites in children occur on the head and neck, which is particularly dangerous due to the location of the bite. The primary risk relates to the cessation of the cervical carotid artery continuity because this leads to certain death. The cause of wounds on these parts of the body is usually associated with children's short stature. Also, children often play with dogs and bring their heads close to the dog's, thus making their lips, nose, and cheeks the central "target area" (7, 8). Head bites are especially risky if they are caused by a rabid animal because the rabies virus spreads through the nervous system reaching the brain, and the disease develops fast. Especially dangerous are dog bite injuries to human genital organs (9, 10). Although there is no clearly identifiable target group in the sense of specific occupations, mail carriers often seem to be more vulnerable. Morgan and Palmer (11) estimate that 5.000 postal workers seek medical assistance due to dog bites in the United Kingdom every year. Despite numerous studies, it is difficult to prove exactly why dogs bite, at what point, and what profile of person is particularly at risk. There are several reasons for the aggressive behavior in dogs, and most important are the protection of their puppies, guarding of their territory, and the search for food. A high population density as well as the number of people moving about in some areas are the most common factors that lead to an increase in dog aggression and the number of bites (12-16). Almost half of all dog bites occur within the family where the dog lives. Dogs may bite their owners, close family members, or neighbors (4, 17). A significant number of dogs of known owners attack people in urban and

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public areas due to the irresponsible behavior of their owners (18, 19). Some dog breeds (Bull Terrier, German Shepherd Dog, Cocker Spaniel, Pit Bull, Collie, Rottweiler, Doberman Pinscher, and Siberian Husky) have been identified as more aggressive dog breeds than others (4, 20-23). However, all dogs may exhibit aggressive behavior under certain circumstances (22). Alongside registered dogs, in countries undergoing transition and the regions with a war in their past, there is also a population of stray dogs. After their owners abandon them, dogs who are used to a certain lifestyle find themselves in a very confusing state. The loss of human support causes a number of problems, from territorial status to food security. Existential threats activate self-preservation mechanisms and a return to natural patterns of behavior, resulting in various unwanted events. Uncontrolled movement of dogs on roads causes traffic accidents, dogs usurp peace in the communities by barking, and they often attack people in a crowd, while their bites cause physical and mental harm (18). Several hundred different types of bacteria have been isolated from the canine oral cavity, and in their saliva, on their tongues, and gums (24-29). These are related to dogs of known owners who had, to a greater or lesser extent, health and veterinary care. A higher quantitative and qualitative representation of the bacterial population in the oral cavity of the stray dog population without any veterinary supervision is to be expected. After a dog bite, what usually occurs are minor lesions, for which no medical help is required. Dogs have rounded teeth and during the bite, their jaws pressurize, which may cause significant damage to the tissue (on the skin, muscles, tendons, blood vessels, nerves) (14). Dog bites may lead to severe infections of the wound with systemic complications and lead to prolonged disabling if not treated properly (26, 30-33). Microorganisms isolated from infected bite wounds are similar to those isolated from the oral cavity (34). The importance of the risk of infection of bite wounds is particularly emphasized: an infection by rabdovirus from the Lyssavirus family and by tetanus spores, as well as the subsequent development of these diseases. For these reasons, all dog bites should be treated in a timely and adequate manner (15).

**TABLE 1.** The number of stray dogs, their density in relation to the canton area, and the density of population in FBδH. Study performed in the period 2008–2009 (18)

| Cantons | Cantonal area km² | Population density | Number of stray dogs | Density of stray dogs |
|---|---|---|---|---|
| Tuzla | 2649 | 187.9 | 4500 | 1.69 |
| Zenica-Doboj | 3.334 | 119.9 | 10 000 | 2.99 |
| Herz-Neretva | 4.401 | 51.5 | 3300 | 0.75 |
| Sarajevo | 1.276 | 329.9 | 4000 | 3.13 |
| Total | 11660 | Average 172.3 | Total 21800 | Average 2.14 |

**Epidemiological Aspects of Dog Bites in the Federation of Bosnia and Herzegovina**

The bites of different animals are not uncommon, and almost as a rule, they pose a problem to both the person who was bitten and the health system. Approximately 90% of animal bites are caused by dogs and cats (35). In Bosnia and Herzegovina (B&H), like in most areas of the world, the largest number of bites is inflicted by dogs and cats. According to the current Law on the Protection of the Population from Infectious Diseases in the FBδH, every animal bite must be registered by the ordinating physician and reported to the primary-level epidemiological services. From this point, an application is sent to the epidemiological services at the secondary level (the Public Health Department of the Canton) and is further transmitted to the tertiary level (the Public Health Institute of the FB&H) (36). However, many bitten persons, especially those with minor injuries, do not seek medical help, so many bites remain unregistered. As a result, the actual number of people injured is certainly much higher than the number of registered bites. These injuries are caused by dogs living alongside humans as domestic animals or pets. However, there is another category of dogs, which are those who do not have an owner, so-called street dogs or stray dogs. The number of these dogs in B&H increased considerably in the post-war period. Their presence is evident, primarily in urban, but also in rural areas of B&H, and this has an adverse effect on the health of the population (18). One of the health risks are bites by this dog category. Although there is no difference between the bites of stray dogs and the bites of dogs that have an owner, the bites of stray dogs are more problematic when it comes to the potential risk of rabies because these dogs are often not

**TABLE 3.** Attack rates by cantons in FBδH. Study performed in the period 2008–2009 (12)

| Cantons | Number of stray dogs | Number of people bitten | Attack rate |
|---|---|---|---|
| Tuzla | 4500 | 1749 | 38.9 |
| Zenica-Doboj | 10 000 | 796 | 1.26 |
| Herz-Neretva | 3300 | 243 | 7.36 |
| Sarajevo | 4000 | 1613 | 40.32 |
| Total | 21800 | 4401 | Average 20.18 |

**TABLE 2.** Number of people bitten, presented by canton in FBδH (18)

| | CANTONS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Tuzla | | Zenica-Doboj | | Herz-Neretva | | Sarajevo | | |
| Year | N | Bites/100000 inhab | N | Bites/100000 inhab | N | Bites/100000 inhab | N | Bites/100000 inhab | Total |
| 2013 | 458 | 102 | 209 | 57 | 62 | 28 | 419 | 101 | 1148 |
| 2014 | 450 | 101 | 165 | 45 | 51 | 22 | 483 | 117 | 1149 |
| 2015 | 410 | 92 | 242 | 66 | 75 | 33 | 385 | 93 | 1112 |
| 2016 | 431 | 96 | 180 | 49 | 55 | 24 | 326 | 79 | 992 |
| Total | 1749 | Average | 796 | Average | 243 | Average | 1613 | Average | 4401 |

N: number of people bitten

**TABLE 4.** Comparison of a 4-year period of dog bites in Sarajevo (B&H), Zagreb (Croatia), and Belgrade (Serbia) (12, 19, 38)

| | Sarajevo | | | Zagreb | Belgrade | | |
|---|---|---|---|---|---|---|---|
| Year | Stray dog bites | Owned Dogs' bites | Total dog bites | Total dog bites | Stray dog bites | Owned dogs' bites | Total dog bites |
| 2003 | 58 | 68 | 126 | 889 | 1694 | 748 | 2442 |
| 2004 | 71 | 60 | 131 | 902 | 1345 | 580 | 1925 |
| 2005 | 99 | 60 | 159 | 865 | 1509 | 1056 | 2565 |
| 2006 | 119 | 63 | 182 | 802 | 1482 | 947 | 2429 |

**TABLE 5.** Bacterial microflora in 50 infected dog bite wounds in humans (26, 29, 46)

| Type of bacteria | Percentage of wound presence (%) | Type of bacteria | Percentage of wound presence (%) |
|---|---|---|---|
| *Pasteurella* | 50 | *Escherichia coli* | 6 |
| *Streptococcus* | 46 | *Klebsiella* | 4 |
| *Staphylococcus* | 46 | *Lactobacillus* | 4 |
| *Neisseria* | 32 | *Citrobacter* | 4 |
| *Corinebacterium* | 12 | *Flavobacterium* | 4 |
| *Moraxella* | 10 | *Micrococcus* | 4 |
| *Enterococcus* | 10 | *Proteus mirabilis* | 4 |
| *Bacillus* | 8 | *Capnocytophaga* | 2 |
| *Pseudomonas* | 6 | *Eikenella corrodens* | 2 |
| *Actinomyces* | 6 | *Flavimonas* | 2 |
| *Brevibacterium* | 6 | *Stomatococcus* | 2 |

vaccinated, and if they are, the vaccination data are difficult to find. In addition, these dogs cannot be placed under the 10-day veterinary supervision, while this is done with dogs who have an owner. In some FB&H cantons, during the 1996–2005 period, there were 1260 stray dog bites and three bites inflicted by dogs with known owners, that is, 6.9% more bites inflicted by dogs of known owners compared to the number of bites from stray dogs (37). The bites of dogs who have owners indicate the insufficient education of their owners. The percentage of dog bites, in relation to whether the dogs are stray animals or have owners, changed in the FB&H over the 2006–2010 period, where the percentage increased in favor of stray dogs. The reason for this was an enormous increase in the population of stray dogs following the Animal Welfare Act, with the local government bodies being unprepared to provide enough shelters for these abandoned animals (12, 37). The following table I shows data on stray dogs in several cantons in FB&H.

