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# The protective effect of L-carnitine supplementation on retinopathy of prematurity: A retrospective cohort study

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**Ethics Committee Approval**
This research was approved Baskent University Institutional Review Board (Project No: KA-22/54, Date: January 25, 2022).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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## Abstract

**Background/Aim:** Retinopathy of prematurity (ROP) is important morbidity in premature infants and is the most common preventable cause of blindness in childhood. Carnitine is a key molecule in energy metabolism and in oxidation of fatty acids, which are the main structural component of phospholipid membrane of the brain and retinal cells. Premature babies are born with insufficient carnitine pools. This study aimed to evaluate the effect of carnitine replacement on ROP in premature infants aged < 34 gestational weeks.

**Methods:** Premature infant records between 2014 and 2019 were retrospectively examined. All premature infants at gestational ages < 34 weeks were included. Data from the two groups who received/did not receive carnitine supplementation in total parenteral nutrition (TPN) over two consecutive time periods and whose ROP examination was complete were recorded retrospectively. Fifty-eight out of 125 infants were in the carnitine group, and 67 formed the non-carnitine group (CG and NCG, respectively). The morbidity data from subjects, especially those with ROP, who received (CG) and did not receive carnitine (NCG), were compared.

**Results:** Of the 125 infants enrolled, no significant differences in gestational age ($P$ = 0.323) or birth weight ($P$ = 0.597) between the groups was found. The Cox regression analysis revealed that carnitine replacement in the premature infant was a protective factor for ROP ($P$ = 0.045, B = −0.933, hazard ratio 0.393, 95% confidence interval 0.158–0.978).

**Conclusion:** Carnitine supplementation may offer protection from developing ROP during exposure to oxygen in premature infants.

**Keywords:** L-carnitine supplementation, Prematurity, Retinopathy of prematurity

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## Introduction

Retinopathy of prematurity (ROP) is most important cause of preventable blindness in children [1]. Recent advances in neonatal intensive care have led to an improvement in survival rates for premature infants [2]. Consequently, the incidence of ROP has increased correspondingly. ROP is characterized by abnormal neovascular development in the retina of premature infants. These abnormal blood vessels cause a tractional retinal detachment, which is the main cause of visual impairment and blindness in ROP patients [3]. The main factors associated with ROP are low gestational age, low birth weight, and prolonged exposure to supplementary oxygen following delivery [4].

Carnitine is essential for long-chain fatty acid transport through the cell membrane and both the mitochondrial outer and inner membranes into the mitochondrial matrix in which fatty acid oxidation occurs. So, carnitine is crucial for energy production in tissues dependent upon fatty acid oxidation [5]. Fatty acids, such as docosahexaenoic acid (DHA), are important for nervous system development because these compounds are an integral component of the phospholipid membrane of the brain and retinal cells. DHA is accumulated in the rapidly developing brain and retina, especially during the last trimester of pregnancy and the first 24 months of life with ongoing accumulation throughout childhood [6–9]. High DHA concentrations in the retina and brain gray matter suggest that these fatty acids have important roles in retinal and neural functions. Animal studies have shown that the depletion of DHA from the retina and brain results in reduced visual function and learning deficits. Preterm babies, who suffer from numerous morbidities, including retinal and neurological issues, have more carnitine and essential fatty acid insufficiency due to preterm birth and nutritional problems. These data suggest that preterm infants are especially under risk because of imbalances in dietary fatty acid.

In the human body, fatty acids are mostly found in the central nervous system and then continues to the retina. It is known that premature babies born before the last trimester especially have insufficient levels of fatty acids and insufficient carnitine storage [10]. Consequently, carnitine plays a very important role in the transport of fatty acids from the cell cytosol into mitochondria, especially in premature babies. Our hypothesis was that replacement of carnitine in diets for premature babies with insufficient carnitine and fatty acid pool due to preterm birth and feeding difficulties may offer protection against ROP. This study aimed to evaluate the effect of carnitine replacement on ROP in premature infants < 34 gestational weeks.

## Materials and methods

This double-center, retrospective cohort study was conducted at Baskent University Konya Hospital and Gaziantep Medical Park Hospital. This research was approved by Baskent University Institutional Review Board (Project No: KA-22/54). Premature infant records between 2014 and 2019 were retrospectively examined. All premature infants at gestational ages < 34 weeks were included. Clinical data and demographic information, including gestational age, gender, weight; antenatal steroid administration, respiratory distress syndrome (RDS), the day of oxygen exposure, bronchoalveolar dysplasia, maximum fractional inspired of oxygen, intraventricular hemorrhage (IVH), hemodynamically significant patent ductus arteriosus, treatment options of patent ductus arteriosus, whole blood cell count at admission, maximum C-reactive protein (CRP) levels during hospitalization, total days of parenteral nutrition, necrotizing enterocolitis, frequency of hypothyroidism, frequency of sepsis, frequency of ROP and stages, and duration of hospital stay were obtained from the medical records of the enrolled infants. Infants with comorbid complex congenital heart disease, genetic abnormalities, and other severe deformities were excluded from the study. Subjects were classified into two groups through matched case-control. Of the 125 infants enrolled, 58 were in the carnitine group (CG), and 67 were in the non-carnitine group (NCG) as shown in Table 1. The cases in the CG were older (2014–2017) than the NCG (2017–2019). To avoid selection bias, the characteristics of patients, such as gestational week and birth weight, were similarly chosen. Also, the cases were grouped as ROP and non-ROP to provide statistics to evaluate the association between carnitine replacement and premature retinopathy. Baseline characteristics (Table 3) and weight gain (Table 4) were examined based on ROP grouping.

In the CG, carnitine was given 25mg/kg by IV route in the first 6 hours of life with total parenteral nutrition (TPN) [11]. Carnitine supplementation was continued until TPN was stopped. Carnitine supplementation was not continued during enteral feeding. NCG did not receive any carnitine support. The first assessment of the state of patent ductus arteriosus in both groups was made on the third day of life. Treatment and follow-up of the patent ductus arteriosus were done as per our pediatric cardiology guidance. Cranial ultrasound was performed in the first week of life and at the end of the first month of life for staging IVH and cystic encephalomalacia, respectively. Ophthalmological examinations were initiated at the fourth week of life or at the 31$^{st}$ week of gestational age (whichever came later) and were repeated weekly or biweekly using the schedule for follow-up recommended by the American Academia of Pediatrics and American Academy of Ophthalmology [12].

**Sample size calculation**

The known incidence of ROP in infants < 34 weeks is 66% [13]. On the open access website, it was calculated that the administration of L-carnitine would lead to a reduction in ROP by 20%, with a power of 80%, a confidence interval (CI) of 95%, and 58 cases for each group at a 1:1 ratio (https://clincalc.com/stats/SampleSize.aspx).

**Statistical analysis**

Descriptive statistics of scale variables were presented as mean (standard deviation (SD)) or median (range) as appropriate. Demographic and clinical continuous variables were compared using the two-independent Student's t-test for normally distributed values and the Mann–Whitney U test for non-normally distributed values. Z-scores of skewness, kurtosis, and Shapiro–Wilk statistics were used to understand whether the continuous variables were normally distributed. Categorical variables were compared using Fisher's exact test. The univariate analyses to identify variables associated with ROP were investigated using chi-squared, Fisher exact, Student's T-, and

Mann–Whitney U tests where appropriate. Univariate Cox regression analyses were used to calculate the hazard ratios and 95% CI to determine the relationship between ROP and carnitine replacement during the period of total oxygen exposure. Statistics related to carnitine replacement (weight gain and weight gain in the ROP infants) were applied because a close relationship between carnitine and energy metabolism exists as shown in Tables 2 and 4. For all tests, the level of statistical significance was set at $P = 0.05$. SPSS 25 was used for all data analyses.

## Results

Of the 125 infants enrolled, 58 (46%) and 67 (54%) were CG and NCG infants, respectively. No significant differences in gestational age, birth weight, gender, antenatal steroid administration, oxygen duration, TPN duration, number of packed red blood cells, maximum CRP levels, the transition time to fully enteral nutrition, whole blood cell indices, except for mean platelet volume (MPV), surfactant instillation, and bronchopulmonary dysplasia (BPD) (any stage) between the groups (Table 1). In the NCG, mechanical ventilation per day was significantly higher ($P = 0.001$), while the total oxygen exposure did not change between the groups. Although BPD at any stage did not differ between groups, when the subgroups were combined, moderate or severe BPD rates were found to be significantly higher in CG. This result, which was unexpected, was explained based on the finding that gestational week (28 (2) versus 30 (2); $P < 0.001$) and birth weight (1060 (206) versus 1420 (340) g; $P < 0.001$) were significantly lower in the moderate-severe BPD group. In other words, babies with lower birth weight and lower gestational age were collected in the group of moderate to severe BPD with receiving carnitine. While no difference was observed between the groups in terms of severe intracranial hemorrhage, grade I-II IVH was observed more in the NCG babies, but it was not significantly different from the CG infants ($P = 0.121$). As proven, sepsis was significantly higher in the NCG babies ($P = 0.025$), and congenital hypothyroidism was significantly higher in the CG babies ($P = 0.025$). Approximately two times more ROP was observed in the NCG, but this difference was not significant ($P = 0.089$). Similarly, advanced stage and laser-requiring ROP were higher in the NCG infants, but this difference was not at the statistically significant level ($P = 0.340$). The Cox regression analysis, in which the time variable was selected as total oxygen exposure day, revealed that carnitine replacement in the premature infant was a protective factor for ROP ($P = 0.045$, B = −0.933, HR: 0.393, 95% CI 0.158–0.978) as shown in Figure 1. Figure 1 and Cox regression results demonstrate that carnitine supplementation in premature infants protects against ROP development by approximately 2.5 times.

Figure 1: Figure shows a statistically significant decrease in retinopathy of prematurity (ROP) in premature infants who were supplemented with carnitine.

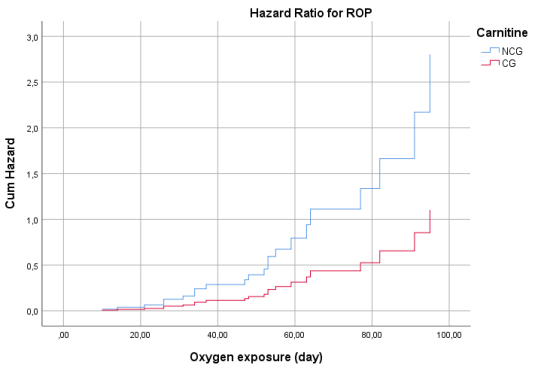


Table 1: Baseline characteristics of study groups

| | Carnitine (n = 58) | Non carnitine (n = 67) | *P*-value |
|---|---|---|---|
| GW, mean(SD) | 29.6 (2.08) | 30 (2.34) | 0.323 |
| BW, mean(SD) | 1357 (312) | 1389(376) | 0.597 |
| Mechanical ventilation; day; median (min-max) | 2 (0–107) | 6 (0–47) | 0.001 |
| O2; day; median (min-max) | 25 (0–107) | 13 (1–116) | 0.099 |
| TPN; day; median (min-max) | 9 (0–107) | 10(0–50) | 0.438 |
| The number of packed RBC; median (min-max) | 0.5 (0–6) | 0 (0–5) | 0.200 |
| Max CRP (mg/dl), median (min-max) | 5.87 (0.1–291.6) | 4.5 (0.2–299) | 0.907 |
| The transition time to full enteral nutrition; day median (min-max) | 11.5 (0–110) | 12 (3–52) | 0.870 |
| WBC; median (min-max) | 9.39 (2.2–47.3) | 9.38 (2.59–38.1) | 0.812 |
| ANS median (min-max) | 2.6 (0.13–48.9) | 2.2 (0.05–15.4) | 0.064 |
| ALS median (min-max) | 5.15 (0.93–45.5) | 5.28 (1.05-31.9) | 0.946 |
| MPV median (min-max) | 7.3 (6.07–9.2) | 6.5 (4.88–9.7) | 0.007 |
| Plt median (min-max) | 235 (28–670) | 229 (48–396) | 0.823 |
| Gender; male; n (%) | 30 (44.8) | 30 (51.7) | 0.438 |
| Antenatal steroid administration; n (%) | 13 (22.4) | 31 (46.3) | 0.005 |
| Surfactant administration | 17 (29.3) | 23 (34.8) | 0.510 |
| Respiratory support; n(%) | 34 (58.6) | 58 (86.6) | 0.001 |
| RDS; n (%) | 34 (58.6) | 58 (86.6) | <0.001 |
| BPD; n (%) | 21 (36.2) | 20 (29.9) | 0.450 |
| BPD moderate and severe; n(%) | 15 (25.9) | 1 (1.5) | <0.001 |
| HsPDA; n (%) | 12 (20.7) | 6 (9) | 0.062 |
| IVH any grade; n (%) | 1(1.7) | 8(11.9) | 0.037 |
| IVH grade1-2; n (%) | 1 (1.7) | 6 (9) | 0.121 |
| Pnmtx; n (%) | 0 | 2 (3) | 0.499 |
| Rop; n (%) | 7 (12.1) | 16 (23.9) | 0.089 |
| Laser or Anti-VEGF requiring ROP; n (%) | 4 (6.9) | 8 (11.9) | 0.340 |
| NEC; n (%) | 11 (19.3) | 6 (9) | 0.095 |
| Proven sepsis; n (%) | 14(24.1) | 29(43.3) | 0.025 |
| Hypotiroidy; n (%) | 7(12.1) | 1(1.5) | 0.024 |
| Exitus; n (%) | 5(8.6) | 1(1.5) | 0.095 |

GW: Gestational week; BW: Birth weight, TPN: Total Parenteral Nutrition, RBC: Red Blood Cell, CRP: C reactive protein, WBC: White Blood cell, ANC: Absolute neutrophil count, ALC: Absolute lymphocyte count, MPV: Mean platelet volume, Plt: Platelet, RDS: Respiratory distress syndrome, BPD: Bronchopulmonary dysplasia, hsPDA: Hemodynamically significant patent ductus arteriosus, IVH: Intraventricular hemorrhage, ROP: Retinopathy of prematurity, NEC: Necrotizing enterocolitis.

When groups were examined in terms of weight gain, the first eight weeks of weight gain were significantly higher in the NCG ($P = 0.019$) as shown in Table 2. MPV was only significantly higher in the CG ($P = 0.007$). During the grouping in terms of ROP, surfactant use, BPD, mechanical ventilation support rate, TPN duration, the transition time for full enteral feeding, proven sepsis, hypothyroidism, total oxygen exposure, number of packed red blood cell transfusion, and maximum CRP levels were significantly higher in the group with observed ROP (Table 3). The reason for these results was the lower birth weights and gestational weeks of the cases in the group with observed ROP. Except for the significantly lower absolute neutrophil count (ANC) in the ROP group, no differences between the groups in whole blood indices were observed. Weight gain in the first eight weeks and weight gain between the second and fourth weeks were significantly higher in the non-ROP group (Table 4).

Table 2: The effect of carnitine replacement on weight gain

| | Carnitine (n = 58) | Non carnitine (n = 67) | *P*-value |
|---|---|---|---|
| BW; mean(SD) | 1357 (312) | 1389 (376) | 0.597 |
| Weight gain in the first 2 weeks median (min-max) | 30 (−190–550) | −40 (−300–350) | 0.053 |
| Weight gain in the first 4 weeks; mean(SD) | 213.4 (180.4) | 207.5 (148.3) | 0.858 |
| Weight gain in the first 8 weeks; mean(SD) | 506.6 (267.2) | 684.6 (232.6) | 0.019 |
| Weight gain between 2nd and 4th week; mean(SD) | 198.2 (163.9) | 250.8 (126.5) | 0.081 |
| Catch-up birth weight day; median (min-max) | 17 (0–27) | 17 (5–43) | 0.299 |

BW: Birth weight, SD: Standard deviation

All findings found as risk factors for ROP seem to be related to lower birth weight and lower gestational age. In addition, the significant difference in TPN duration and transition time to full enteral feeding between ROP and non-ROP groups is another impressive finding revealing the relationship between nutrition and ROP.

Table 3: Retinopathy statistics

| | ROP (n = 23) | Non-ROP (n = 102) | *P*-value |
|---|---|---|---|
| Gender; male; n (%) | 9 (39.1) | 51 (50) | 0.346 |
| Carnitine; n (%) | 7(30.4) | 51(50) | 0.089 |
| Antenatal steroid administration; n(%) | 8 (34.8) | 36(35.3) | 0.963 |
| Surfactant administration n(%) | 12 (52.2) | 28 (27.7) | 0.024 |
| Respiratory support; n(%) | 22 (95.7) | 70 (68.6) | 0.008 |
| BPD; n (%) | 17 (73.9) | 24 (23.5) | <0.001 |
| BPD moderate and severe; n(%) | 4 (17.4) | 12 (11.8) | 0.466 |
| HsPDA; n (%) | 2 (8.7) | 16 (15.7) | 0.523 |
| IVH any grade; n (%) | 4(17.4) | 5(4.9) | 0.059 |
| IVH grade1-2; n (%) | 2(8.7) | 5 (4.9) | 0.612 |
| Pnmtx; n (%) | 1 (4.3) | 1 (1) | 0.338 |
| NEC; n (%) | 5 (21.7) | 12 (11.9) | 0.095 |
| Proven sepsis; n (%) | 18(78.3) | 25(24.5) | <0.001 |
| Hypotiroidy; n (%) | 4(17.4) | 4(3.9) | 0.037 |
| Exitus; n (%) | 1(4.3) | 5(4.9) | 1 |
| GW mean(SD) | 27.7 (1.7) | 30.2(2.06) | <0.001 |
| BW mean(SD) | 1025 (256) | 1453 (316) | <0.001 |
| Mechanichal ventilation; day; median (min-max) | 17 (0–91) | 3 (0–107) | <0.001 |
| O2; day; median (min-max) | 52(10–116) | 12.5 (0–107) | <0.001 |
| TPN; day; median (min-max) | 24 (11–91) | 4.5(0–107) | <0.001 |
| The number of packed RBC transfusion; median (min-max) | 1 (0–5) | 0 (0–6) | 0.001 |
| Max CRP (mg/dl), median (min-max) | 28.4(0.2–9) | 4 (0.1–299) | 0.027 |
| The transition time to full enteral nutrition | 25 (12–93) | 10 (0–110) | <0.001 |
| WBC median (min-max) | 7.5(4.37–8.1) | 9.46 (2.59–47.3) | 0.093 |
| ANC median (min-max) | 1.6(0.79–48.9) | 2.4 (0.05–48.9) | 0.028 |
| ALC median (min-max) | 4.9(1.99–45.5) | 5.4 (1.05–45.5) | 0.975 |
| MPV median (min-max) | 7.07(5.88–8.70) | 6.6 (4.88–9.7) | 0.319 |
| Plt median (min-max) | 228(48.7–336) | 227 (92.4–396) | 0.751 |

GW: Gestational week; BW: Birth weight, TPN: Total Parenteral Nutrition, RBC: Red Blood Cell, CRP: C reactive protein, WBC: White Blood cell, ANC: Absolute neutrophil count, ALC: Absolute lymphocyte count, MPV: Mean platelet volume, Plt: Platelet, RDS: Respiratory distress syndrome, BPD: Bronchopulmonary dysplasia, hsPDA: Hemodynamically significant patent ductus arteriosus, IVH: Intraventricular hemorrhage, ROP: Retinopathy of prematurity, NEC: Necrotizing enterocolitis

Table 4: Weight gain between groups with and without retinopathy

| | ROP (n = 23) | Non-ROP (n = 102) | *P*-value |
|---|---|---|---|
| BW; mean (SD) | 1025 (256) | 1453 (316) | <0.001 |
| Weight gain in the first 2 weeks; median (min–max) | −40 (−210–550) | −30 (−200–340) | 0.835 |
| Weight gain in the first 4 weeks; mean (SD) | 118.6 (130.2) | 235.6 (162.1) | 0.002 |
| Weight gain in the first 8 weeks; mean (SD) | 565 (210.5) | 655.7 (285.5) | 0.235 |
| Weight gain between 2nd and 4th week, mean (SD) | 114.7 (123.3) | 261.7 (133.8) | <0.001 |
| Catch-up birth weight day; median (min-max) | 17.5 (5–31) | 17 (7–43) | 0.201 |

BW: Birth weight, SD: Standard deviation

## Discussion

Carnitine transports fatty acids chains into the mitochondrial matrix, thus allowing the cells to derive energy from the stored fat reserves via β-oxidation of fatty acids [14]. DHA and eicosapentaenoic acid also play a role in anti-inflammatory eicosanoid/docosanoid synthesis, signaling events, gene expression, and cytokine expression. Infants with very low birth weight have low carnitine levels with impaired ketogenesis and are dependent upon parenteral nutrition for a prolonged period. Carnitine is accepted as essential for such infants [10]. Studies have shown that fatty acid oxidation is impaired when tissue carnitine levels fall below 10% of normal. Therefore, relative carnitine deficiency disrupts fatty acid oxidation, thus reducing its use, and problems associated with impaired energy and growth may be observed [10, 15]. Studies in which plasma carnitine levels were evaluated in preterm babies with respiratory distress syndrome (RDS) and the effects of carnitine supplementation on RDS were investigated. The researchers found that plasma carnitine levels were lower in premature infants with RDS and that carnitine supplementation in these infants caused a decrease in the duration of mechanical ventilation and surfactant requirement [16, 17]. Moreover, in some congenital fatty acid oxidation defects due to a genetic mutation that codes for long-chain acyl CoA dehydrogenase (LCAD), retinopathy, which is the unlikely pathophysiology of ROP, could be observed. Therefore, we examined the relationship between the risk of ROP and carnitine supplementation in this study.

In an experimental animal study that investigated the effects of L-carnitine on oxygen-induced retinopathy, the authors found that L-carnitine supplementation offered protection against retinopathy development, a finding that is in line with our findings [18]. Although ROP was observed to be approximately two times higher in the group that did not receive carnitine, this difference was not significant in our study. However, when the time variable was placed in the model, the observed significant difference suggested that the protective effect of carnitine became evident over time. Contrary to our hypothesis, an *in vitro* study showing that agents, such as etomoxir (irreversibly blocks fatty acid oxidation) that block fatty acid oxidation can stop angiogenesis in endothelial cells [19]. In contrast to the results of this study, a study in which the results of 75 LCAD-deficient patients were published reported that retinopathy was present in 43% of cases [20]. Simply put, retinopathy is a common finding in congenital fatty acid metabolism disorders. Additionally, in their study investigating the association of targeted metabolomic levels with ROP in premature infants, Yang et al. [21] found high serum glycine and malonyl carnitine levels to be predictive serum markers associated with the emergence of ROP. Consequently, conflicting results on the effect of carnitine on different retinopathy types can be found in the literature.

In a recently published systematic review on prophylactic administration of L-carnitine on the parenteral nutrition of premature infants, it was suggested that carnitine supplementation may help increase carnitine levels. However, no relevant improvements in the lipid profile, increase in weight gain, decrease in morbimortality, and reduction of hospital stay were found [22]. Results such as growth, weight gain, hospital

duration, ventilator dependency, and lipid profiles have been emphasized as primary outcomes in these studies. In our study, it was found that while carnitine replacement in premature infants led to a decrease in mechanical ventilation duration, weight gain had no positive effects and even had negative effects in the subsequent postnatal weeks. As in our findings, in a metanalysis that examined the effect of the carnitine on adult weight loss in obese patients, they concluded that receiving the carnitine resulted in weight loss [23]. Similarly, weight gain was shown to be higher in the group that did not receive carnitine at the end of the second and eighth weeks. In their randomized controlled study investigating carnitine supplementation on RDS, Ozturk et al. [16] showed that carnitine-supplemented babies spent less time on mechanical ventilation and had less surfactant requirements. Also, Korkmaz et al. [17] found that premature babies with RDS had low serum carnitine levels. Such effects may be related to the role of carnitine on the energy metabolism of respiratory muscles through fatty acid oxidation.

ROP is a disease specific to premature babies, and the greatest risk factors for ROP development are low birth weight, low gestational age, and the. need for oxygen to maintain life. The Cryotherapy for Retinopathy of Prematurity (CRYO-ROP) study demonstrated that ROP is a disease that occurs in 66% of infants born < 1250 g and in 82% of infants born under 1000 g [24]. Moreover, in the WINROP study, which is a web-based screening software tool and was used to investigate ROP risk factors, low weight gain associated with serum insulin-like growth factor 1 (IGF-1) level was found to be one of the other risk factors [25]. Other specific postnatal factors that increase the risk for ROP include sepsis, blood transfusion, and IVH [26]. Similar to the findings in these studies, in our study, gestational week, birth weight, mechanical ventilation rate, total duration of oxygen exposure, BPD rates, proven sepsis rates, maximum CRP levels, and packed red blood cell transfusion rates were found to be significantly higher in the ROP group. Similarly, it was thought that the low ANC observed in the ROP group was related to the infants susceptible to sepsis, one of the risk factors for ROP. ANC significantly correlated with sepsis and CRP levels. Almost all results appear to be related to individuals with chronic lung disease associated with low birth weight and individuals susceptible to sepsis due to their vulnerability and weak immune response to pathogen microorganisms. Another result that we did not encounter in our study that has been found in other studies was the significantly higher number of congenital hypothyroidism cases in the group with observed ROP. This finding was not expected. When congenital hypothyroidism cases were examined in detail, it was observed that birth weight (1068 versus 1395 g) was significantly lower and oxygen exposure duration (51 versus 24 days) were significantly higher in those with hypothyroidism. The main reason for this result seems to be related to low birth weight. So, this result was interpreted as a confounding factor. Studies examining the relationship between weight gain, somatic growth factors, such as insulin-like growth factor 1 (IGF-1), and ROP have shown an inverse relationship between weight gain and ROP [27, 28]. IGF-1was used as a surrogate marker for postnatal growth in another study. Although IGF-1 levels were not measured in our study, significantly more ROPs in those with poor postpartum weight gain were observed similar to these studies. It was noticed that the differences in weight gain were significant in the first four weeks. Weight gains in the first four weeks and between the second and fourth weeks were significantly lower in the group with ROP.

The present study had some limitations. First, it was a retrospective study. It could have been a prospective randomized controlled study to predict risk factors associated with ROP, especially with respect to understanding the effects of carnitine replacement on ROP. Second, a different period may have influenced the results of such different approaches.

**Conclusions**

In conclusion, carnitine supplementation may offer protection against ROP development during exposure to oxygen in premature infants through the relationship between carnitine and angiogenesis, less mechanical ventilation and complications, and less exposure to oxygen. To explain these interactions, further prospective randomized controlled studies with a larger sample size should be conducted to understand the relationship between the supplementation of carnitine and ROP in premature infants.

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# Fear of coronavirus in intensive care nurses: A cross-sectional study

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**Ethics Committee Approval**
The study was approved by the COVID-19 Studies Scientific Committee of Turkish Ministry of Health and Kırklareli University Health Sciences Institute Scientific Research Ethics Committee (number: 69456409-199-E.14455, protocol number: 2020/251 and date: 06.10.2020). All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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## Abstract

**Background/Aim:** The COVID-19 pandemic has had negative effects on healthcare workers and caused burnout and psychosocial problems, endangering their mental health. This study aimed to reveal the COVID-19 fears of intensive care nurses during the pandemic.

**Methods**: The data of this cross-sectional study were collected with the participation of 128 nurses working in the intensive care units of two public hospitals between October 2020 and November 2020. The Nurse Identification Form and the Fear of COVID-19 Scale were used in data collection. The online survey forms were delivered to the nurses through WhatsApp groups to protect the health of both researchers and participants during the COVID-19 pandemic.

**Results**: The nurses' mean COVID-19 fear scale score was found to be 22.7 (5.1). The mean scale scores differed at statistically significant levels, depending on the education status and the number of patients per nurse in one single shift ($P < 0.05$).

**Conclusion**: The COVID-19 pandemic is a cause of stress and fear for nurses and other healthcare workers. During the COVID-19 pandemic process, it is crucial to assess the fears and coping skills of intensive care nurses; the nurses should also be supported physiologically, psychologically, and sociologically. New studies are needed to reveal the effectiveness of support services related to emotion management and infection control in nurses.

**Keywords:** Coronavirus, Fear, Intensive care unit, Nurse, Pandemic

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## Introduction

The novel coronavirus disease 2019 (COVID-19) first appeared in Wuhan, China, with respiratory symptoms (i.e., fever, cough, and shortness of breath) and was spreading rapidly throughout the world by January 30, 2020 [1]. The World Health Organization (WHO) declared it a "Public Health Emergency of International Concern (PHEIC)" [2]. Cases continued to increase; the number of cases was reported to be 162,773,940 as of May 17, 2021 [3]. It was reported that healthcare workers accounted for 14–35% of these cases [4]. The U.S. Center for Disease Control and Prevention (CDC) reported that the number of healthcare workers infected with COVID-19 in a population of 14.717.298 people, 19.06% of whom were healthcare workers, was 335,312; and the number of healthcare personnel who lost their lives was 257.831 as of January 3, 2021 [5]. According to the Turkish Ministry of Health, 7,428 healthcare workers were infected with COVID-19 in that country as of April 29, 2020 [6].

The symptoms of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV2) were mild in some patients and severe enough to require intensive care support in others [3]. According to the report of the Novel Coronavirus Pneumonia Emergency Response Epidemiology Team in China, approximately 5% of individuals diagnosed with COVID-19 require treatment in intensive care units (ICUs) for varied problems (e.g., respiratory failure, septic shock, and/or multiple organ dysfunction/failure) [7]. Being in contact with patients who have COVID-19 in risky care environments, such as ICUs, has brought high exposure to the infection agent for nurses, which causes them to experience fear [2, 8]. Like all healthcare workers, intensive care nurses have concerns and fears about lack of access to appropriate personal protective equipment, being infected with coronavirus and then infecting family members, the possibility of not being able to be tested quickly for COVID-19 when necessary, the possibility of not being able to meet their personal and family needs if they become infected, childcare problems caused by increased working hours and school closures, and deficiencies in access to up-to-date information [9].

The COVID-19 pandemic has had negative effects on healthcare workers, causing burnout and psychosocial problems and endangering the mental health of workers [10]. It is important to first reveal the causes of fear to determine approaches to support the psychological and mental health of healthcare workers [9].

This study aimed to reveal the COVID-19 fears of intensive care nurses during the pandemic.

## Materials and methods

### Research questions

What is the fear level of intensive care nurses during the COVID-19 pandemic?

Which nursing practices have increased the fears of intensive care nurses during the COVID-19 pandemic?

### Study design and participants

The data of this cross-sectional study were collected with the participation of 128 nurses working in the ICUs of two public hospitals (level 1, level 2, and level 3) between October 2020 and November 2020. The universe of the study consisted of 152 intensive care nurses who were actively working in the ICU of one of two public hospitals. In the present study, the purpose was to reach nurses working in all ICUs (152) without using any sampling methods. The sampling of the study consisted of 128 intensive care nurses who volunteered to participate in the study. The power of the study in representing the universe was 84.2%. The study included nurses working actively in ICUs during the study process who volunteered to participate. Nurses who were on leave during the study process and other healthcare professionals who worked in the ICU were not included in the study.

### Data collection tools

The Nurse Identification Form and the Fear of COVID-19 Scale (FVC-19S) were used in data collection.

### Nurse Identification Form

The form was created by the researchers (two intensive care nurses, one academic nurse, and another academic nurse with 10 years of intensive care nursing experience) based on the literature [11, 12].

The form consisted of 16 questions on sociodemographic characteristics (i.e., age, gender, educational status, working in an ICU, professional experience duration, intensive care experience time, weekly working hours, shift type, number of patients cared for in one shift), and COVID-19-related experiences (COVID-19 patient care status, test status, status of infection with COVID-19, being able to access PPE, access to care filters, the aspiration system used, and practices increasing the risk of infection).

### The Fear of COVID-19 Scale (FCV-19S)

The scale was developed by Ahorsu et al. [12] in 2020and adapted to the Turkish language by Bakioglu et al. [13]. The scale consists of one single dimension and seven items The scale does not have reverse items and takes the form of a 5-point Likert scale. "I strongly disagree" is scored with 1 point, and "I strongly agree" is scored with 5 points. The total score that can be received from all the items of the scale reflects the level of coronavirus fear experienced by the individual. Total scores can range between 7 and 35. High scores show that the individual experiences high coronavirus fear. The scale questionnaire takes 10 minutes to fill out. The Cronbach Alpha Coefficient was calculated as 0.88 in the original study [13] and 0.76 in this study.

### Data collection

The online survey forms were delivered to the nurses through WhatsApp groups to protect the health of both researchers and participants during the COVID-19 pandemic. The participants were informed about the study on the first page of the survey form, and their approval to participate in the study was verified. The participants also filled in the data collection forms after providing consent on the written consent form.

### Ethical considerations

The ethical approval required for conducting the research was obtained from the Kırklareli University Health Sciences Institute Scientific Research Ethics Committee (number: 69456409-199-E.14455, protocol number: 2020/251 and date: 06.10.2020), and permission was obtained from the public hospitals. An electronic informed consent form was

presented on the first page of the online survey. The nurses were electronically informed on the first page of the survey that their participation was voluntary and that they could withdraw from the survey at any time.

**Statistical analysis**

Data were analyzed using IBM SPSS Statistics 22.0 (IBM, Armonk, NY, USA). The data were gathered by using descriptive statistics, such as mean (standard deviation), percentage, and frequency. The Kolmogorov Smirnov test was used to test the compatibility of the data with normal distribution. The Independent Sample t-test and ANOVA were used in cross-group comparisons. The Spearman Correlation test was used to examine and determine relations between the variables. The statistical significance value was taken as $P < 0.05$.

## Results

The mean age of the nurses in the study was 29.7 (6.6) years, and 82.8% were women. Also, 67.2% of the participating nurses worked in level 3 intensive care, and the mean weekly working time was 49.6 (10.1) hours. A total of 80.5% of the nurses provided care to patients who were infected with COVID-19; 94.5% were tested for COVID-19 (positive molecular RT-PCR on nasopharyngeal swab) at least once, and 21.4% of those tested were positive. The rate at which the nurses had access to personal protective equipment was found to be 88.3%. It was found that the mean scale score was different at statistically significant levels depending on the education status and the number of patients per nurse in one single shift ($P < 0.05$) (Table 1).

Table 1: Comparison of the nurses' COVID-19 fear according to individual variables and their experiences of COVID-19 (n = 128)

| Variables | | n(%) | FCS Mean (SD) | *P*-value |
|---|---|---|---|---|
| Age (years) | | 29.7 (6.6) | | 0.394 |
| Mean (SD) (Min-Max) | | (20-48) | | r = 0.024 |
| Gender | Female | 106(82.8) | 22.8 (4.9) | 0.354 |
| | Male | 22(17.2) | 21.7 (6.1) | t = -0.930 |
| Education | High school | 21(16.4) | 25.2 (5.9) | 0.013 |
| | Bachelor's degree or higher | 107(83.6) | 22.2 (4.8) | t = -2.517 |
| Working ICU | Level 1 | 12(9.4) | 22.5 (6.6) | 0.748 |
| | Level 2 | 30(23.4) | 23.3 (5.4) | F = 0.291 |
| | Level 3 | 86(67.2) | 22.5 (4.8) | |
| Shift type | Solo night shift | 9(7) | 23.5 (2.0) | 0.292 |
| | Day and night shift | 119(93) | 22.6 (5.3) | t = 1.086 |
| Weekly working (hours) Mean (SD) | | 49.6 (10.1) | | 0.309 r = -0.045 |
| Professional experience duration (month) mean (SD) | | 71.1 (68.1) | | 0.087 r = 0.121 |
| Experience duration in ICU (month) Mean (SD) | | 64.1 (117.7) | | 0.120 r = -0.088 |
| Number of patients cared for in one shift Mean (SD) (Min-Max) | | 2.5 (0.7) (1-5) | | 0.217 r = 0.007 |
| COVID-19 patient care status | Yes | 103(80.5) | 21.2 (4.2) | 0.104 |
| | No | 25(19.5) | 23.0 (5.3) | t = -1.636 |
| COVID 19 PCR test status | Yes | 121(94.5) | 22.8 (5.1) | 0.332 |
| | No | 7(5.5) | 20.8 (5.1) | t = 0.975 |
| Status of infection with COVID-19 | Yes | 26(20.3) | 21.8 (5.2) | 0.368 |
| | No | 95(74.2) | 23.0 (5.1) | F = 1.009 |
| | Not tested | 7(5.5) | 20.8 (4.8) | |
| Being able to access PPE | Yes | 113(88.3) | 22.4 (5.1) | 0.193 |
| | No | 315(11.7) | 24.3 (4.9) | t = -1.308 |
| Being able to care filters | Yes | 106(82.8) | 22.6 (5.3) | 0.733 |
| | No | 22(17.2) | 23.0 (4.2) | t = -0.034 |
| The aspiration system | Open aspiration | 80(62.5) | 23.1 (4.5) | 0.254 |
| | Closed aspiration | 48(37.5) | 21.9 (6.0) | t = -1.148 |

r: Spearman correlation analysis, t: independent sample t test, F: ANOVA, $P < 0.05$

The mean score on the Fear of COVID-19 Scale for the nurses was found to be 22.7 (5.1) (Table 2).

A total of 55.5% of the nurses were afraid of losing their lives due to coronavirus. In addition, 69.6% were uncomfortable about the disease, and they had symptoms of stress and anxiety associated with the disease (36.7% had insomnia, 28.3% tachycardia, and 25.8% sweating in the hands); 59.3% of them experienced tension when they saw stories and news about the coronavirus on social media (Table 3).

Table 2: Nurses' mean scores on the fear of COVID-19 scale (n = 128)

| Scale | Mean (SD) | Min – Max |
|---|---|---|
| The fear of COVID-19 scale | 22.7 (5.1) | 11-35 |

Table 3: Nurses' responses to the items of the fear of COVID-19 scale (n = 128)

| Items | Strongly disagree | Disagree | Undecided | Agree | Strongly agree |
|---|---|---|---|---|---|
| I am most afraid of coronavirus. | 3(2.3) | 18(14.1) | 21(16.4) | 62(48.4) | 24(18.8) |
| It makes me uncomfortable to think about coronavirus. | 5(3.9) | 21(16.4) | 13(10.2) | 55(43.0) | 34(26.6) |
| My hands become clammy when I think about coronavirus-19. | 21(16.4) | 47(36.7) | 27(21.1) | 22(17.2) | 11(8.6) |
| I am afraid of losing my life because of coronavirus-19. | 7(5.5) | 25(19.5) | 25(19.5) | 48(37.5) | 23(18) |
| When watching news and stories about coronavirus-19 on social media, I become nervous or anxious. | 6(4.7) | 21(16.4) | 29(22.7) | 55(46.0) | 17(13.3) |
| I cannot sleep because I'm worrying about getting coronavirus-19 | 2(1.6) | 56(43.8) | 23(18.0) | 22(17.2) | 25(19.5) |
| My heart races or palpitates when I think about getting coronavirus-19 | 21(16.4) | 44(34.4) | 27(21.1) | 24(18.8) | 12(9.4) |

This study determined that the three riskiest interventions that caused fear of COVID-19 for nurses were intubation, invasive aspiration, and inhaler treatment (77.3%, 74.2%, and 72.7%, respectively) (Table 4).

Table 4: Practices increasing the risk of COVID-19 infection in nurses (n = 128)

| Practices | Yes | | No | |
|---|---|---|---|---|
| | n | % | n | % |
| Intubation | 99 | 77.3 | 29 | 22.7 |
| Invasive aspiration | 95 | 74.2 | 33 | 25.8 |
| Inhaler drug application | 93 | 72.7 | 35 | 27.3 |
| Cardiopulmonary resuscitation (CPR) | 81 | 63.3 | 47 | 36.7 |
| Tracheostomy care | 63 | 49.2 | 65 | 50.8 |
| Nasogastric / urinary catheterization and care | 61 | 47.7 | 67 | 52.3 |
| Prone positioning | 56 | 43.8 | 72 | 56.2 |
| Noninvasive aspiration | 56 | 43.4 | 73 | 56.6 |
| Taking anamnesis | 50 | 39.1 | 78 | 60.9 |
| Transfer for medical viewing | 43 | 33.6 | 85 | 66.4 |
| Delivery of valuable items to patients' relatives | 41 | 32.0 | 87.0 | 68.0 |
| High-flow oxygen treatment | 34 | 26.6 | 94 | 73.4 |
| Hemodialysis / hemofiltration therapy | 28 | 21.9 | 76.9 | 78.1 |
| Central venous pressure measurement and catheter maintenance | 27 | 21.1 | 101 | 78.9 |
| Others (feeding, blood sampling, body cleansing) | 12 | 9.4 | 116 | 90.6 |

## Discussion

A total of 80.5% of the intensive care nurses provided care for patients infected with COVID-19, and 94.5% were tested for COVID-19 at least once. Of those tested, 21.4% were positive for the coronavirus. Quigley et al. [14] determined that healthcare workers had a three-times higher risk of becoming infected with COVID-19 than the general population. Bandyopadhyay et al. [15] reported that the rate of infected nurses was 38.6% in their global studies. Kambhampati et al. [16] found that nurses had the highest share (36.3%) of the healthcare workers hospitalized. Semerci et al.'s [17] study reported that 18.9% of participating oncology nurses provided care for patients with COVID-19, and 2.7% were positive for SARS CoV-2. Lombardi et al. [18] found that 8.8% of healthcare workers were infected. A study by Eyre et al. [19] found that

COVID-19 positive rates were lower in intensive care workers because of the low-level difficulty they have in accessing PPE.

That study found that those who did not acquire PPE experienced more COVID-19 fear, even if no statistically significant differences were detected. Hendy et al. [20] found that nurses who had trouble acquiring PPE were more stressed. Likewise, Luceño-Morene et al. [21] found that accessibility to PPE affected anxiety levels. Pouralizadeh et al. [22] identified the key factor affecting anxiety during the COVID-19 pandemic as PPE deficiency. A study by Halcomb et al. [23] was reported that 40% of participating nurses could not access gowns, 45.4% could not access P2/N95 masks, 22.1% could not access surgical masks, and 28.6% could not access goggles. PPEs are crucial elements in providing patient care, and the lack of this safety equipment triggers fear in healthcare professionals [9, 23]. In this study, the high rate of accessing PPEs, at 88.3%, might explain the different results.

High school graduate nurses were more fearful of COVID-19 in the present study. Labrague and De los Santos [11] reported that educational status did not affect anxiety around COVID. However, the nurses who participated in the study had at least undergraduate degrees, which is considered to be effective and necessary for intensive care nurses in dealing with stress and fear management.

The study also found that the fear of COVID-19 among nurses increased as the number of patients increased per nurse in one single shift. Likewise, another study also found that nurse-to-patient ratio > 1:3 nurses experienced more stress [20]. It was reported in a study with a sampling of healthcare workers that an increase in workload triggered burnout due to fear of COVID-19 [25]. It was found that nurses had more time for the care of COVID-19 patients in the ICU, and it was recommended that the ratio of nurses to patients should be 1:1 [26]. Since an increase in the number of patients the nurses care for in one single shift also increases the risk of infection, this is considered to be a factor triggering the fear of COVID-19.

The mean score of the nurses in terms of the fear of COVID-19 was above average levels at 22.7 (5.1). A study by Labrague and De los Santos [11] found a COVID-19 fear scale score average of 19.92 (5.25) (5-35) in frontline nurses. In a study conducted with registry nurses as the sampling, it was reported that nurses experienced high COVID-19 fear levels (7.72 (2.21) in a 10-point rating) [27]. Gonzales-Gill et al. [28] reported that 37.5% of the nurses working in ICUs and emergency departments feared becoming infected. A study conducted in Wuhan reported that nurses (63.2%) experienced high levels of fear [24]. The fact that the intensive care nurses had fears in the face of this disease with high infection rates might have stemmed from their constant contact with COVID-19 patients.