Table 1 and 2 show that the largest number of bites is in areas with a higher population density, which corresponds to the results by Heath and Chomel (13).

The frequency of dog bites in the mentioned cantons of the FB&H differed. It ranged from 1.26 (in the Zenica–Doboj Canton) to 40.2 (Sarajevo Canton) (Table 3). A similar situation occurred in Belgrade (Serbia) where the average number of dog bites was 148.48 per 100,000 inhabitants in the 2003–2006 period (19). The reasons for the higher dog bite rate per 100,000 inhabitants in relation to Sarajevo Canton should be seen in the fact that Belgrade has a greater population density, as well as a larger number of dogs, both those of known owners and stray dogs, which ultimately leads to a greater number of bites (Table 4). In Zagreb (Croatia), during the 1996–2005 period, a total of 10.177 people were physically inspected after injuries (bites, scratches) inflicted by dogs, cats, jackals, and other domesticated and exotic animals. In the observed period, 2003–2006, dogs bit, on average, 864.5 persons a year (38) (Table 4).

Reports from the Greek province of Macedonia indicate a high incidence of dog bites, much higher than that involving other animals (cats, horses). In this study, during the 1989–2009 period, the geriatric population of people aged >65 years was the most vulnerable to dog bites (39). In a similar study from 2006–2010, 328 cases of dog bites were recorded in western Turkey. Out of this number, 48% were in the population of young people aged 0–18 (9).

**The Most Susceptible Groups**

Anybody may be bitten, but there are still people who are at greater risk. People who own dogs, especially if they have a larger number of domestic dogs, are at greater risk of bites than those who do not own them. Men are more commonly bitten than women (40). This ratio is different in different studies. In the aforementioned research in India, 64% of the people bitten were men (36, 41, 42). From the aspect of age groups, children aged 5–9 years are at especially high risk, because they often play with dogs, both with their own and those of unknown owners. Apart from being the most often bitten group, children often hide the fact that they were bitten, so medical care may be given much later (35, 43). In an Indian study, the largest number of those bitten (47.5%) were children aged 2–18 years. The largest number of bites to children occur during their outdoor activities (cycling, playing with a ball, running). The location of the bite is often associated with the age of the child, where younger children are more likely to have head injuries, while in the older children, lower extremities are mainly involved (44). The same data were obtained from a study conducted in Italy (45). The greatest number of bites is unprovoked, but they also occur at the time when the dog is feeding or caring for its puppies (42). Dog bites happen throughout the year, but the largest number of bites occurs in the summer, accounting for half of all bites (15).

**The Ethiopathogenesis of Bite Wound**

Microorganisms that exist in the canine oral cavity, together with saliva, enter the avulsionated tissue under pressure, immediately after the bite, and most commonly in an extremity—the hands, legs, head, or neck—whereby even a small number of bacteria with high virulence can cause an infection immediately after the bite. The force produced by the dog's eyeteeth during the bite varies among breeds, from 310 kPa to almost 31,790 kPa in specially trained dogs (11). Along with the surface damage to the skin, muscles, tendons, blood vessels, and nerves are also

often damaged. In deeper bite wounds, there is a risk of contamination from tetanus spores and consequent tetanus, especially if the wound is not treated adequately (14, 15). However, the greatest risk associated with dog bites is the risk of rabies in the case of an injury from a rabid animal (16). It is estimated that approximately 55,000 people die from rabies every year. There were no cases of human rabies in the researched areas of the FB&H (18). Talan et al. (46) investigated bacterial microflora in 50 infected dog bite wounds in humans (Table 5). The most commonly isolated aerobic bacteria were *Pasteurella* (50%), *Streptococcus* (46%), *Staphylococcus* (46%), *Neisseria* (32%), and *Corinebacterium* (12%).

Bite wounds in people who have a poor immune status due to a previous illness, or who have been diagnosed with diabetes or a peripheral circulation disease, are often infected. In infected and fresh bite wounds (less than 8 hours after the bite and when the wounds were not clinically infected), it was found that they were polymicrobial with a wide combination of aerobic and anaerobic bacteria (26, 46, 47).

**Clinical Symptoms of Bite Wounds**

Usually there is some damage to the skin, which may or may not be accompanied by bleeding. According to the research conducted by Talan et al. (46), of the 50 patients with bite wounds examined, 60% of the wounds were with only an eyeteeth imprint, 10% of the wounds had smaller perforations with lacerations, and 30% were combinations of both. Bruising occurs around the bite wound. Most infections were purulent but appeared without the abscess formation (58%); followed by nonpurulent wounds with cellulitis, lymphangitis, or both (30%); and abscesses (12%). Limited and painful movements of the fingers and joints occur if the extremities are bitten, together with swelling and redness in the area of the injury (30).

**Basic Principles of a Bite Wound Treatment**

Although all bite wounds carry the risk of infection, abundant rinsing of the wound significantly reduces the risk of infection (4). At home, tap water and soap are used to rinse wounds. This is mostly done as self-help in the case of an adult or as first aid in the case of a child. After that, medical assistance should be sought. As the initial part of the bite wound treatment, it is necessary to evaluate the size and depth of the wound; the degree of damage to the surrounding tissue or the nerves; and any damage to the tendons, bones, and joints. As needed, complete wound inspection and debridement should be performed, if necessary, under local or general anesthesia, as well as the removal of any foreign bodies, most often the dog's teeth (11). All bite wounds should be thoroughly washed, first with 3% hydrogen peroxide ($H_2O_2$) solution, followed by injection of povidone iodine solution (Isobetadine) using a 20 mL syringe (30). To reduce the potential risk of tetanus, it is necessary to postpone the wound closure wherever possible, or leave the wound open. It is necessary to lift and immobilize the wounded extremity to prevent edema (11). Superficial abrasive wounds should be thoroughly rinsed but not stitched. Patients are to be prescribed antibiotics. The antibiotic of choice is amoxicillin-clavulanate (Augmentin) (30). Patients should be assessed for the tetanus immunization status and treated with immunization or immunoglobulins if necessary. The necessity of rabies prophylaxis should be assessed on a case-by-case basis.

**Treatment of an Infection Resulting from a Dog Bite**

Although a relatively small number of bite wounds become infected, in larger wounds and in immunocompromized patients, it is necessary, as a measure of precaution, to prescribe antibiotics that are effective against Pasteurella, anaerobes and staphylococci, and these should be modified according to the culture of the results. Empirical imipenem with cilastatin (500 mg four times a day, intravenously) and clindamycin (900 mg four times a day, intravenously) are used for very severe infections. For patients with severe allergic reactions to penicillin, ciprofloxacin (400 mg twice a day, intravenously) plus metronidazole (500 mg three times a day, intravenously) replaces imipenem (11). The treatment of cellulitis usually lasts for 10–14 days, of tenosynovitis 3 weeks, of septic arthritis 4 weeks, and of osteomyelitis 6 weeks. The use of oral antibiotics in therapy when the C-reactive protein concentration is <50 mg/L is a pragmatic approach, which has proved effective in practice (11).

## CONCLUSION

In FB&H, the presence of a large population of stray dogs is evident. Bites from stray dogs are more problematic than those of registered dogs and are becoming an increasing problem. Due to the report deficit on the number of stray dogs, dogs of known owners, and the bites of those from the Southeast Europe region, we did not have the opportunity to adequately compare the existing results from the FB&H. The biggest risk for dog bites is actually the possibility of rabies or tetanus. Patients should be assessed for the tetanus immunization status and treated with immunization or immunoglobulins if necessary. In the FB&H, for a number of years, no cases of rabies have been registered in humans, but occasionally, rabies is registered in animals, most often wild. That is why each individual is treated on a case-by-case basis, and the risk of rabies and the post-exposure protection are individually evaluated and determined. Dog bites are important for both the health and veterinary systems, and it is necessary to address this problem with the coordination of veterinary and health care services

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# Antioxidants used in Restorative Dentistry

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**ABSTRACT**

In recent years, antioxidants have been successfully used in dentistry because of their beneficial effects on human health. The most important effect of antioxidants known is to neutralize the harmful effects of free radicals. Free radicals can cause destruction of cell membranes and DNA, DNA mutations, lipid peroxidation, and other diseases. Although not routinely used, the use of antioxidants continues to expand worldwide, and studies are conducted to determine the effective use of these products. In the field of restorative dentistry, antioxidants are used for bonding procedures after bleaching, remineralization, hypersensitivity, and pulp capping. This review highlights the use and clinical significance of antioxidant therapy in these treatment modalities of restorative dentistry.

**Keywords:** Antioxidant, restorative dentistry, dental bleaching, remineralization, pulp capping, dentin hypersensitivity

## INTRODUCTION

The term antioxidant is used for molecules that reduce the damaging capacity of free radicals using their scavenging property. They are stable enough for donating an electron to a rampaging free radical and neutralizing it (1). Free radical species were defined by Halliwell et al. (2) as "any species capable of independent existence that contains one or more unpaired electrons." They can be in the form of reactive oxygen species (ROS) or reactive nitrogen species. ROS can lead to tissue damage by different mechanisms, such as DNA damage, lipid peroxidation, protein damage, and oxidation of important enzymes (3).

Antioxidants that are helpful in inhibiting the negative effects of ROS can include the following:

1. The "scavenging" or "chain-breaking" antioxidants, such as alpha tocopherol (vitamin E), ascorbic acid (vitamin C), beta carotene (vitamin A), urate, and bilirubin (4). These antioxidants scavenge the active radicals to suppress chain initiation and/or break the chain propagation reactions (1).

2. Thiol-containing antioxidants named as "preventative antioxidants," such as albumin, transferrin, lactoferrin, ceruloplasmin, haptoglobin, and ascorbic acid. These are large proteins by nature (4). These antioxidants also suppress free radical formation by reducing hydroperoxides and hydrogen peroxides ($H_2O_2$) to alcohol and water without forming free radicals, respectively (1).

3. Enzyme antioxidants that function by catalyzing the oxidation of other molecules, such as superoxide dismutase, catalase, and glutathione systems (1, 4).

Antioxidants were used in studies related to industrial processes in the late 19th and 20th centuries. In the field of biology, the first research focused on the use of antioxidants in preventing the oxidation of unsaturated fats (1).

Antioxidants are also widely used in dentistry. Eugenol with its scavenging, preventing effect and presence of enzyme activator for antioxidant action has been used effectively for toothache. Epigallocatechin-3-gallate in green tea reduces the risk of dental caries and plaque formation with its scavenging effect (4).

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In this review, the effects of antioxidants in the treatment methods of restorative dentistry including bonding procedures after bleaching, remineralization, hypersensitivity, and pulp capping were compiled.

**Bond strength after bleaching**

Dental bleaching is a cosmetic procedure that is often requested in clinical practice. The bleaching procedure can be classified into two groups as vital bleaching and nonvital bleaching (5).

Vital tooth bleaching techniques are further divided into two groups as home and office bleaching done with carbamide peroxide and $H_2O_2$, respectively (6). Bleaching agents can cause different side effects, such as pulpal sensitivity, microleakage in restorations, external root resorption, and changes in the composition of tooth structure (7-9). Vital dental bleaching agents contain $H_2O_2$ or carbamide peroxide ranging from 3% to 40% (5).

Lasers and lights are used to initiate the dental bleaching procedure under a photochemical reaction. As a result of this chemical interaction, an increase in the release of hydrogen released from $H_2O_2$ is observed (10). $H_2O_2$ is an agent that decomposes and creates unstable free radicals, such as perhydroxyl radicals, hydroxyl radicals, superoxide anions, and perhydroxyl anions, when it diffuses into the tooth, thus causing oxidation (11).

Many studies have shown that carbamide peroxide and $H_2O_2$ can negatively affect the bonding strength of the composite to the tooth structure (12-14).

Some methods, such as delayed bonding (wait time, 24 h to 3 weeks), removal of the enamel, the use of acetone-based adhesives, alcohol application on the tooth surface, and the use of antioxidants, are proposed to counteract the decreasing bond strength after bleaching (15-17).

Nowadays, some studies have proven that the use of antioxidants, such as sodium ascorbate, alpha tocopherol, grape seed, and proanthocyanidin, can eliminate the harmful effects caused by free radicals after dental bleaching and can increase the bond strength of composite resins (18, 19).

Aloe vera, pomegranate peel, grape seed extract, green tea, and sodium ascorbate were used in a study to evaluate the effects of different antioxidants on shear bond strength to home-bleached enamel. The untreated group was determined as the control group. No significant differences were observed between the shear bond strength of the control group and the experimental groups treated with different antioxidants (20).

It was shown in a study comparing the effectiveness of 10% sodium ascorbate and 5% proanthocyanidin agents on the bond strength after bleaching that the use of antioxidants before bonding on bleached surfaces reverses the harmful effect of bleaching agents and increases bond strength (21).

The benefits of three herbal antioxidants, such as 5% grape seed extract, 5% pine bark extract, and 5% pomegranate peel extract, on the recovery of reduced bond strength were investigated. As a result, it was found out that the use of antioxidants, especially 5% pine bark extract application after bleaching totally neutralizes the deleterious effects of bleaching on enamel surface and increases the SBS significantly (22).

An *in vitro* study compared the effects of 35% sodium ascorbate application as an antioxidant and 1-week delayed bonding procedure on microtensile bond strength after bleaching. According to the results, 35% sodium ascorbate application showed similar bonding strength to 1-week delayed bonding procedure. In this study, both delayed bonding and immediate bonding procedures were found as time-saving applications that restored the bond strength similar to the original bond strength (23).

Alpha tocopherol, 10% ascorbic acid, and hesperidin were used for evaluating the effects of antioxidants on microshear bond strength after chemical and laser bleaching. Hesperidin and ascorbic acid applications showed no significant differences compared with the control group that exhibited the highest microshear bond strength value. Alpha tocopherol showed the lowest microshear bond values. The results of the study showed that immediate bonding procedures could be applied after laser-assisted bleaching without the need for antioxidant application since laser-assisted bleaching did not induce surface morphological changes (24).

An in vitro study examined the shear bond strengths of composite resins to bleached enamel using 10% sodium ascorbate, 10% alpha tocopherol, 10% grape seed extract, and 10% guava seed extract as antioxidants. The results showed that guava seed extract was the most effective antioxidant increasing the bond strength. With the use of these antioxidants, the bonding strengths of the bleached enamel were effectively increased (25).

Dikmen et al. (26) investigated the effects of some antioxidants, such as proanthocyanidin, Accel, and noni fruit juice, on the microtensile bond strength of a single etching adhesive system on dentin treated by sodium hypochlorite (NaOCl). No significant differences were found among the antioxidant-treated groups. The NaOCl group showed lower microtensile bond strength than the other groups.

Nonvital bleaching includes several techniques, such as walking bleaching or nonvital power bleaching. The most commonly used procedure is walking bleaching. The mixture of sodium perborate and water is placed in a pulp chamber, and this application is maintained until the desired color is achieved (5). Antioxidants can be used, such as vital bleaching technique, for immediate restoration. According to an *in vitro* study, sodium ascorbate was applied as an antioxidant after nonvital bleaching, the shear bond strength of the composite to the bleached dentin was evaluated, and the effects were compared with calcium hydroxide, $Ca(OH)_2$, a buffering agent. The results of the study showed that sodium ascorbate showed a significant increase in the bond shear strength of composite, whereas $Ca(OH)_2$ showed failure (27).

Aslan et al. (28) compared the effect of immediate cementation with delayed cementation-on the bond strength of fiber posts after intracoronal bleaching with 35% carbamide peroxide. Researchers divided the experimental groups into 5 as; no bleaching, immediate cementation, immediate cementation after

antioxidant application (Sodium ascorbate), 14-days delayed cemetation and 14 days delayed cemetation after sodium ascorbate application. The 14-day delayed bonding was reported to be more effective than the use of sodium ascorbate application.

**Remineralization**

In 2010, remineralization was defined by Cochrane et al. (29) as "the process whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralized enamel to produce net mineral gain."

Proanthocyanidins are found in grape seeds and grape seed extracts. Silva et al. (30) compared the remineralization effect of grape seed extract and fluoride under cariogenic challenge on enamel and dentin. The samples that were treated with grape seed extract and fluoride showed statistically higher remineralization than the untreated group (30).

Hesperidin is a citrus flavonoid antioxidant. Hiraishi et al. (31) compared the effect of hesperidin on the remineralization of dentin lesions with chlorhexidine. Their study showed that hesperidin enhances remineralization by protecting the collagen structure (31).

According to a study in 2017, researchers used two antioxidants after bleaching and observed their effects on enamel structure and hydroxyapatite crystal growth. In the study, catalase and sodium ascorbate were used as antioxidants, and the test groups were examined after 72 h. The results showed that using antioxidant after bleaching can increase the remineralization capability of saliva, but the topographical properties do not reverse to the initial form (32).

Catalase is an antioxidant that provides remineralization in the fluoride-mediated enamel microstructure, removes free radicals effectively, and affects enamel stiffness. Thakur et al. (33) aimed to neutralize the free acids by applying catalase to teeth treated with 37% hydrogen peroxide as whitening treatment and to restore the surface hardness by topical fluoride application. The surface hardness of whitened enamel was found to significantly increase with catalase and fluoride applications.

**Dentin hypersensitivity**

Dentin hypersensitivity is a short and sharp pain arising from exposed dentin in response to a thermal, evaporative, tactile, osmotic, or chemical stimulus and cannot be associated with any other form of dental pathology or defect. The most widely accepted theory behind the mechanism of dentin hypersensitivity is the hydrodynamic theory often attributed to Brannstrom (34-36). According to this theory, the abrupt movement of fluid within the dentinal tubules causes an outward flow of fluid from the pulp to open dentin surface, causing hypersensitivity (34). The treatment modalities of hypersensitivity include occluding the dentinal tubules or impeding or diminishing neural transmission (34, 35). For this purpose, several strategies, such as lasers, iontophoresis, dentin sealers, and soft tissue grafting, are used. The use of toothpastes is widely preferred in delivering the desensitizing agents (37). Desensitizing agents, such as potassium, fluoride, hydroxyapatite, copal varnishes, and $Ca(OH)_2$, or dentin bonding agents are used (34,38). There has been a growing interest in the use of natural products and antioxidants for the treatment of dentin hypersensitivity (37, 38). According to the results of a study, a dentifrice containing antioxidants, such as ferulic acid, silymarin, and phloretin, in addition to sodium monofluorophosphate, nano-hydroxyapatite, and potassium nitrate, reduced dentinal hypersensitivity in a 2-day to 2-week period (34).