It was determined in the study that 55.5% of the nurses were fearful of losing their lives due to coronavirus, and 69.6% were uncomfortable about the disease. Also, the symptoms of stress and anxiety associated with the disease were detected in nurses (36.7% experienced insomnia, 28.3% tachycardia, and 25.8% sweating in the hands). Hu et al. [24] found that nurses experienced fear of losing their lives to the disease. Another study reported that intensive care nurses experienced fear of death over the course of the pandemic [29]. Yifan et al. [30] determined that 31.4% of the intensive care nurses had an increase in angina and heart rates, and 30.7% had dyspnea during the pandemic. Studies with healthcare workers as the sampling showed that sleep quality decreased and more sleep problems occurred during the COVID-19 pandemic [31, 32]. Sunjaya et al. [33] found that healthcare workers showed symptoms of depression, such as loneliness, insomnia, and difficulty in concentrating. Increased heart rates, sweating, tension, and the idea that something unwanted will happen are common symptoms of anxiety. The symptoms are limited with time in most cases and disappear when the associated event ends [34]. The study results showed that the COVID-19 pandemic has been a cause of stress and fear for nurses and other healthcare workers.

It was found that 59.3% of nurses experienced tension when they saw stories and news on coronavirus on social media. Tayyib and Alahosatimi [27] identified fear and stress of seeing the news on social media as predictive in nurses. It was determined in another study that disinformation on social media created fear in society, with the panic effect manifesting during the course of the pandemic [35]. Therefore, social media news about COVID-19 has psychological effects on society and on nurses.

In the present study, the three riskiest interventions that contribute to the fear of COVID-19 infection for nurses were intubation, invasive aspiration, and inhalation treatment (77.3%, 74.2%, and 72.7%, respectively). Yifan et al. [30] determined that ICU nurses who cared for COVID-19 patients with pneumonia were exposed to infection risk from each patient 11 times during nursing initiatives and interventions (e.g., intubation, open suction, close suction, etc.). Since aerosol-forming procedures (e.g., intubation, cardiopulmonary resuscitation (CPR), NIV, tracheostomy, inhaler drug application, prone positioning, high-flow oxygen treatment, etc.) increase the risk of infection [36], it can be said that intensive care nurses experience fear during aerosol-forming procedures. To decrease intensive care nurses' fears during aerosol-forming procedures, nurses should be informed about performing these practices on COVID-19 patients.

**Limitations**

In this study, the COVID-19 fears of intensive care nurses, who have a special place in the care of COVID-19 patients during the pandemic, which is a topic of current interest, were evaluated, and a valid and standardized scale was used to measure the fear of coronavirus.

However, the fact that the results were limited to the sample affected their generalization. The results should not be generalized to all intensive care nurses since the study was conducted in two small cities and two separate centers where the study took place had different conditions (physical characteristics, equipment availability, patient population, etc.). Research results should be interpreted with this situation in mind.

**Conclusion**

Nurses are concerned about the COVID-19 pandemic. During a pandemic process, the fears and coping skills of intensive care nurses must be identified, and the nurses should be supported physiologically, psychologically, and sociologically.

Necessary measures must be taken for intensive care nurses who are high school graduates or who care for an increased number of patients as they face increased fear due to the risk of infection and spread of the disease during the COVID-19 pandemic. Preventable factors should be considered. Information must be provided at certain periods to keep the information of nurses up-to-date on emotion management and infection control. Emphasis should be made on considering news on the scientific and official sites, not on social media, to avoid an infodemic.

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# Public awareness of first aid treatment in acute burns

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All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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## Abstract

**Background/Aim:** Burn injury is a global public health concern. First aid in burns can reduce morbidity and mortality by stopping the burning process and reducing the size and ultimate depth of the burn injury. The aim of this study is to assess the knowledge about first aid for burns in an urban population in Malaysia.

**Methods**: We conducted a cross-sectional study using questionnaires to assess the knowledge about first aid for acute burns in our single tertiary main national referral unit for burn injuries. A total of 100 respondents were interviewed. Respondents were voluntary outpatients in the surgical outpatient department.

**Results**: Twenty-two percent of the respondents complied with World Health Organization (WHO) recommendations. Other methods used were toothpaste (5.6%), soy sauce (4.4%), traditional oils (3.3%), aloe vera gels (2.2%), and a variety of creams (3.3%). Twenty-five percent agreed that the best information in first aid is through a first aid course; 15% chose a phone application, 14% chose a website, and 12% chose a television advertisement. The recommended first aid treatment (running tap water for ≥ 20 minutes) has proven beneficial in reducing tissue temperature and severity of injury.

**Conclusion**: In 2019, 91% of the Malaysia population had access to the internet, which offers fast and reliable information on first aid for acute burn injuries. The majority of our population still lacks knowledge about first aid treatment for acute burns. Implementation of education regarding burn first aid should target all populations in Malaysia through different community health campaigns, with collaboration between government and non-governmental agencies.

**Keywords:** Awareness of first aid treatment, Acute burn, Malaysia population, Running tap water

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## Introduction

A burn is an injury to the cutaneous or other organic tissue primarily caused by heat or friction, radiation, contact with chemicals, electricity, and radioactivity. According to the World Health Organization (WHO), burns are a global public health risk issue, accounting for an estimated 180,000 deaths each year. The majority of burn deaths take place in low- and middle-income populations, and an estimated two-thirds occur in the South-East Asia regions and Africa. Major burns are a common cause of morbidity, including prolonged stay in hospital, disability, and disfigurement, often with resulting stigma and rejection by the public [1].

In Malaysia, the number of burn cases is increasing in trend [2, 3]. The British Burn Association (2018) recommends cooling an acute burn wound with running tap water for 20 minutes [4]. This will delay the burn progression and improve outcomes in terms of healing and final cosmetic appearance [5]. Several studies on knowledge of first aid in burns from European populations such as London [6] and Australia [7] support these outcomes. However, such studies in the Asian population are limited. No published study on the knowledge of first aid for burns in Malaysia to date can represent the urban population. Thus, this review aimed to assess the awareness of adequate first aid and treatment for burns among the urban population in Malaysia.

## Materials and methods

This is a cross-sectional observational study conducted at a single tertiary main national referral center for burn injuries, the surgical outpatient department in Hospital Sungai Buloh, Selangor, Malaysia, using a previously validated questionnaire (Appendix 1) [8]. Minor alterations of the questionnaire were done to facilitate Malaysian responses. Respondents were recruited by convenience sampling (n=100) from outpatients attending the plastic surgery clinic from January 2019 to March 2019. A non-probability convenience sample was used, and the sample size was estimated using a 95% confidence interval (CI), 5% absolute precision, with 5% expected to practice the recommended first aid technique of holding their burn wound under running cool water—preferably tap water—for more than 20 minutes [3, 9]. Inclusion criteria included non-burn-related outpatients aged between 18 and 65 years old who were able to give informed consent to participate in the study. Respondents who were medical personnel and those who were not Malaysian were excluded.

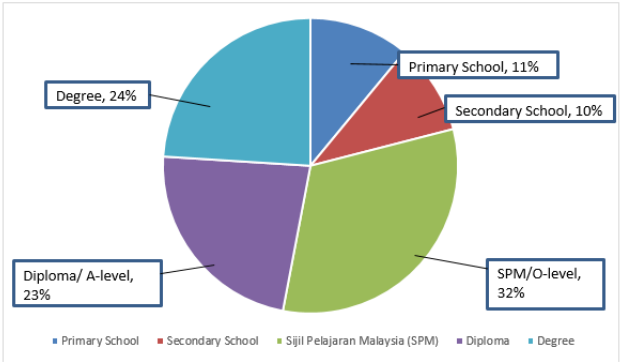
### Statistical analysis

Microsoft Excel 2016 was used to generate data in this study. Simple pie charts were constructed using Microsoft Excel 2016 based on the data we collected from the questionnaire to show the results.

## Results

In this study, 100 respondents were included. Their ages ranged from 15 to 65 years old. Seventy-nine percent had an O-level or its equivalent qualification, and 24% were degree holders (Figure 1). The mean age of the sample was 37.5.

Figure 1: The respondents' education level




From this study, 90% claimed that they had practiced first aid. In general, 73% used running tap water as a first-aid treatment for burns. Only 24% of the respondents reported cooling a burn wound for 20 minutes or more as recommended (Figure 2). Other methods used were applying toothpaste (5.6%), soy sauce (4.4%), traditional oils (3.3%), aloe vera gels (2.2%), and a variety of creams (3.3%) (Figure 3). The majority of respondents do not cover a burn wound (83%) when going to a hospital for treatment. Others indicated using a clean dressing (11%), towels (4%), cling film (1%), and ice packs (1%).

Figure 2: Duration of holding the burn wound under running tap water

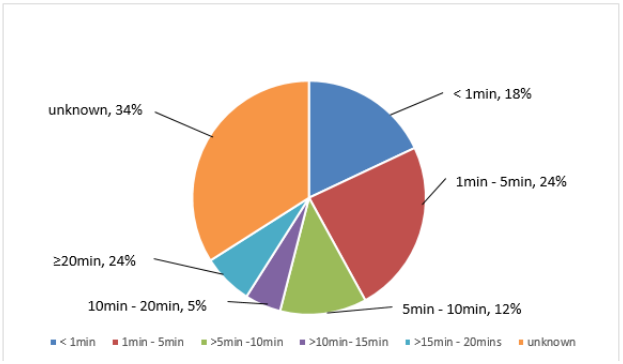
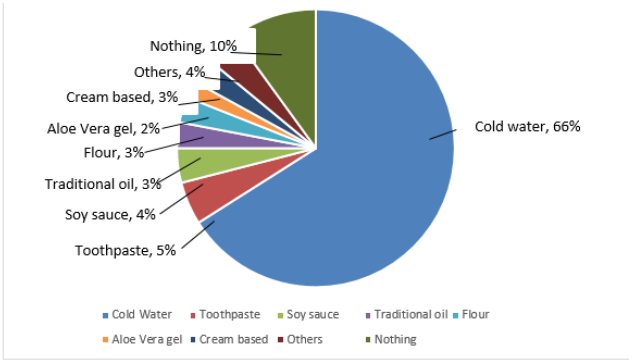
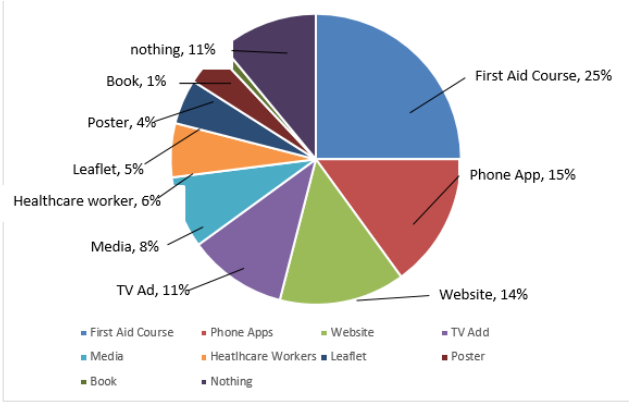



Figure 3: Materials applied during first aid




In the study, most respondents acquired their source of information regarding first aid treatment from family members (45%). Eighty-four percent of respondents were interested in educating themselves on the best method of first aid for burns. Twenty-five percent agreed that the best medium for this information would be a first aid course, followed by 15% choosing a phone application, 14% choosing a website, and 12% opting for a television (TV) advertisement (Figure 4).

Figure 4: Best medium for information




## Discussion

The recommended first aid treatment (running tap water for ≥ 20 minutes) has proven beneficial in reducing tissue temperature and severity of injury [10, 11]. It has been found to be significantly associated with improvement in re-epithelialization time and the healing process. Cooling with running tap water can slow the progressive evolution of the burn wound by impeding coagulation and inflammation, reducing swelling and depth of injury, providing pain relief, and cleansing the wound [12]. It also reduces the need for grafting and promotes faster healing [13]. Cooling burn wounds for 20 minutes or more showed superior improvement in the histological analysis of burn depth compared with those treated for 5 and 10 minutes only [14].

However, our public survey showed that our population still has a poor understanding of proper first aid practice, with only 24% of respondents using the correct methods. The rate of proper first aid practices in other high-income countries ranges from 12% to 22% [15]. The recommended first aid practice for burn wounds was followed by 5% of the population of the east coast of Peninsular Malaysia [3]. In two other Third World countries, Saudi Arabia and South Africa, 5.8% and 26% of the population, respectively, practiced the recommended method [16, 17]. Our survey demonstrated a greater percentage of correspondents practicing proper first aid. This could be because the populations involved in the study were from an urban, upper-middle-class area.

A study conducted in New South Wales indicated that people there received information on first aid treatment mainly from a first aid book (42%) and the internet (33%) [7]. In Malaysia, current statistics showed 29 million people using the internet in 2019, with an estimated population penetration of 90% [18]. The public survey we conducted found the majority of participants have internet access, which is the best medium for information for them on burn first aid. This study has the limitation that our population currently is still lacking in knowledge of first aid treatment for burn injuries.

### Conclusion

Advertisements on social media websites can improve public knowledge and awareness. This information can be easily accessible through smart devices. Proper first aid is simple, cheap, and accessible. Implementation of burn first aid education should target all populations in Malaysia through community health campaigns that can be held in shopping malls and at general public gatherings. However, in the light of the recent COVID-19 pandemic, social distancing is now a new norm. Social media platforms have become the best tool for disseminating information and imparting first aid knowledge about burns to the general public. Therefore, a collaboration between the Ministry of Health, the Minister of Communications, and the Fire and Rescue department holds the key to creating awareness with regards to first aid treatment among fellow Malaysians.

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Appendix 1

**Questionnaire for First Aid Awareness among the Public**

1. How old are you?

☐ <15 ☐ 16-20 ☐ 21-25 ☐ 26-30 ☐ 31-35 ☐ 36-40
☐ 41-45 ☐ 46-50 ☐ 51-55 ☐ 56-60 ☐ 61-65 ☐ >65

2. Gender: ☐ Male ☐ Female

3. Ethnicity

☐ Malay ☐ Chinese ☐ Indian ☐ Other……

4. Are you a parent or grandparent?

☐ Parent ☐ Grandparent

5. If so, how many children do you have, and how old are they?

……

6. What is your occupation?

……

7. At what age did you leave school? Did you do any further training?

☐ O-level/SPM ☐ A-Level/Diploma ☐ Degree ☐ Further training

Please provide details on further training

……

8. Has your child previously had a burn injury?

☐ Yes ☐ No ☐ N/A

9. What do you know about first aid for burns?

☐ Cold water ☐ Cool water ☐ Ice ☐ Toothpaste
☐ Cream ☐ butter ☐ Oil
☐ Other……..

If water: ☐ Running water ☐ Still

For how long:

☐ 1 minute ☐ 5 minutes ☐ 10 minutes ☐ 15 minutes ☐ 20 minutes

What would you cover the burn with?

☐ Nothing ☐ Cling film ☐ Clean dressing ☐ Other……

10. Where did you learn/ hear about this first aid?

☐ Course ☐ Media ☐ School ☐ Family/friends ☐ Healthcare worker
☐ Internet Others……

11. How long ago did you learn this?

☐ Within last year ☐ In the last 5 years ☐ In the last 10 years
☐ >10 years ago

12. Would you be interested in learning more about first aid for burns?

☐ Yes ☐ No

13. If yes, how would you like to receive this information?

☐ First aid course ☐ TV ad ☐ Leaflet ☐ Phone application
☐ Healthcare worker ☐ Website ☐ Poster
☐ Other……

14. Would you find a leaflet like this helpful?

☐ Yes ☐ No

15. Any other comments?

……

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# Interleukin-1 beta gene polymorphisms in patients with fibromyalgia syndrome

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**Ethics Committee Approval**
Ethics approval for the study was obtained from Ethics Committee of Erenköy Psychiatric and Neurological Diseases Training and Research Hospital (Date: 14.07.2014, Number: 13/160). All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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## Abstract

**Background/Aim:** Fibromyalgia syndrome (FMS) has been linked to a genetic background. Although there are conflicting results, it has been suggested that cytokines play a role in FMS etiology. Interleukin (IL)1β is a cytokine that has been linked to FMS symptoms and has been detected in the skin of FMS patients. We aimed to determine the possible relationship between IL1β -31 and IL1β -511 polymorphisms in FMS.
**Methods:** In this cross-sectional study, we included patients who were diagnosed with FMS according to the American College of Rheumatology classification criteria. IL1β -31 (rs 1143627) and IL1β -511 (rs 16944) polymorphism genotyping was conducted in FMS patients (n = 33) and healthy controls (n = 41) using real-time polymerase chain reaction (RT-PCR).
**Results**: IL1β -511 variations in patients with FMS and control groups were significantly different ($P$ = 0.010). The frequency of the IL1β -511 heterozygote AG genotype was significantly higher in controls ($P$ = 0.028). Additionally, the frequency of the IL1β -511 wild type A allele was significantly higher in the control group ($P$ = 0.003). The IL1β -31 genotypes and allele frequencies were not significantly different between the groups.
**Conclusion**: The IL1β -511 wild type A allele could be a risk-reducing factor for FMS. The present study suggests that genetic variations of the IL1β gene could play an important role in FMS etiology.

**Keywords:** Interleukin-1β, Polymorphism, Fibromyalgia syndrome

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## Introduction

Fibromyalgia syndrome (FMS) is a chronic, painful disorder that is characterized by widespread musculoskeletal pain, tenderness, fatigue, sleep disorders, mood disorders, and cognitive problems [1]. The prevalence of FMS is between 2% and 8%. First-degree relatives of patients with FMS are more likely to have FMS and other chronic pain states than relatives of individuals without FMS. Because of this familial aggregation, FMS is considered to have a genetic background [2]. Genes associated with the frequency of chronic pain conditions or pain sensitivity are involved in the regulation of neurotransmitters and other inflammatory pathways that modulate pain sensitivity [3].

FMS was shown to have a 50% heritability. Serotonin transporter gene (SLC6A4), catechol-O-methyltransferase (COMT) short nucleotide polymorphism rs4818, dopamine receptor D4, monoamine oxidase, β-2 adrenergic receptor, and guanosine triphosphate cyclohydrolase are candidate genes that are associated with FMS pathogenesis [4]. However, Feng et al. [5] reported two nonsense mutations associated with high specific cytokine levels.

Despite the controversies about FMS etiology, it has been proposed that cytokines may play a role in the syndrome's etiology. Several studies showed increased interleukin (IL)1β, IL6, IL8, and tumor necrosis factor (TNF)-α levels in FMS [6-10]. However, other studies showed no significant differences in IL1β in FMS patients [11-13].

IL1β is a somnogenic cytokine that is normally produced in response to infection, injury, or immunologic challenge [14], and it has influences on the neuroendocrine, autonomic, limbic, and cortical areas of the central nervous system (CNS) that regulate sleep [15]. IL1β is associated with hyperalgesia, fatigue, fever, sleep, and myalgias that may be relevant to FMS [16]. IL1β is detected in the skin of FMS patients, suggesting an inflammatory background for pain induction [17].

Because IL1β polymorphism has not been investigated adequately in FMS, we aimed to determine the relationship between IL1β -31 and IL1β -511 polymorphisms and FMS in a Turkish sample.

## Materials and methods

### Participants

Thirty-three patients with FMS and 41 control group participants were recruited from the Erenköy Physical Therapy and Rehabilitation Hospital outpatient polyclinic. All patients were diagnosed with FMS by their physiatrist, according to the American College of Rheumatology classification criteria [18]. Ethics approval for the study was obtained from the Ethics Committee of Erenköy Psychiatric and Neurological Diseases Training and Research Hospital (Date: 14.07.2014, Number: 13/160). After a detailed explanation of the study, written informed consent was obtained from all participants.

### Genotyping

Genomic DNA was extracted from whole blood using an iPrep Purification Instrument (Invitrogen, Life Technologies, Carlsbad, CA, USA). Isolated DNA samples were analyzed spectrophotometrically using a NanoDrop 2000 (Thermoscientific, Waltham, MA, USA). IL1β -31 (rs 1143627) and IL1β -511 (rs 16944) were analyzed using Taqman assays (Thermoscientific). Allelic discrimination was performed using a 7500 Fast Real-Time Polymerase Chain Reaction (RT-PCR) (Applied Biosystems, Foster City, CA, USA) by interpreting the fluorescent data from hybridizing probes (VIC/FAM).

### Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 23.0 version (IBM Corp., Armonk, NY, USA) was used for statistical analysis in the study. Patients and control group participants were compared, and differences in the serum levels were identified using a Student's *t*-test or a one-way ANOVA to compare the allele distributions and genotypes. The significance level was accepted as $P < 0.05$.

The goodness-of-fit test was used to check for deviations from the Hardy–Weinberg equilibrium (HWE) in all genotypic distributions. The Haploview bioinformatics software package 4.2 was used to measure linkage disequilibrium (LOD) between variants using D0 and r2 values (https://haploview.software.informer.com/4.2/).

The statistical power was evaluated using G*power software 3.1.9.4 version (G*power, University of Dusseldorf, Dusseldorf, Germany), and 55.9% sample power was obtained in the post hoc power analysis (effect size d = 0.5, alpha error probability = 0.05).

## Results

We examined IL1β -511 (rs16944) and IL1β -31 (rs1143627) polymorphisms in FMS patients in the current research. The genotype and allele frequencies for IL1β polymorphisms in the patient and control groups are presented in Tables 1 and 2. Genotype and allele frequency distributions of the IL1β gene variations were consistent with the HWE.

Table 1: The distribution of IL1β -511 polymorphism and allele frequencies in the patient with fibromyalgia and control groups

| SNP | Control (n = 41) n (%) | Fibromyalgia (n = 33) n (%) | *P*-value | Chi square | Odds Ratio | 95% CI |
|---|---|---|---|---|---|---|
| IL1β -511(rs16944) | | | | | | |
| Genotype | | | 0.010* | 9.253 | | |
| AA | 10 (24.4%) | 8 (24.2%) | 0.988 | 0.000 | 0.992 | 0.341-2.888 |
| AG | 29 (70.7%) | 15 (45.5%) | 0.028* | 4.846 | 0.345 | 0.132-0.901 |
| GG | 2 (4.9%) | 10 (30.3%) | 0.816 | 0.054 | 1.138 | 0.384-3.737 |
| Allelle | | | | | | |
| A | 39 (47.56%) | 31 (45.96%) | 0.003* | 8.699 | 0.118 | 0.024-0.596 |
| G | 33 (52.44%) | 35 (53.04%) | 0.988 | 0.000 | 1.008 | 0.346-2.935 |

n: Number of individuals, SNP: single nucleotide polymorphism, CI: confidence interval, * $P < 0.05$ was denoted as statistically significant

Table 2: The distribution of IL1β -31 polymorphisms and allele frequencies in the patient with fibromyalgia and control groups.

| SNP | Control (n = 41) n (%) | Fibromyalgia (n = 33) n (%) | *P*-value | Chi square | Odds Ratio | 95% CI |
|---|---|---|---|---|---|---|
| *IL1β a -31 (rs1143627)* | | | | | | |
| Genotype | | | 0.546 | 1.696 | | |
| GG | 7 (17.1%) | 8 (24.2%) | 0.446 | 0.581 | 1.554 | 0.498-4.851 |
| GA | 24 (58.5%) | 15 (45.5%) | 0.263 | 1.255 | 0.590 | 0.234-1.489 |
| AA | 10 (24.4%) | 10 (30.3%) | 0.569 | 0.324 | 1.348 | 0.482-3.772 |
| Allellic count | | | | | | |
| G | 51 (62.19%) | 30 (45.45%) | 0.198 | 1.660 | 0.590 | 0.214-1.380 |
| A | 31 (37.81%) | 36 (54.55%) | 0.446 | 0.643 | 0.643 | 0.206-2.008 |

n: Number of individuals, SNP: single nucleotide polymorphism, CI: confidence interval, * $P < 0.05$ was denoted as statistically significant

IL1β -511genotypic frequencies in FMS patients and in the control group were significantly different ($\chi2$ = 9.253, *P* = 0.010). The frequency of the IL1β -511 heterozygote AG genotype was statistically higher in controls compared to patients ($\chi2$ = 4.846, *P* = 0.028; odds ratio [OR] = 0.345, 95% confidence interval [CI] = 0.132–0.901). Although there was no significant difference in the variant G allele frequency between the study groups ($\chi2$ = 0.000; *P* = 0.988), the wild type A allele frequency was significantly higher in the control group ($\chi2$ = 8.699; *P* = 0.003). Our results established that carrying the A allele decreased the FMS risk 0.1-fold (OR = 0.118, 95% CI = 0.024–0.596). Thus, carrying the A allele could be a risk-reducing factor for FMS.

There were no significant differences between the groups regarding the frequency of the IL1β -31 genotype ($\chi2$ = 1.696, *P* = 0.546) or allele (G allele *P* = 0.198, A allele *P* = 0.446), and there were no significant differences in the homozygote mutant (AA) and homozygote wildtype (GG) genotype carriers between the groups (*P* = 0.114). However, allele frequency analysis showed that most of the individuals in the control group carried the wild type G allele (62.19%), while most of patients with FMS had the variant A allele (54.55%), but this difference was not statistically significant.

Table 3 shows the serum IL1β (pg/mL) levels according to IL1β -31and IL1β -511 genotypes and alleles in FMS patients. There were no statistically significant differences between the groups or concerning the IL1β genotypes. The small sample size may account for these results.

We showed that the distribution of the IL1β -511 (rs16944) genotype in the FMS and control groups (*P* = 0.010) is statistically significantly different in the current analysis. Thus, we analyzed the effect of the IL1β genotype polymorphism frequency on the somatization data before and after treatment (Table 4). There was a significant difference between the genotypes according to the somatization data (*P* = 0.039). Eighty percent of the patients with the GG genotype were in the "strong" group before treatment. Moreover, most of the individuals carrying the G allele were in the "strong" group (*P* = 0.024). Although the results are not statistically significant, those who carry the G allele seem to be less responsive to treatment.

Haplotype analysis for IL1β -31and IL1β -511 is shown in Table 5. However, the high-pairwise D′ value was 0.92 (Figure 1), and the frequency of the haplotype created by the risk alleles was not significantly different in FMS patients compared to controls.

Table 3: Serum IL1β (pg/mL) levels according to IL1β -31and IL1β -511 genotypes in the patients with fibromyalgia

| Fibromyalgia (n = 30) | First | Second | Difference | *P*-value |
|---|---|---|---|---|
| IL1β -511(rs16944) | | | | |
| Genotype | | | | |
| AA | 8.89 | 7.11 | 4.84 | 0.484 |
| AG | 6.03 | 5.83 | 0.744 | 0.744 |
| GG | 9.42 | 6.82 | 0.784 | 0.78 |
| Allelle | | | | |
| A | 7.08 | 6.30 | 0.76 | 0.441 |
| G | 9.42 | 6.82 | 2.60 | 0.550 |
| IL1β a -31 (rs1143627) | | | | |
| Genotype | | | | |
| GG | 9.34 | 7.69 | 1.60 | 0.436 |
| GA | 6.04 | 5.66 | 0.30 | 0.527 |
| AA | 9.42 | 6.82 | 2.60 | 0.817 |
| Allelle | | | | |
| G | 7.08 | 6.30 | 0.76 | 0.645 |
| A | 7.16 | 6.16 | 0.99 | 0.545 |

n: Number of individuals, SNP: single nucleotide polymorphism, CI: confidence interval, * *P* < 0.05 was denoted as statistically significant

Table 4: Comparison of Somatization data before and after treatment due to IL1β -511 genotypes in the patients with fibromyalgia

| IL1β-511(rs16944) Genotype | AA | AG | GG | A Allele | G Allele |
|---|---|---|---|---|---|
| Phqsom | | | | | |
| none | 0 | 0 | 0 | 0 | 0 |
| low | 4 (50%) | 2 (33.3%) | 0 (0%) | 6 (26.1%) | 2 (8%) |
| mild | 2 (25%) | 6 (60%) | 2 (20%) | 8 (34.8%) | 8(32%) |
| strong | 2 (25%) | 7 (41.2%) | 10 (80%) | 9 (39.1%) | 15 (60%) |
| *P*-value | 0.039* | | | 0.068 | 0.024* |
| Phqsom 2 | | | | | |
| none | 3 (42.9%) | 1 (7.7%) | 1 (16.7%) | 4(20%) | 2 (10.5%) |
| low | 1 (14.3%) | 5(38.5%) | 0 (0%) | 6 (30%) | 5 (26.3%) |
| mild | 1 (14.3%) | 4 (30.8%) | 3 (50%) | 5 (25%) | 7 (36.8%) |
| strong | 2 (28.5%) | 3(23.1%) | 2(33.3%) | 5(25%) | 5(26.3%) |
| *P*-value | 0. 284 | | | 0.410 | 0.266 |

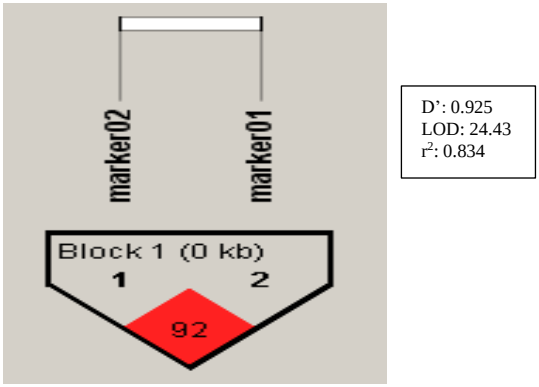
n: Number of individuals, * *P* < 0.05 was denoted as statistically significant

Table 5: The distribution of IL1β -511 polymorphism and allele frequencies in the patient with fibromyalgia and control groups

| Haplotype Associations | Frequency | Case, Control Ratios | Chi Square | *P*-value |
|---|---|---|---|---|
| rs 1143627 A: rs 16944 G | 0.499 | 0.530, 0.479 | 0.404 | 0.525 |
| rs 1143627 G: rs 16944 A | 0.457 | 0.469, 0.449 | 0.063 | 0.802 |
| rs 1143627 A: rs 16944 A | 0.025 | 0.000, 0.041 | 2.664 | 0.102 |
| rs 1143627 G: rs 16944 G | 0.019 | 0.000, 0.031 | 1.975 | 0.159 |

* *P* < 0.05 was denoted as statistically significant

Figure 1: Linkage disequilibrium plot of IL1β -511(rs16944) and IL1β a -31 (rs1143627) single nucleotide polymorphisms




Marker 01: rs16944, Marker 02: rs162601143627, Red represents a high-pairwise D' value, LOD: Linkage disequilibrium

## Discussion

FMS pathophysiology is multifactorial, and it remains unclear. Altered pain processing, hormonal influences, autonomic dysautonomia, and immunological and genetic factors play a role in FMS pathogenesis [19]. Several studies have proposed that there is a relevance between pro-inflammatory cytokines and clinical FMS symptoms such as sleep disorders, hyperalgesia, and fatigue [20, 21].

Although pro-inflammatory cytokines are known to be related to inflammatory responses, there is no information about their function in FMS pathogenesis [22]. Most research has concentrated on pro-inflammatory cytokine serum levels and their receptors. Because epidemiological and molecular studies are not sufficient, the possible association between IL1β -31 and IL1β -511 polymorphisms in patients with FMS has, therefore, been investigated in the present research.

Salemi et al. [17] investigated the role of IL1β in neurogenic inflammation. They examined skin biopsies in patients with FMS using immunohistochemistry and RT-PCR. They found that IL1β mRNA and cytokine expression was increased in patients with FMS. Cytokines were detected in skin biopsies in

healthy individuals. Their study demonstrated the immunoreactivity of IL1β in FMS patients.

To understand the genetic basis of FMS, many single nucleotide polymorphisms (SNPs) in proinflammatory cytokine genes have been investigated [23]. However, in our study there were no significant differences between the groups regarding the IL1β -31 genotype frequency. Hall et al. [24] indicated that IL1β -31 promoter gene polymorphism could alter IL1β gene transcription and subsequently IL1β protein serum levels. The promoter region of the IL1β gene variant at the -31 position in the as IL1β -31 polymorphism has promoter sequences that have a potential source of polymorphisms that affect gene expression. The IL1β -31 gene mutant allele variant in the TATA box region promotes IL1β transcription and accelerates the inflammatory reaction.

**Limitations**

The small sample size in our study limits the generalizability of our results to the general population. To clarify and confirm this relationship, further studies with a larger sample size are required.

**Conclusion**

IL1β -511 genotype frequencies between the FMS and control groups were significantly different. Homozygote wildtype (AA) and heterozygote (GA) genotypes were significantly higher in the control group. Furthermore, wild type A allele was significantly higher in the control group. Our results indicates that carrying the A allele could be a risk-reducing factor for FMS. To the best of our knowledge, the present study established, for the first time, the association between IL1β gene polymorphism and FMS in a Turkish sample.

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# Are P-glycoprotein (ABCB1/MDR1) and endothelial nitric oxide synthase (eNOS) polymorphisms related to severity of the coronary artery disease?

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**Ethics Committee Approval**
The approval of the Çanakkale Onsekiz Mart University, Faculty of Medicine Ethics Committee was obtained for this study (09.04.2014/07-04).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

**Conflict of Interest**
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## Abstract

**Background/Aim:** Atherosclerotic cardiovascular disease is one of the most common causes of morbidity and mortality in developed countries. Genetic and environmental factors are associated with atherosclerosis development. Some single nucleotide polymorphisms have also been directly related to atherosclerosis, for example, polymorphisms that reduce nitric oxide (NO) levels and/or activity have been linked to atherosclerotic diseases. However, the multidrug resistance gene 1 (MDR-1) polymorphism is related to repeated cardiovascular events. This study aimed to investigate the relationship between MDR-1 and endothelial NO synthase (e-NOS) polymorphism and severe coronary artery disease (CAD).
**Methods**: In total, 90 patients presenting with acute coronary syndrome were included in this cross-sectional study. Patients with at least > 70% stenosis in ≥ 2 coronary vessels were defined as severe CAD (Group 1), while those with this same level of involvement in < 2 vessels were diagnosed with single-vessel disease (Group 2). MDR 3435C-T and eNOS T-786C were determined by polymerase chain reaction (PCR) amplification. Comparison of parametric and nonparametric values between the two groups was performed using the Student's t- or Mann–Whitney U test. Categorical variables were analyzed using the $\chi^2$ test. Risk estimations for the association of severe CAD with the polymorphisms were calculated using odds ratio (OR) and 95% confidence intervals by comparing the genotypic combinations.
**Results**: Baseline demographic parameters were similar in both groups, except for the presence of diabetes mellitus and glucose level. T allele of MDR was 42% and 40% in groups 1 and 2, respectively (OR = 1.12). The C allele of eNOS was 34% and 30% in groups 1 and 2, respectively (OR = 1.16). Fourteen and 15 patients (40% and 27%, respectively) had both T and C alleles in patients in groups 1 and 2, respectively (OR = 1.77). All *P*-values were > 0.05.
**Conclusion**: This study is the first one that shows that MDR1 and e-NOS polymorphisms are frequent in patients with ≥ 2 vessel disease and may be associated with severe CAD.

**Keywords:** MDR-1, eNOS, Coronary artery disease, Polymorphism, P-glycoprotein

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## Introduction

Atherosclerotic cardiovascular disease is one of the most common causes of morbidity and mortality in people in developed countries. Atherosclerosis is a chronic, multifocal, immuno-inflammatory, and fibro-proliferative disorder that results from lipid accumulation in medium and large vessels. Endothelial dysfunction is an important change that is often seen in the pathogenesis of atherosclerosis [1, 2]. Many molecules and cytokines play important roles in endothelial functions. Genetic and environmental factors can affect endothelial functions through different mechanisms. Nitric oxide (NO) is a vasoactive substance synthesized from L-arginine via NO synthase. NO affects vascular small vessel tonus and also plays a protective role in atherogenesis because it causes a decrease in low-density lipoprotein (LDL) oxidation, promotes adhesion of thrombocytes and leukocytes to the endothelial surface, and leads to an increase in migration and proliferation of small vessel cells [3, 4]. Genetic polymorphisms on the endothelial NO synthase (eNOS) gene can cause a decrease in the release of NO and/or a deficiency in NO activity [5, 6]. A thymidine mutation in which replacement by cytosine at the nucleotide 786 (T-786C) gene can lead to a significant reduction in eNOS gene promoter activity and is associated with hypertension (HT), acute coronary syndrome (ACS), and coronary vasospasm [7, 8].

P-glycoprotein (Pgp) functions as an adenosine triphosphatase (ATP)-dependent pump of metabolic residual products, xenobiotics, many drugs, and some inflammatory mediators out of the cell [9]. The Pgp-encoded multidrug resistance gene 1 (MDR-1) 3435C-T polymorphism was shown to be related to repeated cardiovascular events in patients who are being treated with clopidogrel [10]. However, the relationship between MDR-1 polymorphism and atherosclerosis remains unclear.

In this study, the association between MDR-1, e-NOS polymorphisms, and severe coronary artery disease (CAD) was investigated.

## Materials and methods

### Patient population

In total, 90 patients with ACS who were admitted to the hospital and who underwent coronary angiography between November 2014 and April 2015 (mean age: 62 (9) years) were consecutively enrolled in this cross-sectional study. Diagnosis of ACS was defined as unstable angina pectoris (UA), non-ST-segment elevated myocardial infarction (NSTEMI), and/or ST-segment elevated myocardial infarction (STEMI) according to the European Society of Cardiology guidelines [11,12]. Possible biases were avoided by including all consecutive patients who fulfilled the specified diagnostic criteria within the specified time interval.

### Definitions

A complete history, physical examination findings, risk factors for atherosclerotic heart disease, and current medications were recorded. Patients who were treated with antihypertensive drugs or those whose baseline blood pressure exceeded 140/90 mmHg were diagnosed with HT. Diabetes mellitus (DM) was defined as a fasting blood glucose level > 126 mg/dl or the use of anti-diabetic medication. Hyperlipidemia was defined as a total cholesterol level > 200 mg/dl and/or an LDL level > 160 mg/dl. Patients with known atherosclerotic disease, visible coronary artery plaque(s) on coronary angiography, peripheral artery disease, malignancy, renal or hepatic insufficiency, and/or chronic inflammatory disease, and those who were younger than 18 or older than 90 years of age were excluded from the study. All participants agreed to take part in the research, and the approval of the Çanakkale Onsekiz Mart University, Faculty of Medicine Ethics Committee was obtained for this study (09.04.2014/07-04).

### Coronary angiography

Coronary angiography was performed with a femoral approach using Judkins catheters and the contrast agent, Iopramide (Ultravist-370, Bayer Schering Pharma, Germany) along with angiographic equipment (GE Medical Systems, Innova 2100, USA). Two independent cardiologists quantitatively evaluated the severity of coronary atherosclerotic lesions based on at least three projections in all patients. Cohen's kappa was calculated for intra-observer agreement test ($K = 0.94$; $P = 0.001$). Any patients with > 70% lumen stenosis in at least two projections in ≥ 2 vessels were described as having severe CAD (Group 1), while those with this same level of involvement in < 2 vessels were diagnosed with the single-vessel disease (Group 2).

### Laboratory analysis

All routine biochemical tests were carried out with the Cobas 6000 Integra (Roche Diagnostics, IN, USA) auto-analyzer device using the chemiluminescence method. Venous blood was collected in 6-ml ethylenediaminetetraacetic acid (EDTA) tubes for isolation of the genomic DNA and stored at –20 °C. A total of 90 DNA samples were genotyped by real-time polymerase chain reaction (PCR) analysis.

The total genomic DNA was extracted using MagnaPure Compact (Roche) and Invitek kit extraction techniques (Invitek®; Invisorb spin blood, Berlin, Germany). Target genes were amplified by real-time PCR (qPCR), LightCycler 2.0 methods (Roche). LightCycler FastStart DNA Master HybProbes, master mix (water, PCR-grade, $MgCl_2$, stock solution, primer mix, HtbProbe mix), and template DNA from patients were used for real-time amplification for each target gene. MDR 3435C-T and eNOS T-786C were determined by PCR amplification.

The amplification protocol for eNOS T-786C consisted of a denaturation step of 10 min at 95 °C. The amplification conditions for 40 cycles consisted of several steps: (1) denaturation at 95°C for 5 s, (2) annealing at 62 °C for 10 s, (3) extension at 72 °C for 6 s, (4) melting curve step with denaturation at 72 °C for 30 s, (5) annealing at 95 °C for 20 s, (6) melting at 40 °C for 1 s, and (7) cooling step at 40 °C for 30 s.

The amplification protocol for MDR 3435C-T consisted of a denaturation step of 30 minutes at 95°C. The amplification conditions for 45 cycles consisted of several steps: (1) denaturation at 95 °C for 5 s, (2) annealing at 55 °C for 5 seconds, (3) extension at 72 °C for 8 s, (4) melting curve step with denaturation at 95 °C for 30 s, (5) melting at 40 °C for 2 s, (6) 80 °C 0.1 s, and (7) cooling step at 40 °C for 30 s. A software program (LightCycler 2.0, Roche) was used for the

detection of the mutated profiles for target single nucleotide polymorphism (SNP) analysis.

**Statistical analysis**

All statistical studies were carried out with the SPPS program (version 19.0, SPSS, Chicago, Ill., USA). Quantitative variables were expressed as median (minimum–maximum), and qualitative variables were expressed as percentages. All measurements were evaluated with the Kolmogorov–Smirnov test and the Shapiro–Wilk test, and a comparison of parametric and nonparametric values between the two groups was performed with the Student's t- or Mann–Whitney U test. Categorical variables (risk factors and polymorphisms) were analyzed using the $\chi^2$ test. Risk estimations for the association of severe CAD with the polymorphisms were calculated using odds ratio and 95% confidence intervals (CIs) by comparing the genotypic combinations.

## Results

Clinical and laboratory findings for the subjects are given in Table 1. Baseline demographic parameters were similar in both groups, except for the presence of DM and glucose level were higher in group 1. When coronary artery involvement was examined, stenosis rates of > 70% for each vessel, and the number of patients with the three-vessel disease was higher in group 1. Genotype properties and allele frequencies are given in Table 2. The T alleles of MDR were 42% and 40% in groups 1 and 2, respectively (OR = 1.12; *P* = 0.70). The C alleles of eNOS were 34% and 30% in groups 1 and 2, respectively (OR = 1.16; *P* = 0.63). Fourteen and 15 patients (40% and 27% in groups 1 and 2, respectively) had both T and C alleles (OR = 1.77; *P* = 0.21).

Table 1: Baseline demographic parameters of patients

| Clinical characteristics | ≥ 2 vessel disease (n = 35) | < 2 vessel disease (n = 55) | *P*-value |
|---|---|---|---|
| Male/Female | 21/14 | 41/14 | 0.25 |
| Diabetes mellitus, n (%) | 12 (48) | 18 (27.7) | 0.06 |
| Hypertension, n (%) | 18 (72) | 36(55.4) | 0.15 |
| Hyperlipidemia, n (%) | 11 (44) | 18(27.7) | 0.138 |
| Smoking, n (%) | 13 (52) | 38(58.5) | 0.58 |
| Family history, n (%) | 2 (8) | 6 (9.2) | 0.85 |
| Age, mean (SD) | 63 (9) | 61 (8) | 0.17 |
| Glucose, (mg/dl) | 132 (84–280) | 110 (72–263) | 0.012 |
| LDL, (mg/dl) | 109 (53–200) | 110 (45–194) | 0.551 |
| Troponin I, (ng/ml) | 1.0 (0.11–25) | 1.43 (0.1–25) | 0.402 |
| Hemoglobin, (g/dl) | 12.75 (9.3–17.1) | 13.7 (8.3–18) | 0.17 |
| Creatinine, (mg/dl) | 0.9 (0.5–2) | 0.8 (0.5–1.7) | 0.38 |
| eGFR, mean (SD) | 80.8 (25.6) | 89 (26.4) | 0.151 |
| Vessel stenosis ≥70% | | | |
| LAD, n (%) | 10 (28.6) | 15 (25.5) | 0.02 |
| Cx, n (%) | 6 (17.1) | 5 (9.1) | <0.001 |
| RCA, n (%) | 5 (14.3) | 5 (9.1) | <0.001 |
| Gensini score | 94 (30–174) | 52.7 (10–132) | 0.001 |

SD: standard deviation, LDL: Low density lipoprotein, eGFR: Estimated glomerular filtration rate, LAD: Left anterior descending artery, Cx: Circumflex artery, RCA: Right coronary artery.

Table 2: The polymorphic SNPs, genotypic, and allele frequencies of the target MDR-1 and eNOS genes in both groups

| Gene/Genotype | Patients | | *P*-value | OR | CI (95%) |
|---|---|---|---|---|---|
| | ≥ 2 vessel disease (n:35) n/% | < 2 vessel disease (n:55) n/% | | | |
| MDR-1 3435C<T | | | | | |
| C/C | 9/25.7 | 19/34.5 | | | |
| C/T | 22/62.9 | 28/51.0 | | | |
| T/T | 4/11.4 | 8/14.5 | | | |
| Alleles | | | | | |
| C | 40/0.571 | 66/0.600 | | | |
| T | 30/0.429* | 44/0.400 | 0.70 | 1.12 | (0.61–2.06) |
| eNOS T786C | | | | | |
| T/T | 16/45.7 | 28/51.0 | | | |
| T/C | 14/40.0 | 20/36.3 | | | |
| C/C | 5/14.3 | 7/12.7 | | | |
| Alleles | | | | | |
| T | 46/0.657 | 76/0.691 | 0.63 | 1.16 | (0.61–2.02) |
| C | 24/0.343** | 34/0.309 | | | |
| MDR-1 T + eNOS C alleles, patient (%) | 14 (40)*** | 15 (27) | 0.21 | 1.77 | (0.72–4.37) |

OR: odds ratio, CI: confidence interval, *: The T allele for MDR-1 3435C<T; OR = 1.12 (0.61–2.06), *P*=0.70, **: The C allele for eNOS T786C; OR = 1.16(0.61–2.02); *P* = 0.63, ***: The T allele for MDR-1 + C allele for eNOS T786C were significant; OR = 1.77 (0.72–4.37); *P*=0.21

## Discussion

This study showed that coexistence of the eNOS T-786C and MDR-1 3435C-T polymorphisms may be related to the severity of CAD in ACS patients.

Endothelial dysfunction is an important mechanism in atherosclerosis pathogenesis. NO is one of many molecules that regulate endothelial functions. NO has a regulatory effect on vascular tonus; also, reduced activity of NO results in an increase of leukocyte adhesion, vascular small muscle cell migration and proliferation, and platelet aggregation [5–7, 13]. The T786C variation of the eNOS gene is associated with a reduction in gene promoter activity and therefore a decrease in NO levels. Fatini et al. [14] reported that the eNOS T-786 C/C genotype is related to a 9.1-fold higher risk of CAD in patients with homocysteinemia. Tangurek et al. [15] found that the incidence of eNOS T-786C polymorphism was 2.1-fold higher in patients with CAD than it was in controls; this trait was also related to CAD severity. Other studies have suggested that the T-786C CC genotype is an independent risk factor for both the presence and severity of atherosclerosis [13], and this risk is currently increasing in younger patients [16]. In our study, the C allele frequency for eNOS was higher in patients with severe CAD, but the difference was not statistically significant. This non-significant result may have been caused by our study's small sample size.

The MDR-1 (also called ABCB1) gene encodes Pgp, which is responsible for cellular efflux of many medications, inflammatory mediators, and metabolic remnants. Pgp also affects the absorption of drugs along the gastrointestinal tract [10]. Platelet-activating factor (PAF) is a pro-inflammatory molecule that stimulates the release of inflammatory mediators from endothelial and inflammatory cells [17]. A high PAF level or inappropriate PAF transport can cause an increase in inflammatory mediators and endothelial dysfunction [18]. Endothelial dysfunction is one component in the process of atherosclerosis onset [19]. PAF levels were found to be higher in patients with atherosclerosis and acute myocardial infarction than in controls [20–22]. The body's PAF level is regulated by metabolization or transmembrane transport mechanisms that include the Pgp transporter system. Because Pgp is responsible for the efflux of some phospholipid molecules, such as PAF,

PAF levels are related to Pgp expression and activity [23]. It is known that the SNP of MDR-1 can cause an increase in Pgp expression and activity. A few studies evaluating the relationship between CAD and MDR-1 polymorphisms can be found in the literature. Ayaz et al. [24] reported that the PAF levels are not related to MDR-1 polymorphism in both the CAD and control groups. Most MDR-1 studies have investigated antiplatelet resistance in atherosclerotic CAD and acute ischemic events. Spiewak et al. [25] demonstrated that C3435T polymorphism of the MDR-1 gene may influence platelet function as assessed using CADP-CT with PFA-100 in patients with ACS who were treated with percutaneous coronary intervention with stenting. A subgroup analysis of the TRITON-TIMI 38 study reported repeated coronary events in patients who required antiaggregant treatment [10]. To our knowledge, no study evaluating the presence of both eNOS and MDR-1 polymorphisms in ACS patients has been published.

We evaluated two polymorphisms of eNOS and MDR-1 that are associated with endothelial functions. The presence of the C allele in both eNOS and MDR-1 appears to be related to the severity of CAD in patients with ACS.

**Limitations**

Some limitations to this study should be discussed. First, our study population consisted of a small sample size because our funding was limited. The results will most likely be statistically significant if the number of patients is high enough. Second, whether inflammatory and coagulation markers were affected by polymorphisms was not examined. Last, our study population includes ACS patients, but a healthy control group was not included. More studies are needed to evaluate different genetic mechanisms and environmental factors in atherosclerosis pathogenesis.

**Conclusion**

This study showed that MDR1 and e-NOS polymorphisms frequently occur in patients with ≥ 2 vessel diseases and may be associated with severe CAD.

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# Investigation of the association between HLA-G polymorphisms and obesity

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**Ethics Committee Approval**
Ethics approval for the study was obtained from Gaziantep University Ethics Committee, 01.08.2018, 2018/176.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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## Abstract

**Background/Aim:** Obesity is a global public health problem seen worldwide, with an increasing prevalence over time. Obesity is a multifactorial disease affected by both genetic and environmental factors. It is accompanied by many other diseases, the most important of which are immune system disorders. Induction of suppression of HLA-G molecule T and B lymphocytes is associated with natural killer cells and antigen-presenting cells. The HLA-G gene shows functional polymorphisms in regulatory regions. The HLA-G gene is slightly more conserved compared to other HLA genes, both in the same population and among different populations. The aim of this research is to determine the association of HLA-G gene polymorphisms (14 bp insertion/deletion 3 'UTR (rs66554220), rs41557518, and rs1063320) with obesity.
**Methods:** Fifty normal (BMI ≤ 30) individuals having no obesity-related chronic disorder, and 50 obese (BMI ≥ 30) individuals were included in the study. After DNA isolation, PCR and PCR-RFLP methods were used for genotyping.
**Results:** The genotype frequencies of HLA-G polymorphisms (rs66554220, rs41557518, and rs1063320) in the normal and obese groups were compared, and as a result, no significant difference was found ($P > 0.05$).
**Conclusion:** No significant association was found between rs66554220, rs41557518, and rs1063320 polymorphisms and obesity.

**Keywords:** Obesity, HLA-G, Polymorphism

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## Introduction

Obesity presents significant risks to society's health, and its incidence is rising rapidly. The complicated impacts of this disease, including interactions between genetic and environmental susceptibility, are difficult to be treated and prevented [1]. Obesity is the result of chronic excess in energy intake compared to expenditure, resulting in extravagant quantities of triglycerides being stored in adipose tissue [2]. The surplus energy is collected and stored in the fat cells that will be enlarged (hyperplasia) or multiply (hypertrophy), which is obesity's pathological lesion. Enlarged fat cells cause clinical issues due to obesity, either due to the weight or mass of the excess fat or due to increased free fatty acid and peptide secretion from enlarged fat cells. In turn, obesity causes other diseases, like diabetes mellitus (DM), heart diseases, gallbladder disorder, some cancers, and osteoarthritis [3].

Body mass index (BMI) is the most frequently used measure of obesity; it is defined as the weight of an individual in kilograms (kg) divided by the height of the individual in meters squared ($kg/m^2$) [4]. The World Health Organization (WHO) categorizes an obese individual with a BMI $\geq$ 30 $kg/m^2$, and individuals having BMI $\geq$ 40 $kg/m^2$ are defined as extremely obese. The etiology of obesity is multifactorial; there is a complicated interaction between genetics, hormones, and the environment [5]. The genetics of obesity result from structural changes, deletions, or mutations influencing genes that encode proteins engaged in the regulation of appetite and metabolism, and are transferred under X-linked models or autosomal Mendelian traits [6]. Twin studies have shown that genetic inheritance leads to 40–75% of situations of obesity [7].

Obesity is categorized as syndromic, monogenic, or polygenic obesity. Syndromic obesity is seen together with dysmorphic characteristics, hyperphagia, other symptoms of hypothalamic impairment, cognitive delay, and organ-specific defects [8]. Pleiotropic syndromes occur when a single gene affects two or more unconnected phenotypic characteristics; for example, Bardet-Biedl syndrome and chromosomal rearrangements that involve obesity usually involve Prader-Willi, WAGR, and Sim-1 (single-minded gene) syndromes [9]. Significant genes such as the leptin gene (LEP), leptin receptor gene (LEPR), pro-opiomelanocortin gene (POMC), and melanocortin 4 receptor gene (MC4R) are associated with obesity [10]. Obesity is described as an excessive adiposity, with several associated comorbidities, particularly immunity dysfunction. Changes in the function of immune cells and inflammation in obesity play an important part in almost all obesity pathophysiological impacts [11]. Modified circulating concentrations of inflammatory cytokines, for example, tumor necrosis factor alpha (TNF-α), interlukine-6 (IL-6), and C-reactive protein (CRP), were described in both overweight and obesity in adults [12, 13].

The gene region encoding the tissue antigens of the immune system has been defined as the main tissue compatibility component, major histocompatibility complex (MHC), and also named as "human leukocyte antigen" (HLA) genes. HLA genes are members of the HLA class Ib antigens, and are located on chromosome 6p21.3 [14]. HLA-G is expressed in fewer tissues and is less polymorphic than the other molecules in class I. A few researchers have shown that in preeclampsia and recurrent spontaneous abortion cases, decreased mRNA and protein levels were seen compared to normal placentas [15, 16]. HLA-G may have different effects, either helpful in inflammatory and autoimmune diseases, or risky in some cases such as tumors and infectious diseases [17]. HLA-G genes have polymorphic locations, which present at 5' UTR (upstream regulatory region) and 3' UTR (untranslated region) compared to the protein coding region. Polymorphisms show distinct effects in these regions. Gene transcriptions are influenced in 5' UTR while mRNA processing and stability are influenced in the 3' UTR [18]. The HLA-G polymorphic 3'UTR has an essential function in arranging the expression of the HLA-G gene [19]. The 14bp ins/del (5′ ATTTGTTCATGCCT-3′) polymorphism contains a deletion (del) or insertion (ins) of 14 base pairs in the +2960 site in exon 8 [20]. The ins allele is linked with an alternative splicing where 92 bp is deleted, shifting the stability of mRNA and decreasing the concentrations of HLA-G [21]. The 14 bp ins/del (5′ ATTTGTTCATGCCT-3′) polymorphism and soluble concentrations of HLA-G are associated with diverse diseases involving autoimmune disorders, recurrent abortions, certain cancers, as well as inflammatory diseases, along with coronary heart disease (CHD) [22–24].

Obesity is a disease characterized by hormonal and metabolic differences and is associated with more than one genetic, environmental, and metabolic factor. Obesity is accompanied by many diseases, the most important of which are immune system disorders. The aim of this research is to determine the association between HLA-G gene polymorphism and obesity by analyzing the 14 bp insertion/deletion 3' UTR rs66554220, rs41557518, and rs1063320 polymorphisms in obese and healthy subjects.

## Materials and methods

This study, started in September 2018 and completed in September 2019, was confirmed by The Ethics Committee of Gaziantep University on 01.08.2018 with ethical approval number 2018/176, and the study was carried out in accordance with the Declaration of Helsinki. When the post-hoc power analysis was performed, the power = 0.73 was obtained, the effect size (OR) = 0.34, and alpha = 0.05. Fifty obese individuals (BMI $\geq$30 $kg/m^2$) and 50 normal (BMI $\leq$30 $kg/m^2$) individuals participated in the study. The samples were collected in the Department of General Surgery, Faculty of Medicine, SANKO University.

**DNA Isolation**

Blood samples (4 ml) were collected in ethylene diamine tetra acetic acid (EDTA) tubes and stored at -20°C in a refrigerator until DNA isolation. The Thermo Fisher Scientific Pure Link Genomic DNA mini kit was used for isolating whole genomic DNA.

**Amplification and Genotyping of the HLA-G Gene**

The ingredients for the rs66554220, HLA-G 14 bp ins/del polymorphism in the 3'UTR, 1597 ΔC, rs41557518 the cytosine deletion at codon 130 in exon 3, tagging the HLA-G *01:05N null allele, and the rs1063320 (+3142GΔC) polymorphisms of the HLA-G gene were roughly the same (2–

2.5 µl of Buffer, 2 µl of dNTP, 0.3 µl of forward, and 0.3 µl reverse primer) and a total volume of 25 µl. Reaction conditions were 94°C for 5 min, 35 cycles at 94°C for 30 s, 55–58°C for 30 s, 72°C for 30 s, and final extension at 72°C for 5 min.

The rs66554220 polymorphism is genotyped directly after polymerase chain reaction (PCR). The rs41557518 and rs1063320 polymorphisms are genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method; The PPUMI and BaeGI enzymes were used to digest the PCR products. The sample fragments were run in agarose gel electrophoresis, and genotyping was done according to the band sizes. The sizes of the digestion products (bp) and genotypes for rs41557518 and rs1063320 polymorphisms are given in Table 1.

Table 1: Restriction products and genotypes of HLA-G gene polymorphisms

| Polymorphisms | rs66554220 | | rs41557518 | | rs1063320 | |
|---|---|---|---|---|---|---|
| Restriction Products (bp) | del/del | 210 bp | HLA-G *01:05N (+/+) | 504 bp | CC | 406 bp |
| | ıns/ıns | 224 bp | HLA-G *01:05N (-/-) | 389 bp, 115 bp | GG | 316 bp, 90 bp |
| | del/ıns | 210/224 bp | HLA-G *01:05N (+/-) | 504 bp /389 bp / 115 bp | CG | 406 bp /316 bp / 90 bp |

**Statistical analysis**

The allele and genotype frequencies were determined by direct counting. The genotype frequencies of HLA-G polymorphisms in the normal and obese groups were compared by chi-square ($\chi^2$) test, and statistical significance was accepted at the $P<0.05$ level. The SPSS statistical software package version 22.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses.

## Results

The mean age of obese patients was 41.64 (10.62) years. The obese group consisted of 25 (50%) male and 25 (50%) female patients with a mean weight of 114.568 (18.86) kg, mean height of 2.86 (0.258) $m^2$, and mean BMI of 40.1 (6.66). The mean age of the control group was 42.14 (11.27) years. Similar to the obese group, the control group also consisted of 25 (50%) males and 25 (50%) females. The mean weight and height of the control group were 60.46 (8.94) kg and 2.775 (0.26) m, respectively. The mean BMI for the control group was calculated as 21.7 (2.04). As expected, there is a significant difference in the mean weight of the obese group [114.568 (18.86) kg] and that of the control group [60.46 (8.94) kg]. After comparison of the genotype frequencies for rs66554220, rs1063320, and rs41557518 polymorphisms in the groups, no significant difference was found ($P > 0.05$) (Table 2).

Table 2: Genotype distribution for HLA-G gene polymorphisms

| rs66554220 | Control (n=50) n (%) | Obese (n=50) n (%) | $\chi^2$ (*P*-value) |
|---|---|---|---|
| del/del | 16 (32) | 15 (30) | 1.02 (0.60) |
| del/ins | 26 (52) | 23 (46) | |
| ins/ins | 8 (16) | 12 (24) | |
| rs1063320 | | | |
| CC | 7 (14) | 7 (14) | 1.19 (0.55) |
| CG | 27 (54) | 22 (44) | |
| GG | 16 (32) | 21 (42) | |
| rs41557518 | | | |
| HLA-G*01:05N (+\+) | 3 (6) | 2 (4) | 1.08 (0.58) |
| HLA-G*01:05N (+\-) | 27 (54) | 23 (46) | |
| HLA-G*01:05N (-\-) | 20 (40) | 25 (50) | |

## Discussion

Obesity is a globally important issue worldwide, being related to so many chronic diseases such as hypertension, cardiovascular diseases, and type 2 diabetes [25]. In general, obesity depends on the accumulation of fat, insufficient physical activity, defects in the endocrine system and homeostasis, and unconscious food consumption. Another important reason for improving obesity is the genetic background [26]. There are numerous research studies in the literature investigating the relationship between genetic factors and obesity. Obesity is identified as low-level systemic inflammation, especially in some tissues containing adipose tissue and the liver. Extreme fat accumulation in the body may cause changes in the functions and numbers of some cells of the immune system like mast cells, neutrophils, and T and B lymphocytes [27]. The HLA-G gene is a member of a non-classical class I, located in the human major histocompatibility complex, which plays a role in modulating the immune system [28]. Due to the relationship between obesity and the immune system, the present study aimed to investigate for the first time the association of the HLA-G gene polymorphisms (rs66554220, rs41557518, and rs1063320) with obesity.

Study groups consisted of obese and control individuals. PCR/PCR-RFLP methods were used to analyze three HLA-G polymorphisms. Statistically, no significant relation was seen between the analyzed HLA-G gene polymorphisms and obesity. The 14-bp insertion/deletion polymorphism (rs66554220) containing the 3' UTR region is crucial because of the gene expression regulation, RNA stability, and alternative splicing of HLA-G [29].

HLA-G may negatively affect the regulation of the human immune response. The results of a meta-analysis study about the relationship between many cancers and rs66554220 have shown that 14-bp ins/del polymorphism may have a role in cancer risk [30]. Rheumatoid arthritis (RA) is an autoimmune disease. HLA-G 14 bp ins/del and +3142G > C polymorphisms were detected in RA patients in an Iranian population. It is concluded that there was no statistically significant difference between 14 bp ins/del and RA; however, there were significant associations between the +3142G > C variant and a predisposition to RA [31].

Marzuillo et al. [32] studied HLA-G 14 bp in obese children and adolescents. They have reported an association between the ins/ins genotype and the homeostasis model assessment. Many case-control studies have shown inconsistent results that some of the ins alleles were significantly associated with decreased or increased risk, and additionally, some have shown no significant association [33].

Chen et al. [34] studied the relationship between HLA-G rs16375 and rs41557518 polymorphisms and esophageal cancer patients in the Kazakh and Han nationalities in Xinjiang. It is indicated that there was an increased risk of EC in patients with the HLA-G (rs16375) 14bp del genotype (−14bp/−14bp) and the HLA-G rs41557518 0105N genotype (C/C) compared with HLA-G 14bp del genotype (+14bp/+14bp) and the 0105N genotype (C/C) in the Kazakh, but in the Han, there was not.

The HLA-G gene exhibits characteristic properties. HLA class I genes present a large amount of exonic polymorphic

sites in spite of the HLA-G gene having restricted polymorphic sites and casual dispersion across the exons and introns [35].

The HLA-G gene is 4170 bp in size and consists of 8 exons. Four isoforms, HLA-G1 to -G4, are membrane attached, and three isoforms, HLA-G5 to -G7, are soluble. The HLA-G gene isoforms are moderated by alternative splicing of HLA-G mRNA [36, 37].

**Limitations**

Obesity is a complex disease having many causes and effects. HLA-G still may act in the development of obesity through different pathways. It should be considered that there may be differences in genotype distributions between populations.

Therefore, the association of obesity and HLA-G gene is controversial and needs to be examined by further studies with large sample sizes and in different ethnicities.

**Conclusion**

There is no study in the literature about the effects of the HLA-G gene on obesity. The present study aimed to determine the association between obesity and the HLA-G gene polymorphisms (14 bp insertion/deletion 3 'UTR (rs66554220), rs41557518, and rs1063320). We found no statistical association between obesity and rs66554220, rs41557518, and rs1063320 polymorphisms. Obesity is a multifactorial disease related to so many factors such as genetics, diet, and environment, among others, in addition to ethnic differences that should also be considered.

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# A comparison of the features of RT-PCR positive and negative COVID-19 pneumonia patients in the intensive care unit

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**Ethics Committee Approval**
Approval for the study was granted by the Clinical Research Ethics Committee of Afyonkarahisar Health Sciences University (decision no:187, dated:2021). All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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## Abstract

**Background/Aim:** COVID-19 is a serious disease, primarily affecting the respiratory system. The disease spreads from person to person and has become a global health problem of great concern worldwide. The aim of this study was to compare the clinical and laboratory characteristics and the mortality rates of suspected and confirmed COVID-19 cases admitted to the intensive care unit with severe pneumonia.
**Methods:** A retrospective case-control study examination was made of 397 patients diagnosed with suspected or confirmed COVID-19 who were followed up in the intensive care unit (ICU) of Afyonkarahisar Health Sciences University Medical Faculty Hospital between 20 March 2020 and 31 December 2020. The cases were compared in respect of demographic, clinical and laboratory characteristics, prognosis, and mortality rates.
**Results:** 397 patients comprised of 37 (9.3%) with suspected COVID-19 and 360 (90.7%) confirmed COVID-19. No difference was determined between the suspected and confirmed cases in respect of age, gender, and comorbidities ($P < 0.05$). Malignancy was determined in 14 (37.8%) and in 33 (9.2%) of the suspected and confirmed COVID-19 cases, respectively. $PaO_2$ and $PaO_2/FiO_2$ values of the confirmed COVID-19 patients were found to be significantly lower than those of suspected COVID-19 cases ($P = 0.027$ and $P = 0.018$, respectively). No statistically significant difference was determined between the mortality rates of suspected and confirmed COVID-19 patients (59.5% and 56.1%, respectively, $P = 0.731$).
**Conclusion:** When the blood analyses of 397 patients who were hospitalized in ICU with an initial diagnosis of severe COVID-19 pneumonia, regardless of COVID-19 RT-PCR test results, were compared, the LDH and CK values were determined to be significantly high, whereas PaO2 and PaO2/FiO2 values were significantly low. Since the sensitivity of RT-PCR test is low especially in cancer patients, it leads to false negative tests in a significant proportion of patients in acute phase of the disease. Therefore, the majority of patients with COVID-19 are not detected by this test, and clinical symptoms, as well as CT scans, are important to identify patients with COVID-19. Since COVID-19 infection has similar initial symptoms to other pneumonias, it is recommended to study other respiratory viral agents in patients with a negative RT-PCR test.

**Keywords:** COVID-19 pneumonia, Intensive care unit, mortality, RT-PCR

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## Introduction

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing the coronavirus disease 2019 (COVID-19) first emerged in Wuhan, China, in December 2019, and the disease was defined as an infectious disease affecting the human respiratory system [1]. From January 2020, the disease spread rapidly across the globe, was reported as a severe threat to human life, and was declared a global pandemic by the World Health Organisation in March 2020 [2].

SARS-CoV-2 infection can be asymptomatic, cause mild upper respiratory tract infection or severe viral pneumonia that can result in respiratory failure or death [3]. Although the disease is basically characterised by lung inflammation, it may also cause damage to the gastrointestinal system, the liver, and the nervous system [4-5].

In the diagnosis of COVID-19, the reverse-transcriptase polymerase chain reaction (RT-PCR) method is often used, based on the amplification of the virus in nasal and oropharyngeal smears taken from infected individuals. However, there are views that the sensitivity of the RT-PCR test is insufficient in the diagnosis of COVID-19 as some infected patients can have at least one false-negative result [6].

As the PCR test does not provide sufficiently rapid results and sensitivity falls to 50%-70% especially in samples with a low viral load, non-contrast computed tomography (CT) of the lungs is frequently used at the diagnosis stage. In a study in China, CT examination was found to be 88% diagnostic and was positive in 97% of PCR-positive patients [7]. Therefore, clinical, laboratory, and thorax CT findings of the patient should be evaluated together for the diagnosis of the disease [8, 9].

The aim of this study was to compare the clinical and laboratory characteristics and the mortality rates of suspected COVID-19 cases and confirmed RT-PCR COVID-19 cases who were treated in the Intensive Care Unit because of severe pneumonia.

## Materials and methods

This retrospective study was conducted in the COVID-19 Intensive Care Unit of Afyonkarahisar Health Sciences University Medical Faculty Hospital between 20 March 2020 and 31 December 2020. The study was conducted following the Declaration of Helsinki, and patients gave their written consent.

Two groups of patients were included in the study. The first group included patients defined as suspected COVID-19 cases according to the COVID-19 diagnosis, treatment, and follow-up guidelines of the Turkish Republic Ministry of Health, who had severe pneumonia but SARS-CoV-2 could not be determined with the RT-PCR test. The second group was defined as confirmed COVID-19 cases with severe pneumonia and SARS-CoV-2 positivity determined with the RT-PCR test in nasopharyngeal or respiratory samples [10].

All patients were evaluated in respect of demographic data, comorbidities, clinical and laboratory findings, prognosis, and mortality rates. The data were retrieved from the hospital's electronic patient records system. At the end of the study, the evaluated data were compared between the suspected and confirmed COVID-19 patient groups.

### Statistical analysis

Data obtained in the study were analyzed statistically using SPSS vn. 26.0 software (IBM Corpn, Armonk, NY, USA). The patients were separated into two groups as suspected and confirmed COVID-19 cases (according to the RT-PCR test result as positive and negative). In the comparison of categorical variables between the groups, Chi-square test was used. Conformity of continuous variables to normal distribution was assessed visually with histograms and with the analytical method of the Kolmogorov-Smirnov test and the Shapiro-Wilk test. Continuous variables showing normal distribution were stated as mean (standard deviation) values, and those not showing normal distribution, as median and interquartile range values. In the comparison of continuous variables between the groups, the Independent Samples t-test and the Mann Whitney U-test were used as appropriate to the parametric assumptions. A two-tailed *P*-value of < 0.05 was accepted as statistically significant.

## Results

Evaluation was made of a total of 397 patients 37 (9.3%) comprised of COVID-19 cases and 360 (90.7%) confirmed cases. No difference was determined between the suspected and confirmed cases in respect of age, gender and comorbidities (hypertension, diabetes, heart failure, coronary artery disease, asthma, COPD, chronic liver disease, chronic renal failure) ($P < 0.05$). Malignancy was determined in 14 (37.8%) suspected and in 33 (9.2%) confirmed COVID-19 cases. The frequency of malignancy was determined to be statistically significantly higher in suspected cases ($P < 0.001$). The comparison of the comorbidities between groups is shown in Table 1.

Table 1: Comparison of the groups according to gender and comorbidities

| | Possible COVID-19 | Definitive COVID-19 | *P*-value |
|---|---|---|---|
| Female sex (n-%) | 11-29.7 | 114-31.7 | 0.809* |
| Median age (interval) | 69 (31-92) | 68 (27-95) | 0.639** |
| Hypertension (n-%) | 17-45.9 | 168-46.7 | 0.933* |
| Diyabetes mellitus (n-%) | 9-24.3 | 118-32.8 | 0.357* |
| Congestive heart failure (n-%) | 11-29.7 | 94-26.1 | 0.696* |
| Coronary artery disease (n-%) | 5-13.5 | 94-26.1 | 0.111* |
| Asthma- Chronic obstructive lung disease (n-%) | 10-27 | 69-19.2 | 0.279 |
| Liver disease (n-%) | 0 | 7-1.9 | 0.392* |
| Cerebrovascular disease (n-%) | 0 | 15-4.2 | 0.379* |
| Chronic kidney failure (n-%) | 2-5.4 | 30-8.3 | 0.755* |
| Malignancy (n-%) | 14-37.8 | 33-9.2 | < 0.001* |

Fisher'sexact test, ** Mann-Whitney U test

In comparison of two groups mentioned, in respect of initial symptoms and physical examination findings, $PaO_2$ and $PaO_2/FiO_2$ values of the suspected COVID-19 patients were found to be significantly higher than values of confirmed COVID-19 cases ($P = 0.027$ and $P = 0.018$, respectively). No significant difference was determined between the groups in respect of the other findings evaluated. The initial symptoms and physical examination findings of the suspected and confirmed COVID-19 patients are shown in Table 2.

In the comparison of the laboratory values of the patients, D-dimer, procalcitonin and troponin levels were determined to be statistically significantly higher in suspected COVID-19 cases ($P < 0.05$). In the same group, LDH, creatinine kinase, potassium, magnesium, albumin, and fibrinogen levels were found to be significantly lower ($P < 0.05$). The comparison of the laboratory values of the groups is shown in Table 3. Bacterial growth was determined in the blood and tracheal

aspirate cultures of 10 (27%) suspected COVID-19 cases, divided in 6 tracheal aspirate and 4 blood cultures.

Table 1: Comparison of the groups according to clinical symptoms and physical examination findings

| | Possible COVID-19 | Definitive COVID-19 | *P*-value |
|---|---|---|---|
| Fever (n-%) | 6-16.2 | 34-9.4 | 0.244* |
| Shortness of breath (n-%) | 37-100 | 358-99.4 | 1* |
| Dry cough (n-%) | 27-73 | 213-59.2 | 0.114* |
| Fatigue (n-%) | 6-16.2 | 83-23.1 | 0.412* |
| Myalgia (n-%) | 1-2.7 | 26-7.2 | 0.494* |
| Headache (n-%) | 1-2.7 | 19-5.3 | 1* |
| Systolic pressure(mmHg) | 130 (90-145) | 130 (110-210) | 0.881 |
| Diastolic pressure (mmHg) | 78 (50-89) | 74.5 (90-157) | 0.086 |
| Heart rate (/min) | 89 (69-116) | 87 (62-174) | 0.397 |
| Breath rate(/min) | 26 (20-32) | 26 (18-45) | 0.408 |
| $SpO_2$ (%) | 87 (55-93) | 87 (56-98) | 0.451 |
| $PaO_2$ (%) | 64.5 (50-120) | 61.2 (25-120) | 0.027 |
| $PaO_2/FiO_2$ | 120 (80-240) | 110 (58-1120) | 0.018 |
| SOFA | 5 (1-9) | 4 (1-9) | 0.115 |
| GKS | 13 (3-15) | 12 (3-15) | 0.958 |

SOFA: Sequential Organ Failure Assessment, GKS: Glasgow coma scale

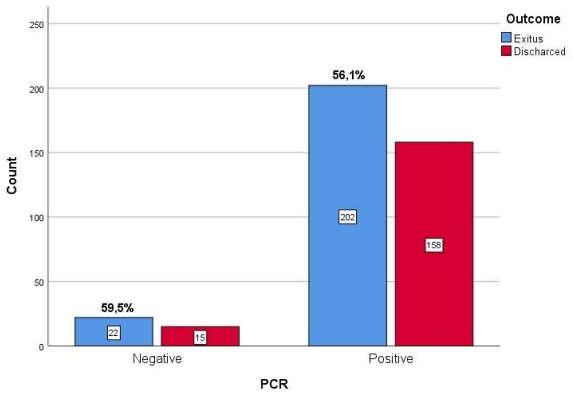
Table 3: Comparisons of the laboratory values of the patients

| | Suspected COVID-19 | Confirmed COVID-19 | *P*-value |
|---|---|---|---|
| WBC ($10^3$/uL) | 10140 (1400-24550) | 9005 (1290-12980) | 0.430* |
| Lymphocyte ($10^3$/uL) | 570 (90-1670) | 690 (74-1310) | 0.116* |
| Hemoglobin (g/dL) | 11.6 (6.6-19.6) | 12.75 (6.4-16.2) | 0.008* |
| Platelet ($10^3$/uL) | 223 (6-611) | 209 (16-883) | 0.702* |
| C-reactive protein, (mg/dL) | 13.78 (10.7) | 13.16 ( 8.9) | 0.877** |
| Procalcitonin (ng/mL) | 3,15 (7.5) | 2.33 (11.9) | 0.026** |
| Lactate (mg/dL) | 16 (7-45) | 18 (6-120) | 0.370* |
| d-Dimer (µg/mL) | 6.59 (14.7) | 3.61 (9.3) | <0.001** |
| Ferritin (ng/mL) | 453 (6-9600) | 733 (28-4021) | 0.093* |
| Lactate dehydrogenase (LDH) (U/L) | 326 (65-1430) | 470 (17-1910) | <0.001* |
| Creatinine kinase (CK) (IU/L) | 68 (32-757) | 106.5 (12-9345) | 0.001* |
| Aspartate aminotransferase (AST) (U/L) | 32 (11-945) | 43 (11-707) | 0.054* |
| Alanine aminotransferase (ALT) (U/L) | 24 (3-489) | 25 (6-321) | 0.136* |
| Gamma glutamyl transpeptidase (GGT) (U/L) | 50 (8-581) | 45.5 (4-718) | 0.459* |
| Alkaline phosphatase (ALP) (U/L) | 86 (36-813) | 71.5 (23-416) | 0.031* |
| Creatinine (mg/dL) | 1.87 (3.1) | 1.39 (1.6) | 0.953** |
| Glomerular filtration rate (eGFR) (mL/dk/1.73 m²) | 68.16 ( 31.7) | 69.44 (28.1) | 0.667** |
| Sodium (mmol/L) | 137.7 (6.6) | 137.2 (7.1) | 0.261** |
| Potassium (mmol/L) | 4.28 (0.7) | 4.51 ( 0.7) | 0.05** |
| Magnesium (mg/dL) | 2.05 ( 0.36) | 2.21 (0.47) | 0.032** |
| Phosphorus (mg/dL) | 3.33 (0.6) | 3.46 (0.8) | 0.107** |
| Albumin (g/dL) | 3.01 ( 0.5) | 3.14 (0.5) | 0.035** |
| Fibrinogen (mg/dL) | 461.2 (155.2) | 523.5 (150.4) | 0.018** |
| Sedimentasyon (mm/h) | 54 (5-103) | 65 (2-124) | 0.097* |
| Troponin (ng/mL) | 0.125( 0.3) | 0.056 (0.2) | 0.001** |

*Mann-Whitney U test, ** t test

When the prognoses of the two groups were evaluated, the mortality rate was determined to be 59.5% in suspected and 56.1% in confirmed cases. The mortality rates were found to be similar ($P$ = 0.731). The mortality rates of two groups are shown in Figure 1.

Figure 1: Mortality rates of the suspected and confirmed COVID-19 patient groups




## Discussion

Although PCR tests are mostly accurate for the diagnosis of SARS-CoV-2 infection, they are suboptimal for upper respiratory tract samples [11]. Previous studies have reported that COVID-19 basically involves the lower respiratory tract and rates of nucleic acid determination with nasopharyngeal smear are low (30%-50%) [12]. Lei et al. [13] stated that infected patients can be overlooked when only nucleic acid tests are used. In some studies that have evaluated antibody response in COVID-19 patients using serological tests, it is stated to be useful for the diagnosis of patients with negative RT-PCR results [14, 15]. Serological tests have been developed using spike (S) and nucleocapsid (N) high antigenic structure proteins as the target. In most people, measured antibodies develop within days or weeks of the onset of symptoms [16], and therefore serological tests are limited to the early stage of infection [17].

Song et al. [18] recommended thorax CT as a tool that can be used for screening suspected COVID-19 patients. In a study of 205 COVID-19 patients by Wang et al. [19], 1070 samples were collected and the rates of positive determination in those samples were as follows; the highest positivity rate was in bronchoalveolar lavage fluid samples (14/15, 93%), followed by sputum (72/104, 72%), nasal smears (5/8, 63%), and pharyngeal smears (126/398, 32%). According to the results from that study, it was stated that more accurate results could be obtained with the use of thorax CT together with RT-PCR testing.

Shah et al. [20] reported that of a total of 316 patients, 31 other respiratory tract viruses were determined in 283 COVID-19-negative patients. In the current study, 9.3% of the cases had a negative RT-PCR test result for COVID-19. As there were not sufficient facilities available for COVID-19 antibody testing or for other respiratory tract viral agents in negative patients, these were not studied as a limitation of the study.

In this study, the confirmed COVID-19 patients who were in the Intensive Care Unit (ICU) with clinical findings of severe COVID-19 infection and thorax CT findings consistent with COVID-19 pneumonia, were compared with suspected COVID-19 patients in respect of age, gender, comorbidities, symptoms, blood tests, and mortality rates. No difference was determined between the groups in respect of age. When comorbidities were evaluated, only the frequency of malignancy was found to be significantly higher in the suspected COVID-19 patients. Assad et al. [21] reported that of 302 cancer patients who presented with suspected COVID-19, 247 (81.8%) were SARS-CoV-2 RT-PCR negative, and 55 (18.2%) were determined as positive. In cancer patients in particular, serological tests have low sensitivity [22]. Therefore, in the vast majority of cancer patients, COVID-19 cannot be determined with serological tests, and it is important to identify COVID-19 patients with both CT scans and clinical symptoms.

The most common symptoms of COVID-19 are fever, dry cough, shortness of breath, and listlessness [23]. The clinical symptoms of both groups in the current study were similar, with the most common symptoms of shortness of breath and cough. As these symptoms can be caused by other respiratory tract viruses, and acute respiratory tract infections are common, it is difficult to bring this pandemic under control.

In patients infected with COVID-19, acute phase reactants show pathological changes with hematological, biochemical, and coagulation tests. Of the hematological changes, lymphopenia, leukocytosis, leukopenia, and mild thrombocytopenia may be seen [24].

Although there was no difference between the current study groups in the lymphocyte values, they were reduced in both groups. This result demonstrates the similarity of COVID-19 to many other respiratory tract infections, triggering of the natural immune response by inflammation and the destruction of lymphocytes after infection [25, 26].

Previous studies have reported that an excessive immune response plays an important role in severe influenza or the pathogenesis of SARS [27]. Elevated CRP, which is an acute phase reactant, may be linked to an excessive immune response [28]. In the current study, elevated CRP levels were determined in both suspected and confirmed COVID-19 patients.

In the suspected COVID-19 cases of the current study, the leukocyte and procalcitonin levels were higher than those of the confirmed COVID-19 cases. The reason for this could be that the potential for bacterial infection could be high in the suspected COVID-19 group. There was determined to be nosocomial bacterial infection in the suspected COVID-19 patients in the current study. Prompt determination of bacterial infections is very important to be able to start antibiotic treatment in time during treatment.

Some studies have reported abnormalities of varying degrees in the liver function tests of COVID-19 patients [29, 30]. In a study that compared the level of markers related to liver functions in patients with and without COVID-19, it was reported that the liver function test levels were significantly higher in the COVID-19 patients, suggesting acute liver damage [31]. In the current study, the liver function test markers (ALT, AST, GGT) were observed to be significantly high in both groups.

LDH is an enzyme that contributes to energy production with the conversion of lactate to pyruvate, and present in almost all cells of the body, with highest in the heart, liver, lungs, muscles, kidneys and blood cells. LDH is a marker of acute and chronic tissue damage, and accepted as an inflammatory marker [32]. In a case series in literature, it was reported that LDH was high in cases with cardiac involvement, those with a severe clinical course, and mortal cases [33]. Shi et al. [34] reported that an elevated LDH level was an independent risk factor for exacerbation in mild COVID-19 patients. Poggiali et al. [35] reported that LDH could be a precursor marker in the evaluation of respiratory function ($PaO_2$ / $FiO_2$) and respiratory failure in COVID-19 patients.

Consistent with findings in literature, LDH and CK values of the confirmed COVID-19 patients in ICU were found to be statistically significantly high. Furthermore, the $PaO_2$ value and $PaO_2$ / $FiO_2$ ratio were found to be statistically significantly lower than those of suspected COVID-19 patients. In a study by Zheng et al. [36], the viral load of patients with severe COVID-19 was significantly higher than patients with mild COVID-19, and it was reported that high viral load could be a risk factor for severe disease.

In a study by Ke et al. [37] that compared patients who were PCR(+) and had clinical symptoms with patients who were diagnosed from clinical symptoms despite a negative PCR test, the BUN and D-dimer levels were reported to be significantly higher in the clinically diagnosed group than in the PCR(+) group, and the hemoglobin level was lower. In the current study, the creatinine level was higher in the suspected COVID-19 group than in the confirmed COVID-19 patients, the D-dimer level was statistically significantly higher and the hemoglobin level was statistically significantly lower. In addition the potassium, magnesium, and albumin values were significantly low and the troponin level was significantly high in the suspected COVID-19 group compared to the confirmed COVID-19 group. These results were thought to be due to the greater number of patients with malignancy, COPD, and CRF in the suspected COVID-19 patient group.

The mortality rates were similar in both groups of this study. However, the higher mortality rate in the suspected COVID-19 group can be explained by the fact that there was a significantly higher number of patients with malignancy in that group.

**Conclusions**

When the blood analyses of the 397 patients hospitalized in ICU with an initial diagnosis of severe COVID-19 pneumonia were compared with positive and negative COVID-19 RT-PCR tests, LDH and CK values were determined to be significantly high and the PaO2, and PaO2/FiO2 values were significantly low in confirmed cases group. Since the sensitivity of RT PCR test is low especially in cancer patients, it leads to false negativity in a significant proportion of patients in the acute phase of the disease. Therefore, the majority of patients with COVID-19 are not detected by this test, and clinical symptoms, as well as CT scans, are important to identify patients with COVID-19. Since COVID-19 infection has similar initial symptoms to other infections, it is recommended to study other respiratory viral agents in patients with a negative RT-PCR test.

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# Effect of warming different intravenous fluids on maternal and neonatal outcomes during cesarean section - comparison of crystalloids and colloids

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**Ethics Committee Approval**
The study protocol was approved by Zekai Tahir Burak Women's Health Training and Research Hospital's Ethical Committee (2011-KAEK-19, Approval code: 155/2017).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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## Abstract

**Background/Aim:** Fluid warming is a useful method to prevent maternal and neonatal hypothermia. Because colloids stay in the intravascular space longer than crystalloids, their protective effect against hypothermia may be more emphasized. The aim of this study is to compare the effects of using warmed colloids or crystalloids on maternal and neonatal core temperatures, neonatal blood gas values, and maternal thermal comfort scores.

**Methods:** After ethical approval, 220 ASA I or II pregnant women, scheduled for cesarean section with spinal anesthesia were enrolled in the study. Patients were assigned to receive hydroxyethyl starch (group HES) or Ringer's lactate solution (group RL) throughout the intraoperative study period. Once the patient entered the room, fluids were actively warmed with fluid warmer to 41 °C in both groups. Measurement of maternal core temperature (MT) and thermal comfort score (TCS) were performed before starting intravenous fluid administration (control), at the time of delivery of the neonate (delivery), 15th, 30th minutes, and at the end of the surgery. Tympanic temperature of the neonates was measured 1 minute after delivery. Blood gas samples from the umbilical artery of the neonates at the 1st minute and Apgar scores at 1st and 5th minutes after delivery were evaluated.