Propolis, a bee product, has been a striking natural agent for the treatment of dentin hypersensitivity (38). Sankari et al. (39) stated that propolis occludes dentinal tubules and reduces dentinal hypersensitivity of periodontally involved teeth. Purra et al. (38) compared the desensitizing effect of propolis with 5% potassium nitrate and distilled water. Their results showed that propolis is the most effective desensitizing agent in the intermediate relief of sensitivity.

Madhawan et al. (36) compared the clinical efficiency of propolis with sodium fluoride, casein phosphoprotein-amorphous calcium phosphate fluoride, and distilled water and found that propolis is the most rapid agent in treating dentinal hypersensitivity. Another study pointed out that propolis showed the most rapid decrease of dentin hypersensitivity compared with hydroxyapatite, sodium fluoride, and potassium nitrate. The authors interpreted that the anti-inflammatory action of propolis stimulates reparative dentin formation that is able to reduce dentin permeability (40).

**Pulp capping**

When dental pulp is exposed either traumatically or because of caries, direct pulp capping technique is used for protecting pulpal health and function, allowing the patients to retain their teeth longer and at lower costs than root canal treatment, which is an alternative invasive technique (41). For this purpose, a wide range of materials have been used (42, 43). These materials should control infection, prevent microleakage, promote hard tissue formation, and should be handled easily (42).

Some of the materials used for direct pulp capping are zinc oxide eugenol (ZOE), glass ionomer cement or resin-modified glass ionomer cement, adhesive systems, $Ca(OH)_2$, and mineral trioxide aggregate (MTA). Eugenol has been used for many years in dentistry as liners, bases, cements, or temporary restorations. However, capping is questionable. Since the cytotoxicity of ZOE is high, controversy exists for its use in direct pulp capping therapies (44).

$Ca(OH)_2$ has been accepted as the gold standard in vital pulp therapies (41). When it is applied to the exposed pulp tissue, it induces hard tissue formation that is an important advantage in vital pulp therapies (42). However, high solubility in oral fluids, lack of adhesion, and degradation after acid etching are some of the undesirable effects of this material (43). Additionally, the high pH (12.5) causes the destruction of cell membranes and protein structures. The reparative dentin formed by $Ca(OH)_2$ results in a porous and incomplete barrier structure, and the formation of extensive dentin causes the obliteration of pulp chamber (42, 43).

As stated previously, the high pH value also made $Ca(OH)_2$ a toxic material and prone to dissolve soft tissue that causes

chronic inflammation and cell necrosis. Al-Shaher et al. (45) found that propolis, an antioxidant agent, has superior properties than $Ca(OH)_2$ and does not produce pulpal inflammation, necrosis, and infection while inducing tubular and high qualified dentin production.

Mineral trioxide aggregate is a pulp capping agent that consists of calcium oxide, tricalcium silicate, dicalcium silicate, tricalcium aluminate, and bismuth oxide. Although $Ca(OH)_2$ and MTA have many similarities, such as antibacterial property, radiopacity, or biocompatibility, MTA has some advantages, such as containing iron or sealing ability (44).

The results of a study that compared the effects of ProRoot MTA (Dentsply Caulk Milford, DE, USA) (MTA material), Dycal (Dentsply Caulk Milford) ($Ca(OH)_2$ material), and propolis showed that the response of dental pulps to propolis was comparable to $Ca(OH)_2$ and MTA (43).

Ahangari et al. (42) investigated the effect of propolis as an alternative material to $Ca(OH)_2$ on dentin regeneration and on the potential role of dental pulp stem cells. They reported that although more stem cells were found in the $Ca(OH)_2$ control group at each time point, the propolis group showed superior properties over $Ca(OH)_2$ with respect to the prevention of the formation of inflammation, infection, and necrosis and inducing the formation of higher quality tubular dentin. The anti-inflammatory property of propolis aids to inhibit the synthesis of prostaglandins and helps the immune system by promoting phagocytic activity, stimulating cellular immunity, and increasing the healing effects on epithelial tissue. Additionally, the elements of propolis, such as iron and zinc, are effective in the synthesis of collagen, and flavonoids regulate the immune system response (43, 46). According to the results of a study, propolis flavonoids, as direct pulp capping agents, can stimulate reparative dentin and may delay the formation of pulp inflammation (46). Parolia et al. (43) reported that the response of dental pulps to propolis as a pulp capping agent is comparable to that of ProRoot MTA (Dentsply Caulk Milford) and Dycal (Dentsply Caulk Milford). Sabir et al. (46) reported that propolis delays pulpal inflammation and stimulates reparative dentin formation.

Glass ionomer cements/resin glass ionomer cements and adhesive systems are cytotoxic when they are placed directly on the pulp tissue. These two agents showed chronic inflammation when used as direct pulp capping agents (44, 47). N-acetylcysteine (NAC) showed significant antioxidant effect by inhibiting glutathione depletion (48). Goldberg et al. (48) reported that NAC induces reparative dentin formation in a rat molar model. The effect of NAC in reducing cytotoxicity and in inducing mineralized tissue conductivity with resin-modified glass ionomer cements was examined. NAC detoxifies and functionalizes resin-modified glass ionomer cements by detoxification and antioxidant cell proliferation (49).

Catalase, as an oxidoreductase of $H_2O_2$, has been used as one of the enzymatic defense mechanisms of the body against toxic oxygen species or reactive oxygen intermediates. Alaçam et al. (50) reported that perforated dog teeth treated with the topical application of catalase antioxidant enzyme showed better healing in the 90-day period than the teeth treated without catalase.

## CONCLUSION

Although there are studies showing the benefits of antioxidant use in restorative dentistry including bonding procedures after bleaching, remineralization, hypersensitivity, and pulp capping, studies must be conducted on reducing the risk of dental caries and plaque formation to determine the most appropriate material and methods for the exact regeneration of dentin–pulp complex and to reduce the undesirable effects caused by dentin hypersensitivity. Most of the studies proved that the use of antioxidant agents improves the bond strength after bleaching. As a result, the use of antioxidants can open a new possible treatment modality for restorative procedures to prevent the negative effects of ROS.

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Case Report

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# Cerebral Malaria with Corpus Callosum Splenium Lesion

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Malaria is still an important public health problem especially in less developed and developing countries. Cerebral malaria is one of the most severe complications of malaria. Mortality is high, and some neurocognitive problems may be observed in surviving patients during the long-term follow-up. A 27-year-old man was admitted to the emergency department with fever, vomiting, diarrhea, muscle pain, fatigue, headache, progressive drowsiness, and seizure. His cranial magnetic resonance imaging revealed focal T2 hyperintensity in the corpus callosum splenium. His blood smears showed diagnostic crescent-shaped gametocytes for *Plasmodium falciparum* malaria. His physical and neurological examinations were normal at discharge. Corpus callosum splenium involvement is really rare, but if observed, it is suggestive for diagnosis in patients with suspected cerebral malaria. Malaria is not only a problem in endemic areas, but it is a problem worldwide in this globalization age. We all have to be familiar to the systemic and also central nervous system complications of malaria.

**Keywords:** Malaria, cerebral malaria, corpus callosum splenium

## INTRODUCTION

Annually, over 500 million clinical malaria cases have been observed worldwide, thereby causing over 1 million deaths. Cerebral malaria is one of the most severe complications of infection with *Plasmodium falciparum* malaria. Mortality is high, and some neurocognitive problems may be observed in surviving patients during the long-term follow-up. Cerebral malaria is seen in 2% of malaria cases. It mainly affects children in endemic areas. Cerebral malaria frequency is rarer in adults than in children (1, 2).

Cerebral malaria may cause diffuse involvement in the brain; therefore, wide spectrum neurological manifestations can be observed. Altered consciousness and coma are the most common neurological manifestations, followed by seizures. In addition to transient extrapyramidal, neuropsychiatric manifestations, and focal motor, sensorial signs may occur. Approximately one-third of patients with cerebral malaria die (2, 3).

Cranial magnetic resonance imaging (MRI) can be normal despite the neurological signs. However, various reports of MRI in cerebral malaria have revealed focal or diffuse signal changes in the centrum semiovale, corpus callosum, basal ganglia, cortex, cerebellum, and brainstem (2). Here we report a case of a well-treated adult patient with cerebral malaria who has a corpus callosum splenium lesion.

## CASE PRESENTATION

A 27-year-old man was admitted to our emergency department with fever, vomiting, diarrhea, muscle pain, headache, and progressive drowsiness. The patient is originally from Turkey and studying in Cyprus for 2 years. He had regular work-related travel history to Sierra Leone. He had never used any prophylactic medicine for malaria. On physical examination, tachycardia, icterus, hepatosplenomegaly, and anemia were found. His body temperature was 38.3 °C. On neurological examination, he was lethargic and had a Glasgow Coma Scale score of 6. He had meningeal irritation signs with neck stiffness. Additionally, convulsions with nystagmus, generalized tonic spasms, and apnea were observed. Cranial MRI was performed urgently and revealed focal T2 hyperintensity in the corpus callosum splenium that showed diffusion restriction in diffusion-weighted imaging (DWI) and *apparent diffusion coefficient (ADC).* Additionally, any contrast enhancement in the parenchyma and meninges

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was not observed (Figure 1). On electroencephalography (EEG), generalized severe background slowing was found (Figure 2a).