**Results:** Maternal tympanic temperatures, maternal thermal comfort scores were higher at all measurement values other than control measurement in group HES. Neonatal tympanic temperatures ($P$ = 0.051), neonatal umbilical artery cord pH ($P$ < 0.001) and $pO_2$ ($P$ = 0.001) values were higher and $pCO_2$ ($P$ < 0.001) and $HCO_3$ ($P$ < 0.001) values were lower in group HES.

**Conclusion:** Colloids are more effective than crystalloids in terms of maternal and neonatal temperatures and thermal comfort scores. Even if there was no difference between Apgar scores in our study, for neonates with potential vulnerabilities, better umbilical artery cord values may provide clinical advantages.

**Keywords:** Hypothermia, Crystalloids, Colloids, Maternal core temperature, Neonatal core temperature, Apgar score

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## Introduction

Hypothermia occurs in 30-60% of parturients who delivers with cesarean section with neuroaxial anesthesia and maternal hypothermia contributes to hypothermia of the newborn [1, 2]. Neonatal hypothermia is commonly defined as a core temperature below 36.5°C [3]. Hypothermia has an impact on neonatal mortality and morbidity, especially in preterm and low birth weight infants [4].

Neuroaxial anesthesia has been shown to demonstrate a negative correlation between block levels and core temperature by inhibiting vasomotor function which results in peripheral vasodilatation [5].

In the study in which intestinal temperature was measured in 28 patients who underwent spinal anesthesia for elective cesarean section, hypothermia was observed in half of the patients, and baseline values could not be restored in 8 of these patients during the 8-hours follow-up [6].

Infusion of warmed intravenous fluids is used to prevent hypothermia. Use of colloids instead of crystalloids may be more effective in cesarean section operations, since colloids are four times long lasting in the intravascular space [7].

Yokoyama et al. [8] showed that using prewarmed (41°C) intravenous fluids reduced the occurrence of maternal hypothermia during cesarean section. In that study, maternal core temperature, neonatal Apgar scores, and blood gas values have been shown as significantly improved with warmed colloids.

The aim of this study is to compare the effects of using either warmed colloids or warmed crystalloids on maternal and neonatal core temperatures, neonatal blood gas values and maternal thermal comfort scores.

## Materials and methods

The study protocol was approved by Zekai Tahir Burak Women's Health Training and Research Hospital's Ethical Committee (2011-KAEK-19, Approval code: 155/2017) and study was conducted according to the Helsinki Declaration principles.

After Ethics Committee approval, 220 ASA I or II pregnant women, scheduled for cesarean section with spinal anesthesia were enrolled in the study. An informed written consent was taken from the patients before participation. Exclusion criteria were refusal or contraindication for regional anesthesia, age under 18 or over 40 years, body weight over 100 kg and body height below 150 cm, pregnancy with gestational age under 36 weeks and multiple gestations, fetal anomaly, placental invasion anomalies, history of preeclampsia/eclampsia, and abnormal values of fibrinogen, coagulation tests, hemoglobin, hematocrit and platelet.

Using a computer-generated randomization list, patients were assigned to receive hydroxyethyl starch (6% Voluven® 130/0.4, Fresenius, Pharma Austria GmbH, Graz, Austria) (group HES) or Ringer's lactate solution throughout the intraoperative study period (group RL). Patients in the group HES received hydroxyethyl starch 130/0.4 10 mL/kg/h and group RL received Ringer lactate solution 20 mL/kg/h. The volume regimen was based on the presumption that hydroxyethyl starch and Ringer lactate solution show different volume effects. As soon as the patient entered the room, fluids were actively warmed with fluid warmer (en Flow® Controller Model 121, USA) to 41°C. The safety of usage and stability of HES that had been warmed has been shown in previous studies [9].

With patients in the sitting position, spinal anesthesia was performed with 26 gauge atraucan (atrau-com®, Egemen International, Izmir) needle. A standard solution of 12 mg hyperbaric bupivacaine was injected in 30 seconds to cerebrospinal fluid. After the procedure, the patients lied supine with 20° tilt to left to prevent hypotension caused by aorta-caval pressure.

Hypotension was defined as a decrease of 20% or more below baseline mean arterial pressure value and treatment was achieved with ephedrine bolus of 10 mg until the mean arterial pressure returned to normal values. Bradycardia was defined as heart rate < 50 beats/min and treatment was achieved with atropine 0.5 mg.

Measurement of maternal core temperature and thermal comfort score was performed before starting intravenous fluid administration (control), at the time of delivery of the newborn (delivery), 15$^{th}$, 30$^{th}$ minutes and at the end of the surgery. Tympanic temperature of the neonate was measured 1 minute after delivery. A blood gas sample from the umbilical artery was obtained and analyzed by a blood gas analyzer. Apgar scores were evaluated 1 and 5 minutes after delivery by a pediatrician.

**Statistical analysis**

Statistical analyses were performed using SPSS Software (Version 21.0, SPSS Inc., IL, USA. Continuous data were expressed as mean(SD) (range). After determining normal distribution using Kolmogorov-Smirnov test for quantitative data, analysis were performed using either Student's t-test or Mann–Whitney U-test. $P < 0.05$ was considered significant.

The primary outcome was the difference in mean Tc between study groups at the time of delivery of newborn (Tcdel) Secondary outcomes were all Tc measurements, neonatal Tc, neonatal arterial blood pH. The sample size of study was calculated based on a performed pilot study of 20 patients (n = 10 in each group). A clinically significant difference in Tcdel between study groups was 0.2 and groups had a standard deviation (SD) of 0.4 and 0.6. The minimum sample size required to detect a significance difference was calculated as at least 104 in each group, (208 in total), considering type I error (alfa) of 0.05, power (1-beta) of 0.8, effect size of 0.39 and two-sided alternative hypothesis.

## Results

There was no statistically significant difference between the demographic characteristics of patients in group HES and group RL. Duration of anesthesia-induction, duration of surgery and cord-clamping time were not different between two groups. The amount of administered intravenous solutions was 390.0 (107.4) mL for group HES, and 646.6 (255.9) mL for group RL (Table 1).

In neonatal umbilical artery cord pH analysis, the pH ($P < 0.001$) and $pO_2$ ($P = 0.001$) values were higher, and $pCO_2$ ($P < 0.001$) and $HCO_3$ ($P < 0.001$) values were lower in group HES when compared to group RL. The Apgar scores at 1$^{st}$ and 5$^{th}$ minute were not different between two groups. Tympanic

temperature of the neonates at birth was also higher in group HES ($P$ = 0.051) (Table 2).

Table 1: The demographical and surgical characteristics of patients

| | Group HES n = 110 mean(SD) (min-max) | Group RL n = 110 mean(SD) (min-max) | $P$-value |
|---|---|---|---|
| Height (cm) | 161.8(4.3) (157-170) | 161.4(6.8) (150-170) | 0.935 |
| Weight (kg) | 84. (13.2) (67-108) | 80.3(12.7) (60-107) | 0.461 |
| Age (years) | 32.0(4.1) (27-38) | 31.1(4.9) (23-42) | 0.683 |
| Gestational age (weeks) | 38.7(0.3) (38-39) | 38.7(1.1) (36-40) | 0.261 |
| Infused intravenous fluid (ml) | 390.0(107.4) (250-500) | 646.6(255.9) (400-1100) | 0.007* |
| Anesthesia-surgery initiation duration (min) | 7.4(2.6) (5-13) | 7.1(1.8) (5-11) | 0.849 |
| Duration of surgery (min) | 40.1(7.7) (30-55) | 48.2(16.9) (27-87) | 0.216 |
| Cord clamping time (min) | 5.8(1.8) (3-9) | 6.2(3.0) (4-10) | 0.412 |

*$P$ < 0.05

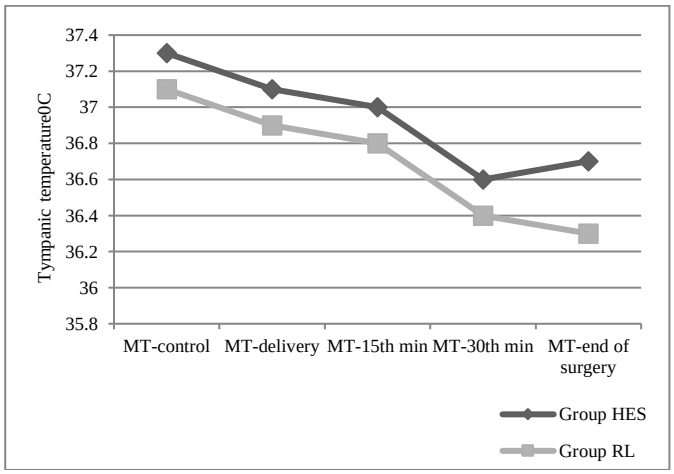
Table 2: Neonatal outcomes

| | Group HES n = 110 mean(SD) (min-max) | Group RL n = 110 mean(SD) (min-max) | $P$-value |
|---|---|---|---|
| Apgar (1st min) | 8.7(0.4) (8-9) | 8.5(0.7) (7-9) | 0.765 |
| Apgar (5th min) | 9.9(0.3) (9-10) | 9.8(0.3) (9-10) | 0.892 |
| Tympanic temperature (ºC) | 37.9(0.3) (37.5-38.5) | 37.7(0.9) (34.6-39.1) | 0.051* |
| pH | 7.34(0.04) (7.26-7.41) | 7.32(0.04) (7.22-7.38) | < 0.001* |
| $pO_2$ (mmHg) | 24.2(6.9) (16.4-35.8) | 20.3(4.8) (12.4-30.6) | 0.001* |
| $pCO_2$(mmHg) | 40.5(6.7) (33.6-52.4) | 43.6(7.2) (31.1-56.9) | < 0.001* |
| $HCO_3$ (mmol/L) | 21.4(2.7) (18.2-27.9) | 22.1(2.6) (15.4-26.3) | < 0.001* |
| BE (mmol/L) | -3.25(2.8) (-6.6- -2) | -3.45(2.3) (-9.5- -1) | 0.865 |

*$P$ < 0.05

Maternal core temperatures at delivery ($P$ = 0.017), at 15th minute ($P$ < 0.001), at 30th minute ($P$ = 0.002) and at the end of the surgery ($P$ < 0.001) were higher in group HES when compared to group RL (Table 3) (Figure 1).

Figure 1: Maternal core temperatures (MT)




When the groups were compared in terms of thermal comfort score, TCS-delivery ($P$ < 0.001), TCS -15th minute ($P$ = 0.004), TCS-30th ($P$ = 0.001) and TCS- end of surgery ($P$ = 0.017) were higher in group HES (Table 3) (Figure 2).

There was no difference between the groups in terms of maternal hemodynamic variables and ephedrine use (Table 4).

Figure 2: Maternal thermal comfort scores (TCS)

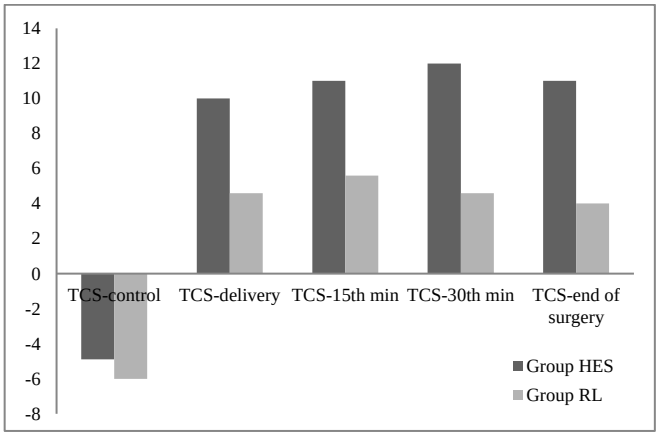



Table 3: Maternal tympanic temperature and thermal comfort score

| | Group HES n = 110 mean(SD) (min-max) | Group RL n = 110 mean(SD) (min-max) | $P$-value |
|---|---|---|---|
| Maternal temperature (ºC) -control | 37.3(0.4) (37.6-37.9) | 37.1(0.3) (36.3-37.4) | 0.230 |
| Maternal temperature (ºC) -delivery | 37.1(0.6) (36.3-38.1) | 36.9(0.4) (36.1-37.5) | 0.017* |
| Maternal temperature (ºC) -15th minute | 37.0(0.6) (35.9-37.8) | 36.8(0.3) (36.1-37.5) | < 0.001* |
| Maternal temperature (ºC) -30th minute | 36.6(0.6) (35.7-37.6) | 36.4(0.4) (35.7-37.6) | 0.002* |
| Maternal temperature (ºC) -end of surgery | 36.7(0.8) (35.5-37.7) | 36.3(0.5) (35.6-37.3) | < 0.001* |
| TCS-control | -4.9(15) (-20-20) | -6.0(9.8) (-20-10) | 0.857 |
| TCS-delivery | 10(14) (0-40) | 4.6(1.7) (-10-40) | < 0.001* |
| TCS- 15th minute | 11(15.2) (0-40) | 5.6(12.9) (-10-40) | 0.004* |
| TCS-30th minute | 12.0(16.8) (0-40) | 4.6(11.8) (-10-40) | 0.001* |
| TCS- end of surgery | 11.0(17.9) (-10-40) | 4.0(11.2) (-10-40) | 0.017* |

*$P$ < 0.05, TCS: Thermal comfort score

Table 4: Maternal hemodynamic variables and ephedrine use

| | Group HES n = 110 mean(SD) (min-max) | Group RL n = 110 mean(SD) (min-max) | $P$-value |
|---|---|---|---|
| MAP (mmHg) -control | 94.5(9.3) (81-106) | 98.4(8.8) (73-108) | 0.311 |
| HR (beat/min) -control | 90.4(12.1) (75-111) | 99.2(15.5) (78-137) | 0.103 |
| MAP (mmHg) -start of surgery | 67.5(12.0) (56-97) | 74.2(14.1) (43-98) | 0.08 |
| HR (beat/min) -start of surgery | 105.2(22.5) (86-162) | 93.6(21.9) (64-129) | 0.397 |
| MAP (mmHg) -delivery | 79.4(14.5) (60-102) | 78.6(11.2) (57-97) | 0.987 |
| HR (beat/min) -delivery | 97.3(25.3) (76-162) | 94.8(16.5) (65-132) | 0.723 |
| Amount of ephedrine used (mg) | 10.6(4.1) (5-20) | 12.5(7.0) (10-30) | 0.057 |

*$P$ < 0.05, MAP: Mean arterial pressure, HR: Heart rate

## Discussion

Pregnant women undergoing cesarean section are susceptible to hypothermia. There are many reasons causing heat loss during cesarean section under neuroaxial anesthesia. Vasodilation below sensory block causes heat loss, due to decreased core-periphery temperature gradient and redistribution of the blood [10, 11]. Neuraxial anesthesia also results in reduction of thermoregulatory vasoconstriction and shivering thresholds above the level of the block by approximately 0.5°C [12].

Sultan et al. [13] reported improved umbilical artery cord pH by active warming in a meta-analysis yet the rest of the neonatal outcomes were remained unchanged. Maternal temperature decrease was smaller in this warmed group. During cesarean delivery, patients may receive liters of intravenous fluids intra-operatively to minimize spinal hypotension. Fluid warming may be effective in the cesarean delivery to reduce any

decrease in core temperature and reduce the degree of heat loss from core to periphery.

When colloids are administered, the most of the solution is expected to remain in intra vascular space, and heat distribution may have occurred in a limited area such as the heart, trunk, and vascular space. In last trimester, 85% of the heat produced by the fetus exits by the placenta [14]. Therefore, we hypothesized that using warmed intravenous colloid solutions instead of crystalloid solutions may enhance the fetus to receive warm blood supply through the placenta. The oxyhemoglobin dissociation curve shifts to the right with warmed blood [15]. Thus neonates in the warmed colloid group may have received higher oxygen supply before birth.

In another study using warmed colloids, Apgar scores at one minute and umbilical artery pH were reported significantly higher in the warmed colloid group than the unwarmed colloid group [8].

The main finding of this study is that maternal tympanic temperature, maternal thermal comfort score and the neonatal tympanic temperature, pH, $pO_2$ values were higher and $pCO_2$ and $HCO_3$ values were lower in group HES when compared to group RL. There was no difference between Apgar scores.

Although neonatal umbilical artery cord pH and tympanic temperature of the neonates at birth were significantly higher in group HES than group RL, the difference between measurements was not very high. Still, these small differences may be clinically useful for neonates with potential neonatal vulnerabilities such as prematurity, cardiac abnormalities, low birth weight and fetal distress, and may alter Apgar scores.

As seen in the literature, our study of comparing the effects of using either warmed colloids or warmed crystalloids on maternal temperatures and maternal thermal comfort scores and neonatal outcomes was the first study that planned in this way.

Limitations of the study include not measuring the time between patient's entrance to the room and the onset of anesthesia. However, no difference was found between other measured durations. Therefore, there is a need for further studies to clarify the effect of the timing of the warming.

**Conclusion**

Choosing the warmed fluid given to pregnant women during cesarean section as colloids is more effective against hypothermia by providing superiority to crystalloids in terms of maternal and neonatal temperatures and thermal comfort scores. Although there is no difference in terms of Apgar scores in this study, we think that the difference in neonatal umbilical artery cord pH in favor of group HES may have a positive effect on neonates with risk factors.

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# Outcomes after eversion of sac and subtotal excision of sac in cases of primary hydrocele

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**Ethics Committee Approval**
This study was approved by the Research Ethics Committee of AFMC affiliated to MUHS India (Number-13, May 2016).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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No conflict of interest was declared by the authors.

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## Abstract

**Background/Aim:** Hydrocele is a common disease worldwide, and an effective treatment for it is surgery. The commonly performed procedures for its treatment are a) excisional technique — subtotal excision of the sac and b) Jaboulay's procedure — eversion of the hydrocele sac. This study aimed to evaluate the merits and demerits of eversion of the sac versus subtotal excision in terms of complications, postoperative hospital stays, and recurrence rate.

**Methods:** This prospective study was conducted at a large surgical center. Patients who presented with scrotal swelling and were diagnosed as primary hydrocele cases constituted the study population (100 in all). Subtotal excisions or eversions of the sac were performed depending on the type of hydrocele encountered preoperatively. Follow-up was done at 1week, 1 month, 3 months, and 6 months postoperatively.

**Results:** Eversion of the sac was done in 70 cases, and subtotal excision was done in 30 cases. Incidence of edema, intense pain, and hematoma formation was higher in the group undergoing excision of the sac compared to those undergoing eversion of the sac. The average postoperative hospital stay was 3–5 days in the eversion group. The average postoperative hospital stay was 7–9 days in the subtotal excision group. No recurrence was noted in either of the study groups during the stays study period.

**Conclusion:** Eversion of the sac was associated with fewer postoperative complications, minimal tissue handling, and good hemostatic control compared to subtotal excision of the sac. Patients who underwent eversion of the sac received earlier discharge than those undergoing a subtotal excision of the sac for primary vaginal hydrocele.

**Keywords:** Hydrocele, Eversion of sac, Subtotal excision of sac

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## Introduction

Hydrocele is one of the most common diseases worldwide. Since the olden days, surgical procedures have been applied to treat hydrocele. The surgical procedure commonly used for the treatment of hydrocele is a radical operation in which the parietal layer of the tunica vaginalis is completely removed, and its cut edges are sutured posteriorly [1].

Vaginal hydrocele is the most common morbidity in men due to Wuchereria bancrofti infection. Diagnosis is straightforward on clinical examination most of the time, but when in doubt, ultrasound is a useful tool to differentiate these swellings from other causes of swelling in the scrotum. The only effective treatment for hydrocele is surgery, i.e., hydrocelectomy (subtotal excision of the sac) [2]. Two of the commonly performed surgeries are:

*Excisional Method (Hydrocelectomy):* If the sac wall (tunica) is thick or the hydrocele is multi-loculated or very large, the excisional method is preferred. This reduces the chances of recurrence and avoids strangulation of the cord. However, although it seems to result in the lowest recurrence rate, the procedure has the highest complication rate [3]. Great care must be taken to stop bleeding after subtotal excision of the wall (hydrocelectomy) because hemorrhage from the cut edge can cause a large scrotal hematoma even if the wound is drained. In excision of the sac (hydrocelectomy), the whole of the sac is excised, and the remaining edge is sutured by continuous catgut or electro-cauterized to prevent hemorrhage. This procedure is preferred in a hydrocele with thickened sac, as in filariasis [4].

*Eversion of the sac (Jaboulay's procedure)*—This is most useful for recent-onset hydrocele with thin sac tissue, in which placement of the testis in a pouch is an alternative carrying a lesser risk of hemorrhage [5]. The sac is opened, everted, and the edges sutured behind the testis. This procedure is associated with a reduced risk of recurrence but may have an increased risk of hematoma [6].

Minimal access hydrocelectomy is done through a 2 cm incision, and the outcome in terms of morbidity reduction and recurrence rate has been found to be satisfactory. This procedure requires minor dissection and minimal manipulation during treatment. It also has been found to result in virtually no recurrence and minimal complications and requires a short operative time [7].

Various options and benefits are available for tackling primary hydrocele; this study was designed to evaluate the merits and demerits of eversion of the sac versus subtotal excision of the sac, with the objective of comparing the complication rate, hospital stay and recurrence rate.

## Materials and methods

**Type of study:** This was a hospital-based prospective observational study of 100 patients who presented with scrotal swellings at a large surgical center. Informed consent was obtained from all patients before the study, and the steps of both operative procedures were explained. The total duration of the study was two years.

**Inclusion criteria:** This study included all patients with hydrocele, scrotal swelling incorporating the testis, who tested positive for trans-illumination, and where it was possible to get above the swelling at the base of the scrotum.

**Exclusion criteria:** Those hydrocele cases that had an accompanying hernia or varicocele were excluded. The provisional diagnosis made in all cases was hydrocele of tunica vaginalis–primary variety (idiopathic).

**Preoperative workup:** The diagnosis was confirmed by scrotal ultrasonography after the scrotal examination. Preoperative laboratory investigations (complete blood counts, blood sugar, viral markers) were done and were within normal limits. In doubtful cases, filarial etiology was ruled out by repeated night peripheral blood smear for microfilaria. A preoperative anesthesia checkup was done; after receiving fitness from the anesthesiologist, informed consent for surgery was also obtained.

The indications of hydrocele surgery were as follows: a) Medically ineligible for employment due to untreated hydrocele; b) Interference with work; c) Interference with sexual function; d) Interference with micturition due to the penis getting buried in the scrotal sac; e) Negative impact on the patient's family; f) Dragging pain; g) Liability to trauma given the nature of the patient's work or mode of transport such as cycling; h) Possible effect on the testis of long-standing hydroceles. All patients were subjected to operative treatment under spinal anesthesia. Surgical procedure depended upon what type of hydrocele was encountered preoperatively. Postoperatively, cases were kept in the wards for at least 24 hours. Cases were followed up at 1 week, 1 month, 3 months, and 6 months.

**Statistical analysis**

Data were entered into a Microsoft Excel sheet for analysis. Continuous variables were presented as categorical data, as numbers, percentages, pie charts, and bar diagrams, and Fisher's exact test was used. This study was approved by the Research Ethics Committee of the Armed Forces Medical College (AFMC), affiliated with the Maharashtra University of Health Sciences (MUHS) India (Number-13, May 2016.

## Results

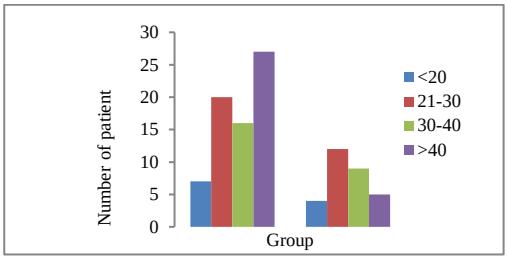
A total of 100 patients who satisfied the inclusion criteria were included in the study. Most of them were from socioeconomically depressed classes. In terms of occupation, the maximum number were manual laborers, followed by farmers and soldiers.

**Age:** The patients' age varied from 12 to 75 years. The mean (SD) was 39.51 (17) years in the eversion of sac group and 30.07 (7.9) years in the excision of sac group. The maximum incidence was in the age groups between 21 and 30 years and over 40 years, as presented in Table 1 and Figure 1.

Table 1: Age group wise distribution

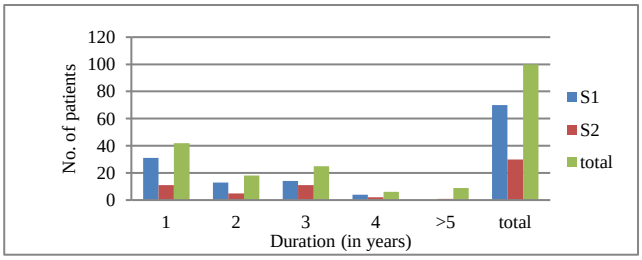
| Age group (Years) | S1(Eversion of sac) | S2(Subtotal excision of sac) | Total |
|---|---|---|---|
| ≤ 20 | 7(10%) | 4(13.3%) | 11 |
| 21 – 30 | 20(28.5%) | 12(40%) | 32 |
| 31 – 40 | 16(22.8%) | 9(30%) | 25 |
| > 40 | 27(38.5%) | 5(16.6%) | 32 |
| Total | 70 | 30 | 100 |

Figure 1: Age group wise distribution




**Presenting complaints:** All patients complained of scrotal swelling, with gradual onset and slowly increasing in size, which started from the bottom of the scrotum. Only 53 patients complained of mechanical discomfort due to swelling. There was no history suggestive of filariasis, tuberculosis, or syphilis in any patient, whereas 22 patients had a history of mild trauma. The duration of the swelling was less than 1 year in 46 cases (46%), followed by 3 years in 16 cases (16%), 2 years in 15 cases (15%), and 33 cases with a duration of 4 years and above. The majority of the patients presented within 1 year of onset of symptoms (Figure 2).

Figure 2: Duration of scrotal swelling



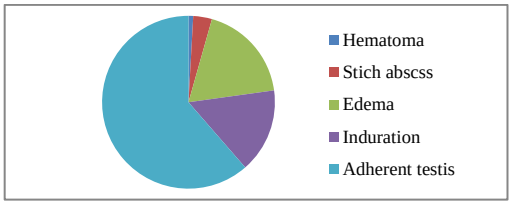

**Physical examination:** Eighty-eight patients had unilateral scrotal swelling (right side in 59% of cases and left side in 29%). Twelve patients had bilateral scrotal swellings. The size of the swelling varied from 7 x 4 cm to 13 x 8 cm. It was purely scrotal in all cases, either oval or pyriform in shape, and it was possible to get above the swelling. Three hydroceles had a protruding appearance. The surface was smooth, except in eight cases that had a constricting ring in the center. The skin was normal in all cases except for the loss of scrotal rugosities. There was no associated hernia. The penis was deviated to the opposite side in eighteen cases. The hydrocele was non-tender. In 30 cases, the swelling was tense and cystic, whereas all other cases were uniformly fluctuant. Testis could not be felt separately in any of the patients. Epididymis could be felt separately in a few cases. Trans-illumination was positive in all cases. There was no significant regional lymphadenopathy. All hydroceles were of the tunica vaginalis type, and the sac was unilocular and thin in all except 18 cases where it was slightly thickened. Testis and epididymis were normal. Small hydroceles were treated by drugs and in cases of moderate-sized hydroceles partial excision and eversion of the sac was performed.

Eversion of the sac was done in 70 cases, where the patients had a small swelling and a thin sac. The procedure was simple, and the operation could be performed rapidly. No tissue dissection was performed, no reactionary edema occurred, bleeding was minimal, and recurrence was unlikely as the tunica cannot surround the testis again. No drainage tube was inserted in these cases. There was mild hematoma in one case, which subsided completely within two weeks' time. Four patients developed stitch abscesses, which were controlled with antibiotics. None of them developed scrotal abscesses. Mild induration of the wound was present in 18 cases. Two of these cases resulted after stitch abscess formation. Induration subsided completely when they were followed up at the end of 4 weeks. At the time of discharge, the testis were adherent to the anterior wall of the scrotum in all cases. The size of the testis was slightly bigger than normal in 12 cases due to a pre-existing thick sac, as presented in Table 2 and Figure 3. The average postoperative hospital stay was 3.5 days. There have been no recurrences so far.

Table 2: Complications of eversion of sac

| Complications | No of cases | Percentage |
|---|---|---|
| Hematoma | 1 | 1.4 |
| Stitch abscess | 4 | 20 |
| Edema | 21 | 30 |
| Induration of scrotum | 18 | 25.7 |
| Adherent testis | 70 | 100 |

Figure 3: Complication in eversion of sac

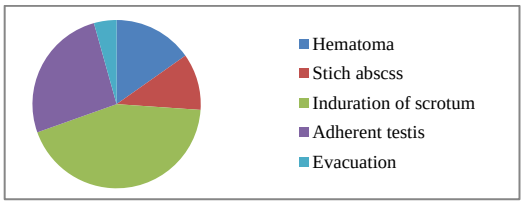



Subtotal excision of the sac was done for moderate and large-sized hydroceles. The sac was partially excised and everted, and sutured with continuous Monocryl™ sutures. Dependent drainage was maintained in all cases. Of the total 30 cases, seven developed a hematoma. Five of them were mild and subsided within a few days. Other patients were asymptomatic at the end of four weeks. Two patients required a surgical evacuation of the hematoma. Five patients developed stitch abscesses, and two developed minimal gaping of wounds. None developed a scrotal abscess. Induration of the wound was present in 20 cases. All patients had mild pain for approximately two days. The testis was adherent in 12 cases. Patients who complained of postoperative pain for a longer duration are presented in Table 3 and shown in Figure 4. The average postoperative hospital stay was 7.2 days. There have been no recurrences during the study period.

Table 3: Complications of subtotal excision of sac

| Complications | No of cases | Percentage |
|---|---|---|
| Hematoma | 7 | 23.3 |
| Stitch abscess | 5 | 16.6 |
| Induration of scrotum | 20 | 66.6 |
| Adherent testis | 12 | 40.0 |
| Evacuation | 2 | 6.66 |

Figure 4: Complication of subtotal excision of sac




**Comparison of the eversion of sac (S1) and excision of sac (S2) groups:** The wound complications occurring in S1 and S2 were as follows: a) Edema. Of the 100 patients, 49 in the sac eversion group had no edema, whereas four patients in the sac excision group and 21 in the sac eversion group had mild edemas. Thirteen patients in the sac excision group and one patient in the sac eversion group had severe edemas (Figure 5). It is statistically significant using Fisher's exact test ($P < 0.001$). b) Hematoma: Of the 100 patients, no hematomas developed in 69 patients of the sac eversion group and 23 patients in the sac

excision group. One patient (1.4%) developed a hematoma after eversion of the sac and seven patients (23.3%) following excision of the sac (Figure 6). It is statistically significant using Fisher's exact test ($P$ = 0.001). c) Pain was another complication: Among the 100 patients, mild pain was experienced by 52 patients after eversion of the sacand 18 patients after excision of the sac; furthermore, moderate pain was experienced by 18 patients following eversion of the sac and by seven patients after excision of the sac; five patients had severe pain (Figure 7). It is statistically significant using Fisher's exact test ($P$ < 0.001). Complications and the average hospital stay in eversion of the sac (S1) and for subtotal excision of the sac (S2) are presented in Table 4.

Figure 5: Wound edema in S1 and S2 group

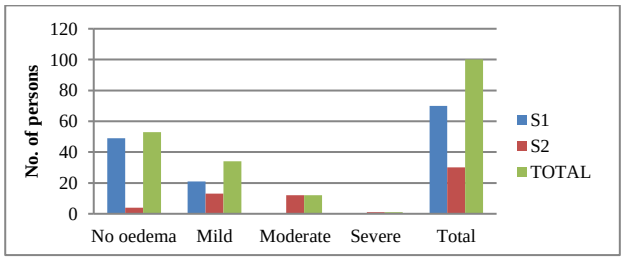



Figure 6: Hematoma formation in S1 and S2 group

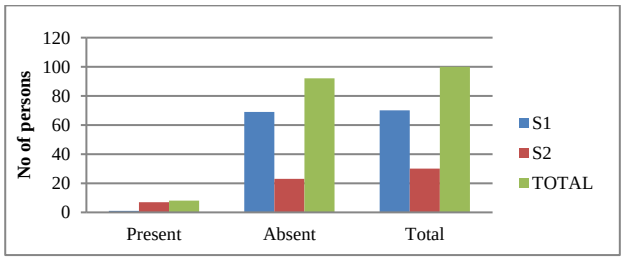



Figure 7: Intensity of pain in S1 and S2 group

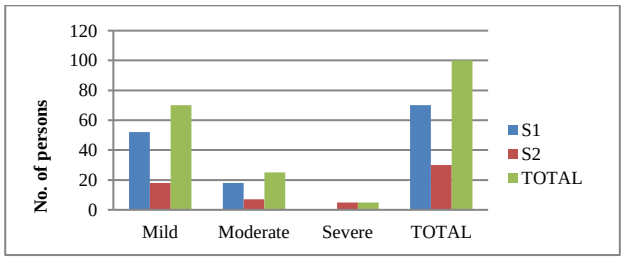



Table 4: Complications and average hospital stay in S1 and S2

| | Eversion of sac (n = 70) | Subtotal excision of sac (n = 30) |
|---|---|---|
| Hematoma | 1.4% | 23.3% |
| Stitch abscess | 11.6% | 16.6% |
| Induration of scrotal wound | 25.7% | 66.6% |
| Adherent testis | 100.0% | 40.0% |
| Average post-operative hospital stay (days) | 3.5 days | 7.2 days |

## Discussion

Radical operative treatment for vaginal hydrocele has been practiced since the time of Volkmann (1876). The standard treatment for hydrocele is surgical and is widely accepted. The disadvantage of surgical treatment is a high incidence of complications, such as hematoma and infection, although these complications can be minimized with careful surgery. The main postoperative complication appears to be the formation of scrotal hematoma, which predisposes to other complications such as infections and scrotal abscesses, among others. Hydrocele affects all age groups; the age group maximally affected in the present series is between 20 and 40 years (57%). In the study by Undre et al. [8], 66.1% of patients belonged to this age group, and 26.7% of patients had bilateral scrotal swelling, whereas, in 38.1%, the swelling was on the left side. In Jachhowski's study [9], bilateral scrotal swellings were present in 41.2% of patients, with 35.3% of cases having swellings on the left side. In the present study, 88% of cases had unilateral swelling, and 12% had bilateral swelling. General examination revealed no abnormalities. On clinical examination, most of the swellings were oval in shape or globular. Swellings of the scrotum were more common on the right side (59%) than the left side (29%), which is comparable to the study reports of Agbakwuru et al. [10]. Bilateral swellings were found in 12% of cases, and the scrotal examination revealed a vaginal hydrocele. In most cases, scrotal rugosities were lost in the hydrocele. It was possible to get above the swelling. Fluctuation and trans-illumination were positive in all cases. The preoperative blood examination revealed neither leukocytosis nor high eosinophil count. Erythrocyte sedimentation rate was within normal limits. The routine urine examination was also within normal limits. The diagnosis was confirmed by scrotal ultrasonography after scrotal examinations.

All cases were subjected to operative treatment under spinal anesthesia. The sac was thin in most cases, thick in some cases, and there was no underlying demonstrable pathology in the testis, epididymis, or spermatic cord. The fluid was clear and amber-colored, with 12 patients having more than 200 ml of fluid. A corrugated rubber drainage tube was used in cases treated by subtotal excision of the sac, which was removed after 48 hours. All patients had mild to moderate severity of pain postoperatively for 24–48 hours. The pain lasted longer in patients who had some complaints such as hematoma and infection, and nine patients (9%) developed a fever postoperatively. However, five patients (5%) had a fever for only one day, which was considered a reactionary fever. In the four patients whose fever lasted more than 48 hours, a local examination of the wound revealed induration, hematoma, and stitch abscess in these cases. None developed a scrotal abscess. A pus culture revealed staphylococcus aureus coagulase positivity, with three cases resistant to the drugs amoxicillin and clavulanate potassium combination and one case to streptococcus. Patients were discharged on the third or fourth postoperative day, and sutures were removed between the sixth and seventh postoperative day in the outpatient department.

At the time of discharge, in all patients except those who had hematomas, the size of the scrotum on the operated side had reduced considerably. Those who developed hematomas (8%) were kept in the hospital until the disappearance of pain and fever and the control of infection. They were followed up at intervals of 15 days. In all these cases, the swelling disappeared by the end of 6 weeks. The eversion of the sac was performed for small-sized hydroceles [11]. Hydroceles 6 cm x 9 cm size or less were operated by this method. This method is simple and can be performed rapidly, avoiding mobilization of the sac from the surrounding scrotal layers, and thus the postoperative hematoma is eliminated. The only difficulty we encountered during the operation was the replacement of the testis into the scrotum, in a few cases. This was easily overcome by extending the skin incision slightly. No drainage tube was used in these cases. This is an added advantage in preventing infection. Recurrence is also

unlikely as the tunica cannot surround the testis. On the other hand, Reddy and Srinivas [12] separated tissues from the sac for a distance of 1 cm around, to facilitate the easy return of the testis into the scrotum. Of the 100 patients, 70 were treated by eversion of the sac, and one of them (1.4%) developed a mild hematoma postoperatively. This may have been due to the blood going in from the cut edges of the skin or from accidental injury to the vessels by the needle while plicating the sac; the hematoma subsided completely at the end of 2 weeks. None required evacuation. Only four patients developed an infection in the form of a stitch abscess. None developed a scrotal abscess. Mild induration of the scrotal wound was evident in 18 cases. At the time of discharge, the testis was adherent to the anterior wall of the scrotum in all cases. This adherence really hinders the protective mechanism of the testis to trauma. However, so far, no cases have been reported with any complaints. In six cases that had slightly thicker and bigger sacs intraoperatively, the size of the testis was bigger than its fellow counterpart. Lord [13], in his original work, treated 22 cases by this method. The incidence of hematoma, infection, and recurrence was nil. Efron et al. [14], in his series of 29 cases, had the same results, except for one patient who had a large testicular mass at the time of discharge due to plication of the sac; preoperatively, this patient had chronic epididymitis and a thick-walled hydrocele. In the present study, we noticed similar findings in six cases. The diameter of the testis did not regress during the three months of follow-up. Reddy's study [12] of 675 cases where Lord's technique was employed led to the conclusion that the complications such as hematoma and infection were negligible, although three cases had a recurrence. There has been no recurrence in the present study. The average postoperative hospital stay was 3 to 5 days. The civilian cases were discharged home, and the soldiers were discharged to their respective units with the recommendation of light duties for 15 days. From the third week onwards, the soldiers were able to resume their normal duties without any discomfort.

The subtotal excision of the sac is also a simple technique. However, complications like hematomas are very common due to the separation of the sac from the surrounding tissues despite meticulous hemostasis and the use of a dependent corrugated rubber drainage tube. This is due to oozing from the small blood vessels during the separation of the sac. Oozing may continue into the layers of loose scrotal tissue, giving rise to a hematoma. The use of monopolar diathermy is relatively contraindicated, as there is a theoretical risk of damage to the testicular artery because the entire current has to pass between the body and the scrotum through a narrow isthmus consisting of the neck of the scrotum and the spermatic cord on each side [13]. After the subtotal excision of the sac, 23.3% of cases developed a mild to moderate-size hematoma, whereas in the study by Efron [14], 30% of cases had developed a hematoma. Two patients required evacuation; moderate-sized hematoma took nearly a month to regress completely. Associated pain and weight of the scrotum added more to the discomfort. The other complications, such as stitch abscess and induration, were also proportionately high when compared with the eversion of sac technique, although no patient developed a scrotal abscess. The incidence of hematomas was high in larger-sized hydrocele (12%) containing more than 200 ml of hydrocele fluid. These patients usually stayed more than 7 days in the hospital postoperatively. The incidence of stitch abscesses was also high in large-sized hydroceles. The average postoperative hospital stay was 4.6 days, whereas, in the series by Efron et al. [14], the average hospital stay was 14 days. In that study, patients took at least 15 days to resume their normal duties, and this delay was mainly due to pain and swelling of the scrotum. In the present study, the hospital stay after eversion of the sac was slightly lower than in the other studies. In other studies, patients went home on the sixth or seventh postoperative day, whereas in the present study, most of the patients went home on the third or fifth postoperative day.

**Limitations**

The most important limitation of this study is the small sample size of patients undergoing subtotal excision of the sac compared to those undergoing eversion of the sac, as it has an inherent selection bias for the surgical patient. Even so, the current study presents a unique evaluation of outcomes after eversion of the sac and subtotal excision of the sac in cases of primary hydrocele.

**Conclusion**

A radical operation for hydrocele of the tunica vaginalis constitutes a fair percentage of operations carried out in any general hospital in India. Operation on hydrocele is regarded as a minor operation and is usually done by junior doctors, the common complication being hematoma, which increases the postoperative morbidity. Hence a truly effective operation should be such that it gives the best result even when performed by junior doctors. Eversion of the sac is therefore advocated because of its simplicity, effectiveness, and rapidity, which, due to minimal tissue handling and good hemostatic control, virtually eliminates postoperative complications such as hematomas and infections, while not requiring a drainage tube. The only limitation of this technique is that it can be employed only for small hydroceles with thin sacs. Subtotal excision of the sac, which has stood the test of time, still holds good and is indicated for larger hydroceles. To conclude, in the present study, the primary vaginal hydrocele was the most common cystic swelling of the scrotum and, when treated surgically, showed good results.

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# Reconstruction of large abdominal wall tissue defect using vacuum assisted wound closure

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**Ethics Committee Approval**
Ethical Clearance was obtained from institutional ethical committee, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University on 17.07.2017 (D. No. 642/FM).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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## Abstract

**Background/Aim:** Abdominal wall defects may result from trauma, burn, necrotizing soft tissue infection or complications of abdominal surgeries. Reconstruction poses a great challenge for the surgeon in cases of large abdominal wall defects with lack of surrounding tissue. Abdominal wall defects lack a good functional and aesthetic impact and early reconstruction is prudent for better outcomes. This study evaluates the results of Vacuum assisted wound closure (VAC) therapy in patients with large anterior abdominal wall tissue defect which could not be closed primarily otherwise.

**Methods:** This case series included 20 patients with partial thickness, anterior abdominal wall tissue defects. All patients had suffered an acute trauma. Wound debridement was done and VAC therapy dressing was applied in systematic manner. Dressing was changed every 3 or 4 days and continued up to 9-14 days.

**Results:** Twenty patients underwent VAC therapy with an average age of 32 years (range, 25-52 years). Indications comprised tissue loss after acute trauma due to road traffic accident, burn and other trauma leading to anterior abdominal wall soft tissue defect. VAC was used for an average of 12 days, with an average negative pressure of 125 mm Hg. Healthy granulation tissue was formed in all patients. Subsequently split thickness skin grafts were applied in these patients. This results in early recovery as well as decreased morbidity in all patients.

**Conclusions**: All patients had good tolerance to Vacuum-assisted closure. It offers many benefits comprising fewer dressing changes and an earlier return to daily activities. Patients with large abdominal defects can benefit from this procedure.

**Keywords:** VAC therapy, Split skin grafting, Abdominal wall tissue defect

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## Introduction

Successful restoration of substantial large soft tissue defects, especially of abdominal wall, has always been considered challenging for reconstructive surgeons. The morbidity associated with delayed abdominal wall wound healing, imposes a significant psychological and financial burden both for patient and society. So achieving early success in initial stage of reconstruction of abdominal wall is very critical and warranted [1-3].

Methods adopted for reconstruction of abdominal wall depend on the location, extent (layers involved), size, etiology as well as types of wound. For better understanding and communication among reconstructive surgeons, abdominal soft tissue defect can be classified by anatomical zones and by extent or depth of tissue loss.

Abdominal wall defect has been assigned zones depending on the location of defect. Three anatomical zones have been designated (Figure 1(a) and 1(b)) [4].

Zone 1: Midline defects with extension across the midline
Zone 2: Upper quadrants defect of the abdomen
Zone 3: Lower quadrants defect of the abdomen

Depending upon depth, there are three types of abdominal wall defects [5]:

1. Involvement of only skin and subcutaneous tissue;
2. Involving of muscle and fascia only; and
3. Full thickness defects involving skin, subcutaneous tissue, muscle, and fascia.

Figure 1a: Anatomical Zones of Abdominal wall:- Zone 1

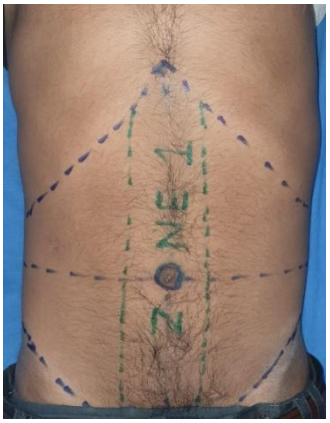
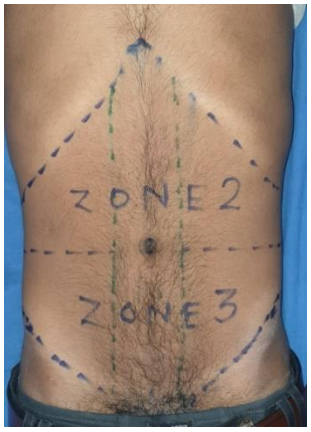



Figure 1b: Anatomical Zones of Abdominal wall:- Zone 2 and 3




There are various clinical conditions which can lead to abdominal wall defect requiring replacement including traumatic injury, radiation-associated wounds, oncologic resection, superficial soft tissue infection, and septic evisceration, burn or complications of various surgeries [1, 5].

Reconstruction can be done by primary closure when wound can be approximated easily and that too without tension, flap cover needed when appropriate tissue is available, bioprosthetic mesh can be used along with flap when there is full thickness loss, skin grafting can be done when there is partial thickness loss and also wound is clean [4].

Reconstruction of large abdominal wall defects with no surrounding tissue pose a great challenge for the reconstructive surgeon. It becomes more challenging in the cases when the wound is grossly contaminated. Such a large abdominal wound requires daily dressing till the wound becomes healthy for cover. This traditional method of wound management takes longer time which adds to morbidity of patients and financial burden to family and society [1].

In this era of wound management, the use of vacuum assisted wound closure therapy for management of complicated wound has been well documented. Vacuum-assisted closure (VAC) therapy includes the use of negative pressure to augment conditions for wound healing. It can be effectively applied during early management of acute trauma patients. It not only stimulates granulation tissue formation due to application of micromechanical forces but also provides a closed moist environment with removal of excess fluid [6, 7]. Since ideal reconstruction can only be performed on a non-edematous, clean wound with no sign of active infection and with minimal co-morbidity, vacuum assisted wound therapy methods can be good option in early stage of large partial soft tissue defect in abdominal region. Keeping this aspects of early wound management in consideration, we applied Vacuum assisted wound closure methods in large abdominal defects which otherwise could not be primarily closed and the depth of wound included only skin and subcutaneous tissue.

Ultimately, the main goal of such reconstruction is early recovery with improved patients' health related quality of life. Successful abdominal wall reconstruction with VAC has shown positive influence on patients' pain, physical as well as social functioning, and rejoining of work.

## Materials and methods

This case series was conducted in department of plastic and reconstructive surgery, at Jawaharlal Nehru Hospital, Aligarh Muslim University. Ethical Clearance was obtained from institutional ethical committee, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University on 17.07.2017 (D. No. 642/FM). After informed consents taken, vacuum assisted wound closure dressings were applied to 20 patients who fulfilled the following inclusion criteria:

- Acute wound either due to trauma or burn
- Partial thickness loss of anterior abdominal wall
- Age over 12 years.

Exclusion criteria of this study were patients with:

- Polytrauma
- Unstable condition
- Chronic wounds

Preliminary patients' data were noted in all patients who fulfilled above mentioned criteria and Vacuum assisted wound closure device were applied in systematic manner. Clinical examination of wound was done during dressing change at every 3rd / 4th day or when the canister was filled. This was continued for 10-14 days until healthy granulation tissue formed. And wound became fit for skin grafting.

**Vacuum Assisted Wound Therapy technique:**

1. **Wound Preparation:** After taking microbiological culture swab from wound, adequate surgical debridement of wound was done and hemostasis was achieved. Peri-wound area was cleaned and dry.
2. **Foam placement:** Specially designed sterile, open-cell foam placed in wound cavity, covering all areas of wound cavity.
3. **Sealing with drapes:** Wound cavity along with 3-5cm of peri-wound area was sealed with adhesive drapes.
4. **Negative pressure application:** After attachment of tubing to dressing and connecting it with machine, a controlled, uniform negative pressure of 125mm Hg was applied.

## Results

Twenty patients received VAC therapy. Among these patients, 16 had suffered road traffic accident (RTA), 2 patients suffered heavy machine injury at work place and 2 patients had sustained thermal burn. Average length of VAC therapy was use was 12 days (10-14). In all the patients vacuum-assisted closure therapy was continued until wound becomes ready for skin grafting. After VAC therapy, skin grafting was performed to cover granulation tissue in all the 20 cases. The average age of patients was 32 years (range, 22–52 years). There were 15 male and 5 female (Table 1, 2). No complication occurred that could be directly attributed to VAC therapy, such as, a deep infection or bleeding. Two of the representative case of this study has been shown below with their details of study.

Table 1: Patients and VAC therapy details

| | | |
|---|---|---|
| Total cases | 20 | |
| Average age | 32 years (range 22-52 years) | |
| Sex | Male | 15 (75%) |
| | Female | 5 (25%) |
| Etiology | Road traffic accident | 16 (80%) |
| | Heavy machine injury | 2 (10%) |
| | Thermal Burn | 2 (10%) |
| Average duration of VAC therapy | 12 days (10-14 days) | |
| Pressure used in VAC therapy | -125 mm Hg | |
| Average length of stay at hospital | 20 days (17-24 days) | |
| Largest size of wound covered | 21 x 20=420 cm² | |
| Complications due to VAC dressing | Nil | |

Table 2: Patients demographic details

| Case | Age (years) | Sex | Mode of injury | Size of wound (cm2) | Number of VAC dressing | Total Days of VAC therapy done | Total duration of hospital stay(Days) |
|---|---|---|---|---|---|---|---|
| 1 | 26 | M | Machine injury | 420 | 5 | 14 | 24 |
| 2 | 17 | M | RTA | 240 | 3 | 9 | 20 |
| 3 | 40 | M | RTA | 330 | 5 | 14 | 23 |
| 4 | 25 | F | RTA | 99 | 3 | 10 | 18 |
| 5 | 52 | M | RTA | 180 | 3 | 9 | 18 |
| 6 | 34 | F | Burn | 330 | 4 | 12 | 21 |
| 7 | 32 | M | RTA | 380 | 4 | 13 | 21 |
| 8 | 28 | M | Machine injury | 361 | 3 | 10 | 20 |
| 9 | 42 | M | Burn | 285 | 3 | 9 | 19 |
| 10 | 38 | F | RTA | 273 | 4 | 11 | 20 |
| 11 | 24 | M | RTA | 252 | 4 | 10 | 22 |
| 12 | 33 | M | RTA | 204 | 3 | 9 | 17 |
| 13 | 25 | F | RTA | 352 | 4 | 12 | 21 |
| 14 | 23 | M | RTA | 336 | 5 | 14 | 24 |
| 15 | 27 | M | RTA | 168 | 4 | 11 | 20 |
| 16 | 31 | M | RTA | 414 | 4 | 12 | 18 |
| 17 | 24 | M | RTA | 224 | 3 | 9 | 17 |
| 18 | 34 | M | RTA | 360 | 3 | 10 | 20 |
| 19 | 33 | F | RTA | 400 | 4 | 13 | 23 |
| 20 | 48 | M | RTA | 288 | 3 | 10 | 20 |

M: Male, F: Female, RTA: Road traffic accident

### Representative case 1

26 years old man suffered heavy machine injury while working in lock making factory. There was avulsion of skin and subcutaneous tissue from anterior abdominal wall leading to a defect of about 21 cm x 20 cm (420 cm²). Initially patient was admitted under emergency surgical team. Patient was transferred to plastic surgery side for reconstruction, once he was stabilized, on 6th day. VAC dressing which was applied, changed on every third day and continued for 14 days, followed by split-thickness skin grafting. Patient was discharged on day 24th in stable condition with good graft take (Figure 2).

Figure 2a: Condition of wound before VAC application in a case of post road traffic accident abdominal wall defect

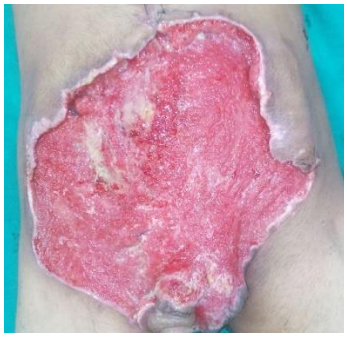


Figure 2b: VAC therapy being applied

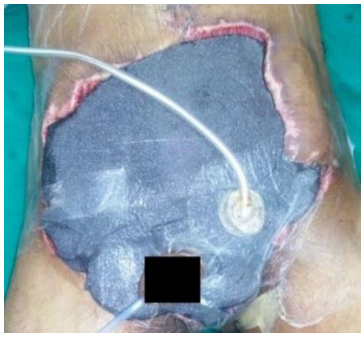


Figure 2c: After VAC therapy, the contour has improved

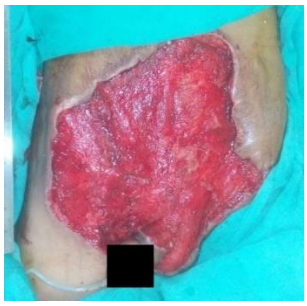


Figure 2d: After split thickness skin grafting

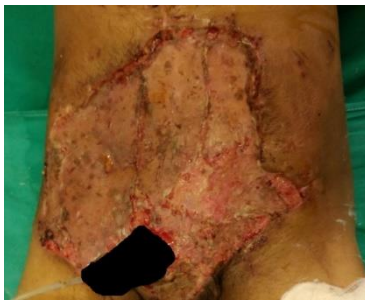
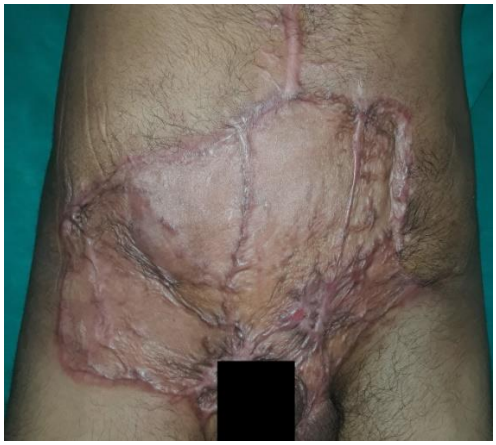


Figure 2e: After 1 month follow up



**Representative case 2**

This patient was a 17 years boy who had road traffic accident. There was anterior abdominal wall defect with skin and subcutaneous tissue loss along with abrasion and friction burn present. The wound was debrided to remove gross contamination then VAC dressing was applied once infection was controlled and it was continued for 9 days. After VAC therapy, split-thickness skin grafting was done and then on day 20th, patient was discharged in satisfactory condition (Figure 3).

Figure 3a: Condition of wound at presentation in emergency department in a patient with history of RTA

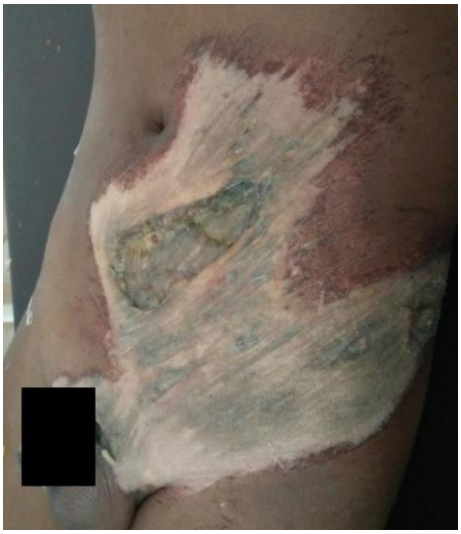
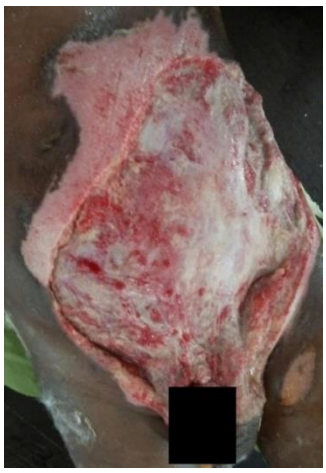


Figure 3b: Condition of wound after debridement

Figure 3c: After VAC therapy



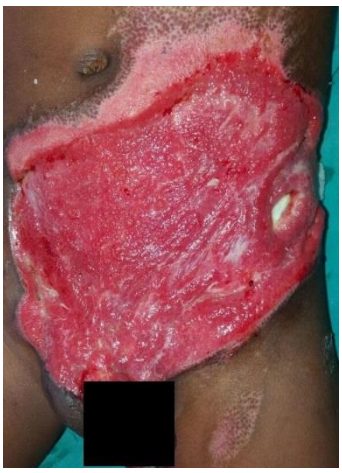
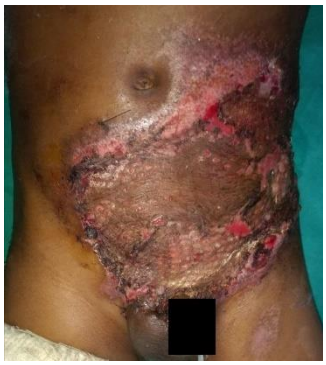
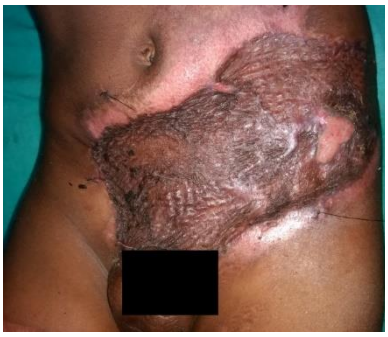


Figure 3d: After split thickness skin grafting

Figure 3e: After 20 days in follow up





## Discussion

Trauma surgeons often come across abdominal wall soft tissue defect in emergency department. The management of this defect depends on site, size, depth, contamination, availability and conditions of surrounding tissue. In cases where the thickness of wound consists only of skin and subcutaneous tissue, early coverage of wound is warranted otherwise persistent infections in wound may lead to involvement of deeper tissue. This will lead to delay in reconstruction which will cause ill health to patients and monetary burden to family as well as society. It has been found in literature that infection in local tissue prolong the inflammatory phase, decreases oxygenation, causes collagenolysis and thus retard the progress of wound healing [8].

The biggest problem in large tissue defect in abdominal region is the availability of local tissue for coverage. At the time of presentation in emergency, usually the wound is grossly contaminated which preclude early coverage with skin grafting. Even if wound is clean and early skin grafting is done, it will lead to contour deformity because of cavity defect in wound at the time of presentation. So successful reconstruction requires optimal wound condition, which is the goal to reach as soon as possible. The main aim of reconstruction in trauma patient is early recovery with minimal complication and better aesthetic appearance with optimal functionality of reconstructed area. Conventional treatment methods such as frequent wet dressing will be painful and protracted [9].

Vacuum assisted wound closure (VAC) therapy has been considered an effective method in wound preparation for early coverage. As has been seen in this study that VAC therapy not only reduces duration for granulation tissue formation but also removes hematoma, exudate and pathogens. Sealing of wound with VAC dressing provides evenly moist environment and avoids contact to the atmosphere & thus intrusion of pathogens. VAC therapy also reduces size of wound with early obliteration of cavity defect.

Webb et al. [10] in their study reported a “Ilizarovian” effect of VAC on wound leading to healthy healing granulation tissue formation. In our study also we can clearly appreciate the early and healthy granulation tissue formation due to mechanical deformation effect of VAC therapy. de Alcântara Jones et al. [11] used vacuum assisted closure system in infected wounds where in 19 patients they observed that healthy granulation tissue which was free from any infection was obtained with a significantly reduced size of lesion.

Labler et al. [12] in his study found that VAC therapy may trigger accumulation of neutrophils and angiogenesis due to raised IL-2 and VEGF levels. We also found that there was augmentation in wound healing phase with early cavity coverage with healthy tissue with decreased infective foci. DeFranzo [13] also reported that patients treated with Negative Pressure Wound Therapy showed faster granulation formation than simple wet dressing. Z. Ali et al. [14] observed earlier appearance of granulation tissue in patients treated with VAC as compared to the conventional dressing group. They also found that complete (100%) granulation was achieved earlier and in higher proportions in VAC group as compared to the conventional group. Morykwas et al. [15] found a significant increased rate of

granulation tissue formation in both continuous and intermittent VAC application.

On comparison with previous experiences of conventional wound management at our center, we found that VAC therapy has significantly reduced the wound closure time by 30-50%. Average period of VAC treatment was 12 days and that of total hospitalization was 20 days. The longest duration of vacuum assisted closure therapy was 14 days. In DeFranzo et al. [2] study of VAC application on abdominal wound, the average time of VAC application was of 13 days. The longest duration of VAC therapy in their study was 96 days.

Although this treatment method is more expensive when we consider the cost of each dressing especially in developing countries like India but it reduces the costs of hospital stay for a longer time, in comparison with conventional dressing and multiple surgical debridement sessions. Flack et al demonstrated an overall lower cost of care (US$52,830 versus US$61,757 per person) for patients treated with VAC therapy compared with advanced dressings [16].

After removal of VAC therapy, early wound coverage was done with split thickness skin grafting in all our patients. In DeFranzo et al. [2] study 28 out of 63 patients; split thickness skin grafting was done.

Patients returned to their daily life as early as possible with minimum morbidity. Early return to family and society decreases their financial and psychological burden.

This study showed that early and aggressive wound management with VAC therapy creates aesthetically pleasing functional abdominal wall where patient can swiftly merge in the society and his profession with better chance for early recovery in one hospital stay.

However, smaller sample size and lack of control group are the limitations of this study which needs to be considered.

**Limitations**

Sample size is small due to the cost of the dressings. The study with large sample size will establish the definitive role of NPWT therapy in managing large abdominal wounds.

**Conclusion**

VAC therapy is a worthwhile adjuvant therapy in the treatment of partial thickness, large abdominal wall soft tissue defect which cannot be primarily closed or covered with flap. It not only facilitates the rapid granulation tissue formation but also controls of infection. Eventually, it reduces the expenses of treatment by decreasing the duration of treatment and hospital stay.

**Acknowledgements**

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# How has the initial lockdown and reopening due to the COVID-19 pandemic affected physical activity level and well-being in Turkey?

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**Ethics Committee Approval**
The ethical approval was gained from Burdur Mehmet Akif Ersoy University Ethics Committee for Non-invasive Research on the date 13th May 2020 and the number was GO-2020/121.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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## Abstract

**Background/Aim:** The worldwide spread of COVID-19 caused changes in daily routines. During the lockdown, parks and gyms were closed and access to outdoor areas was limited. After reopening, many restrictions were removed. Since this process may have altered physical activity and well-being levels, this study aimed to explore how physical activity (PA) levels and well-being of Turkish citizens were affected by the initial COVID-19 lockdown and the reopening.
**Methods:** In this prospective, cross-sectional study, participants were asked to complete an online survey twice: first in May 2020 (when initial strictest partial lockdown procedures were applied) and second in July 2020 (after most restrictions had been removed). The online survey consisted of questions related to demographics, PA levels, and well-being. Categorical questions (expressed as either day per week or min per day) were used to assess vigorous and moderate PA, time spent sitting, etc. The WHO-5 Well-Being Index was used to assess well-being.
**Results:** The mean age and the body mass indexes of 94 individuals (52 females, 42 males) were 36.16 (10.04) years and 25.14 (3.82) kg/m$^2$, respectively. During both lockdown and after reopening, low levels of PA and well-being levels above the low mood limit (< 50) were identified. No significant change was found in either any of the PA-related variables or the well-being of participants between the initial and final assessments ($P > 0.05$).
**Conclusion:** Our results showed that PA levels and well-being of Turkish citizens remained unchanged after the reopening process when compared to the initial lockdown due to COVID-19. These findings may also suggest that reopening was not a sufficient stimulant factor to alter physical activity and well-being. Despite all the negative effects brought on by the pandemic, it is essential to adopt approaches to keep PA levels and well-being high.

**Keywords:** Physical activity, COVID-19, Well-being

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## Introduction

In December 2019, a pandemic viral disease had its first cases in the city of Wuhan, China. This disease was given the name COVID-19; the World Health Organization (WHO) declared it an outbreak [1]. In a short time, COVID-19 spread all over the world and became a global health threat [2].

Given the powerful transmissibility of the disease, response efforts by the Turkish government were directed to restrain the spread of the virus. A lockdown, imposed in March 2020 when the first case was detected in Turkey, included limiting social distance, closing schools, and prohibiting gathering in public places.

The closure of parks and gyms and limiting recreational activities affected the daily routines of citizens [3]. Most of the lockdown strategies were said to have led to a decrease in the physical activity (PA) level of the population [4]. The pandemic caused a reduction in energy expenditure and time spent on outdoor sports activities. Lifestyles substantially changed due to the containment measures, with the consequent risk of sedentary behaviors [5].

In addition to the negative effect of the pandemic on PA habits, mental health may also have been substantially changed due to the containment measures [6]. It was reported that prolonged self-isolation negatively impacted psychological response, thus promoting post-traumatic stress symptoms and anxiety [7]. Concerns about financial uncertainty, job loss, and other factors caused by the COVID-19 lockdowns have also negatively affected people's well-being [3, 6]. Although quarantine and physical isolation measures were thought to have a positive effect in protecting peoples' general health by preventing and mitigating the transmission of the virus, the implementation of these restraint measures may have a long-lasting and wide-ranging negative psychosocial impact [8]. Continuously watching, hearing, and reading about COVID-19 on TV, in newspapers, on the internet, etc., may have affected mental health [5]. Many people may have become anxious, angry, uncertain, and depressed due to the effects of the outbreak [7, 8].

The initial partial lockdown was ended in June 2020, and a new period termed the "new normal" began. Within the scope of the new normalization process, parks, outdoor areas, gyms, cafes, restaurants, and libraries were opened, working from home was ended, and bans on traveling were lifted. After reopening, citizens returned to their daily routines, with some legal restraints and personal precautions such as wearing a mask, attention to social distancing, and hygiene practices [9].

Although previous studies conducted in various countries (Canada, Portugal, Australia, and Italy) have demonstrated the impact of physical isolation on the physical and mental health of the population during the global pandemic, only limited knowledge is available on the consequences of the initial partial lockdown and reopening process on PA level and well-being [3, 6, 10, 11]. Other than one study that examined the effects of the lockdown and reopening [12], nearly all other related research examined the effects of the lockdown only without investigating the effects of reopening [3, 11, 13–17].

Therefore, we intended to explore the impact of the initial COVID-19 lockdown and reopening on the PA levels and the well-being of individuals. To the best of our knowledge, no published paper has reported these effects in the Turkish population. We hypothesized that PA levels and well-being of Turkish citizens would change after the initial strict, partial lockdown was ended when compared to the initial COVID-19 lockdown period in Turkey.

## Materials and methods

### Design

This prospective study was performed between May 2020, when the initial strictest partial lockdown procedures were implemented by the national government, and July 2020, when most of the restrictions were removed (the so-called "controlled social life" period). The study was conducted in accordance with the ethical principles of the 1975 Declaration of Helsinki. Approval was received from the ethics committee for non-invasive research at our local university. All participants provided online informed consent before participation.

### Participants

Inclusion criteria were: (i) age between 18 and 65 years, (ii) able to mobilize independently, (iii) able to read, write, and understand the country's native language, (iv) willing to participate in the study. Exclusion criteria were: (i) COVID-19 diagnosis, (ii) any findings that could be related to COVID-19, (iii) hospitalization for any reasons, (iv) mental and cognitive disorders that could prevent filling out the questionnaire. Virtual snowball sampling was used for the recruitment of participants. To spread the word and refer participants to the study link, social media communications (Facebook, Instagram, Twitter) were used. Participants were asked to complete the questionnaire using an online survey platform (Google Forms) twice: in May 2020 (initial assessment) and July 2020 (final assessment).

### Outcome measures

The online survey consisted of questions related to demographics, PA level, and well-being. Demographic characteristics included age, gender, and education status. The presence of any chronic illnesses was also captured.

PA levels were assessed with categorized questions measuring activities performed in the previous week. The part of the questionnaire related to the PA level contained questions on the number of days with vigorous PA per week, duration of vigorous PA per day, number of days with moderate PA per week, duration of moderate PA per day, number of days with at least 10 minutes walking in the corridor and time spent sitting or lying down per day [18].

The WHO-5 Well-Being Index was used to measure well-being. The WHO-5 is a brief self-reported scale of current mental well-being [19]. It contains five positive expressed sentences. The sentences are: "I have felt cheerful and in good spirits," "I have felt calm and relaxed," "I have felt active and vigorous," "I woke up feeling fresh and rested," and "My daily life has been filled with things that interest me." Respondents were asked to rate how well each of the five statements applied to them when considering the last 14 days. Each of the five sentences was scored from 5 (all of the time) to 0 (none of the time). The scores were summed to obtain a total raw score

ranging from 0 to 25 (0 is absence of well-being and 25 is maximal well-being). Then, the raw score was multiplied by four in order to translate it to a percentage scale from 0 (absent) to 100 (maximal). The highest scores indicated a strong sense of well-being [20].

**Statistical analysis**

Statistical analyses were performed using SPSS software version 25.0 (IBM Corporation). Descriptive statistics were summarized as counts and percentages for categorical variables. To determine whether variables were normally distributed, the Kolmogorov-Smirnov test and visual methods (histograms, probability plots) were used. The non-normally distributed and ordinal variables were presented as median (minimum-maximum) and interquartile range (25–75%). The Chi-square test or Fisher's exact test, as appropriate, was used to compare the proportions in different groups. The McNemar-Bowker test was used to evaluate the change in stage-based variables of PA between the initial and the final assessments. To compare the change in non-normally distributed and ordinal variables, the Wilcoxon test was used. A 5% type-I error level was used to infer statistical significance ($P < 0.05$).

## Results

In total, 102 individuals responded to surveys at both time intervals. Surveys of four individuals who did not meet the inclusion criteria were excluded. Surveys of four individuals were also discarded due to missing item responses. The final analyses were done on surveys obtained from 94 individuals.

The mean age and the body mass index (BMI) of 94 individuals (52 females, 42 males) were 36.16 (10.04) years and 25.14 (3.82) kg/m$^2$, respectively. The majority of individuals (43.6%) held bachelor's degrees, and 19 individuals (20.2%) had reported a history of chronic illnesses such as hypertension, diabetes mellitus, asthma, hypercholesterolemia, hepatitis B, coronary artery disease, and breast cancer. The distribution of demographic characteristics of surveyed participants is presented in Table 1.

Table 1: Distribution of demographic characteristics of surveyed participants

| n=94 | Number (n) | Percent (%) |
|---|---|---|
| Gender | | |
| Female | 52 | 55.3 |
| Male | 42 | 44.7 |
| Education Status | | |
| Primary Education | 5 | 5.3 |
| High School | 3 | 3.2 |
| Associate Degree | 10 | 10.6 |
| Bachelor's Degree | 41 | 43.6 |
| Master's Degree | 24 | 25.5 |
| Doctorate | 11 | 11.7 |
| Chronic Illness | | |
| Yes | 19 | 20.2 |
| No | 75 | 79.8 |

The BMI of the individuals remained stable after reopening ($P > 0.05$). The results showed that while 77.7% of participants did not do any vigorous PA during the lockdown, this proportion was 73.4% after reopening. Also, 54.3% of participants reported not doing any moderate PA during the lockdown, a proportion that was 48.9% after reopening. As variables related to PA level assessed through categorical questions expressed in min per day were analyzed, no significant change was observed in the number of individuals between the initial and final assessments ($P > 0.05$). Table 2 shows the results of the McNemar-Bowker test.

Table 2: Changes in the stage-based variables of physical activity level

| | | 1st Assessment | | 2nd Assessment | | *P*-value |
|---|---|---|---|---|---|---|
| | | n | % | n | % | (McNemar-Bowker Test) |
| Vigorous PA, min per day | None | 73 | 77.7 | 69 | 73.4 | 0.495 |
| | <15 | 1 | 1.1 | 5 | 5.3 | |
| | 16-30 | 7 | 7.4 | 5 | 5.3 | |
| | 31-45 | 7 | 7.4 | 6 | 6.4 | |
| | 46-60 | 3 | 3.2 | 5 | 5.3 | |
| | 61-90 | 1 | 1.1 | 3 | 3.2 | |
| | 90< | 2 | 2.1 | 1 | 1.1 | |
| Moderate PA, min per day | None | 51 | 54.3 | 46 | 48.9 | 0.853 |
| | <15 | 7 | 7.4 | 8 | 8.5 | |
| | 16-30 | 19 | 20.2 | 21 | 22.3 | |
| | 31-45 | 10 | 10.6 | 11 | 11.7 | |
| | 46-60 | 3 | 3.2 | 6 | 6.4 | |
| | 61-90 | 1 | 1.1 | 1 | 1.1 | |
| | 90< | 3 | 3.2 | 1 | 1.1 | |
| Time Spent Sitting, min per day | <15 | 4 | 4.3 | 3 | 3.2 | 0.152 |
| | 16-30 | 9 | 9.6 | 11 | 11.7 | |
| | 31-45 | 7 | 7.4 | 12 | 12.8 | |
| | 46-60 | 9 | 9.6 | 13 | 13.8 | |
| | 61-90 | 8 | 8.5 | 15 | 16.0 | |
| | 90< | 57 | 60.6 | 40 | 42.6 | |

PA: Physical Activity, n: number, % percent

No significant change was found in any of the PA-related variables or the well-being of participants between the initial and final assessments ($P > 0.05$) (Table 3).

Table 3: Changes in the assessed variables of participants

| | 1st Assessment | | 2nd Assessment | | *P*-value |
|---|---|---|---|---|---|
| | Median (min-max) | IQR (25-75%) | Median (min-max) | IQR (25-75%) | |
| Vigorous PA, days per week | 0 (0-7) | 0-0 | 0 (0-7) | 0-1 | 0.723 |
| Moderate PA, days per week | 0 (0-7) | 0-2 | 1 (0-7) | 0-2 | 0.812 |
| Time Spent walking in corridor, days per week | 0 (0-7) | 0-3 | 0 (0-7) | 0-5 | 0.316 |
| WHO-5 Well-Being Index Score | 64 (0-100) | 48-76 | 64 (8-100) | 52-80 | 0.383 |

IQR: Interquartile range, COVID-19: Coronavirus disease 2019, PA Physical activity, WHO-5: The 5-item World Health Organization, Wilcoxon Signed Rank Test

## Discussion

The findings of our study illustrated that the PA levels and well-being of Turkish citizens did not show a significant difference between the initial strict partial lockdown period and the period after the reopening. We also found that during both lockdown and post-reopening, the PA levels of participants were low and their well-being was above the low mood limit (< 50). These findings may also suggest that reopening changes were not sufficient enough to stimulate a change in PA behavior and well-being.

The stressful context of mandatory behavioral changes, including staying at home and social isolation, was thought to influence both physical and mental health during the COVID-19 pandemic. Since regular PA provides benefits to immunologic responses as well as enhanced mood and coping with stress, encouragement to be physically active has become more essential than ever for both the physical and mental health of the population during the COVID-19 pandemic [7, 11].

Doing moderate-intensity exercise has been reported to act as an important adjuvant to stimulating the immune system [21, 22]. Results of a recent study based on a large Biobank cohort also showed that regular physical activity could lower the risk of severe COVID-19 infection and hospitalization [23]. Generally speaking, it is universally accepted that people who are in self-quarantine should try to be physically active and exercise as much as they can at home without changing their routines [4]. Nowadays, many scientific communities have

highlighted the benefits of doing exercise or increasing PA levels during COVID-19 restrictions [3–6, 11, 14, 17, 24].

Some studies have provided useful insights into the effects of COVID-19 lockdowns on PA and well-being [3, 6, 11, 14, 17]. Lesser et al. [3] showed that inactive Canadian people became less physically active, while active people became more physically active during COVID-19 restrictions. The PA level of Canadians was found to be strongly related to their well-being. The authors suggested that during public health restrictions, outdoor PA should be encouraged to increase well-being [3].

Maugeri et al. [11] investigated the PA level of Italians before and during the COVID-19 quarantine. To assess PA levels and psychological well-being, they used IPAQ and the Psychological General Well Being Index, respectively. Results of their study showed that PA levels significantly decreased during the pandemic. They thought that this phenomenon could be due to a sharp change in everyday routine and that people who remained at home spent much more time in low-intensity activities. They also found that the reduction of PA levels was related to lower well-being. They highlighted that maintaining regular PA was an important step toward improving physical and mental health [11].

Faulkner et al. [17] evaluated PA and the well-being of individuals from four different countries (United Kingdom, Ireland, New Zealand, and Australia) within the first two to six weeks of government-mandated COVID-19 restrictions. They used IPAQ-SF and the WHO-5 Well-Being Index for the assessments. Their findings demonstrated that participants whose exercise behavior was negatively affected by COVID-19 restrictions reported lower well-being.

In a study surveying, 1491 adults in Australia, 21% of participants reported increased PA while 49% reported declined and 30% reported unchanged PA. Moreover, negative changes in PA were associated with higher depression, anxiety, and stress symptoms. Authors highlighted the utilization of health-promotion strategies directed at adopting or maintaining positive health-related behaviors to address increases in psychological distress during the pandemic [14]. Similarly, Gornicka et al. [25] surveyed 2381 Polish adults and found that the PA levels of 38% remained unchanged while 43% reported decreased PA.

Unlike previous studies, which examined the effects of the lockdown only, a more recent study by Ding et al. investigated the effects of both lockdown and reopening on daily steps. To measure daily step count, they used a social fitness plugin of the most popular mobile social media application in China. They found a sharp decline in daily step counts upon the lockdown of an average of 3796 steps, followed by a significant trend of an increase of 34 steps/day until the end of the lockdown. After reopening, they found that the increasing trend to be continued, but at a slower rate of five steps per day. They concluded that the COVID-19 lockdown led to a sustained period of reduced physical activity despite later increases in step count and suggested that PA should be encouraged and facilitated by government and health professionals [12].

In our study, we measured PA levels and well-being both during the lockdown and after reopening, as Ding and colleagues did; however, we did not measure these variables pre-lockdown. This was due to the prospective design of the study and the inventories we used to measure outcomes. As a result, we found that PA levels and well-being of Turkish citizens did not change after reopening as compared to the initial strict partial lockdown period. In other words, reopening did not cause a significant change in PA levels and the well-being of individuals.

Akçay et al. [24] also investigated the effect of COVID-19 on PA levels of Turkish citizens. They assessed PA levels only during the lockdown, using IPAQ-SF, and compared the percentages with the national and global prevalence, which had been reported by previous studies. They found 70% of the participants to have insufficient PA levels and an increased inactivity rate due to the COVID-19 pandemic. This PA percentage was twice the global value. They suggested that preventive measures should be taken in a timely manner to avoid the risk of diseases that may stem from inactivity.

A study by Akçay et al. [24] showed that the PA level of Turkish citizens during the initial lockdown was lower than usual, and our study showed that reopening had no impact on the PA levels of Turkish citizens. Considering these results together, it can be inferred that the PA levels of Turkish citizens, which were already low during the lockdown, remained the same after reopening, though they were expected to change according to the hypothesis of the study. Unlike the results of Ding et al. [12], which reported a slight but significant increase in the PA levels of Chinese citizens post-reopening, we did not find a significant change in either PA levels or well-being. We suggest that future studies should investigate the possible reasons for these results. Perceived barriers to PA and psychosocial factors such as self-motivation and social influence during the COVID-19 pandemic, which may have an influence on PA levels, should be examined.

A common feature of the above studies was the utilization of an online survey in early 2020 pre- and during the strictest public health restrictions. We also used an online survey to collect data; however, we conducted this study using a prospective design. In other words, we first examined the PA and well-being levels of participants from Turkey during the initial, strictest partial lockdown and then re-assessed these variables after most of the restrictions had been removed (the so-called “controlled social life” period).

To the best of our knowledge, ours is the first study to investigate the effects of reopening after the initial strict partial lockdown during the COVID-19 pandemic in Turkey on PA levels and well-being. Therefore, the timing of the data collection was expedient for accurately measuring PA levels and well-being during and after the public restrictions due to the pandemic.

**Limitations**

There are also some limitations to this study. First, all data were collected using a self-reported online survey. However, self-reported data is subject to some biases and limitations. Second, the findings of our study revealed that reopening was not a sufficient stimulant factor to alter people’s physical activity and well-being. However, it should be kept in mind that PA levels and well-being might be affected by education level, marriage status, chronic illnesses, etc. The fact that these confounding factors were not analyzed in the present study could be another limitation.

## Conclusion

Our results showed that PA levels and well-being of Turkish citizens remained unchanged after the reopening process as compared to during the initial COVID-19lockdown. These findings may also suggest that reopening was not a sufficient stimulant factor to alter people's physical activity and well-being. Despite all the negative effects brought on by the pandemic, it is essential to adopt approaches to keep PA and well-being at a high level.

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# Serum markers, morphological index, RMI, and ROMA in preoperative diagnosis of ovarian cancer

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**Ethics Committee Approval**
This study was approved by the Malatya Clinical Research Ethics Committee, 22.03.2017 and 2017/35. All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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No conflict of interest was declared by the authors.

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## Abstract

**Background/Aim:** Ovarian cancer is the second most common gynecologic malignancy worldwide and is the deadliest among gynecological cancers. It is important that this cancer, which is usually diagnosed in advanced stages, is referred to a gynecologist oncologist without delay. An ideal screening method does not yet exist. Although CA 125 is still the most used tumor marker, it cannot detect early-stage ovarian cancer. Also, CA 125 is not specific for ovarian malignancy. Therefore, new serum markers, such as HE4, and more complex algorithms, like ROMA and RMI, have emerged. Here we evaluate the preoperative potential of patients with adnexal mass to have a malignant or benign mass with morphological index, CA 125, HE4, RMI, and ROMA tests.

**Methods**: This study is a prospective cohort study. A power analysis was done before starting the study. The sample size was at least 80 when the Type I error was set at 0.05, and the confidence interval was 95%. We included into the study 84 patients admitted to our clinic because of pelvic mass and underwent operation between March 2016 and October 2018. To homogenize the benign and malignant groups, 42 patients were collected from each group. CA 125 and HE4 levels of the samples were studied by the electrochemiluminescence method. ROMA and RMI values were calculated, and the data were entered into SPSS. Data were analyzed using SPSS 22.0 statistical package program.

**Results**: Each of the CA 125 ($P = 0.002$), HE4 ($P < 0.001$), morphological index ($P < 0.001$), ROMA ($P < 0.001$), and RMI ($P < 0.001$) tests has been successful in differentiating malignant masses from benign masses. In the malignant-benign differentiation of adnexal masses preoperatively, CA 125 was the test with the lowest sensitivity, and RMI had the highest sensitivity. However, in the ROC analysis, the morphological index has a higher area under the curve.

**Conclusion**: Although CA 125 is still the most frequently used marker in the preoperative evaluation of adnexal masses, it has low specificity and sensitivity, especially in premenopausal patients. The use of new tumor markers (e.g., HE4) and other algorithms (e.g., ROMA and RMI) is supported by our findings and the literature. However, here we show that an expert ultrasonographic evaluation with morphological index alone could be effective.

**Keywords:** Ovarian cancer, Pelvic mass, RMI, ROMA, CA 125, HE4, Morphological index

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## Introduction

Ovarian cancer is gynecological cancer with a high mortality rate and is usually diagnosed in an advanced stage. Besides, about 239,000 new cases are reported annually; it is estimated that one out of every 75 women will have ovarian cancer in their lifetime. One out of every 100 women diagnosed with ovarian cancer, which is ranked seventh among female cancers, will lose their life due to this disease and/or its complications [1]. Since cytoreductive surgery constitutes the most important component in treating epithelial ovarian cancer, true diagnosis of the patients in the preoperative period is very important.

Today, multiple methods, such as pelvic examination, imaging methods, and biochemical markers in serum, are used to diagnose ovarian cancer. Neither ultrasound, which gives information about the structure and size of the mass, nor serum markers (such as CA 125, HE 4), are insufficient for malignant-benign differentiation [2]. For this reason, combined tests came into use, in which clinical data are evaluated [such as the risk of malignancy index (RMI) and Risk of Ovarian Malignancy Algorithm (ROMA), and CA 125, HE 4, and the ultrasonographic features of mass] [3].

In addition, the morphological index, in which the ultrasonographic findings of the mass are evaluated (volume and structure properties of the mass), is used in the preoperative evaluation of the adnexal masses [4].

In this study, we discuss the effects of serum CA 125, HE 4, RMI, ROMA, and Morphological Index on the preoperative malignancy prediction of the patients who were diagnosed with adnexal mass and whose pathology results were finalized after surgery.

## Materials and methods

### Patient group

In our study, 84 patients who were admitted to our clinic between March 2016 and October 2018 were not pregnant, without any known cancer disease, liver or kidney failure and operated for pelvic mass were included. The study was prospectively defined and conducted in a single center. Our study has been approved by the ethics committee of Inonu University Faculty of Medicine. Consent was obtained from the patients who were planned for operation. This research was supported by the Scientific Research Projects department, with the 2018/869 project number.

### Data collection

Detailed anamnesis was obtained from patients; age, menopausal status, parity, family history, and history of known disease were examined and recorded. A single clinician took patients to a transvaginal ultrasonography examination using an IC5-9-D 7 MHz transducer of the Voluson E6 (GE Healthcare, Milwaukee, WI, USA) ultrasound device. In the ultrasonography, wall structure, septa thickness, presence of solid area, whether being bilateral, the presence of intraabdominal metastasis, and acid of the mass were evaluated. Blood (10 ml) was collected from the patients in routine biochemistry tubes on the morning of the operation. These blood samples were centrifuged at 3500 rpm, and the separated serum was transferred into microcentrifuge tubes. Samples were stored at -80°C until analysis. On the analysis day, samples were thawed at room temperature and transferred to conical bottom polypropylene tubes and vortexed for homogenization. The prepared samples were analyzed using the electrochemiluminescence method with CA 125 and HE4 kits in Roche brand (Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim) e601 model device.

### Evaluation of the data

In the ultrasonography examination, the characteristics of the masses were evaluated according to the scoring system developed by Depriest et al. [5], and morphological indices were calculated. By force of this scoring system, the volume, width, length, and height of the mass were calculated by multiplying with the coefficient of 0.523. The volumes under 10 $cm^3$ have received 0, 1, 2, 3, or 4 points (10–50 $cm^3$, 1 point; 50–200 $cm^3$, 2 points; 200–500 $cm^3$, 3 points; > 500 $cm^3$, 4 points). In addition, if the wall was thinner than 3 mm and flat, the score was 0; if it was thicker than 3 mm and flat, the score was 1 point; if it had a papillary projection smaller than 3 mm, the score was 2 points; if it has a papillary projection bigger than 3 mm, the score was 3 points; and if the solid areas were dominant, the score was 4 points. The masses without septa received 0 points; septa thinner than 3 mm received 1 point; septa between 3 mm and 10 mm received 2 points; 10 mm and more solid masses received 3 points; and totally solid masses received 4 points. According to this scoring system, the masses were evaluated with a score between 0 and 12 in total.