His laboratory results were as follows: hemoglobin 8.7 g/dL, white blood cells 16,000/mL, platelets 59,000/mL, erythrocyte sedimentation rate 65 mm/h, C-reactive protein 20 mg/dL, aspartate aminotransferase 80 U/L, alanine aminotransferase 149 U/L, lactate dehydrogenase 1640 U/L, gamma-glutamyl transferase 88 U/L, total bilirubin 5.64 mg/dL, indirect bilirubin 5.07 mg/dL, glucose 108 mg/dL, urea 49 mg/dL, creatinine 1.03 mg/dL, sodium 136 mmol/L, and potassium 3.2 mmol/L. Hepatitis markers for hepatitis B and C and human immunodeficiency virus infections were all negative. On urine examination, a dark, hematuric urine with many erythrocytes was found. On abdominal ultrasonography, hepatosplenomegaly and focal spleen infarction were reported.

His blood smears showed acanthocytosis, ring-shaped trophozoites, and schizonts in erythrocytes, ruptured schizonts, and crescent-shaped gametocytes that resemble *P. falciparum* infection (Figure 3).

Antimalarial treatments with intravenous artemether and lumefantrine, quinine, and tetracycline tablets were started, and the patient regained consciousness within 3 days. Antiepileptic medication with levetiracetam was also started. Control EEG was performed on week 1 and showed normal background activity (Figure 2b).

All his neurological examination and detailed neurocognitive evaluation were normal at discharge. Consent was obtained from the patient to report the features of this case.

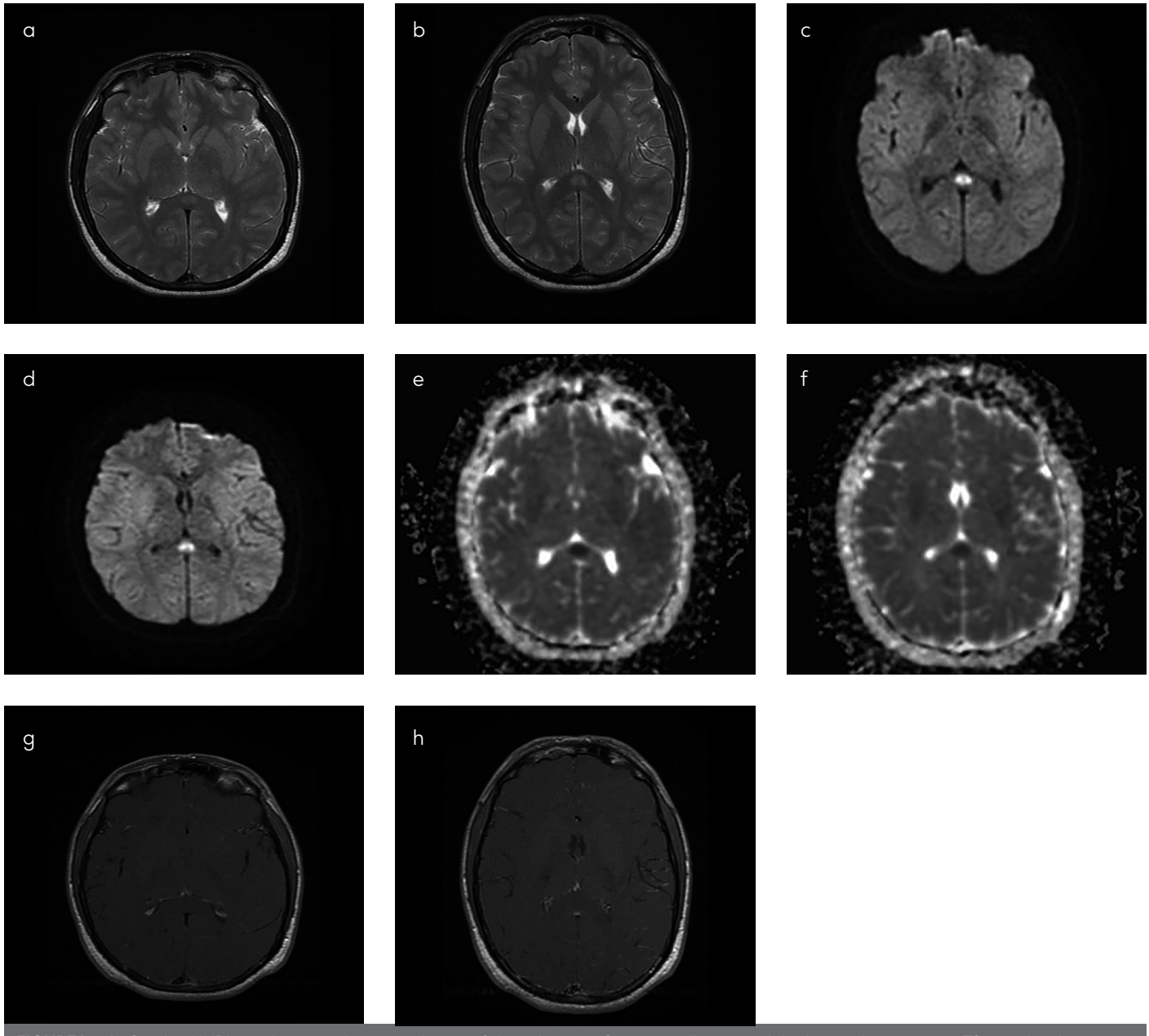


**FIGURE 1. a-h.** Cerebral MRI at admission showing a lesion of the splenium of corpus callosum with high signal intensity on T2-weighted sequence (a, b), DWI (c, d), and low signal intensity on ADC map images (e-f). Lesion does not reveal contrast enhancement on TI-weighted sequence (g, h)

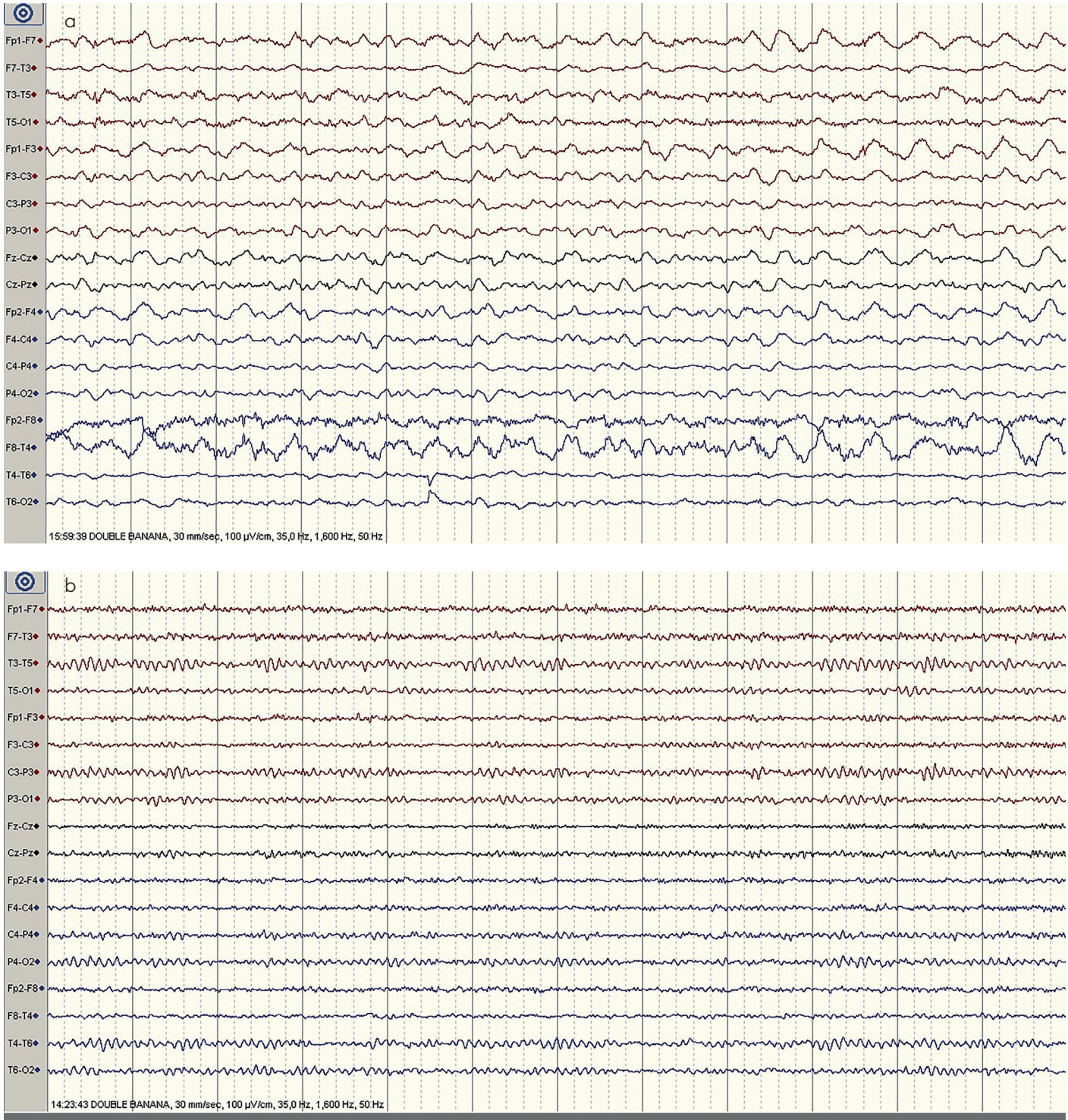


**FIGURE 2. a, b.** Initial EEG shows generalized severe background slowing with 3 Hz delta waves (a). Control EEG shows normal background activity (b)

## DISCUSSION

Cerebral malaria is one of the most severe complications of infection with *P. falciparum* malaria. The pathogenesis of cerebral malaria has been explained by two mechanisms: vascular sequestration of parasitized erythrocytes and potential cerebral toxicity by cytokines. Parasitized red blood cell sequestration in brain small capillaries results in impaired perfusion and local release of inflammatory factors. Released cytokines trigger vasodilatation, cerebral edema, and ischemia (1-4).

This pathomechanism results in cerebral swelling, intracranial hypertension, brainstem involvement, arterial–venous infarction, and retinal changes or papilledema.

The widespread involvement in the central nervous system causes a wide spectrum of clinical findings, such as confusion, seizures, and problems in posture, ocular movements, and respiratory patterns. The clinical hallmark of cerebral malaria is impaired consciousness that may progress to coma. Our patient also had coma with the systemic involvement of malaria. After

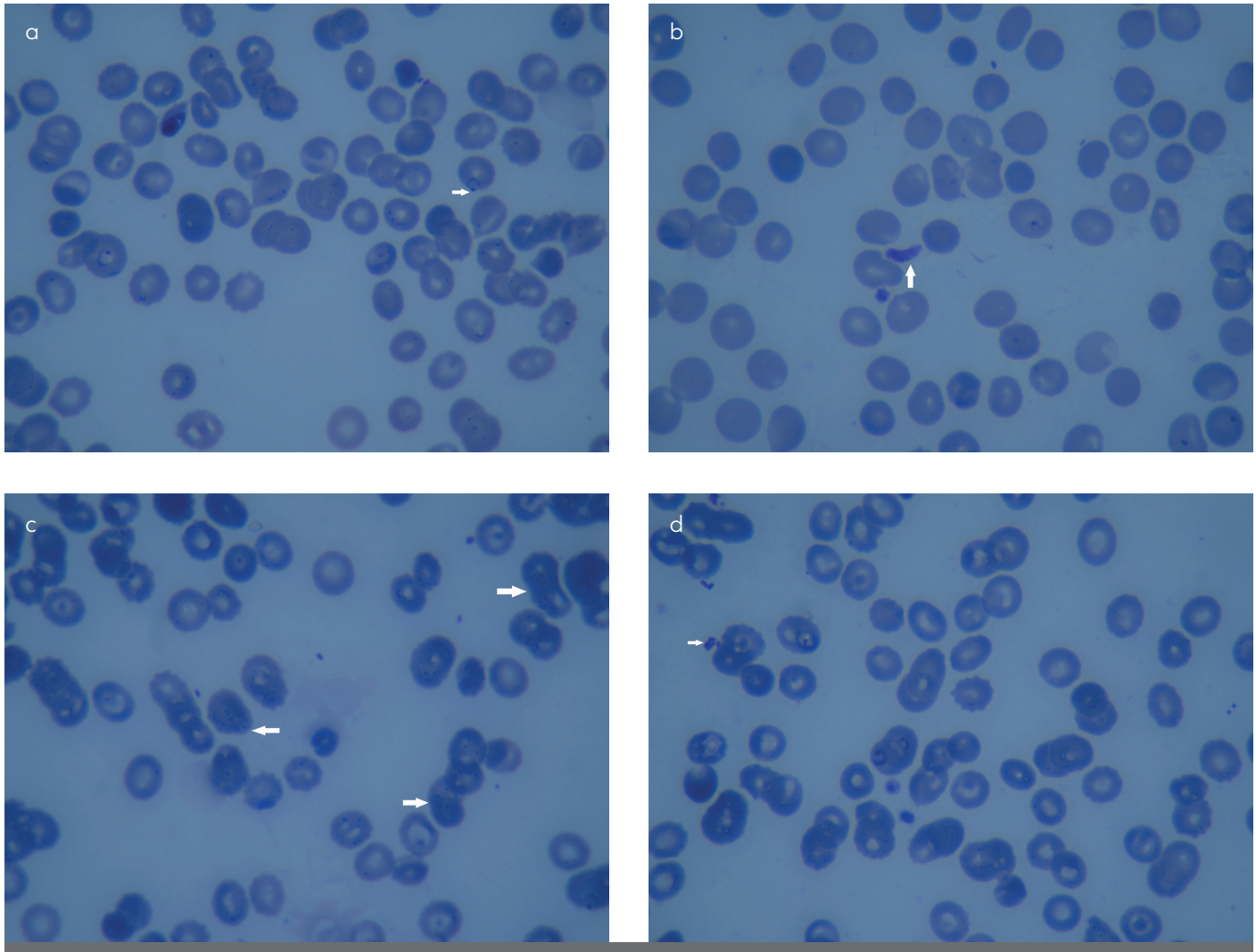


**FIGURE 3. a-d.** Blood smear shows acanthocytosis, ring-shaped trophozoites (a), schizonts in erythrocytes (c), ruptured schizonts (d), and crescent-shaped gametocytes (b)

exclusion of all possible other reasons for coma, this patient was diagnosed with cerebral malaria.

Cranial MRI can be normal despite the neurological signs. However, various reports of MRI in cerebral malaria have revealed focal or diffuse signal changes in the centrum semiovale, corpus callosum, basal ganglia, thalamus, cortex, and brainstem. Pontin and upper medulla myelinolysis and cerebellar demyelination have also been reported. Microinfarcts or microbleedings of the cerebral hemispheres, brainstem, cerebellum, and venous thrombosis or infarcts can be observed. The hyperintensities on T2-weighted images were considered to be due to edema, ischemia, toxic injury, or gliosis. DWI changes usually do not occur in a vascular distribution because of local ischemia due to a high metabolic demand, resulting from reduced blood flow, focal hypoglycemia, seizures, or hypoxia (2-5).

The largest published prospective imaging study on cerebral malaria includes 120 Malawian children. The researchers summarized all the MRI features of cerebral malaria. T2 signal changes in the corpus callosum were reported in nearly half of the study population. They also observed that corpus callosum involvement was associated with positive DWI changes, and some cases had predominantly diffuse involvement of the splenium. They report that corpus callosum DWI abnormalities were observed in 6 of 32 cases without retinopathy and in 52 of 120 with retinopathy. Additionally, 5 in 6 of retinopathy negative cases and 38 in 52 of retinopathy positive cases with corpus callosum involvement had splenium predominance (4).

These studies also reported corpus callosum involvement without any diffusion restriction in DWI. Distinctively, our case had acute splenium diffusion restriction in MRI in the early period of his disease. Cytotoxic edema via excitotoxic and inflammatory injury is most likely the main mechanism in corpus callosum splenium involvement in malaria. Fever, seizures, and ischemic injury due to vascular sequestration of infected erythrocytes are the other discussed mechanisms (2-4, 6, 7).

The transient lesions of the corpus callosum splenium have also been described in patients with viral infections, antiepileptic drug toxicity or withdrawal, high-altitude cerebral edema, metabolic disorders, Wernicke encephalopathy, Marchiafava–Bignami disease, hemolytic uremic syndrome, and traumatic axonal injury. The researchers hypothesize that these reversible lesions are due to intramyelinic edema in this area (2, 4, 6, 7).

Corpus callosum splenium involvement is rare, but if observed, it may be suggestive for diagnosis in patients with suspected cerebral malaria. Cerebral malaria should be considered in the list of possible causes of reversible lesion of the corpus callosum splenium. Our knowledge regarding adult cerebral malaria is very limited. Increasing case reports help to improve our knowledge with regard to imaging findings in adult cerebral malaria.

Malaria is not only a problem in endemic areas, but it is a problem globally in this globalization age. We all have to be familiar to the systemic and also central nervous system complications of malaria. Early diagnosis and effective treatment prevent radiological sequel, early mortality, and long-term cognitive dysfunction.

**Informed Consent:** Written informed consent was obtained from the patient who participated in this study.

**Peer-review:** Externally peer-reviewed.

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Case Report

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# Refractory Pseudotumour Cerebri in a Pediatric Case

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**ABSTRACT**

Pseudotumour cerebri (PTC) is traditionally defined as increased intracranial pressure (ICP) >200 mm$H_2O$ with non-focal neurological findings, except the sixth-nerve palsy, and normal cerebrospinal fluid (CSF) composition without brain pathology or evidence of venous thrombosis. A 6-years-old girl was referred to our clinic for blurred vision in her left eye and a progressive headache. Her history was positive for a progressive vision loss in the left eye. Both optic disks were blurred and swollen. The opening pressure of CSF was 310 mm/$H_2O$. Despite the repeated lumbar punctures (LP) and medical treatment, the patient had to undergo the optic nerve sheath fenestration. A consequent shunt procedure had to be performed due to a persistently high CSF level. In this report, we emphasize that if surgical procedures can be applied earlier in refractory PTC cases, better results of visual improvement may be observed. In this report, we emphasize that early surgical treatment in refractory PTC cases results in better visual improvement.