The percentages of ROMA were calculated by formulating the CA 125 and HE4 levels and the menopausal status of the patient. Women older than 50 years of age and who had a hysterectomy were accepted in the postmenopausal period. CA 125 and HE4 values were measured with IU/ml units [6].

**For premenopausal women;**

Predictive Index (PI) = -12.0 + 2.38 × LN [HE4] + 0.0626 × LN [CA 125]

**For postmenopausal women;**

Predictive Index (PI) = -8.09 + 1.04 × LN [HE4] + 0.732 × LN [CA 125]

The percentage of ROMA = calculated as exp (PI) / [1 + exp (PI)] ×100.

The RMI score was calculated by multiplying the patient's ultrasonography score with the menopausal condition and CA 125 value. Ultrasonography scores ranged from 0 to 3. Multiloculated cysts, solid areas, metastases, and presence of acid and bilateral lesions were each calculated as 1 point, and the value was presented as 0 if none of these were present, as 1 if one of these were present, and as 3 if two or more were present in the formula. If the patient is in the premenopausal period, the M score is evaluated as 1, and in the postmenopausal period, the M score is evaluated as 3. The CA 125 value was measured as IU/ml and placed in the formula [6].

### Statistical analysis

Data are given by mean (standard deviation) and number (percentage). The normality of the data distribution was tested using the Shapiro-Wilk test. In statistical analysis, Mann-Whitney U test, Yate's corrected chi-square test, Pearson correlation coefficient and diagnostic tests (e.g., Roc analysis, sensitivity, and specificity) were used where applicable. IBM SPSS Statistics 22.0

program was used in the analysis. P-values < 0.05 was considered statistically significant.

## Results

A total of 84 patients diagnosed with adnexal mass were included in our study. The pathology results of 42 patients were evaluated as benign, and the remaining 42 were evaluated as malignant. The average age of the patients who had benign pathology was 47.8 (15.08) years, and the average age of the patients who had malignant pathology was 51.3 (16.2). The first serum test applied to patients who have adnexal masses was CA 125, a glycoprotein. While 21.4% (n = 9) of the patients with benign pathology had a bilateral mass, 35.7% (n = 15) of the patients with malignant pathology had a bilateral mass. The final pathology results of the patients were compared with acid, which is a finding suggestive of malignant disease. While acid was present in 19% (n = 8) of patients reported as benign due to pathology, it was present in 40.5% (n = 17) of the patients reported as malignant as a result of pathology. When the pathology results of the patients were examined, in the benign patient group, 20 patients (47.6%) were diagnosed with serous cystadenoma, and 12 patients were diagnosed with mucinous cystadenoma (28.5%), and 10 patients (23.8%) were diagnosed with mature cystic teratoma. In the malignant patient group, 28 patients were diagnosed with serous carcinoma (66.6%), and 14 patients were diagnosed with mucinous carcinoma (33.3%). When the performed surgeries were examined, only cystectomy was performed in 3 patients (8%), unilateral salpingo-oophorectomy (USO) in 27 patients (64%), and total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH + BSO) in 12 patients (28%). Only the USO procedure was performed in the malignant patient group in four patients (9%). Seventeen patients underwent TAH + BSO + omentectomy and pelvic paraaortic lymphadenectomy (PPLND) (41%), and 21 patients (50%) underwent cytoreductive surgery. The pathology result of all patients who underwent USO in the malignant patient group was serous carcinoma, and severe intraabdominal tumor involvement was determined. However, neoadjuvant chemotherapy was considered appropriate after surgery because they could not tolerate cytoreductive surgery due to their advanced age and current comorbidities. In patients diagnosed with serous or mucinous carcinoma, TAH + BSO + PPLND + omentectomy was performed if distant organ (such as liver, abdominopelvic peritoneum, spleen) metastasis was not detected. In patients with distant organ metastases, lymphadenectomy was performed after cytoreductive surgery (Table 1).

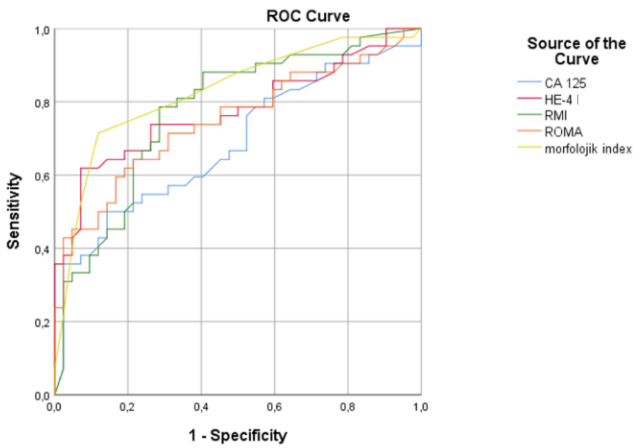
Table 1: Characteristics of patients included in the study

| | |
|---|---|
| Age | |
| Benign patient group | 47.8 (15.08) |
| Malignant patient group | 51.3 (16.2) |
| Bilaterality | |
| Benign patient group | 21.4%, n = 9 |
| Malignant patient group | 35.7%, n = 15 |
| The presence of ascites | |
| Benign patient group | 19%, n = 8 |
| Malignant patient group | 40.5%, n = 17 |
| Pathology | |
| Benign patient group | |
| Serous cystadenoma | 47.5%, n = 20 |
| Mucinous cystadenoma | 28.5%, n = 12 |
| Mature cystic teratoma | 23.8%, n = 10 |
| Malignant patient group | |
| Serous cystadenocarcinoma | 66.6%, n = 28 |
| Mucinous cystadenocarcinoma | 33.3%, n = 14 |
| Surgical procedure | |
| Benign patient group | |
| Cystectomy | 8%, n = 3 |
| USO | 64%, n = 27 |
| TAH+BSO | 28%, n = 12 |
| Malignant patient group | |
| TAH+BSO+PPLND+Omentectomy | 41%, n = 17 |
| Cytoreductive surgery | 50%, n = 21 |
| USO | 9%, n = 4 |

n: number of patients, USO: unilateral salpingooophorectomy, TAH + BSO: total abdominal hysterectomy + bilateral salpingooophorectomy, PPLND: pelvic and para-aortic lymphadenectomy

Each of the CA 125, HE4, morphological index, ROMA, and RMI tests were evaluated preoperatively. For CA 125, the sensitivity was 50% (95% CI: 34.1–65.8), the specificity was 85.7% (95% CI: 71.4–94.5), the positive predictive value was 77% (95% CI: 61.1–88.6), and the negative predictive value was 63.1% (95% CI: 55.2–70.3). For HE4, the sensitivity was 61.9% (95% CI: 45.6–76.4), the specificity was 92.86% (95% CI: 80.5–98.5), the positive predictive value was 89.66% (95% CI: 73.9–96.3), and the negative predictive value was 70.9% (95% CI: 62.1–78.3). For the morphological index, the sensitivity was 71.4% (95% CI: 55.4–84.2), the specificity was 88.1% (95% CI: 74.3–96.03), the positive predictive value was 85.71% (95% CI: 72–93.3), and the negative predictive value was 75.5% (95% CI: 65.3–83.4). For RMI, the sensitivity was 78.5% (95% CI: 63.1–89.7), the specificity was 71.4% (95% CI: 55.4–84.2), the positive predictive value was 73.3% (95% CI: 62.4–81.9), and the negative predictive value was 76.9% (95% CI: 64.4–85.9). For ROMA, the sensitivity was 64.2% (95% CI: 48–78), the specificity was 78.5% (95% CI: 63.1–89.7), the positive predictive value was 75% (95% CI: 61.7-84.8), the negative predictive value was 68.7% (95% CI: 58.7–77.2) (Table 2, Figure 1).

Figure 1: ROC curves of the tests used in the malignant-benign differentiation of adnexal masses in the preoperative period




CA 125: Cancer antigen 125, HE4: Human epididymis protein 4, RMI: Risk of malignancy index, ROMA: Risk of ovarian malignancy algorithm.

Table 2: Sensitivity, specificity, PPV, NPV percentages, area under the ROC curve and *P*-values of CA 125, HE 4, ROMA, RMI tests and morphological index used in preoperative evaluation of adnexal masses. Optimal cut-off values were determined with ROC curves.

| | Optimal cut-off | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | AUC | *P*-values |
|---|---|---|---|---|---|---|---|
| CA-125 | 52.4 | 50 | 85.7 | 77 | 63.1% | 0.685 | 0.002 |
| HE 4 | 83.4 | 61.9 | 92.86 | 89.6 | 70.9 | 0.775 | < 0.001 |
| ROMA | 16.1 | 64.2 | 78.5 | 75 | 68.7 | 0.749 | < 0.001 |
| RMI | 53.7 | 78.5 | 71.4 | 73.3 | 76.9 | 0.775 | < 0.001 |
| Morphological Index | 7 | 71.4 | 88.1 | 85.7 | 75.5 | 0.828 | < 0.001 |

CA 125: Cancer antigen 125, HE 4: Human epididymis protein 4, ROMA: Risk of ovarian malignancy algorithm, RMI: Risk of malignancy index, PPV: Positive predictive value, NPV: Negative predictive value

## Discussion

Although early diagnosis is possible in some tissue and organ cancers, given today's technological potential, especially in epithelial ovarian cancers, there is no possibility of early diagnosis. Since most patients are asymptomatic or have unclear complaints in the early stage, the patients are typically referred to the physician in advanced stages, and the disease appears to have intrapelvic and/or intraabdominal widespread metastases at the time of diagnosis. This has led modern to the use and development of tests that provide early diagnosis [1].

The first serum test applied to patients who have adnexal masses was CA 125, a glycoprotein. However, it has been reported that this serum antigen increases in many physiological and pathological scenarios, including gynecological and non-gynecological causes, making this test not useful for early diagnosis [7]. HE4 is an alternative serum marker used in recent years. HE4 seems a more advantageous serum antigen because it is secreted minimally in normal ovarian tissue and endometriosis but has an increased secretion in ovarian epithelial tumors [6]. In a study in which the CA 125 and HE4 tests were used to differentiate malignant and benign adnexal masses, the sensitivities in detecting malignancy were 83.3% and 90%, respectively [8]. In another extensive study, HE4 was a more sensitive test than CA 125 for detecting malignancy in the adnexal mass [9]. Here we report sensitivities for detecting preoperative malignancy of ovarian pathologies of 61.9% and 50% for HE4 and CA 125.

Although the superiority of the HE4 test over CA 125 in the early diagnosis of ovarian cancer has been presented in our study and similar studies in the literature, combined tests (such as ROMA and RMI) are widely used for this purpose, where the patient's age, ultrasonographic findings of the mass, and both tumor markers are considered. When the studies conducted in the literature about the use of combined tests are examined, it is observed that there are different results Jacobs et al. [10] reported the sensitivity of RMI as 85% and specificity as 97% in a study involving 101 benign and 42 malignant ovarian tumors. In another study conducted by Liest et al. [11], it was shown that ROMA and RMI tests did not have superiority over each other in the preoperative malignant-benign differentiation of the adnexal mass of 784 patients. While in another study conducted on 457 patients with an adnexal mass, the ROMA test was superior to RMI (sensitivity 89% vs. 80.7%) [12]. In another study conducted by Oranratanaphan et al. [13], the ROMA test did not show a significant superiority over the RMI test in the malignant-benign differentiation of the preoperative adnexal masses. In our study, the RMI test was sensitive according to the ROMA test (sensitivity 78.5% vs. 64.2%) in malignancy differentiation.

It is an unquestionable fact that the combined tests used in the preoperative evaluation of adnexal masses are more advantageous than the serum markers used alone. In a study conducted by Karlsen et al. [14] on the comparison CA 125, HE4, ROMA, and RMI tests in preoperative malignancy analysis of adnexal masses in 1218 patients, ROMA and RMI tests had sensitivities close to each other, but the specificity of both tests was higher than that of CA 125 and HE4 tests alone. Similar results were obtained in another study conducted for this purpose; the sensitivity of ROMA and RMI tests was higher than those of CA 125 and HE4 tests [15]. Our study concluded that the sensitivity of the RMI and ROMA combined test was higher than tests relying on other serum markers.

Although various serum markers and advanced combined tests are used in the evaluation of adnexal masses, the morphological index based on totally non-invasive ultrasonographic findings [where tumor size, volume, and content of the mass (septa structure, papillary projection, heterogeneity) are evaluated] might be the best currently available diagnostic method. In the study conducted by Pavlik et al. [16], the sensitivity of ultrasonography in the detection of malignancy was 73.3%, and the sensitivity of detection of benign masses was 91.3%. In another study conducted on 216 patients diagnosed with ovarian cancer and 144 patients whose benign ovarian tumors were detected, it was concluded that neither the HE4 nor the ROMA test alone was superior to the morphological index (ultrasonography) in the preoperative evaluation of the masses [17]. In our study, the morphological index test, which is based on the ultrasonographic examination of the mass, has a sensitivity of 71.4%, which was close to that of the best performing method (the RMI test).

### Limitations

The person's experience performing the ultrasound can be considered a limitation. However, in this study, ultrasound was performed by the most experienced available specialist.

### Conclusion

In conclusion, the early diagnosis of ovarian epithelial tumors, which are rare but deadly, is important. Early diagnosis for this pathology, for which a screening program has not yet been developed for the healthy population, is an important goal. Although serum markers used alone have been used for this purpose, the results were disappointing, necessitating the development of combined tests. Indeed, the superiority of combined tests is supported in our study and the literature. However, besides these invasive and expensive tests, the malignant-benign differentiation of the masses can be performed successfully in the preoperative period by an ultrasound procedure when performed by an experienced physician.

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# Cerebellum and nucleus caudatus asymmetry in major depressive disorder

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**Ethics Committee Approval**
The study was approved by the RTEU Faculty of Medicine Ethics Committee Decision No. 2020/13.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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## Abstract

**Background/Aim:** The relationship between major depressive disorder (MDD) and specific brain regions was investigated using neuroimaging methods. Although the findings show structural hemispheric asymmetry, research has often focused on the specific brain region involved in MDD. This study aimed to investigate asymmetry in the brain regions of MDD patients for the first time with volBrain, which is a fully automated segmentation technique.

**Methods:** Our study was designed as a case-control study. Structural asymmetry was evaluated using the current web-based fully automated segmentation algorithm, volBrain, that analyzes volumetric T1 axial magnetic resonance imaging data. Sixteen cases with MDD and 14 healthy controls were analyzed. For comparison of continuous data between binary groups, an independent T-test was used for data that follow a normal distribution and Mann–Whitney U (MWU) test was used for data that did not follow a normal distribution while categorical data were evaluated using Chi-square test (or Fisher's exact test when needed).

**Results:** There was no significant difference in terms of gender ($\chi 2$ [1, n = 30] = 0.117, $P$ = 0.732), education level (2 [1, n = 30] = 0.002; $P$ = 0.961] and marital status ($P$ = 0.596, Fisher exact chi-square test). However, both groups were found to be similar in terms of age ($P$ = 0.608, MWU test). Right/left nucleus caudatus volume ratios ($P$ = 0.028, MWU test) and right/left cerebellum volume ratios were significantly smaller in the case group ($P$ = 0.006, independent T-test). When the volumes of the right and left parts were compared, only the volume of the right globus pallidus was larger (statistically significant) in the case group ($P$ = 0.008, independent T-test).

**Conclusion:** In line with our hypothesis, our study supports the notion of cortico–striatal–pallidal–thalamic circuit abnormalities in current MDD research and found that some regions in this phase may contain structural asymmetry. In addition, this study contributed to the literature consisting of studies that have examined the relationship between cerebellum and MDD by adding that the cerebellum may show structural asymmetry. The results of our study suggest that research using volBrain may be beneficial to patients with MDD. Current web-based fully automatic segmentation algorithms can restrict both the rater-induced differences in manual segmentation applications and the differences that various segmentation algorithms can create. The challenge of multicenter research can be overcome by using web-based fully automated segmentation volumetry systems and data containing the same standardized magnetic resonance imaging (MRI) acquisition parameters because it is easy for clinicians around the world to access web-based fully automated segmentation volumetry systems. Research on fully automatic segmentation techniques might be the driving force behind fully understanding biological foundations of MDD in the future.

**Keywords:** Asymmetrical, Cerebellum, Depression, Nucleus caudatus, volBrain

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## Introduction

Major depressive disorder (MDD) is one of the psychiatric disorders with a chronic course that is common throughout an MDD patient's life and is accompanied by severe impairment in functionality [1]. Although many comprehensive studies have been conducted, the etiology of MDD remains uncertain, and research concerning this subject is ongoing [2]. Although the factors that play a role in the development of the disorder cannot be revealed exactly, MDD is thought to be a multifactorial disorder that can be induced by the interaction of biological, psychological, and social factors [3]. Many neuroimaging techniques have been used, including structural magnetic resonance imaging (sMRI), functional MRI (fMRI), magnetic resonance spectroscopy (MRS), diffusion tensor imaging (DTI), positron emission tomography (PET), and near infrared spectroscopy (NIRS) in studies examining the biological basis of MDD [4]. Noninvasive neuroimaging studies have shown that various behavioral patterns seen in MDD patients may be associated with structural and functional abnormalities in specific brain regions [5–7].

Although asymmetry is a common aspect of the human brain, asymmetry abnormalities are a condition that is particularly emphasized in MDD patients and can be demonstrated by MRI studies in some psychiatric disorders [8]. Studies conducted using neuroimaging techniques and designed on the basis of a hypothesis based on the relationship between emotions and brain lateralization have shown that structural and functional asymmetries between hemispheres can be found in MDD patients [9–12]. Numerous studies using neurocognitive, electrophysiological, and neuroimaging measurements in MDD patients provide evidence of functional brain asymmetry abnormalities [13]. Although the hypothesis that many cognitive and emotional functions are performed asymmetrically between the left and right hemispheres has a long history, this hypothesis should still be evaluated [9]. Some researchers think that brain asymmetry reveals different clinical manifestations of different psychiatric disorders as a result of similar differences in brain function [14, 15]. In this context, right hemisphere hyperactivity/left hemisphere hypoactivity stands out as a feature frequently detected in MDD neuroimaging studies [14]. Although hemispheric asymmetry abnormalities have been shown in MDD neuroimaging studies, most neuroimaging studies were established to evaluate detailed structural asymmetry in the whole brain [2, 8, 16]. Anatomical studies in MDD patients report that volumetric changes in gray matter are frequently observed in cortical-limbic areas such as the anterior cingulate cortex, dorsolateral prefrontal cortex, orbitofrontal cortex, amygdala, thalamus and putamen [17–20]. A recent sMRI study showed that abnormalities in structural asymmetry seen in MDD patients involve the cortico–striatal–pallidal–thalamic circuit [14]. Structural hemispheric asymmetry can be a biological marker of MDD as studies showing that this asymmetry is present in patients with MDD in remission, and in babies of mothers with MDD have been published [9]. Recent research has increasingly emphasized the relationship between the cerebellum and MDD; nevertheless, studies examining cerebellar dimensions in MDD are very few [21, 22]. While early MRI studies showed that \cerebellar size decreased in patients with MDD, a recent quantitative MRI study did not reveal a statistically significant difference between MDD and control patients [23]. A voxel-based morphometry study that followed the earlier studies indicated that the left cerebellum gray matter volume may decrease in MDD and the left cerebellar hemisphere may play a role in MDD pathophysiology [24].

Although the gold standard for volumetric measurement of brain structures is considered to be manual segmentation techniques, such procedures have a number of limitations due to different concerns, such as differences between the relevant evaluators and the significant need for anatomical and methodological expertise of the assessor [25, 26]. Manual segmentation requires a significant time requirement that can limit the review and standardization of large data sets [25]. To overcome these limitations, several automatic techniques, which can be segmented into regional computer structures, can be used [27, 28]. Several automatic segmentation algorithms, such as volBrain, FIRST, FSL-ANAT, Freesurfer and MRIcloud, were developed to identify MRI brain data analysis in an objective, reliable, and repeatable manner [25, 29, 30]. Studies have been published showing that the closest and highest accuracy rate of manual segmentation measurements can be achieved by volBrain analysis among fully automated segmentation applications [29, 31]. VolBrain is a web-based MRI brain volumetric system that provides rapid volumetric measurements with reference values based on a contrast free three-dimensional T1 gradient echo image [32].

The purpose of this study was to investigate past findings addressing the relationship between MDD and emotions with emphasis on brain lateralization and especially, regional brain asymmetry, which is emphasized to be observed in cortico–striatal–pallidal–thalamic circuits with the newly developed fully automated segmentation technique, volBrain. In addition, due to the lack of adequate research in this field with the increased emphasis on the recent relationship between the cerebellum and MDD, it was also aimed to evaluate possible cerebellar asymmetry using volBrain in MDD patients in our study. As we know, our research is the first study investigating brain volumes using the volBrain method in MDD. For this reason, it is thought that our study will enable the comparison of volBrain findings related to MDD with the results of sMRI study and contribute to the potential use of volBrain in scientific research.

## Materials and methods

### Participants

Within the scope of our study, the cases recorded between September 25, 2016, and January 22, 2020, in the Psychiatric Outpatient Clinic and Picture Archiving Communication Systems (PACS) of Recep Tayyip Erdogan University (RTEU) Education and Research Hospital were retrospectively examined. In our study, data from recorded cases were examined in our hospital automation system between the specified dates. Those cases diagnosed with MDD (ICD-10: F32.0 in the hospital automation system) based on the structured clinical interview questionnaire (SCID-I) according to DSM-IV criteria were included in the study. Criteria for study inclusion for MDD patients included patients between the ages of 18 and

85, literate, without additional psychiatric, neurological, and/or significant physical disease (cancer, diabetes mellitus, liver failure, renal failure, hypertension, endocrine disease, and others), no history of suicide attempts, not pregnant or lactating, not undergoing medical treatment, and no substance use/abuse. PACS included 16 cases with volumetric axial T1 sequence brain imaging data taken with the 3 Tesla GE Discovery Magnetic Resonance (MR) 750W GEM ENAB device. Brain MRIs are normally reported by specialist radiologists working in the Radiology Department. Similarly, healthy (control) subjects included 14 people who were between the ages of 18 and 85 and literate, without additional psychiatric, neurological, or significant physical disease (cancer, diabetes mellitus, liver failure, renal failure, hypertension, endocrine disease, and others), no history of suicide attempts, not pregnant or lactating, not undergoing medical treatment, and no substance use/abuse. PACS included healthy subjects with volumetric axial T1 sequence brain imaging data obtained with the 3 Tesla GE Discovery Magnetic Resonance (MR) 750W GEM ENAB device. Brain MRIs are normally reported by specialist radiologists working in the Radiology Department. A total of 30 (patients and controls) volumetric axial T1 sequence brain imaging data taken with 3 Tesla GE Discovery Magnetic Resonance (MR) 750W GEM ENAB devices were compared using the volBrain analysis algorithm. The study was carried out in compliance with the Declaration of Helsinki. The study was approved by the RTEU Faculty of Medicine Ethics Committee Decision No. 2020/13. All patients in the study were informed about the study, and their written informed consent was obtained. The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

**Measures and Procedures**

**Psychiatric Evaluation and Data Registration Form**: This form was prepared by our group and aimed to evaluate the compliance of the sociodemographic data of the cases according to the inclusion and exclusion criteria. Sixteen patients who had records in the automation system of the hospital and met the inclusion criteria (ICD-10: F32.0 in the hospital automation system) were included in the study.

**Implementation:** The patients who had psychiatric outpatient application records in the automation system of RTEU Faculty of Medicine Training and Research Hospital and who were diagnosed with MDD based on SCID-I during their application and who were thought to meet the study criteria were identified [33]. Patients who had phone numbers accessed in the hospital file information system received a phone call. Patients who chose to participate in the study during the phone call were invited to the psychiatry outpatient clinic to evaluate the study criteria in detail. Patients found to fulfill the study criteria were included in the study.

**Neuroimaging - MRI acquisition parameters:** Cranial MRI examination was performed using 36-channel head coil with 3 Tesla Discovery MR 750W, GEM-70, (General Electric Company, USA). The volumetric T1 axial (AX 3D T1 BRAVO) image obtaining parameters used for research included several parameters: (1) FOV: 24, Fhase FOV: 1.00, (2) Slice Thickness: 1 mm, (3) EN: 9.0, TE: 3.6, (4) Flip Angle: 12, (5) Frequency:288, (6) Fhase:288, (7) NEX: 1.00, and (8) Bandwith:31.25.

**Volbrain Volumetry Report**

The VolBrain system is an internet-based program that analyzes brain sMRI data automatically, reliably, and cantially. Axial T1 DICOM (Digital Imaging and Communications in Medicine) files were converted to the Neuroimaging Informatics Technology Initiative (NIFI-1) format. Whole brain volumetric analysis was performed by uploading compressed T1-weighted images in NIFTI format to the online "volBrain" MRI brain volumetric system. VolBrain created a PDF report that included an automatic MRI analysis of brain data and the two-sided volumes of the structures and volume information about total intracranial cavities. After an average of 12 min of processing time, volumetric measurements of white matter, gray matter, cerebrospinal fluid, cerebrum, cerebellum, nucleus caudatus, globus pallidus, putamen, nucleus accumbens, thalamus, hippocampus, and amygdala were obtained. The resulting volumes were statistically compared between groups and the possibility of volumetric differences was reviewed. A statistical comparison of the obtained data between the groups was obtained by proportioning the volumes of each case as right/left parts.

**Statistical analysis**

The SPSS 21.0 Statistical Package Program was used to evaluate the data. Descriptive analyses of categorical data were obtained, and the results were expressed as numbers and percentages. A chi-squared test (Fisher's Exact chi-squared test as needed) test was used for comparative evaluation of categorical data. Normal distribution eligibility of continuous data was evaluated with the Kolmogorov–Smirnov test. Mean and standard deviation values of the data that follow a normal distribution and median and quarters clearance values of the data that do not follow a normal distribution are given. For comparison of continuous data between binary groups, an independent T-test was used for data following a normal distribution, and Mann–Whitney U (MWU) test was applied to data that did not follow a normal distribution. A statistically significant *P*-value was accepted as $< 0.05$.

## Results

In our study; volBrain results from 30 individuals, including 16 patient cases and 14 in the control group, were evaluated. No significant difference in terms of gender ($\chi 2$ [1, n = 30] = 0.117; $P = 0.732$), education level (2 [1, n = 30] = 0.002; $P = 0.961$])and marital status ($P = 0.596$, Fisher's exact test) were found (Table 1). However, both groups were found to be similar in terms of age ($P = 0.608$, MWU test). In our study, total brain and total white/gray matter volumes in addition to cerebellum, ventricle, nucleus caudatus, putamen, thalamus, globus pallidus, hippocampus, amygdala, and nucleus accumbens volumes were compared separately for right and left between case and control groups. In terms of these variables, no statistical difference was found between the case and control groups except for the right globus pallidus volume ($P = 0.008$, independent T-test), and the findings are summarized in Table 2. However, the right/left ratios of volumes for each brain region mentioned earlier were also compared between groups.

Significant differences between the case and control groups in terms of right/left ratios of cerebellum ($P$ = 0.006, independent T-test) and nucleus caudatus ($P$ = 0.028, MWU test) volumes were noted. The statistical analysis of the right/left ratios of the volumes of the brain regions is summarized in Table 3.

Table 1: Sociodemographic features of case and control groups

| | Case (n=16) Median (IQR) | Control (n=14) Median (IQR) | *P*-value |
|---|---|---|---|
| Age (Years) | 55.5 (31) | 69 (33) | 0.608[a] |
| | Case (n=16) Number (%) | Control (n=14) Number (%) | *P*-value |
| Gender | | | |
| Male | 9 (56.25%) | 7 (50%) | 0.732[b] |
| Female | 7 (43.75%) | 7 (50%) | $\chi^2$: 0.117 |
| Education Level | | | |
| Below high school | 9 (56.25%) | 8 (57.14%) | 0.961[b] |
| High school and above | 7 (43.75%) | 6 (42.86%) | $\chi^2$: 0.002 |
| Marital Status | | | |
| Married | 11 (68.75%) | 10 (71.43%) | 0.596[c] |
| Other | 5 (31.25%) | 4 (28.57%) | |

IQR: inter-quartile range, [a] Mann–Whitney U Test, [b] Chi-Squared Test, [c] Fisher's Exact Test

Table 2: Volumetric data of different brain structures in case and control groups

| | Case (n=16) Mean (SD) | Median (IQR) | Control (n=14) Mean (SD) | Median (IQR) | *P*-value |
|---|---|---|---|---|---|
| WM Volume ($cm^3$) | 539.98 (81.30) | | 521.74 (81.09) | | 0.847[a] |
| GM Volume ($cm^3$) | 658.61 (114.84) | | 643.22 (73.5) | | 0.186[a] |
| Right Cerebrum Volume ($cm^3$) | 526.6 (61.24) | | 514.29 (58.65) | | 0.879[a] |
| Left Cerebrum Volume ($cm^3$) | 519.79 (59.13) | | 507.23 (57.46) | | 0.997[a] |
| Right Cerebellum Volume ($cm^3$) | | 63.88 (9.95) | | 60.65 (7.54) | 0.423[b] |
| Left Cerebellum Volume ($cm^3$) | | 64.08 (8.99) | | 57.3 (8.84) | 0.179[b] |
| Right Ventricle Volume ($cm^3$) | | 9.05 (9.78) | | 7.23 (32.26) | 0.728[b] |
| Left Ventricle Volume ($cm^3$) | | 10.52 (7.49) | | 10.04 (31.87) | 0.951[b] |
| Right Caudate Volume ($cm^3$) | | 3.3 (0.91) | | 3.47 (1) | 0.240[b] |
| Left Caudate Volume ($cm^3$) | 3.25 (0.5) | | 3.32 (0.48) | | 0.965[a] |
| Right Putamen Volume ($cm^3$) | 3.85 (0.76) | | 3.44 (0.69) | | 0.921[a] |
| Left Putamen Volume ($cm^3$) | 3.71 (0.85) | | 3.66 (0.63) | | 0.242[a] |
| Right Thalamus Volume ($cm^3$) | 5.32 (1.24) | | 5.16 (0.76) | | 0.147[a] |
| Left Thalamus Volume ($cm^3$) | 5.34 (1.46) | | 5.27 (0.84) | | 0.078[a] |
| Right GP Volume ($cm^3$) | 0.98 (0.2) | | 0.92 (0.38) | | 0.008[a] |
| Left GP Volume ($cm^3$) | 0.95 (0.32) | | 0.92 (0.3) | | 0.260[a] |
| Right Hippocampus Volume ($cm^3$) | 3.64 (0.61) | | 3.82 (0.53) | | 0.781[a] |
| Left Hippocampus Volume ($cm^3$) | 3.55 (0.69) | | 3.65 (0.37) | | 0.188[a] |
| Right Amygdala Volume ($cm^3$) | 0.54 (0.25) | | 0.54 (0.18) | | 0.209[a] |
| Left Amygdala Volume ($cm^3$) | 0.57 (0.26) | | 0.51 (0.2) | | 0.249[a] |
| Right NA Volume ($cm^3$) | | 0.3 (0.1) | | 0.29 (0.22) | 0.790[b] |
| Left NA Volume ($cm^3$) | | 0.34 (0.06) | | 0.36 (0.14) | 0.697[b] |

SD: standard deviation, IQR: inter-quartile range, WM: white matter, GM: gray matter, GP: globus pallidus, NA: nucleus accumbens, [a] Independent T-Test, [b] Mann–Whitney U Test

Table 3: Asymmetry parameters of case and control groups

| | Case (n = 16) Mean (SD) | Median (IQR) | Control (n = 14) Mean (SD) | Median (IQR) | *P*-value |
|---|---|---|---|---|---|
| Right/Left Cerebrum Ratio | | 1.01 (0.03) | | 1.01 (0.03) | 0.759[a] |
| Right/Left Cerebellum Ratio | 1.01 (0.03) | | 1.02 (0.08) | | 0.006[b] |
| Right/Left Ventricle Ratio | | 0.89 (0.28) | | 0.95 (0.34) | 0.790[a] |
| Right/Left Caudate Ratio | | 0.96 (0.1) | | 1.03 (0.15) | 0.028[a] |
| Right/Left Putamen Ratio | | 0.99 (0.09) | | 0.98 (0.17) | 0.294[a] |
| Right/Left Thalamus Ratio | | 0.98 (0.14) | | 0.99 (0.09) | 0.854[a] |
| Right/Left GP Ratio | | 1.01 (0.28) | | 1.06 (0.19) | 0.552[a] |
| Right/Left Hippocampus Ratio | 1.04 (0.1) | | 1.05 (0.09) | | 0.642[b] |
| Right/Left Amygdala Ratio | | 0.92 (0.2) | | 1.01 (0.33) | 0.052[a] |
| Right/Left NA Ratio | | 0.86 (0.16) | | 0.94 (0.23) | 0.525[a] |

SD: standard deviation, IQR: inter-quartile range, WM: white matter, GM: gray matter, GP: globus pallidus, NA: nucleus accumbens, [a] Independent T-Test, [b] Mann–Whitney U Test

## Discussion

In our study, the volumetric measurements ($cm^3$) of the brain regions and the right/left brain ratios in MDD patients were investigated using volBrain, which is a fully automated segmentation technique. Comparison results of the right/left nucleus caudatus and right/left cerebellum volume ratios were found to be statistically significant. When the total volumes of the brain regions and the volume measurements of each of the right and left parts were compared, it was found that only the right globus pallidus volume was larger compared to the control group. In a recent Enhancing Neuro-Imaging Genetics Through Meta-Analysis (ENIGMA) consortium study in which 2,540 MDD cases and 4,230 control patients were included from 32 data sets in which the volumes of brain regions were compared, the presence of structural brain asymmetries in MDD was investigated. Although that study had a fairly large sample size, no significant difference was found between cerebral cortical and subcortical volumes between groups in the ENIGMA study [8]. Similar to our comprehensive study in the literature, the brain region volume results of our study were similar to the ENIGMA consortium study except that the right globus pallidus volumes of the case group were larger than the control group. This finding is remarkable in terms of representing the suitability of the use of the volBrain system in MDB.

In the ENIGMA study, it was thought that direct comparison of the brain region volumes of the cases may have had a cumulative effect. For this purpose, the comparison of the right/left volume ratios of the brain regions in our study was also designed to reveal the case-specific asymmetry bias. In this context, the differences between the groups of right/left nucleus caudatus and cerebellum volume ratios are remarkable in our study. When our results were analyzed, it was found that the right/left nucleus caudatus and right/left cerebellum ratios of the case group were lower than those of the control group. These results reveal an asymmetry in these brain regions of the case group in which the right parts are smaller than the left ones. The lack of significant differences in the results of our direct volumetric comparisons can be explained by the fact that asymmetry is not secondary to shrinkage or growth, that is, it may be a structural feature. When the meta-analysis of the studies conducted with functional neuroimaging techniques in the literature was analyzed, it was shown that the bilateral

caudatus gave less response to reward stimuli in patients with MDD [6, 34]. Hypoactivation of the right caudate during the processing of positive stimuli in MDD patients has also been reported in previous studies [10]. A recent sMRI study indicated that the total caudate nucleus size is smaller in MDD patients compared to the control group; in addition, the right part of the caudate nucleus shrinks more than the left [12]. In the same study, it was also stated that further studies are needed to investigate inter-hemispheric imbalances, assuming shrinkage in the right caudate nucleus may be due to asymmetry in cortical and subcortical volumes in MDD [12]. MRI-based manual segmentation studies found different results consisting of no decrease or change in nucleus caudatus volumes in individuals with MDD compared to healthy subjects [35, 36]. Our study shows that no change in the nucleus caudatus volume between the groups could be found. Asymmetry was detected when comparing the right/left nucleus caudatus ratios between the groups.

Considering the recent increase in the number of studies examining the relationship between MDD and cerebellum, it is interesting that our study found a significant difference in the right/left cerebellum ratios between the case and control groups. Recent studies emphasize the importance of investigating the relationship between basal ganglia and cerebellum volumes in patients with/without MDD [21, 22, 37]. In this relationship, it is assumed that the basal ganglia and cerebellum affect the cortical activity separately by different thalamic pathways during the first period [38]. However new evidence of the existence of direct subcortical connections between these two structures, it is accepted that they have the capability of affecting motor, cognitive, and limbic functions together [22]. In the first study defining the structural bond between basal ganglion and cerebellum in non-human primates, it was shown that the dentate nucleus of the cerebellum projects to the intralaminar nuclei of the thalamus and then to the striatum and outer globus pallidus using the transneuronal viral tracing method accompanied by marking of the neurons in the dentate nucleus [39]. From this point of view, the results presenting the difference in the right/left cerebellum, nucleus caudatus ratios, and right globus pallidus volumes detected in our study may be important. Direct connections between the basal ganglia and the cerebellum suggest that these regions work together to modulate processes, such as motor control and emotion recognition or expression [40]. In addition, it has been frequently emphasized in recent studies that the basal ganglia and cerebellum have common effects in regulating and exhibiting response selection, and reward feedback [22, 40]. However, as our study showed, neuroimaging studies examining cortico–striatal–pallidal–thalamic circuits in MDD patients indicate the presence of abnormalities concerning these regions [41–43]. The abnormalities of the right globus pallidus and nucleus caudatus (one of the structures of corpus striatum) may reflect the relationship of cortico–sriatal–pallidal–thalamic circuits in patients with MDD, a finding that has been emphasized in previous research. In addition, showing the right/left asymmetry of the cerebellum in our study is thought to shed light on the possible relationship between these circuits and the cerebellum. It is important that our study is the first comprehensive study carried out with the volBrain application in MDD patients.

**Limitations and strengths**

Relatively small sample size, single-center conduct of the study, retrospective design, older age of the patients selected for the study, and confounding effects of drugs can be counted as limitations of this study; however, the use of a fairly new segmentation method can make this study valuable. This information will form an important publically available pipeline for MDD-related structure segmentation, and it is hoped that it will allow researchers to better analyze their data in an easy to use, yet accurate and efficient, manner. This process suggests that the proposed method can be readily applied under different research and clinical conditions although a much larger validation will be required. In addition, the use of a web-based fully automatic segmentation technique, which is accessible, can limit the differences that can be created by various fully automatic segmentation algorithms in addition to the differences in manual segmentation applications due to different raters.

Future cross-sectional multicenter studies should not include different segmentation techniques and MRI acquisition parameters. The challenge of multicenter research can be overcome by using web-based fully automated segmentation volumetry systems and the use of data containing the same standardized MRI acquisition parameters because it is easy for clinicians around the world to access web-based fully automated segmentation volumetry systems.

**Conclusion**

In conclusion, in line with our hypothesis, our study supported the notion of cortico–striatal–pallidal–thalamic circuit abnormalities in current MDD research and found that some regions in this phase may contain structural asymmetry. In addition, this study contributed to the literature by adding information about structural asymmetry in the cerebellum to the studies examining the relationship between cerebellum and MDD. From this point of view, the use of volBrain, which presents volumetric findings compatible with the literature related to MDD, is also promising. Research on fully automatic segmentation techniques could become the driving force behind fully understanding the biological foundations of MDD in the future. Successful implementation of Volbrain in our study suggests that Volbrain may be an important part of clinical applications in many other neuropsychiatric disorders in the near future.

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# The healing effects of *Ganoderma lucidum* on intestinal ischemia-reperfusion damage in rats

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**Ethics Committee Approval**
Kırıkkale University Animal Experiments Local Ethics Committee, 01.03.2017, 17/02.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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## Abstract

**Background/Aim:** Intestinal ischemia-reperfusion (I/R) injury causes serious clinical problems and carries high morbidity and mortality risks. *Ganoderma lucidum* (GL) is known as an anti-inflammatory immunomodulator and antioxidant. This study aimed to investigate the curative effects of GL on intestinal I/R injury in rats.

**Methods:** Twenty-four Wistar-Albino male rats were randomly divided into three groups. After 30/90 minutes of I/R, intestinal and hepatic tissue examples were histologically examined. Biochemical analysis, serum, intestinal and hepatic tissue malondialdehyde (MDA), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) levels were measured.

**Results:** The I/R group had significantly elevated leukocyte, thrombocyte, serum, and intestinal and hepatic tissue MDA levels compared to the sham group ($P < 0.05$). Serum, intestinal tissue, and liver tissue SOD levels were significantly lower in the I/R group than in the control group ($P < 0.05$). In addition, GSH-Px levels measured in serum and liver tissue were lower in the I/R group. However, the use of GL prevented these decreases due to I/R damage. Prior administration of GL considerably alleviated histopathologic changes due to I/R injury in the intestinal and liver tissue samples.

**Conclusions:** Our experimental study showed that I/R injury led to significant oxidative stress by inducing free oxygen radicals in intestinal and hepatic tissues. Serum and tissue MDA, SOD, and GSH-Px levels were considerably useful laboratory parameters in identifying oxidative stress. The protective effects of GL on intestinal I/R injury were promising considering these parameters.

**Keywords:** Antioxidative, Anti-inflammatory, Immunomodulator, Intestinal ischemia-reperfusion, *Ganoderma lucidum*

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## Introduction

The intestinal ischemia-reperfusion (I/R) injury may result from a variety of clinical conditions, such as intestinal obstruction, mesenteric arterial occlusion, hemodynamic shock, and surgical interventions. Factors such as free oxygen radicals, neutrophils activation, and xanthine oxidase enzyme systems seem to be related to tissue damage due to I/R injury [1]. Leukocyte-endothelial interactions lead to excessive overproduction of pro-inflammatory cytokines and free oxygen radicals. Intestinal tissue damage caused by I/R injury triggers local and systemic inflammatory responses. Multiorgan failure is one of the most catastrophic outcomes of intestinal tissue damage [2].

*Ganoderma lucidum* (GL) has been used for various purposes for more than 2,000 years [3]. It has been demonstrated to be effective in cerebral I/R injury [4], renal I/R injury [5], and cardiac I/R injury [6]. It has also been reported that there are effective immunomodulators, antioxidants, as well as more than 100 isolated molecules with chemopreventive and tumoricidal properties in GL [7-9]. Our experimental study aimed to evaluate the possible ameliorative effects of GL on intestinal I/R injury.

## Materials and methods

After obtaining approval from the ethics committee (Kırıkkale University Animal Experiments Local Ethics Committee, 01.03.2017, 17/02), 24 healthy males (250-300 gr) Wistar-Albino rats were randomly divided into three groups: control, I/R injury, and I/R injury with GL treatment (I/R+GL). All rats were kept under a 12-hour cycle (12 hours of dark and light), fed ad libitum for two weeks, and deprived of food 12 hours before the experiment but allowed to drink water. Seven days before the I/R injury, GL treatment group rats were administered with 250 mg/kg body weight GL dissolved in 2 ccs of saline by orogastric lavage. Before the surgical procedure, all rats were anesthetized by intramuscular administration of 5-10 mg/kg xylazine hydrochloride (Rompun®, Bayer-Istanbul), and 50-70 mg/kg dose of ketamine hydrochloride (Ketalar®, Pfizer Istanbul). After shaving the abdomen of all rats, a midline incision was performed, and the abdomen was entered after local cleaning with a 10% povidone-iodine solution and sterile dressing.

The only laparotomy was performed to examine the superior mesenteric artery in the control group of rats. In the I/R+GL and I/R groups, the superior mesenteric artery was clamped for 30 minutes and released for 90 minutes to induce I/R injury. Tissue and blood samples were harvested at the end of 90 minutes, and all animals were decapitated.