**Keywords:** Pseudotumour cerebri, refractory, optic nerve sheet fenestration

## INTRODUCTION

Pseudotumour cerebri (PTC), also known as idiopathic intracranial hypertension, is defined as the presence of papill-edema with a normal neurologic examination, except for cranial nerve abnormalities, no structural brain lesions, and the absence of abnormal meningeal enhancement on neuroimaging, normal cerebrospinal fluid (CSF) composition, and intracranial pressure (ICP) >280 mm in children, or >250 mm in nonobese, nonsedated children (1). Since there are no universally accepted diagnostic criteria for PTC in children, the evaluation and management are usually determined on a case-by-case basis, family, and the clinician together. Most pediatric PTC cases are primary, whereas secondary causes can be listed as medications, such as antibiotics, chronic steroid use, growth hormone replacement, rethynoids, and thyroid replacement or diseases, such as hypervitaminosis A and E, hypovitaminosis D, Down syndrome, Addison disease, chronic kidney disease, and anemia (2, 3). Obesity is one of the most important etiologies of secondary PTC (4). The main goal of the treatment is to prevent the visual loss caused by the elevated CSF pressure. Poor visual acuity and severe optic disk edema at presentation are risk factors for visual loss (3). Medical and surgical methods are recommended for the treatment of PTC, but there is no universally accepted method. In this report, we aimed to present a refractory case of pediatric PTC resulting in permanent visual impairment despite oral medications and surgical procedures.

## CASE PRESENTATION

Written informed consent was obtained from the patient's parents. A 6-years-old girl was admitted to our hospital with blurred vision in her left eye and a progressive headache that had started 10 days before. She was born from non-consanguineous parents with a normal prenatal, natal, and postnatal history. She was not obese or overweight, weighing 33 kg, and with a body mass index of 22 kg/$m^2$. On an ophthalmological examination, both optic disks were blurred and swollen with a visual acuity of 20/200 on the left eye and 20/30 on the right eye. Aside from these findings, her neurological history and examination were completely normal. The blood count and biochemical tests were within the normal

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range, including C-reactive protein, the erythrocyte sedimentation rate, and vasculitis markers such as the rheumatoid factor, anti-nuclear antibody, cANCA, and pANCA. Cranial magnetic resonance imaging (MRI) revealed an empty sella due to invagination to the sella turcica of the suprasellar cistern and accumulation of fluid in the optic nerve sheath. The MRI venography was normal, which was performed to exclude cerebral venous sinus thrombosis. The lumbar puncture (LP) was planned with sedation, and the opening pressure of the CSF was measured at 310 $mmH_2O$. A CSF analysis revealed no abnormalities. The patient was evaluated for primary and secondary causes of PTC using biochemical tests, CSF analysis, and neuroimaging. To rule out sarcoidosis, the level of CSF angiotensin-converting enzyme was measured, which was within a normal range. The patient was initially given 10 mg/kg/day of peroral acetolazolamide. Despite treatment with acetazolamide for 3 days, her complaints continued, the severity of headache increased, and visual acuity was deteriorated to hand motion on both eyes. The cerebrospinal fluid pressure was measured 3 days later, and the opening pressure of CSF under sedation with midazolam was 570 $mmH_2O$, while it dropped to 150 $mmH_2O$ as the closing pressure. The reason for this rapid decrease in the CSF pressure was that the increased flow rate of CSF could not be controlled. Pulse steroid treatment (30 mg/kg/day for 5 days) was initiated, while the search for a center that could perform the planned optic nerve fenestration procedure continued. At the end of the second day of pulse steroid treatment, ICP was measured as 440 $mmH_2O$. Repeated ophthalmological examination revealed the left optic disk pallor. The connection with the surgery center was achieved on the 13th day of the follow-up. On the 15th day of her hospitalization, the optic nerve sheath fenestration (ONSF) was performed on her left eye. The visual acuity in the left eye did not show any improvement after the surgery, although the patient was free of headaches during her follow-up.

About a year later, she was re-admitted to hospital for severe headache and vomiting. The patient had no follow ups for about a year. During this time period, she was on topiramate and acetolazolamide but had quit her medications during the last week. Her right optic disk margins were blurred and swollen in the fundoscopic examination, whereas her left optic disk was totally pale, which was a sign of optic atrophy. Her visual acuity was 20/200 in the right eye and hand motions on left eye. Repeated cranial MRI and MRI venography revealed the same pathologies that were reported previously. The opening CSF pressure was measured as 610 $mmH_2O$. A new treatment consisting of acetolazolamide (10 mg/kg /day) and topiramate (3 mg/kg/day) was initiated. The control CSF pressure after 1 week on medications was measured as 300 $mmH_2O$. After 3 months, she presented with an increased loss of vision in her right eye. Her ophthalmological examination revealed an increase in the right optic disk edema. The opening pressure of the CSF was measured as 730 $mmH_2O$. At that point, an urgent right ONSF was performed. After the second ONSF, the pressure of the CSF persisted at extremely high levels despite the oral medications, and the patient was referred to a neurosurgery clinic for a ventriculoperitoneal shunt (VPS) procedure.

**DISCUSSION**

Pseudotumour cerebri is traditionally defined as increased intracranial pressure (ICP) >200 $mmH_2O$ with non-focal neurological findings, except the sixth-nerve palsy, and a normal CSF composition without brain pathology and no evidence of venous thrombosis (1-3).

The first case was reported in 1893 with an increased ICP of unknown etiology. The term of PTC was first used by Nonne. The term *benign intracranial hypertension* has been widely used to indicate a similar entity, but the visual morbidity of the pressure cannot be called benign, so this term is no longer used in the literature as expected. After 2013, the PTC syndrome was widely accepted as an umbrella term (5). Based on the etiology, PTC can be classified in three groups: primary, secondary, and atypical. Many causes of the secondary PTC have been reported, and PTC in children is often secondary (2, 5).

The main pathophysiologic mechanism is the impairment in the CSF flow dynamics. The CSF hypersecretion or impaired CSF resorption are mainly proposed as mechanisms of PTC. In the literature, several mechanisms due to vitamin A, obesity, hormones, natriuretic peptides, and aquaporin have been reported; however, the exact pathophysiological pathways remain obscure (5, 6).

It has a broad clinical spectrum, ranging from asymptomatic papilledema to reduced consciousness and total loss of vision. Dramatic clinical presentations, such as bilateral abducens palsy in an infant with reduced consciousness and acute blindness, have been reported. The most common symptom is headache (7). Visual changes such as diplopia, transient visual obscurations, loss of visual acuity, or visual field have also been reported. Total visual loss is not common but is the most feared complication of the disease.

In this report, our patient presented with a severe headache and concurrent visual loss. Other presentations such as back pain, pulsatile or non-pulsatile, bilateral and fluctuating tinnitus, diplopia, nocturia, dizziness, photophobia, and radicular pain are also less commonly reported symptoms seen in patients with PTC (7, 8).

MRI findings that suggest an elevated ICP in adults and children with PTC have been described and include flattening of the posterior sclera, distension of the perioptic subarachnoid space, enhancement of the prelaminar optic nerve, tortuosity of the orbital optic nerve, intraocular protrusion of the prelaminar optic nerve, and an empty sella (9). Our patient had an empty sella due to the invagination of the suprasellar cistern to the sella turcica and accumulation of fluid in the optic disk sheath.

The treatment goal of PTC is normalizing the CSF pressure to prevent loss of vision. Treatment modalities in children vary between different centers. The traditional treatment modality is repetitive LP and surgical shunting supported by medical therapy. Repetitive LP is not thought to be a suitable medical option in children with refractory PTC due to patient discomfort. Traditionally, acetolazolamide, which is a carbonic anhydrase inhibitor that reduces the CSF production, is the preferred initial medical treatment, which was also the first choice in our patient (10). Steroid and topiramate therapy are other conventional treat-

ment choices. Severe and medically refractory cases of PTC are generally treated surgically by using ONSF and VPS, which are known to be effective in reducing papilledema and improving or stabilizing visual function in almost all patients. The sequence of treatment modalities is not exactly clear in refractory PTC. Generally, less severe cases are treated medically. A Cochrane Collaboration review found no sufficient data to support the superiority of one type of surgery over the other (11). Shunting procedures have resulted in permanent surgical complications according to previous studies (12, 13). In our study, the ONSF of the left eye was performed immediately when the optic disk pallor was observed to improve vision loss. Clinical experiences support that the unilateral ONSF protects from vision loss in the other eye, too. In our case, opposite to this common belief, this expected protective effect of ONSF was not observed, although it was performed bilaterally in follow ups. Even after these interventions, the patient had to undergo a VPS operation. We believe that the optimal timing of surgical procedures such as ONSF or VPS in refractory cases should be discussed carefully during their follow ups and may be regarded as the first-line choice in these cases. Advanced refractory PTC algorithms are needed based on new clinical evidence from future studies.

**Informed Consent:** Written informed consent was obtained from the parents of the patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept - Ç.G.S., Ö.Y.K.; Design - Ç.G.S.; Supervision - A.A., D.Y.; Resource - Ö.Y.K., H.K.; Materials - Ç.G.S., Ö.Y.K., H.K.; Data Collection and/or Processing - Ç.G.S., Ö.Y.K., H.K., P.A.Ö.; Analysis and/or Interpretation - Ç.G.S., Ö.Y.K., H.K., P.A.Ö.; Literature Search - Ç.G.S., Ö.Y.K., H.K., A.A., D.Y.; Writing - Ç.G.S., Ö.Y.K., P.A.Ö.; Critical Reviews - Ç.G.S., Ö.Y.K., P.A.Ö., A.A., D.Y.

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**Conflict of Interest:** The authors have no conflicts of interest to declare.