**Blood analysis:** The blood samples taken from the rats were centrifuged at 3,000 xg for 10 minutes. The blood samples were sampled in the Eppendorf tubes and kept at -80 C for use on the next working day. On the next working day, the blood samples were brought to room temperature, and the frozen serums were allowed to melt. SOD, MDA, and GSH-Px levels in serum samples were measured by the ELISA method. Also, blood thrombocyte, leukocyte, and hemoglobin levels were studied.

**Biochemical tissue analysis:** Intestinal and liver tissue samples taken for the study were homogenized with PBS (Phosphate Buffer Saline, pH: 7.4) solution. The amount of total protein in all tissue samples was measured by the Bradford method with the aid of a spectrophotometer. The values of MDA, GSH-Px, and SOD parameters in tissue homogenates were measured by the ELISA method in a plate reader (Thermo Scientific Multiskan FC, 2011-06, USA).

**Histopathologic examination of intestinal and liver tissue samples:** Tissue samples taken from the intestine and liver were fixed in 10% formaldehyde. Samples were kept in fixation for 24 hours. After determination, for the evaluation of the ileum, a 1-cm longitudinal section was taken from each resection material and placed on blotter paper, and the tissue was followed. Approximately a 1-cm sample was taken from the liver tissues and dried. It was embedded in paraffin after routine tissue procedures. Sections of 5-micron thickness were taken from paraffin blocks. They were then stained with hematoxylin-eosin. All preparations were examined by the same pathologist under the same light microscope (Olympus BX53F-Japan). Intestinal tissue samples were evaluated histopathologically according to the classification mentioned in the study of Chiu et al. [10]. Histopathologic changes in the liver tissue, such as obstruction, leukocyte infiltration, sinusoidal dilatation, and vacuolar degeneration were evaluated. Histopathologic examination of the samples using the same light microscope was performed by the same pathologist unaware of the study groups.

**Statistical analysis**

All results were statistically analyzed using SPSS 18.0 windows package program. Numeric variables were expressed as mean (standard deviation). The differences among the groups in terms of parameters were analyzed using analysis of variance (ANOVA) with the Tukey post hoc test. In paired comparisons of the groups, the Mann Whitney U and Kruskal Wallis tests were used. $P < 0.05$ was accepted as significant.

## Results

**Hematologic parameters**

The mean leukocyte levels were $6.21x10^3$ uL in the control group, $8.55x10^3$ uL in the I/R group, and $5.56x10^3$ uL in the I/R+GL group. All groups were compared in terms of leukocyte levels. The leukocyte level of the I/R group was significantly higher than that of the control group ($P = 0.001$). When the I/R+GL group and the control group were compared, the difference was found to be statistically insignificant. When the I/R group and I/R+GL were compared, the leukocyte level of the I/R group was found to be significantly higher than that of the I/R+GL group ($P = 0.001$). Platelet mean levels were $894.75x10^3$ uL in the control group, $1066.63x10^3$ uL in the I/R group, and $960.38x10^3$ uL in the I/R+GL group, with the I/R group having significantly high levels ($P = 0.024$). When the I/R group and the I/R+GL group were compared, platelet levels of the I/R+GL group were found to be significantly lower than those of the I/R group ($P = 0.044$) (Figure 1).

Figure 1: A-The leukocyte levels of the study groups. B- The hemoglobin levels of the study groups C- The platelet levels of the study groups. D- Serum MDA levels of the study groups E- Serum GSH-Px levels of the study groups. F- Serum SOD levels of the study groups

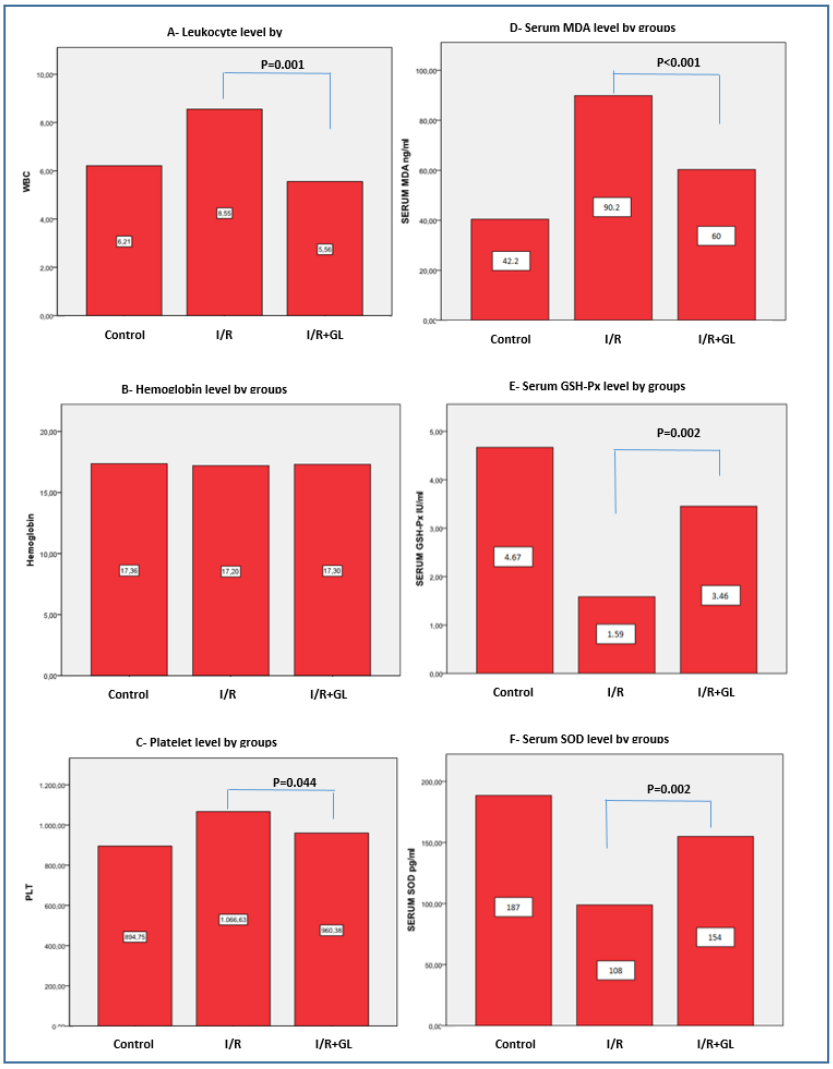
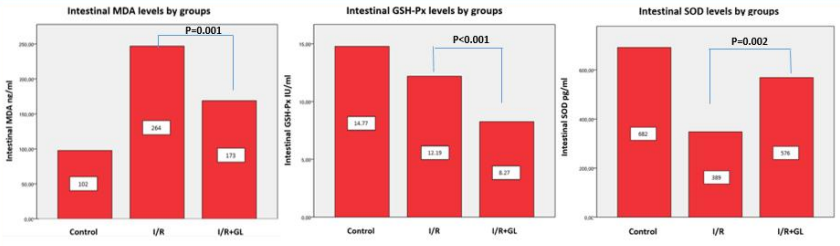
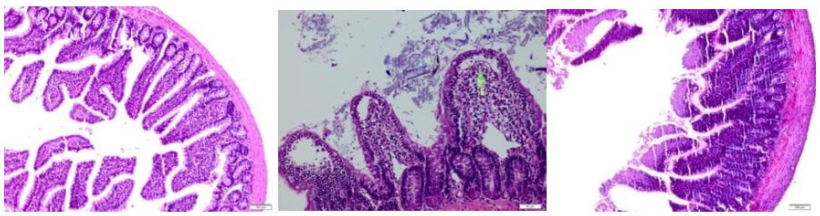



Figure 2: Intestinal tissue MDA, GSH-Px, and SOD levels of the study groups and histopathologic changes according to Chiu's classification of the study groups.



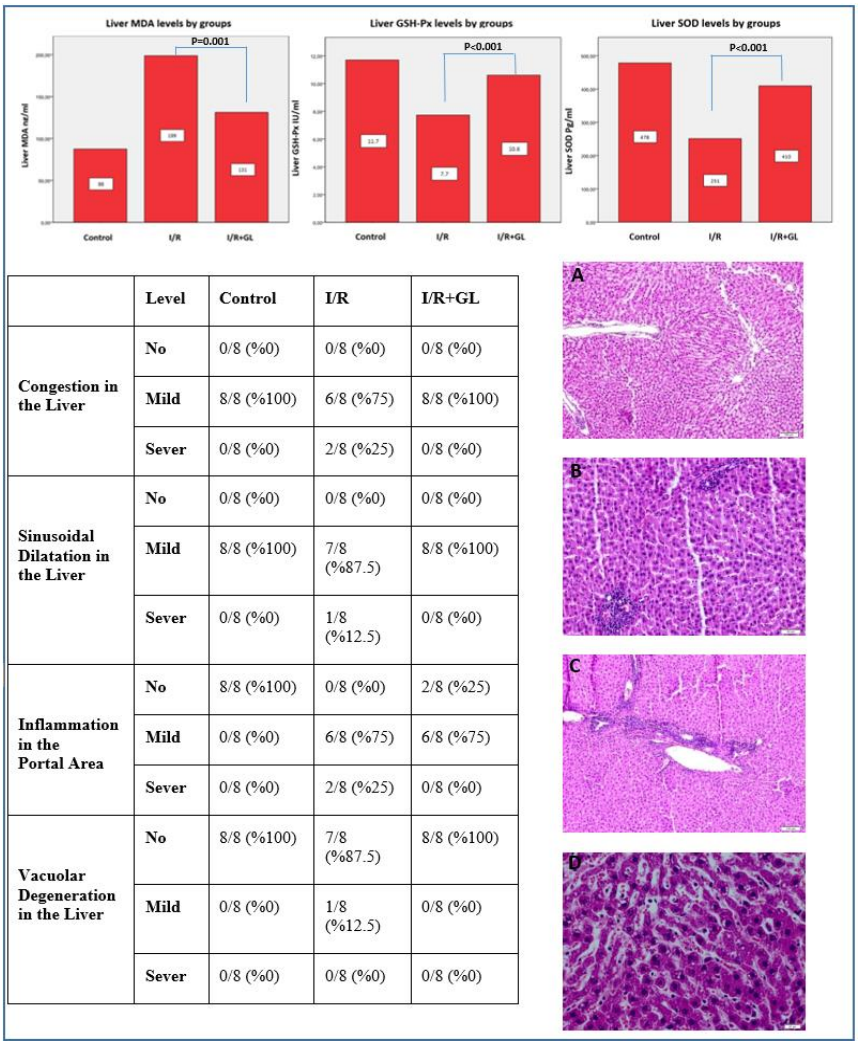

| Groups | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Grade 4 | Grade 5 | Total |
|---|---|---|---|---|---|---|---|
| Countrol | 8 | | | | | | 8 |
| I/R | 0 | 6(%75) | 2(%25) | | | | 8 |
| I/R+GL | 4(%50) | 4(%50) | | | | | 8 |

| Chiu | Mucosal Changes |
|---|---|
| Grade 0 | Normal mucosal villi. |
| Grade 1 | Development of subepitelial Gruenhangen's space, usually at the apex of the villus; often with capillary congestion. |
| Grade 2 | Extension of the subepithelial space with moderate lifting of epithelial layer from the lamina propria. |
| Grade 3 | Massive epithelial lifting down the sides of villi. A few tips may be denuded. |
| Grade 4 | Denuded villi with lamina propria and dilated capillaries exposed. Increased cellularity of lamina propria may be noted. |
| Grade 5 | Digestion and disintegration of lamina propria; hemorrhage and ulceration. |



Chiu Grade 0 Chiu Grade 1 Chiu Grade 2

Figure 3: The MDA, GSH-Px, and SOD levels in the liver tissue samples of the study groups, and histopathologic changes in the liver tissue samples of the study groups. A-İntense sinusoidal dilatation and mild congestion with no portal inflammation (Hematoxylin & eosin staining, x100, original magnification), B-Mild sinusoidal dilatation, congestion, and portal inflammation (Hematoxylin & eosin staining, x200, original magnification), C-Intense portal inflammation, (Hematoxylin & eosin staining, x100, original magnification), D-Vacuolar changes (Hematoxylin & eosin staining, x400, original magnification)




| | Level | Control | I/R | I/R+GL |
|---|---|---|---|---|
| Congestion in the Liver | No | 0/8 (%0) | 0/8 (%0) | 0/8 (%0) |
| | Mild | 8/8 (%100) | 6/8 (%75) | 8/8 (%100) |
| | Sever | 0/8 (%0) | 2/8 (%25) | 0/8 (%0) |
| Sinusoidal Dilatation in the Liver | No | 0/8 (%0) | 0/8 (%0) | 0/8 (%0) |
| | Mild | 8/8 (%100) | 7/8 (%87.5) | 8/8 (%100) |
| | Sever | 0/8 (%0) | 1/8 (%12.5) | 0/8 (%0) |
| Inflammation in the Portal Area | No | 8/8 (%100) | 0/8 (%0) | 2/8 (%25) |
| | Mild | 0/8 (%0) | 6/8 (%75) | 6/8 (%75) |
| | Sever | 0/8 (%0) | 2/8 (%25) | 0/8 (%0) |
| Vacuolar Degeneration in the Liver | No | 8/8 (%100) | 7/8 (%87.5) | 8/8 (%100) |
| | Mild | 0/8 (%0) | 1/8 (%12.5) | 0/8 (%0) |
| | Sever | 0/8 (%0) | 0/8 (%0) | 0/8 (%0) |

**Oxidative stress-related parameters: Serum and tissue MDA levels**

The mean serum MDA level was 42.2 ng/ml in the control group, 90.2 ng/ml in the I/R group, and 60 ng/ml in the I/R+GL group (Table 1). When the control group and the I/R group were compared, the difference was found to be significant ($P < 0.05$). When the control group and the I/R+GL group were compared, the difference was also found to be significant ($P < 0.05$). Serum MDA levels were found to be significantly lower in the GL treatment group ($P < 0.05$). Mean intestinal tissue MDA levels were measured as 101.87 ng/ml in the control group, 263.6 ng/ml in the I/R group, and 173.3 ng/ml in the I/R+GL group (Table I). Intestinal MDA levels were found to be significantly ($P < 0.05$) higher in the I/R group. Intestinal MDA levels of the GL treatment group were significantly ($P < 0.05$) lower than those of the I/R group. Mean MDA levels measured in liver tissues were 87.57 ng/ml in the control group, 198.84 ng/ml in the I/R group, and 131.23 ng/ml in the I/R+GL group (Table 1). The liver MDA level of the I/R group was significantly higher than that of the control group ($P < 0.05$). The liver MDA level of the I/R+GL group was significantly higher than that of the control group ($P = 0.023$). When the I/R group and the I/R+GL group were compared, it was seen that the liver MDA level of the I/R+GL group was lower than that of the I/R group, and this difference was statistically significant ($P < 0.05$) (Figure 1, 2, 3).

**Antioxidant system-related parameters: Serum and tissue SOD and GSH-Px levels**

The mean serum GSH-Px level was measured as 4.67 IU/ml in the control group, 1.59 IU/ml in the I/R group, and 3.46 IU/ml in the I/R+GL group (Table 1). The serum GSH-Px level of the I/R group was significantly ($P < 0.05$) lower, while it was significantly higher in the I/R+GL group ($P < 0.05$). Mean intestinal tissue GSH-Px levels were measured as 14.77 IU/ml in the control group, 12.19 IU/ml in the I/R group, and 8.27 IU/ml in the I/R+GL group (Table I). The I/R group presented significantly lower intestinal tissue GSH-Px levels than the control group ($P < 0.05$). When the control group and the I/R+GL group were compared, $P < 0.05$ was found: the intestinal tissue GSH-Px level of the I/R+GL group was found to be significantly lower than that of the control group. When the I/R group and the I/R+GL group were compared, it was observed that the serum GSH-Px level of the I/R+GL group was lower than that of the I/R group, and the difference was statistically significant ($P < 0.05$). The mean liver tissue GSH-Px levels were measured as 11.7 IU/ml in the control group, 7.7 IU/ml in the I/R group, and 10.6 IU/ml in the I/R+GL group (Table 1). Liver Tissue GSH-Px levels were significantly lower in the I/R and I/R+GL groups compared to the control group. However, liver tissue GSH-Px levels were significantly lower in the I/R group than in the I/R+GL group ($P < 0.05$) (Figure 1, 2, 3)

Table 1: The MDA, GSH-Px, and SOD levels in serum, intestinal tissue, and liver tissue by groups (Control, I/R, I/R+GL)

| | Serum | | | Intestinal Tissue | | | Liver Tissue | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | I/R | I/R+GL | Control | I/R | I/R+GL | Control | I/R | I/R+GL |
| SOD (pg/ml) | 187 | 108 | 154 | 682 | 389 | 576 | 478 | 251 | 410 |
| GSH-Px(IU/ml) | 4.67 | 1.59 | 3.46 | 14.77 | 12.19 | 8.27 | 11.7 | 7.7 | 10.6 |
| MDA (ng/ml) | 42.2 | 90.2 | 60 | 102 | 264 | 173 | 88 | 199 | 131 |

I/R: Ischemia-reperfusion, GL: Ganoderma lucidum, SOD: Superoxide dismutase, GSH-Px: Glutathione peroxidase, MDA: Malondialdehyde.

Mean serum SOD levels were 187 pg/ml in the control group, 108 pg/ml in the I/R group, and 153 pg/ml in the I/R+GL group (Table 1). The serum SOD level of the I/R group was found to be significantly lower than that of the control group ($P < 0.05$). When the control group and the I/R+GL group were compared, $P < 0.05$ was found: a significant difference was noted. The serum SOD level of the I/R+GL group was noted as significantly ($P < 0.05$) higher than that of the I/R group. Mean intestinal tissue SOD levels were measured as 682 pg/ml in the control group, 389 pg/ml in the I/R group, and 576 pg/ml in the I/R+GL group (Table 1). When the control group and the I/R group were compared, a significant difference was noted ($P < 0.05$). When the I/R group and the I/R+GL group were compared, it was observed that the intestinal SOD level of the I/R+GL group was significantly higher ($P < 0.05$). Mean liver tissue SOD levels were measured as 478 pg/ml in the control group, 251 pg/ml in the I/R group, and 410 pg/ml in the I/R+GL group (Table 1). When the control group and the I/R group were compared, a significant difference was observed ($P < 0.05$). When the I/R group and the I/R+GL group were compared, it was observed that the liver SOD level of the I/R+GL group was significantly ($P < 0.05$) higher. (Figures 1, 2, 3)

**Histopathologic changes in the intestinal tissue samples of the study groups**

Changes in the intestinal tissue of the groups were evaluated according to Chui's classification [10]. The villi in all rats in the control group were evaluated as normal (Chui Grade 0). Enlargement of the subepithelial area and capillary congestion at the apex of villi (Chui Grade 1) were observed in six (75%) rats in the I/R group. In two (25%) rats, it was observed that the subepithelial area was enlarged, and the epithelial layer was separated from the lamina propria moderately (Chui Grade 2). While villi were evaluated as normal in four (50%) rats in the I/R+GL group (Chui Grade 0), enlargement in the subepithelial area and capillary congestion in the apex of the villi (Chui Grade 1) were detected in four (50%) rats in the I/R+GL group (Figure 2).

**Histopathologic changes in the liver tissue samples of the study groups**

There was severe and mild congestion in the liver of two (25%) and six (75%) rats, respectively, in the I/R group. All the rats in the I/R+GL group showed mild congestion. In the I/R group, mild sinusoidal dilatation was observed in seven (87.5%) rats, while severe dilatation was observed in one (12.5%) rat. In the I/R+GL group, mild sinusoidal dilatation was observed in all rats. In the I/R group, inflammation in the portal area was severe in only two (25%) rats and mild in six (75%) rats. In the I/R+GL group, two (25%) rats had no signs of inflammation, while 6 (75%) had mild signs of inflammation (Figure 3).

## Discussion

Complement activation, activation of pro-inflammatory and inflammatory cytokines, and the emergence of free oxygen radicals are important steps in the chain of events that trigger each other and can lead to organ damage during an I/R injury. Especially toxic release due to the endothelial relationship with neutrophil activation, xanthine oxidase enzyme system, cytokines, and free oxygen radicals are the main factors responsible for tissue damage [11]. The intestinal tissue is one of the tissues sensitive to I/R injury. An I/R injury in the intestinal tissue carries high morbidity and mortality risks [12, 13]. Many experimental studies have been conducted to explain the complex interactions and reduce the unfavorable effects of I/R injuries. We preferred the intestinal I/R experimental model to evaluate the protective effects of GL in our study.

Ischemia and reperfusion times vary in different studies. Laurens et al. performed a 60-minute ischemia duration followed by a 60-minute reperfusion duration in their experimental study [14]. Yoshida et al. applied a 20-minute and 40-minute ischemia duration followed by 180-minute reperfusion in their experimental I/R model [15]. In our study, we applied 30-min ischemia and 90-min reperfusion to the superior mesenteric artery in rats to induce the intestinal I/R injury.

GL is represented with anti-inflammatory, antidiabetic, immunomodulatory, anti-inflammatory, antibacterial, neuroprotective, antioxidative, and antitumor properties in the literature [16]. It has also been shown as an effective agent against I/R injury with, particularly, anti-inflammatory and immunomodulatory properties. The healing effects of GL were reported in the studies focusing on I/R injuries in tissues such as renal [5], cardiac [6], and cerebral [4]. Our study is the first study to investigate the possible protective effects of GL on intestinal I/R injury in rats. GL was administered orally at a dose of 250 mg/kg in the treatment group of rats. The effectiveness of this dose has been shown in the literature [17].

Hematologic changes such as blood platelet, leukocyte, and hemoglobin levels are commonly used in both experimental intestinal I/R models and clinical follow-up and investigation of the efficacy of therapeutic agents. It has been reported that leukocyte and platelet levels increase in rats with I/R injury [18]. Our findings in our I/R injury group rats were consistent with these findings. WBC levels were found to be significantly high in the I/R group rats, whereas WBC levels in the GL treatment group rats were close to normal levels. Normal WBC levels in the rats administered with GL before I/R injury seem to be related to the immunomodulatory and anti-inflammatory properties of GL. Increased platelet level is an important marker in terms of identifying the degree of tissue damage after I/R injury.

Previous studies showed that I/R injury led to an increase in platelet levels in the early period of the injury. Increased platelet levels seem to be responsible for the damage to the intestinal villi [19]. However, the mechanism of these changes is still unknown. Our results showed that the platelet levels of the GL treatment group animals were lower than those of the I/R group animals.

We used MDA levels as one of the biochemical parameters of I/R injury in our study. Zhong et al. [5] examined

kidney MDA levels as an indicator of I/R injury in a study that investigated the oxidative stress preventive role of GL polysaccharides (GL-PS) in kidney I/R injuries. They found that MDA levels in the group with I/R injury were higher than in the group administered with GL-PS. They reported the positive effects of GL-PS on kidney I/R injury. A study by Zhonghui et al. [20] investigated the effects of GL-PS on I/R injury in skeletal muscles. To evaluate the antioxidant activity of GL-PS, they studied tissue MDA levels as an indicator of lipid peroxidation resulting from ischemia in skeletal muscles, and SOD, GSH-Px, and catalase levels in tissue. They found that GL-PS effectively reduced MDA levels in the skeletal muscle of mice and showed that high dose GL (200 mg/kg) showed a better effect. The authors concluded that GL reduced lipid peroxidation and prevented exercise-induced oxidative damage. In this study, we studied MDA levels in serum, intestinal, and liver tissue samples. The fact that MDA values increased significantly in serum, intestinal, and liver samples in the I/R group compared to the control group showed that I/R injury was fully developed in our experimental model. Serum MDA levels were normal in the GL treatment group rats, and these results showed that GL considerably reduced lipid peroxidation and had a protective effect against tissue damage due to I/R injury. Our study also examined the antioxidant activity of GL treatment by measuring GSH-Px and SOD levels in intestinal and liver tissue samples and serum. Significantly decreased GSH-Px and SOD levels were noted in tissue samples and serum of I/R injury group animals. Oxidative stress was achieved with our experimental model in our study. When all groups were compared in terms of SOD and GSH-Px levels, it was observed that the SOD levels of the GL treatment group were higher than those of the I/R group and closer to those of the control group. The antioxidant activity of GL was attributed to an increase in the antioxidant system enzyme of the SOD level. Our results showed that the administration of GL before I/R injury considerably alleviated the decrease in GSH-Px and SOD levels, especially in the liver tissue and serum.

It has been shown that the release of lysosomal hydrolase enzymes and increased microvascular permeability occur after intestinal I/R injury and lead to edema, bleeding, necrosis, flattening of the villi, and disruption in mucosal integrity [11]. The nature of the injury is related to the type of tissue. Intestinal tissue is the most vulnerable tissue to I/R injury [13, 21]. Our findings were consistent with these findings in the I/R injury group rats. The intestinal venous system drains into the portal venous system. An important part of the oxygenation of the liver tissue is provided by the portal venous system. It is known that mesenteric ischemia can affect the liver by reducing portal vein blood flow [22]. Therefore, in this study, we examined histopathological changes in intestinal tissue as well as serum and liver samples.

It has been reported that GL treatment reduces lipid peroxidation and has a healing effect on tissue ischemic damage. Chen et al. [23] investigated intestinal MDA levels as an indicator of oxidative stress and found that GL-PS reduced intestinal MDA levels in a study in which they examined the healing effects of GL-PS on small intestinal damage in mice developed after the use of methotrexate. Our results showed that GL treatment reduced intestinal tissue damage due to I/R injury.

Lin et al. [24] investigated the healing effects of GL on carbon tetrachloride-induced hepatic fibrosis in rats. They found that carbon tetrachloride induced hepatic fibrosis in rats and significantly increased MDA and hydroxyproline concentrations. They also noted that the GL extract reduced the hepatic MDA and that hydroxyproline levels increased by carbon tetrachloride. In our study, histopathologic examination of the liver tissue samples of the GL treatment group rats showed that liver tissue was considerably preserved from I/R injury. MDA levels were also consistent with these findings.

**Conclusions**

Early diagnosis and treatment of acute mesenteric ischemia remain a challenging problem due to the lack of a reliable, sensitive, and specific parameter. Additionally, there is no therapeutic agent that can be effective in the progression of organ failure caused by a delay in diagnosis.

Our results showed that GL, with its anti-inflammatory, immunomodulatory, and antioxidant properties, had significant healing effects on preventing I/R injury damage in the intestinal tissue and eliminating its unfavorable effects on the liver tissue. Our experimental study is the first to examine the effects of GL on intestinal tissue damage resulting from I/R injury. There is a need for further investigations to identify the biochemical mechanisms of the healing effects of GL on I/R injury.

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# The effects of combined hydroxychloroquine and azithromycin therapy on QRS wave in COVID-19 patients

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**Ethics Committee Approval**
The study was approved by the ethical committee of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital (No: 2020-06/681).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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## Abstract

**Background/Aim:** Hydroxychloroquine and azithromycin are frequently used for the treatment of coronavirus disease 2019 (COVID-19). The use of these medications increases the risk of adverse cardiovascular events. The aim of our study was to investigate the effects of these drugs on the arrhythmogenic electrocardiographic (ECG) markers, QRS duration, and QRS dispersion, and also to evaluate gender differences with respect to these effects.

**Methods:** Between March and June 2020, 107 (54 males, 53 females) patients admitted to our hospital's isolation ward with COVID-19 diagnosis with no history, risk factors, or clinical findings of cardiovascular diseases were included in this prospective cohort study. All participants had a mild illness, and none of them required intensive care unit admission. ECGs of the patients were recorded before starting treatment with combined hydroxychloroquine and azithromycin, and a second ECG was recorded on the next morning following the last dose of the treatment. All ECGs were evaluated by two blinded cardiologists in terms of QRS duration and dispersion.

**Results:** Among study participants, QRS duration was significantly prolonged after treatment with hydroxychloroquine and azithromycin (81.14 (9.11) versus 85.5 (10.48) ms [$P < 0.01$]), and the same pattern was observed with QRS dispersion (36.67 (9.54) versus 40.18 (9.35) ms [$P < 0.01$]). When gender differences were evaluated, male patients also showed significant changes in both QRS duration (82.65 (8.04) versus 87.31 (10.7) ms [$P < 0.01$]), and dispersion (36.93 (8.61) versus 41.31 (9.63) ms [$P < 0.01$]), while in females the difference was statistically insignificant for both QRS duration (79.06 (10.18) versus 82.8 (9.79) ms [$P = 0.056$]), and dispersion (36.31 (10.83) versus 38.62 (8.86) ms [$P = 0.23$]).

**Conclusions:** The combined use of hydroxychloroquine and azithromycin led to an increase in both QRS duration and dispersion in all patients. These changes were more significant in males than in female patients. No clinical effects of these ECG changes were observed in the short-term, and further studies are needed to investigate the possible clinical implications of these drugs during longer follow-up periods.

**Keywords:** Hydroxychloroquine, Azithromycin, COVID-19, QRS wave, Arrhythmia

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## Introduction

In late 2019 a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing the coronavirus disease 2019 (COVID-19) appeared in Wuhan China [1], and on March 11, 2020, the World Health Organization (WHO) declared that this virus had reached pandemic status [2]. In the absence of vaccines or curative medical treatment, COVID-19 exerted an unprecedented global impact on public health and health care delivery. In March 2020, a study claimed that the antiviral effects of hydroxychloroquine combined with azithromycin were found to be efficient against SARS-CoV-2 virus, and thus these drugs became part of the COVID-19 treatment protocols in many medical centers worldwide [3]. Because of the worldwide emergence and rapid spread of COVID-19 the use of these drugs has also increased. The increasing use of these drugs has raised concerns about their safety and the serious cardiovascular side effects associated with their use [4].

The antimalarial drug, hydroxychloroquine, and commonly used macrolide antibiotic, azithromycin, affect ventricular repolarization through alterations in myocardial ionic channels [5]. The earliest implications of these effects involve QT interval prolongation as seen on a surface electrocardiogram (ECG), which may lead to serious ventricular arrhythmias, such as torsade de pointes [6, 7]. Another fact is that hydroxychloroquine is metabolized by cytochrome P450 enzymes and combining it with azithromycin, which inhibits this enzyme, has led to an increase in concerns about the safety of this combination [8, 9].

Another ECG parameter that correlates with the occurrence of ventricular arrhythmias due to disturbances in ventricular repolarization is the QRS duration and its dispersion [10]. QRS dispersion is a novel ECG parameter and is measured as the difference between the maximal and minimal QRS duration using a standard 12-lead ECG. Studies have shown that increased QRS dispersion is associated with adverse cardiovascular events and mortality even in healthy asymptomatic individuals [11].

The effects of combined use of hydroxychloroquine and azithromycin therapy on QT interval were studied earlier [12], but the effects of this therapy on QRS duration and dispersion have not yet been investigated. The aim of our study was to investigate the effects of hydroxychloroquine and azithromycin combination therapy on changes in QRS duration and dispersion values before and after the treatment in patients diagnosed with COVID-19 who had no known history of cardiovascular diseases. Gender differences with respect to the effects of these drugs on QRS duration and dispersion were also investigated.

## Materials and methods

### Study populations

In this prospective cohort study, 107 patients (54 males, 53 females) with a positive COVID-19 polymerase chain reaction (PCR) test fulfilled the inclusion criteria and were evaluated between March 18 and June 8, 2020. Patients included those with new onset mild symptoms who were admitted to the isolation ward following the Ministry of Health's guidelines. Patients with normal arterial oxygen saturation (SpO2) and computed tomography (CT) showed no or very limited pneumonic infiltration.

All patients were admitted to the isolation ward once they had a positive polymerase chain reaction (PCR) test. They were treated for five days with combination therapy consisting of hydroxychloroquine (2 x 400 loading and 2 x 200 maintenance doses) and azithromycin (Day 1: 500 mg and other days 1 x. 250 mg). No antiviral agent or any other treatment was used to treat these patients. All participants were asymptomatic and had a negative PCR test before discharge, and no patients needed transfers to more specialized wards or intensive care units. The mean hospital stay duration was 8.3 (1.15) days. ECGs of the patients were recorded on the first day before starting the treatment and on the morning following the last dose of therapy.

### Exclusion criteria

Patients under the age of 18 and over the age of 65 with a systemic disease, such as hypertension and diabetes mellitus, history of any cardiovascular diseases, the presence of electrolyte disturbance in the routine biochemical tests, impaired liver and/or renal function tests, the presence of anemia or polycythemia, and hypothyroidism or hyperthyroidism were excluded. Patients with clinical findings that indicated the presence of cardiovascular diseases or have abnormalities on the baseline ECG were also excluded. To avoid selection bias, participant eligibility was evaluated separately by two blinded cardiologists, and a patient was excluded after the reports of both investigators matched.

### Electrocardiogram

The 12-lead ECG recordings were obtained while the patient was in the supine position. ECG length was 10 s; therefore, depending on heart rate, 4–6 beats per lead were used. ECG parameters were manually measured using a magnifying Glass (TorQ 150 mm Digital Caliper LCD) and a digital ruler accuracy of 0.01 mm by two blinded cardiologists. The measure of agreement was done using Cohen's kappa method, and the result was found to be within acceptable levels (Cohen's K = 0.17).

QRS duration (expressed in ms) was calculated by measuring the distance from the initiation of the Q or R wave until the end of the R or S wave. QRS dispersion was defined as the difference between the maximal and minimal QRS duration. ECG recordings were obtained at a speed of 25 mm/s, amplitude of 10 mm/mV. Values in mm with the digital caliper, which was 1/100 mm in precision, were calculated as ms multiplied by 40.

### Statistical analysis

The sample size analysis was done with a confidence interval of 95% and type 1 error of 0.05. Statistical power analysis showed a G*power of 0.8937. Continuous variables were examined by Shapiro–Wilk test to check for normality of distribution. Continuous variables are presented as a mean (standard deviation). Categorical data are presented as percentages or frequencies. Student t- and Mann–Whitney U tests were used to compare parametric and non-parametric continuous variables, respectively. Categorical variables were compared using a chi-squared (v2) test. Pearson's correlation test was used to determine the correlation between the variables. A two-tailed *P*-value of < 0.05 was considered statistically

significant. All data were analyzed using SPSS (IBM, Chicago, IL, USA) version 23.0.

### Ethics

All procedures, including the informed consent process, were conducted in accordance with the ethical standards of the National Health and Medical Research Council of Turkey and the Helsinki Declaration. The patients were informed about the study, and they were included after their informed voluntary consent forms were obtained. The study was approved by the ethical committee of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital (No: 2020-06/681).

## Results

Between March 18 and June 8, 2020, 183 patients with a positive COVID-19 PCR test were evaluated, among which 107 patients (54 males, 53 females) fulfilled the inclusion criteria and were analyzed in the study (Figure 1). No significant differences between participants of different genders in terms of sociodemographic and baseline clinical characteristics (Table 1) were noted. Before starting treatment with hydroxychloroquine and azithromycin, a baseline ECG was obtained, and another ECG was obtained on the next morning after the last treatment dose. To avoid any interpretation or confirmation bias, ECG evaluations were done by two blinded cardiologists, and a measure of agreement was done using the Cohen's kappa method. This value was found to be within acceptable levels (Cohen's K = 0.17).

Figure 1: Flow chart showing the number of COVID-19 patients assessed for eligibility, the number of patients excluded and the final number of study participants

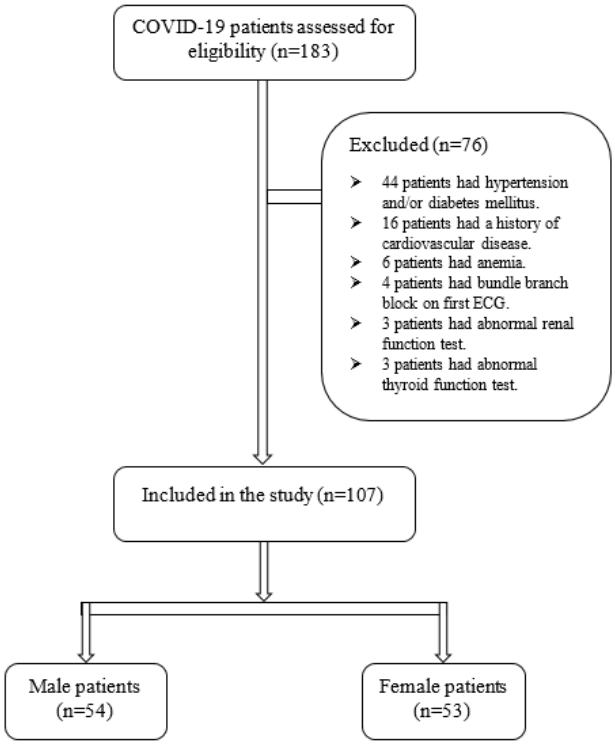



Table 1: The comparison of sociodemographic and baseline clinical characteristics of the study groups

| Variables | Male group (n = 54) | Female group (n = 53) | T-value | *P*-value |
|---|---|---|---|---|
| Age, years | 46.74 (12.52) | 45.88 (13.61) | 0.34 | 0.73 |
| Basal HR, b/m | 78.26 (13.51) | 77.91 (12.45) | 0.13 | 0.88 |
| BMI, kg/m$^2$ | 26.81 (2.54) | 26.37 (3.51) | 0.74 | 0.45 |
| Systolic BP, mmHg | 122.84 (14.62) | 121.53 (11.46) | 0.51 | 0.60 |
| Diastolic BP, mm Hg | 74.88 (7.45) | 72.56 (6.81) | 1.68 | 0.09 |
| Hemoglobin, gr/dL | 14.2 (1.7) | 14.3 (2.1) | 0.27 | 0.78 |
| TC. mg/dL | 171.8 (27.4) | 173.9 (31.7) | 0.36 | 0.71 |
| LDL, mg/dL | 135.8 (22.6) | 137.8 (31.9) | 0.37 | 0.70 |
| Triglyceride, mg/dL | 137.8 (31.9) | 139.5 (41.6) | 0.23 | 0.81 |
| HDL, mg/dL | 31.7 (4.8) | 31.9 (4.7) | 0.21 | 0.82 |
| Calcium, mg/dL | 9.3 (0.8) | 9.4 (0.6) | 0.73 | 0.46 |
| Sodium, mEq/L | 140.5 (1.4) | 140.7 (1.2) | 0.79 | 0.42 |
| Potassium, mEq/L | 4.1 (0.7) | 4.2 (0.5) | 0.84 | 0.39 |
| TSH, mIU/L | 3.3 (1.3) | 3.4 (0.8) | 0.47 | 0.63 |

BMI; Body Mass Index, BP; Blood Pressure, TC; Total Cholesterol, LDL; Low-Density Lipoprotein, HDL; High-Density Lipoprotein, TSH; Thyroid Stimulating Hormone

The overall baseline heart rate was 78.26 (13.51) beats per minute (bpm), and the post-treatment heart rate was 77.91 (12.45) bpm ($P$ = 0.84) as shown in Table 2. The mean QRS duration after treatment was significantly prolonged, with baseline QRS duration being 81.14 (9.11) ms and the post-treatment QRS duration being 85.5 (10.48) ms ($P$ < 0.01) as shown in Table 2 and Figure 2. The mean QRS dispersion value also increased after treatment with a baseline QRS dispersion value of 36.67 (9.54) and a post-treatment QRS dispersion value of 40.18 (9.35) ($P$ < 0.01) as shown in Table 2 and Figure 3. Significant prolongation of QT interval was also observed (Table 2).

Figure 2: Comparison of QRS durations for patients before and after the treatment with hydroxychloroquine and azithromycin.

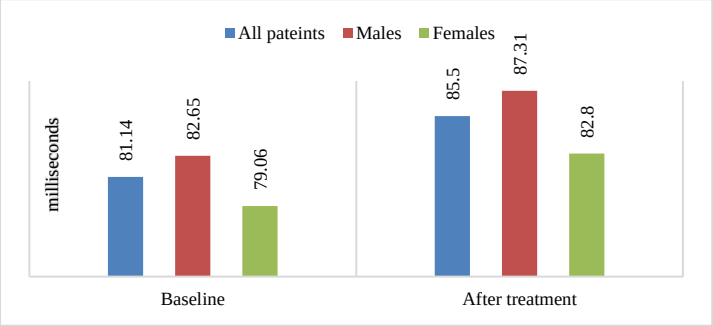
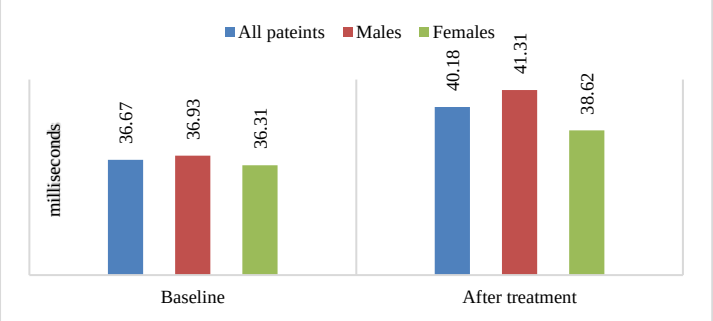



Figure 3: Comparison of QRS dispersions for patients before and after the treatment with hydroxychloroquine and azithromycin.




When comparison was made according to patients' genders, both gender groups showed prolongation of QRS duration and an increase in QRS dispersion values after combined treatment with hydroxychloroquine and azithromycin; however, males were significantly affected more by the treatment than females (Table 2). The mean baseline QRS duration of male patients before the treatment was 82.65 (8.04) ms, and after treatment it was 87.31 (10.7) ms ($P$ < 0.01), while in female patients, the baseline QRS duration was 79.06 (10.18) ms, and after treatment, it was 82.8 (9.79) ms ($P$ = 0.056) as shown in Table 2 and Figure 2. In terms of QRS dispersion, in

male patients, the baseline QRS dispersion was 36.93 (8.61), and after treatment, QRS dispersion was 41.31 (9.63) ($P < 0.01$), while in female patients, the baseline QRS dispersion was 36.31 (10.83), and after treatment, the QRS dispersion was 38.62 (8.86) ($P = 0.23$) as shown in Table 2 and Figure 3. No cases of treatment discontinuation because of serious ECG changes were noted, and none of the patients experienced a life-threatening malignant ventricular arrhythmia during hospitalization. No correlation was found between COVID-19-related signs and symptoms and changes in ECG parameters.

Table 2: The comparison of electrocardiogram (ECG) findings of all patients before and after treatment with combined hydroxychloroquine and azithromycin

| Variables | Before Treatment (ms) | After treatment (ms) | T-value | *P*-value |
|---|---|---|---|---|
| Heart rate | 78.26 (13.51) | 77.91 (12.45) | 0.19 | 0.84 |
| QRS duration (ms) | 81.14 (9.11) | 85.5 (0.48) | 3.24 | < 0.01 |
| - QRS duration Males (n = 54) | 82.65 (8.04) | 87.31 (10.7) | 2.65 | < 0.01 |
| - QRS duration Females (n = 53) | 79.06 (10.18) | 82.8 (9.79) | 1.92 | 0.056 |
| QRS dispersion (ms) | 36.67 (9.54) | 40.18 (9.35) | 2.72 | < 0.01 |
| - QRS dispersion Males (n = 54) | 36.93 (8.61) | 41.31 (9.63) | 2.49 | < 0.01 |
| - QRS dispersion Females (n = 53) | 36.31 (10.83) | 38.62 (8.86) | 1.21 | 0.23 |
| QT duration (ms) | 361.73 (27.08) | 381.67 (32.85) | 4.84 | < 0.01 |
| - QT duration Males (n = 54) | 358.96 (28.62) | 378.86 (34.91) | 3.83 | < 0.01 |
| - QT duration Females (n = 53) | 365.16 (24.96) | 385.36 (29.94) | 3.77 | < 0.01 |
| cQT duration (ms) | 416.22 (36.74) | 435.89 (31.61) | 4.19 | < 0.01 |
| - cQT duration Males (n = 54) | 407.04 (32.92) | 422.35 (35.01) | 2.34 | 0.021 |
| - cQT duration Females (n = 53) | 420.57 (34.13) | 434.35 (36.62) | 2.01 | 0.047 |

## Discussion

The effects of combined hydroxychloroquine and azithromycin treatment on QRS duration and dispersion in COVID-19 patients who had no previous history or clinical findings of cardiovascular diseases were evaluated in this study. Two main findings are reported: (1) the use of hydroxychloroquine and azithromycin led to an increase in both QRS duration and dispersion in all patients and (2) the increases in QRS duration and dispersion were more significant in male than female patients.