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# Delayed Presentation of Diaphragmatic Rupture due to Penetrating Trauma: Acute Mechanical Intestinal Obstruction

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**ABSTRACT**

Although traumatic diaphragmatic ruptures are rare, it is a critical condition that can cause life-threatening complications. Traumatic diaphragmatic rupture may be discovered years after the presentation of the injury, with gastrointestinal or pulmonary symptoms due to a diaphragmatic hernia. Intestinal obstruction due to an isolated diaphragmatic rupture that emerges after a penetrating trauma is highly rare. The present study presents the case of a male patient who underwent laparotomy for intestinal obstruction due to a diaphragmatic hernia caused by a sharp object-induced injury to the thorax 1 year prior to his presentation. We believe that diaphragmatic ruptures are one of the reasons of mechanical intestinal obstruction and that they require urgent surgery.

**Keywords:** Diaphragmatic rupture; ileus; penetrating trauma

## INTRODUCTION

Diaphragmatic ruptures were first defined by Sennertius in 1541, and the first successful diaphragmatic repair was performed by Walker in 1889 (1). Penetrating injuries, such as sharp object-induced injuries and costal fractures, can cause diaphragmatic ruptures. A retrospective study of trauma patients has shown that the incidence of traumatic diaphragmatic rupture is approximately 0.4%–1.2% (2). Overall, 12%–60% of patients with thoracoabdominal trauma who do not require operation cannot be diagnosed in the acute phase. Diaphragmatic ruptures can be diagnosed in the latent phase or by strangulation of the intestinal loop in the diaphragmatic hernia that occurs secondary to the rupture (3). A small diaphragmatic rupture due to sharp objects may not be symptomatic at an early stage. With delay in diagnosis and treatment, progressive abdominal herniation develops because of the pressure difference between the abdominal and thoracic cavities. When abdominal organ herniation develops, respiratory complaints or mechanical obstruction symptoms of the gastrointestinal tract, strangulated or not, are noted. Diaphragmatic ruptures can be diagnosed by chest X-ray, computerized tomography (CT), or diagnostic laparoscopy. In delayed cases, CT is more useful in the diagnosis.

## CASE PRESENTATION

A 25-year-old male patient was admitted to Emergency Clinic for complaints of abdominal pain and no flatulence and defecation for 4 days. There was no history of a previous abdominal surgery. The patient was found to have experienced a sharp object-induced injury to his thorax 1 year prior and had undergone a nonoperative follow-up. Physical examination revealed abdominal distension and widespread tenderness. His blood pressure was 110/80 mmHg, pulse was 74/min, and respiratory rate was 22/min. Hemogram and biochemical examinations revealed no pathology. Abdominal X-ray in an erect standing position revealed an air–fluid level. Posterioanterior (PA) chest X-ray revealed an elevation in the diaphragm, whereas CT revealed that the left diaphragm contours were obscure. Mesenteric fatty tissue and splenic flexure appeared to be herniated into the thorax (Figure 1). The patient was scheduled for surgery with an initial diagnosis of intestinal obstruction. Abdominal exploration revealed a small amount of reactive free fluid in the abdomen. The omentum and splenic flexure were herniated through the 3×2-cm rupture area in the left diaphragm into the thorax. The blood flow of the herniated colonic loop was not impaired; however, the lumen was blocked because of edema. The omentum and splenic flexure were retracted into the abdomen, and the defect was repaired in two lay-

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ers with U stitches using number "0" silk sutures (Silk; *Dogsan Medical* Supplies Industry, Trabzon, Turkey) (Figure 2). A 28-F chest tube was placed in 5. Intercostals spaces and removed on postoperative day 3. The patient was discharged on postoperative day 6 with complete recovery .Informed consent form was obtained from the patient.

## DISCUSSION

Carter and Giuseffi have described the clinical course of diaphragmatic ruptures in the 1950s in three stages: acute phase, latent phase, and obstructive phase (1, 4). The average incidence of diaphragmatic injury was reported to be 3% (0.8%–5.2%) in patients with multiple trauma (5). Overall, 75% of diaphragmatic ruptures occur due to blunt traumas, whereas the remaining 25% occur due to penetrating traumas (6). The left posteromedial tendinomuscular area is the weakest region of the diaphragm during embryological development. Therefore, ruptures on the left side are more frequent, and left-sided diaphragmatic ruptures are relatively more complicated. These complications are due to the intraabdominal organ herniation (5). In particular, stomach and spleen herniation is more frequent, whereas colon herniation is rare. Mechanical intestinal obstruction may be developed as a result of herniation of the lower intestinal system. Diaphragmatic ruptures are organ injuries that are difficult to diagnose. Because diaphragmatic ruptures due to penetrating injuries are small in size, they may not be detected by imaging methods in the absence of herniation. Traumatic diaphragmatic ruptures do not have specific signs and symptoms. Even in cases of normal posttraumatic findings, suspicion and diagnosis of ruptures must be considered to reduce early-stage mortality and morbidity by aiding early diagnosis. In the present study, a definite diagnosis was made and supported by CT of the thorax and upper abdomen. When diaphragmatic ruptures are electively operated, repair through the thorax is possible. However, this approach requires an intraabdominal pathology. For this reason, a transthoracic approach is not preferred in emergency operations. A transabdominal approach should be preferred in emergency operations to ensure adequate exploration of the intraabdominal organs and to have easier access to the diaphragm. Primary diaphragmatic repair with nonabsorbable sutures is generally the preferred method. If the defect is large and primary closure is not possible, it is recommended to close the defect using an appropriate graft (7, 8). Transabdominal surgery was performed in our patient because we detected colon obstruction.

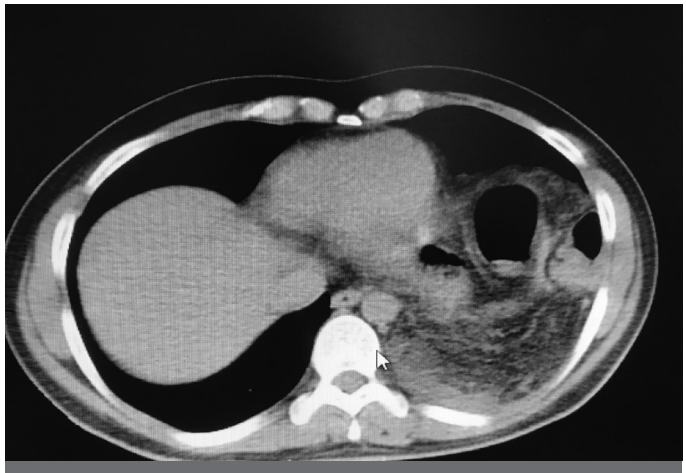

**FIGURE 1.** The left diaphragm contours were not clearly observed on computed tomography. Mesenteric fat tissue and splenic flexure appear to be herniated into the thorax

In conclusion, in patients with a blunt or penetrating trauma history, regardless of the time of injury, mechanical intestinal obstruction due to diaphragmatic ruptures should be considered in differential diagnosis.

**Informed Consent:** Informed consent was obtained from the patients who participated in this study.

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**Conflict of Interest:** The authors have no conflicts of interest to declare.

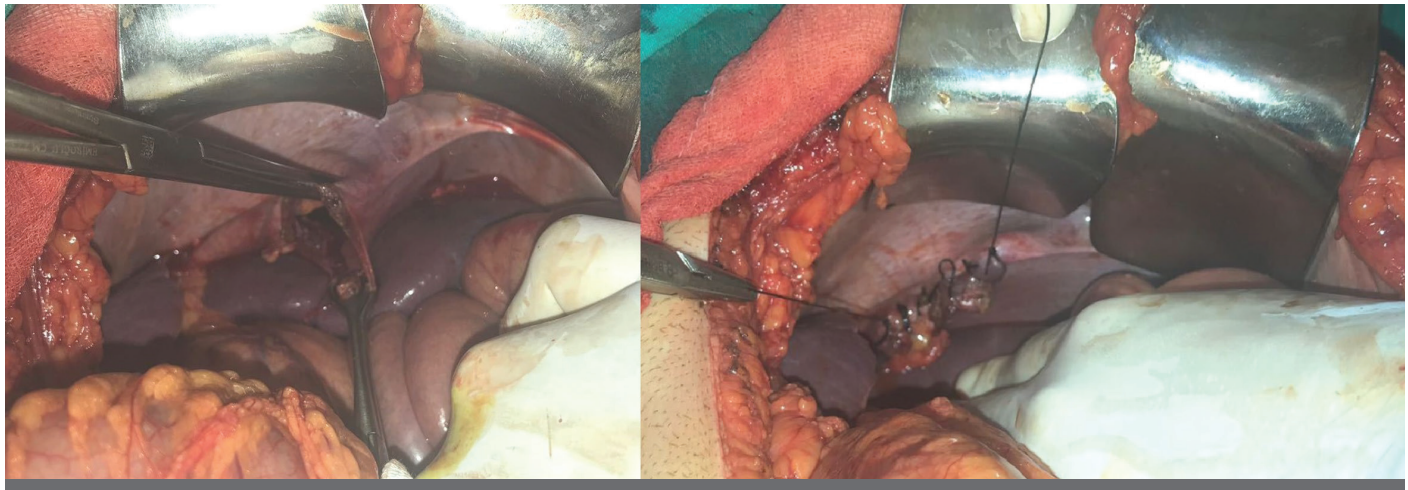

**FIGURE 2.** Left diaphragm defect: 3 cm in diameter. The state of the diaphragmatic defect after being repaired with U stitches using number "0" silk sutures

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