Several authors have explained the physiopathology for ventricular repolarization and depolarization disorders that may lead to ventricular arrhythmias [13]. Some drugs can affect the duration of ventricular repolarization and depolarization via causing changes in the ion channels (especially the potassium channel) of the myocardium and disturbances of the ventricular action potential [14]. Many risk factors, including advanced age, history of heart disease, electrolyte imbalance, diuretic use, kidney and liver failure, can increase the risk of drug-related malignant arrhythmias [15].

During the COVID-19 pandemic, hydroxychloroquine and azithromycin were frequently used due to their anti-inflammatory and immunomodulatory effects [3]. A major concern with the use of these drugs is the risk of arrhythmias. Many studies have shown that the use of these drugs leads to disturbance in ventricular repolarization and as a result, these drugs can cause prolongation of the QT interval leading to serious arrhythmias, such as torsade de pointes and sudden cardiac death [6, 16].

On the other hand, both hydroxychloroquine and azithromycin were found to cause changes in ECG wave durations and alterations in the dispersions of these waves [17, 18]. ECG wave durations and dispersions are non-invasive cardiac markers that are useful for predicting the risk of arrhythmias and sudden cardiac death. The QRS wave represents the ventricular depolarization on the surface ECG, and its relationship with ventricular arrhythmias have been studied for many years. QRS prolongation has been found to be as a predictive parameter for ventricular arrhythmias and sudden cardiac death in many studies [19, 20]. Another more recent parameter used to predict ventricular arrhythmias and sudden cardiac death is the QRS dispersion, which has also shown to be associated with adverse cardiovascular events and cardiac death [21, 22].

QRS dispersion is measured as the difference between maximal and minimal QRS duration on the 12-lead ECG, and it occurs due to the heterogeneity and conduction delays through the ventricle [23]. Kountouris et al. [24] reported that QRS dispersion exhibits a stronger association with left ventricular systolic function than does QRS duration. Recently, an increased QRS dispersion was shown as a prognostic marker of adverse cardiovascular events and mortality among asymptomatic population with no history of cardiovascular diseases in the Multi-Ethnic Study of Atherosclerosis (MESA) study.

As shown in previous studies, increases in QRS duration and dispersion are associated with adverse cardiovascular events and mortality even in individuals with no symptoms or history of cardiac diseases; thus, it is an important non-invasive ECG marker in patients who are at potential risk of myocardial conduction defects because of use of hydroxychloroquine and azithromycin.

In our study, the relationship between the use of hydroxychloroquine and azithromycin in COVID-19 patients with no history or clinical findings of cardiovascular diseases with QRS duration and dispersion was evaluated. The results of our study show that both QRS duration and dispersion after treatment with hydroxychloroquine and azithromycin significantly increased when compared with baseline values. When the evaluation was done based on gender differences, the changes in QRS duration and dispersion were also statistically significant in male patients, while in female patients the difference was statistically insignificant. All study participants received the same doses of both drugs for five days, and the hospitalization periods were similar. No drug- or disease-related complications were observed, and none of the patients required intensive care unit admission.

The results of our study show that the use of combined hydroxychloroquine and azithromycin therapy for COVID-19 patients may affect the duration and dispersion of the QRS wave on the surface ECG, especially in male patients. These changes did not correlate with COVID-19 signs and symptoms.

Although patients experienced QRS prolongation and increases in QRS dispersion during the study period, no cases of treatment discontinuation because of serious ECG changes were reported, and none of the patients experienced a life-threatening malignant ventricular arrhythmia during hospitalization. On the other hand, in our study, the clinical effects of these changes in

short term were evaluated, and longer follow-up periods are needed to show whether these changes have negative clinical implications on longer follow-up periods or are merely benign and reversible conditions.

**Limitations**

This study is subject to the same limitations as other observational studies. The main limitation was the absence of a control group of patients with COVID-19 infections who were not treated with any of these medications. Another import limitation is the small number of patients included in the study because of the focus on choosing patients with no past medical histories. Further work is needed to confirm our findings in a larger group of patients and to investigate the clinical implications of these findings in the long-term.

**Conclusions**

Besides its known effects on QT duration, combined hydroxychloroquine and azithromycin therapy was found to cause significant prolongation of both QRS duration and dispersion in COVID-19 patients with no histories or clinical findings of cardiovascular diseases. These effects show gender differences as ECG changes were more significant in male versus female participants. No clinical effects of these ECG changes were observed in the short-term, but further studies are needed to investigate this combination's clinical implications over longer follow up periods.

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# The assessment of headache and sleep quality in patients with chronic obstructive pulmonary disease

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**Ethics Committee Approval**
Approval was obtained from the Clinical Research Ethics Committee at Selcuk University (Number: 2021/109).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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## Abstract

**Background/Aim:** It is known that sleep disorders and headaches are related. However, there are only very few studies examining this correlation in chronic obstructive pulmonary disease (COPD). The aim of this study was to evaluate the association between sleep disorders, headaches, and hypoxia in COPD patients.
**Methods:** This study was designed as a prospective case-control study with 120 COPD and 83 controls patients of similar age/gender. International classification of headache disorders - version 3 (ICHD III) was used for the diagnosis of headache. Pain intensity was calculated using the visual analog scale (VAS). For the effect of headache, headache activities of daily living index (HADLI) questionnaire was applied. Pulmonary function tests were performed with a PC-based spirometry device. Blood pressure and blood oxygen saturation were measured by fingertip pulse oximetry. Sleep quality was assessed using the Pittsburgh sleep quality index (PSQI). Dyspnea severity was graded according to the modified medical research council (mMRC) scale.
**Results:** Headache was detected in 54 patients (45%) with COPD. The most common type of headache was tension type (66.7%). Patients with headache had higher diastolic blood pressure, severity of dyspnea score, and lower forced expiratory volüme-1 (FEV1), forced vital capacity (FVC) ($P$ = 0.037, $P$ = 0.001, $P$ = 0.001, $P$ = 0.001 respectively). Sleep disturbance was quite common in COPD patients (79.2%). PSQI score was higher in patients with headache ($P$ = 0.006). Patients with poor sleep quality had higher pain severity ($P$ = 0.006). Female gender, high diastolic blood pressure and low forced expiratory volume-1 second (FEV1) increased the risk of headache ($P$ = 0.004, $P$ = 0.02, $P$ = 0.032, respectively).
**Conclusion:** Headache is a complex symptom in patients with COPD and associated with higher diastolic blood pressure and pulmonary dysfunction. These parameters should be evaluated in COPD patients with headache.

**Keywords:** COPD, Headache, Sleep quality, Hypoxia, Pulmonary functions

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## Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease characterized by restriction of airflow during breathing [1]. It is the third most common non-communicable disease, and its incidence is approximately 10% in the population over 40 years old [2]. It is the 5th most common cause of morbidity worldwide [3]. The most important risk factor of the disease is smoking [2]. In spirometry-based studies, the prevalence of COPD was found to be 26% in smokers and 8% in non-smokers [4].

Forced expiratory volume in 1 second (FEV1) can be used to determine the severity of the disease in COPD. FEV1 relative to normal ≥ 80% is called as mild COPD, 50-80% moderate, 30-50% severe and < 30% very severe [3, 5]. Low FEV1 is a risk factor for cardiovascular and cerebrovascular diseases. This impairment in respiratory functions disrupts cerebral and muscular perfusion, leading to fatigue, headache and sleep quality impairment [6, 7].

Sleep disturbances are closely correlated with many chronic diseases. Pittsburgh sleep quality index (PSQI) disturbances are detected in approximately 70% of COPD patients [8]. However, the correlation between them has not been fully revealed. Besides many factors in chronic diseases, headache also negatively affects sleep functions. Headache is most commonly associated with insomnia, obstructive sleep apnea syndrome (OSAS) and restless legs syndrome/periodic limb movement disorder among sleep disorders [9]. The mechanism of the relationship between hypoxia, hypercapnia and sleep disturbance in diseases that cause respiratory dysfunction has not been fully elucidated, but it has been observed that all these factors affect each other [7, 9].

Sleep-related headache is a known fact in both the clinical practice and literature review. However, there are few studies evaluating the relationship between sleep disorders and the headache character associated with chronic hypoxia in COPD patients. Therefore, we aimed to examine sleep disorders, headache characteristics and their relationship with pulmonary function tests (PFT) in COPD patients.

## Materials and methods

### Field of study and ethical approval

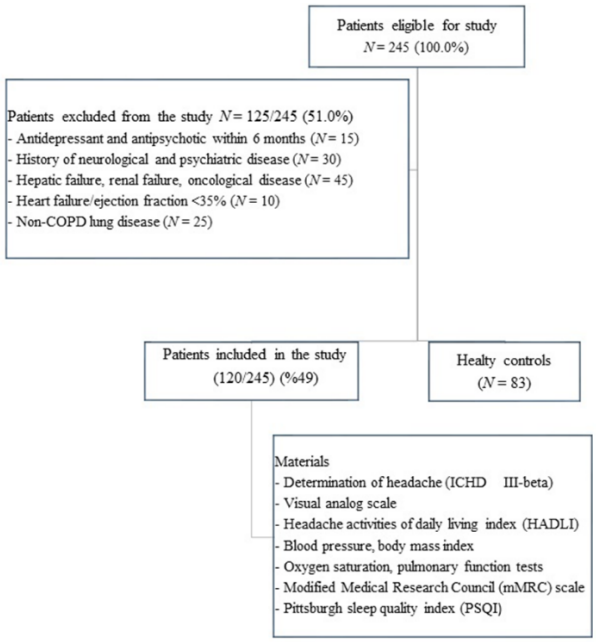
COPD patients who applied to the pulmonary outpatient department were included in the study and all patients were also evaluated in the neurology outpatient clinic. The study was planned as a case-control prospective study. The ethical approval was obtained from Selcuk University clinical research ethics committee.

### Participants and sociodemographic characteristics

Sample size was calculated using the G-power program. In the two-way analysis of variety, it was calculated that a minimum of 120 patients and 80 controls were required for medium effect size. 120 COPD (40-85 years of age, because there is a consensus that COPD cannot be diagnosed < 40 years age) patients and 83 healthy controls were included in the study. The Declaration of Helsinki and good clinical practice guidelines were adhered throughout the study. The objective of the study was explained to the patients participating. Consent form prepared for the study was given to each participant. Participants who had not read and not signed this form were not included in this study.

Diseases and conditions that could affect the study results were excluded. Exclusion criteria were treatment with antidepressants and antipsychotics within 6 months, history of neurological and psychiatric disease (epilepsy, stroke, schizophrenia, depression), hepatic, chronic renal or heart failure, oncological disease and non-COPD lung disease (Figure 1). Patients' age, gender, educational and financial status, living environment, number of children, smoking and alcohol use were questioned. The period of COPD and the treatments applied (beta agonist, corticosteroid, theophylline, home non-invasive mechanical ventilation and oxygen use) were examined. Body weight and height of the patients were measured. Body mass index (BMI) was calculated with [weight (kg) / height$^2$ (m$^2$)].

Figure 1: Patient selection and study design




### Determination of headache and its subtypes

The same neurologist performed detailed neurological evaluation and examination. Patients with normal neurological examination results were included in the study to exclude secondary headache. Brain magnetic resonance imaging (MRI) was requested for patients who were suspected of having cranial pathology in their neurological examination. Patients without cranial pathology included in the study, other patients were excluded. The patient and control group were asked whether they had a headache in the last 3 months. ICHD III-beta version was used for the diagnosis of headache [10]. Pain type, frequency, duration (when using analgesic, without analgesic use), localization, accompanying factors (nausea and/or vomiting, photophobia-phonophobia, sensory symptoms) and presence of aura were questioned. Pain type was grouped as migraine (with or without aura), tension-type headache (TTH), trigeminal autonomic cephalalgia and other headaches. Pain intensity was calculated using the visual analogue scale (VAS) (without the use of analgesics). Pain severity was graded from 0 to 10. The higher the numerical value of the VAS result, the higher the pain intensity was considered [11]. Pain intensity was divided into two groups as over 5, 5 and less. In order to evaluate the effect of headache in daily life, the headache activities of daily living

index (HADLI) questionnaire was applied. This questionnaire composed of 7 questions, each scored between from 1 to 10 (1: it does not affect at all, 10: it affects completely) [12]. The total score ranges between 7-70.

**Pulmonary function and determination of subgroups**

A detailed pulmonary examination was performed by the same pulmonologist. Pulmonary function tests were performed with a PC-based spirometry device (Spirobank Office; Medical International Research (MIR); Rome; Italy) while the patients were sitting in a comfortable and upright position. Patients were asked to close their lips tightly with a disposable mouthpiece for spirometry. Patients were asked to inhale and exhale at maximum. This measurement was repeated 3 times and the best value was obtained. With this test, FEV1, forced vital capacity (FVC) and FEV/FVC values were obtained [13]. Patients based on FEV 1 value divided into 4 groups as ≥ 80, 50-79, 30-49 and < 30 [5].

The severity of dyspnea is rated according to the Modified Medical Research Council (mMRC) scale [14]. This scale has degrees from 0 to 4 and the increase in the degree indicates that the severity of dyspnea increases. Grade 0-2 was named as mild, 3-4 severe dyspnea. Blood oxygen saturation was measured with a fingertip pulse-oximeter (Nonin, Digital pulse oxi-meter, USA).

**Blood pressure measurement**

After the patients rested for 15 minutes, measurements were made 3 times in sitting position and the arithmetic mean was obtained. The cuff size suitable for the arm circumference of each patient was selected. It is measured with a mercury sphygmomanometer. The first and fifth Korotkoff sounds were determined as systolic blood pressure (SBP) and diastolic blood pressure (DBP) [15].

**Evaluation of sleep quality**

Sleep quality was assessed by using the Pittsburgh sleep quality index (PSQI) [16]. This questionnaire consists of 19 questions and 7 subgroups with 0-3 grades. Subgroups included questions about sleep duration, quality, latency and efficiency, sleep disturbance, daytime dysfunction and use of drugs for insomnia. The total score ranges between 0-21. Higher scores indicate worse sleep quality. If the score is above 5, it indicates that the sleep quality is clinically poor [17].

**Statistical analysis**

For data analysis, SPSS 16.0 Package Software (Statistical Package for the Social Sciences Inc.; Armonk, NY, USA) analysis program was used. Normality analysis was performed with Kolmogorov-Smirnov test. Data were expressed as number (n), percentage (%), and mean (standard deviation). Independent sample T, Kruskal Wallis, and Mann Whitney U tests were used for comparison of the means. Nonparametric data were compared with $\chi 2$ or Fisher's exact test. The correlation between numerical data was evaluated using Spearman's Correlation test. R-value = 0.26-0.49: poor and 0.50-0.69: considered to be moderate correlation. A logistic regression analysis was used to predict the factors affecting of headache. Results were evaluated at a 95% confidence interval and a significance level of $P < 0.05$.

## Results

The average age in our study was 63.38 (11.49), there were 120 COPD patients (range 40-85 years old) and the number of male patients was higher (n = 97, 80.8%). There were 83 healthy volunteers of similar age group and gender ($P > 0.05$). Smoking was higher in COPD patients (79.2%, mean (SD): 23.29 (17.91) pcs/day), whereas the use of alcohol was lower (n = 4, 3.3%). Mean BMI was 26.59 (5.04), and blood pressures were 126.75 (18.71) mmHg and 78.11 (12.62) mmHg for systolic and diastolic, respectively. The mean duration of COPD was 7.19 (4.82) years. 112 (93.3%) patients were receiving inhaled B2 agonists, 98 (81.7%) inhaled anticholinergics, 63 (52.5%) inhaled glucocorticoids, and 43 (35.8%) patients were receiving sustained-release theophylline. 30 (25%) patients were using home oxygen and 4 (3.3%) patients were using non-invasive mechanical ventilation. 16 (13.3%) of the patients had a history of COPD in the first degree, 21 (17.5%) in the second- and third-degree relatives.

For the relationship between the treatments used by COPD patients and headache, there was no correlation between the use of inhaled b2 agonists, glucocorticoids, theophylline, and home oxygen and the frequency of headache ($P = 0.729$, $P = 0.362$, $P = 0,168$, $P = 0.836$, respectively). The frequency of headache was higher in patients using inhaled anticholinergics ($x^2 = 10.707$, $P = 0.001$). The frequency of headache in COPD patients was 45% (n = 54). 18 (33.3%) patients with headache were female and 36 (66.7%) were male. Of the headaches, 11 (20.4%) had migraine, 36 (66.7%) had TTH, 3 (5.6%) of them had trigeminal autonomic type headache. Aura was detected in three patients. While VAS score of the patients was 5.38 (1.77), it was 6.25 (1.31) in healthy controls ($P > 0.05$). Headache prevalence was higher in COPD patients ($P = 0.036$). Headache characteristics in patient and control groups were summarized in (Table 1). The diastolic blood pressure of COPD patients with headache was higher ($P = 0.037$). FEV1, FVC values were lower and mMRC scores were higher in patients with headache ($P < 0.001$, $P = 0.001$, $P < 0.001$, respectively). Especially, sleep latency, sleep duration, sleep disturbance and sleep medication use scores were higher ($P = 0.007$, $P = 0.001$, $P = 0.002$, $P = 0.004$, respectively). Total PSQI scores of patients with headache were higher ($P = 0.006$). In the study, sociodemographic characteristics, blood pressure, PFTs and sleep quality of COPD patients with and without headache summarized in (Table 2).

Table 1: Headache characteristics in patients with chronic obstructive pulmonary disease (COPD) and healthy control group

| Parameters | Headache in COPD (n = 54) | | Headache in healthy group (n = 27) | | $X^2$ *P*-value |
|---|---|---|---|---|---|
| | n | % | n | % | |
| Increased with cough | | | | | |
| Yes | 14 | 25.9 | 3 | 11.1 | 2.382 |
| No | 40 | 74.1 | 24 | 88.9 | 0.155 |
| Duration | | | | | |
| Less than 4 hours | 35 | 64.8 | 11 | 40.7 | 4.262 |
| 4 to 24 hours | 11 | 20.4 | 9 | 33.3 | 0.119 |
| More than 24 hours | 8 | 14.8 | 7 | 25.9 | |
| Physical activity | | | | | |
| Increase | 17 | 31.5 | 12 | 44.4 | 3.154 |
| Decrease | 15 | 27.8 | 3 | 11.1 | 0.207 |
| No difference | 22 | 40.7 | 12 | 44.4 | |
| Localization | | | | | |
| Unilateral | 13 | 24.1 | 14 | 51.9 | 6.250 |
| Bilateral | 41 | 75.9 | 13 | 48.1 | 0.023 |
| Pain character | | | | | |
| Throbbing pain | 22 | 40.7 | 23 | 85.2 | 15.68 |
| Dull head pain | 31 | 57.4 | 3 | 11.1 | 0.001 |
| Accompanying symptoms | | | | | |
| Nausea and/or vomiting | | | | | |
| Yes | 23 | 42.6 | 17 | 63 | 2.988 |
| No | 31 | 57.4 | 10 | 37 | 0.102 |
| Photo-phonophobia | | | | | |
| Yes | 14 | 25.9 | 19 | 70.4 | 14.727 |
| No | 40 | 74.1 | 8 | 29.6 | 0.001 |
| Visual | | | | | |
| Yes | 9 | 16.7 | 5 | 18.5 | 0.043 |
| No | 45 | 83.3 | 22 | 81.5 | 1.00 |
| Sensorial | | | | | |
| Yes | 7 | 13.0 | 5 | 18.5 | 0.440 |
| No | 47 | 87 | 22 | 81.5 | 0.522 |
| Visual analog scale | | | | | |
| Mild | 30 | 55.6 | 9 | 33.3 | 3.560 |
| Severe | 24 | 44.4 | 18 | 66.7 | 0.065 |
| Headache type | | | | | |
| Migraine | 11 | 20.4 | 8 | 29.6 | 2.423 |
| Tension | 36 | 66.7 | 14 | 51.9 | 0.850 |
| Trigeminal-autonomic | 3 | 5.6 | 1 | 3.7 | |
| Other | 4 | 7.4 | 4 | 14.8 | |

COPD: Chronic obstructive pulmonary disease, n: Number

Table 2: Characteristics in patients with chronic obstructive pulmonary disease (COPD) according to headache (n = 120). Data are mean (SD) unless otherwise indicated

| Parameters | COPD with headaches (n = 54) | COPD without headaches (n = 66) | *P*-value |
|---|---|---|---|
| Age, years | 62.57 (12.06) | 64.04 (11.06) | 0.488 |
| Disease duration, year | 8.01 (4.78) | 6.51 (4.78) | 0.084 |
| Blood pressure | | | |
| Systole (mmHg) | 129.72 (19.28) | 124.31 (18.01) | 0.192 |
| Diastole (mmHg) | 81.18 (12.19) | 75.60 (12.51) | 0.037 |
| Body mass index | 26.11 (4.86) | 26.98 (5.18) | 0.300 |
| mMRC | 2.81 (0.84) | 1.93 (1.10) | 0.001 |
| $FEV_1$ (%) | 46.64 (16.49) | 59.50 (20.51) | 0.001 |
| FVC (%) | 62.59 (17.54) | 74.48 (20.11) | 0.001 |
| $FEV_1$ / FVC ratio | 74.26 (14.31) | 78.82 (14.57) | 0.088 |
| Oxygen saturation | 88.92 (5.13) | 90.25 (5.43) | 0.115 |
| PSQI (total) | 12.29 (5.98) | 9.16 (5.11) | 0.006 |

COPD: Chronic obstructive pulmonary disease, n: Number, mMRC: Modified medical research council, FEV: Forced expiratory volume, FVC: Forced vital capacity, PSQI: Pittsburgh sleep quality index

The number of COPD patients with sleep quality score above 5 was 95 (79.2%), and the frequency of headache in these patients was 48.4% (n = 46). PSQI total score was 10.57 (5.72) in COPD patients, and 7.89 (4.25) in healthy controls ($P$ = 0.001). Especially in COPD patients, sleep duration was shorter, sleep efficiency and daytime functions were more impaired ($P$ = 0.008, $P$ < 0.001, $P$ < 0.001, respectively). Patients with poor sleep quality had higher headache severity ($P$ = 0.006) and dyspnea severity score (mMRC) was higher in these patients ($P$ = 0.018). Demographic characteristics of COPD patients with sleep quality disorder (PSQI > 5) and without sleep quality disorder (PSQI ≤ 5) were summarized in (Table 3).

Table 3: Characteristics of the patients with chronic obstructive pulmonary disease (COPD) according to sleep quality (N = 120). Data are mean *(*SD) unless otherwise indicated

| Parameters | COPD with poor sleep quality (n = 95) | COPD with good sleep quality (n = 25) | *P*-value |
|---|---|---|---|
| Age, years | 63.61 (11.65) | 62.52 (11.06) | 0.667 |
| Gender, female-male (%) | 22 (%23.3)-73 (%76.8) | 1 (%4)-24 (%96) | 0.042 |
| Disease duration, year | 7.34 (4.99) | 6.60 (4.15) | 0.543 |
| Blood pressure | | | |
| Systole (mmHg) | 126.89 (18.62) | 126.20 (19.43) | 0.877 |
| Diastole (mmHg) | 78.46 (11.99) | 76.80 (14.99) | 0.776 |
| Body mass index | 26.51 (5.09) | 26.88 (4.92) | 0.745 |
| mMRC | 2.46 (1.03) | 1.84 (1.14) | 0.018 |
| $FEV_1$ (%) | 51.86 (18.99) | 60.76 (21.58) | 0.069 |
| FVC (%) | 67.56 (19.96) | 75.08 (19.73) | 0.122 |
| $FEV_1$ / FVC ratio | 76.06 (14.46) | 79.47 (14.99) | 0.240 |
| Oxygen saturation | 89.25 (5.23) | 91.20 (5.45) | 0.078 |
| Headache (%) | 46 (%48.4) | 8 (%32) | 0.178 |
| Painful day/month | 8.17 (6.18) | 9.50 (8.50) | 0.858 |
| Pain frequency/month | 7.39 (5.72) | 9.87 (8.33) | 0.296 |
| Visual analog scale | 5.65 (1.74) | 3.87 (1.12) | 0.006 |
| Restricted Activities | 31.23 (17.24) | 20.00 (9.87) | 0.106 |

COPD: Chronic obstructive pulmonary disease, n: Number, mMRC: Modified medical research council, FEV: Forced expiratory volume, FVC: Forced vital capacity

In COPD patients with headache, as FEV1, FVC, and FEV1 / FVC decreased, sleep quality deteriorated according to Spearman correlation test ($P$ < 0.001, $P$ < 0.001, $P$ = 0.001 r = -0.463, r = -0.426, r = -0.310, respectively). Sleep quality was deteriorated as the mMRC score increased and blood oxygen saturation decreased ($P$ < 0.001, $P$ < 0.001, r = -0.366, r = 0.515, respectively). As the severity of headache increased, sleep quality deteriorated ($P$ < 0.001, r = 0.468) and activities were restricted due to headache scores increased ($P$ < 0.001, r = 600).

Logistic regression model was created with age, gender, blood pressure, mMRC, FEV1, FVC, PSQI to predict headache in patients with COPD. The model achieved a good fit (Hosmer-lemeshow = 0.236, Nagelkerke $R^2$ = 0.294). Gender, diastolic blood pressure, and FEV1 had an effect on headache. Female gender increased the risk of headache by 5.243 times ($P$ = 0.004). Headache risk increased 0.96 times ($P$ = 0.02) when FEV1 decreased by one unit, and 0.37 times increased when diastolic blood pressure increased by one unit ($P$ = 0.032).

## Discussion

Headaches include primary headaches as well as secondary headaches in the second part according to the international classification of headache disorders - version 3 (ICHD-3 beta). This part especially includes the section where the causality relationship with headaches is established. In addition, headache subgroups that are not included in this section, whose causality relationship is not fully confirmed or that are likely to be developed, have been placed in the appendix section [10]. In ICHD-3 beta, section number 10 includes headaches attributed to homeostasis disorder. It has been revealed that headache experienced in this category is not uncommon and its lifetime prevalence is 22% (95% confidence interval, 19-25%) [18]. Headache attributed to hypoxia and/or hypercapnia forms part 10.1, which is the subsection of this section. This subsection includes high-altitude headache (10.1.1), headache attributed to flights (10.1.2), diving headache (10.1.3), and sleep apnea headache (10.1.4) [10]. However, COPD, a major disease with secondary effects of hypoxia and hypercapnia, is not included in this subsection, even in this ICHD-3 beta classification. Therefore, a study was planned to examine the relationship between respiratory functions and headache in COPD.

Although the pathophysiology of the disease remains unclear, cerebral edema that occurs with restriction of intracranial compliance induced by hypoxia, cerebral blood flow and increased venous pressure are blamed among the possible mechanisms [19, 20]. Therefore, placebo double-blind sham controlled magnetic resonance spectroscopy study revealed that hypoxia in migraine is an individual triggering risk factor in patients. According to the sham group, hypoxia caused an increase in the amount of lactate around the occipital cortex and cerebral arteries [21]. Although hypoxia and/or hypercapnia-related headache is classified in ICHD-3, a large-scale epidemiological study conducted in Norway showed no difference between oxygen desaturation and lowest oxygen saturation in patients with and without headache in sleep apnea. Similar headaches were detected in the sleep apnea severity subgroups (mild-moderate-severe) in the same study [22]. However, a limited number of uncontrolled studies have shown that continuous positive airway pressure (CPAP) support reduces the frequency of primary and secondary headaches in patients with sleep apnea syndrome [23, 24]. COPD is a disease that is characterized by inflammation and obstruction of the lung and causes significant morbidity and mortality worldwide. It is also a common serious public health problem [1-4]. The fact that the disease is quite prevalent also brings many accompanying diseases. It has been determined that the frequency of headache in COPD patients is 31.9%. TTH has been seen most frequently (22/38, 57.8%). Headache is particularly high in male COPD patients (female, male 7/31). In this study, a clear relationship between impairment in pulmonary function tests and the presence of headache could not be determined [25]. In our study, the frequency of headache in COPD patients was 45%, and similarly, the most common headache was TTH (36/54, 66.7%). Similar to literature, headache was more prevalent in men (66.7%). Diastolic blood pressure was higher in COPD patients with headache. In addition, the severity of dyspnea, low FEV1 and FVC were found to be associated with headache. Bilateral headache without photo-phonophobia and dull pain type was in the foreground in the patients. However, there was less pain intensity, although headache was more common in COPD patients. These results support the induction of hypoxia in COPD patients with possible mechanisms mentioned earlier (hypoxia-hypercapnia). This situation demonstrates that respiratory functions with impaired obstructive pattern are correlated with headache in COPD patients.

Sleep disorders can accompany many chronic diseases and are also related to the course and severity of the disease. There is also a close correlation between headache and sleep disorders [25]. Cerebral structural abnormalities should be kept in the foreground in headaches that are more pronounced in the morning and persist even if their intensity decreases during the day. Sleep apnea should be considered especially in these pains. This condition is not specific for symptoms and should also suggest other sleep disorders, cervicogenic headaches, analgesic overuse, migraine, and psychiatric disorders [25, 26]. Sleep disturbance is one of the most common symptoms in COPD patients. In the evaluations made using the PSQI scale, it was found that approximately 70% of COPD patients had sleep quality disorders [8]. In another study, this rate was calculated as 73% (final score average = 7.27) [27]. It has been determined that patients with obstructive pulmonary diseases and sleep apnea syndrome have worse sleep quality and this condition is associated with disease severity [28-30]. In our study, sleep quality disturbance was present in 79.2% of COPD patients and this rate is similar to other studies in literature. Sleep disturbance increased as the severity of dyspnea increased in COPD patients. Sleep quality disturbance was higher in COPD patients with headache. FEV1, FVC, FEV1 / FVC, and oxygen saturations were lower in patients with sleep disturbance (but these values were not statistically significant). In addition, headache was severe in patients with sleep quality disturbances. Especially in female COPD patients, sleep quality was worse. In patients with headache, sleep latency was prolonged, sleep duration was decreased, sleep disturbance was severe, and these patients were using more sleeping drugs.

**Limitations**

Blood gas (arterial and/or venous) analysis was not performed for the evaluation of hypoxia. Headache (diagnosed by patients' answers) is a subjective condition. Polysomnography was not performed for the evaluation of sleep disorder. The relationship between headache and brain neuroradiological imaging has not been evaluated.

**Conclusions**

Although there is hypoxia and hypercapnia related headache section in the ICHD-3 beta classification, it is observed that there is no COPD-related headache in the subsection of this group. However, COPD is a very common disease that causes severe disability. In 45% of patients (especially TTH), headache is more frequent than in the normal population. Female gender, high diastolic blood pressure and low forced expiratory volume-1 second (FEV1) increase the risk of headache. Sleep disturbances are quite common in these patients (79.2%). Sleep disturbance is associated with severity of dyspnea and headache. Coexistence of sleep quality disorder and headache is common. For this reason, COPD patients are followed closely, especially in terms of headache and sleep disturbance. Perhaps with larger-scale studies, it will find a place for itself in the subgroup of headaches induced by hypoxia and hypercapnia in the ICHD classification.

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# A systematic assessment of adverse event reporting in selected state hospitals in Sri Lanka

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## Abstract

**Background/Aim:** Patient safety is an integral component of health care. Adverse event reporting plays a key role in ensuring patients' safety. The Sri Lankan Ministry of Health has introduced guidelines and a system of adverse event reporting. Here we assess the pattern of adverse event reporting in selected 46-line ministry hospitals.

**Methods**: The adverse events reported in the year 2019 were analyzed. The frequency of reporting of each event was assessed. The issues in relation to adverse event reporting and root causes were assessed through focus group discussions with selected hospital administrators.

**Results**: Most reported events were "patient falls", contributing to 30.46% of the total. Availability of guidelines, well-established quality management units, and a non-punitive non-fault-finding approach to adverse event reporting and analysis process were identified as strengths of the system. But lengthy paper-based documentation process was recognized as a major weakness.

**Conclusion**: Although the state health sector of Sri Lanka has an established system of adverse event reporting, it is mostly limited to non-clinical events such as falls. Fear of blame and shame among staff and the lengthy paper-based reporting system have negatively affected the process.

**Keywords:** Adverse event reporting, Patient safety, Hospitals, Sri Lanka

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**Ethics Committee Approval**
As this was based on secondary data and no patients involved, ethical clearance was not required. Administrative approval was obtained from Director,Healthcare quality and Safety, Ministry of Health, Sri Lanka.

**Conflict of Interest**
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## Introduction

Consensus has grown globally that learning from patient safety events is vital in making healthcare safer [1]. Patient safety is defined as the absence of preventable harm to a patient during the process of health care and the reduction of risk of unnecessary harm associated with health care to an acceptable minimum, where an acceptable minimum denotes the collective notions of given current knowledge, resources available and the context in which care was delivered weighed against the risk of non-treatment or other options in treatment [2].

It is understood that each point in the process of healthcare carries a certain degree of inherent unsafety [3]. For improvement and assurance of safety in healthcare, every defect should pave the way to improve processes [4].

In 2016, the Sri Lankan Ministry of Health introduced guidelines on adverse event reporting and launched readmission forms [5]. The recognized categories of adverse events to be reported by health care institutions – as per the general circular [5] – are listed in Table 1.

Table 1: Types of adverse events reported by state health care institutions in Sri Lanka, with examples.

| Types | Examples |
|---|---|
| Blood/blood products related | wrong patient/ wrong blood type |
| Documentation related | Wrong/incomplete information |
| Process related | Postponement of surgery |
| Healthcare-associated infections | Surgical site infections/ventilator-associated pneumonia |
| Infrastructure related | Non-fitting trolley/lack of bed railings leading to patient falls |
| Medical equipment related | Computer malfunction/ Breakdown of surgical tools |
| Medication-related | Wrong patient/wrong drug |
| Nutrition-related | Wrong diet |
| Patient accidents | Falls |

Reporting adverse events was expected to facilitate learning and improve safety by generating "alerts" regarding significant new hazards and disseminating "lessons learned" by healthcare organizations from investigating a serious event. Analysis of many reports, which may reveal unrecognized trends and hazards requiring attention, creates insights into underlying systems failures and generates recommendations for "best practices" for all to follow.

In Sri Lanka, the focal body for National Quality Assurance Programme in Health is the Directorate of Healthcare Quality & Safety (DHQS), which is under the administrative purview of the Ministry of Health. Each government healthcare institution has a Quality Management Unit (QMU) to undertake the planning, implementation, and monitoring of the National Quality Assurance Programme with the guidance of DHQS [6]. DHQS conducts quarterly quality performance reviews with the participation of all health care institutions in the country.

The adverse event/incident reporting form introduced by the Ministry of Health Sri Lanka comprises two parts, Part A and Part B. Part A could be completed by any health care worker in their own language. The form is completed immediately after the occurrence of the adverse event, within 24 h and once the area and people are safe. The form is filled out before changing each duty shift. All the adverse events related to clinical management were supposed to be reported by the consultant or a designee. All such reports are seen by the respective consultant or a senior doctor assigned by the consultant. The adverse events associated with the non-clinical management, such as falls, could be reported by the Nursing Sister or a nurse. The nature of the adverse event is expected to be mentioned briefly in the relevant part of the document. The immediate measures are taken to manage the adverse event also need to be mentioned in brief.

Part B is meant to be filled by the Head of the unit. Root causes and contributing factors related to the adverse event are noted after a brief discussion with relevant staff in the unit/ward. Preventive measures could be recommended based on the risk factors, root causes, and contributing factors. The officer completing the form could note the category of the staff member directly involved in an adverse event/incident, but it is not compulsory. The outcome must be mentioned, and the type of adverse event is supposed to be ticked off. A list of adverse events and incidents was provided on the other side of the form (Table 1). A copy of the document is retained in the ward. A separate register is maintained in the ward to record the details of all the adverse events reported.

A copy of the completed adverse event/incident report form is sent to the QMU. The categorization of the adverse event/incident based on the International Classification of Patient Safety [7] was carried out by QMU. It is then sent for the information and authorization to the head of the institution to carry out further root cause analyses, if necessary. The medical officer of QMU analyzes the incident with the relevant consultant and/or other stakeholders. Any criticism or breach of confidentiality at any point in the process is not to be allowed. Instruments and tools recommended for analysis include Why-Why Diagram, Fish-Bone Diagram, and Problem Tree. Preventive actions are recommended and written in the form.

Selected important and serious adverse events could be discussed in the monthly clinical meetings of the hospital to enable learning from experience and prevent such events in future in other wards/units. But it is stressed that no individual should be criticized during any of these proceedings.

A summary of the adverse event/incident is sent to the Directorate of Healthcare Quality & Safety (DHQS) of the Ministry of Health quarterly. DHQS analyzes the events of the report and discusses with the relevant professional colleges as required.

The objectives of the current study were to assess the process of adverse event reporting and analyze the reported events in selected state hospitals in Sri Lanka.

## Materials and methods

The current study was a descriptive mixed-method assessment that included cross-sectional, and retrospective components carried out in June 2020.

During the cross-sectional component, we studied the process of adverse event reporting in the state health service of Sri Lanka. A focus group discussion and a survey of relevant document formats were carried out. There were ten participants in the focus group: five medical administrators, three medical consultants, and two medical officers attached to the quality management units in hospitals. Participants were selected based on convenience. During the focus group discussion, the participants evaluated the current adverse reporting system regarding strengths, weaknesses, opportunities, and threats (SWOT). The qualitative inputs generated were subjected to thematic analysis.

The incidents reported by selected hospitals in 2019 were studied retrospectively. All the line ministry hospitals that participated in quarterly performance reviews conducted by DHQS in 2019 were considered. The hospitals that had not completed the reporting procedure were excluded. Desk review of adverse event reporting forms sent from the selected hospitals was carried out with a checklist designed based on the guidelines and standards for adverse event reporting, as per the General Circular by the Ministry of Health [5].

## Results

Forty-six (46) line ministry hospitals were included in the study. The adverse events reported belonged to seven categories, and it was revealed that the majority (30.46%) of adverse events reported were falls (Table 2).

Table 2. Frequency distribution of adverse events reported from selected hospitals in the year 2019

| Type of adverse event | Reported frequency (Number and percentage) |
|---|---|
| Falls | 3145 (30.46%) |
| Treatment/Diagnosis issues | 671 (6.49%) |
| Drugs/Intravenous infusions/ Blood transfusion issues | 2373 (22.97%) |
| Surgery/Anesthesia-related issues | 128 (1.24%) |
| Laboratory reports related issues | 1415 (13.71%) |
| Labor related issues | 44 (0.43%) |
| Other | 2548 (24.68%) |
| Total | 10324 |

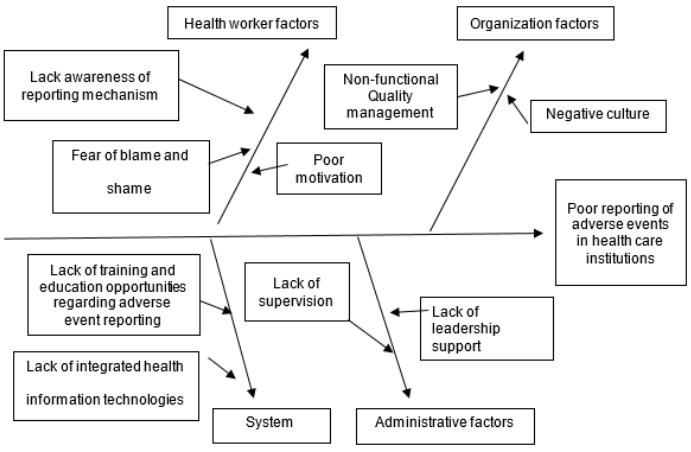
The results of the SWOT analysis of the process of adverse event reporting by health care institutions are depicted in Table 3.

Table 3: SWOT analysis of the current adverse event reporting process

| | |
|---|---|
| **Strengths**<br>Availability of Ministry guidelines in adverse event reporting<br>Availability of well-established quality management units in hospitals networked with the Directorate of Health care Quality and safety<br>The non-punitive non-fault-finding approach in the adverse event reporting and analysis process | **Weaknesses**<br>The lengthy manual documentation process<br>Lack of motivation of staff<br>Lack of forum/platform at the institutional level to discuss adverse events<br>Poor supervision by the Head of the institution |
| **Opportunities**<br>Availability of national quality performance review, which provides a platform for discussion<br>Enthusiasm for professional colleges | **Threats**<br>Blame and shame culture in some settings<br>Fear of litigation |

The overall opinion of the focus group discussion participants regarding the rate of reporting the adverse events was that an actual number of adverse events was far more than reported. The root cause analysis of under-reporting of adverse events was carried out with the participation of the focus group (Figure 1).

Figure 1. Root cause analysis of under-reporting of adverse events




The focus group pointed out that the common belief of health workers was that errors in the health sector were inevitable and mostly unmanageable. The group's opinion was that it has contributed to creating an idea among health workers that incident reporting was 'pointless'. It was also stated that reporting could be discouraged by excessive administrative procedures. The participants stated that health workers were apprehensive about the increased potential for administrators to engage in the regulation of medical quality using reported incident data.

## Discussion

Investigation of critical incidents was reported first in the 1940s by Flanagan as a technique to improve safety and performance among military pilots [8]. The National Patient Safety Agency was established as a Special Health Authority in England and Wales in 2001, which has been responsible for the national reporting and learning system to collect, analyze, and learn from all types of patient safety incidents [9]. Adverse event reporting systems have been a key tool to enhance organizational learning from incidents in a range of high-risk non-health organizations [10], including commercial aviation, the rail industry, and at nuclear power stations. Although adverse event reporting has been instituted in healthcare systems in many countries around the globe, positive experiences similar to those of non-health high-risk organizations are yet to be fully realized.

A successful reporting and learning system to enhance patient safety should be non-punitive for the individuals who report and should not focus on targeting or finding fault with anyone [11]. The successful improvement in patient safety through the analysis of incident reports is less likely without achieving a blame-free culture [12].

The reporting of incidents is most effective when the data collected are analyzed at local, district, and national levels with the participation of professional colleges and scholars through anonymous reporting, meaningful feedback, and ease of reporting [13]. Expertise, adequate resources, and training should be made available to allow for meaningful analysis of reported adverse events to better health services delivery. The recommendations must be disseminated and acted upon by those with the responsibility and mandate to act for the full benefits of adverse event disclosure to be achieved. In the Sri Lankan setup, the establishment of DHQS has been a huge strength in encouraging adverse event reporting and associated further quality improvement activities, which should be wisely utilized to connect all stakeholders in the process.

Although many healthcare organizations worldwide have implemented adverse event reporting systems with the aim of learning from experience to prevent adverse events and medical errors [14], under-reporting of adverse events is a recognized international health concern [15]. If the concerns of the possibility of being punished could be eliminated by guaranteeing legal immunity, the level of reporting would be much improved in the health sector.

Learning from patient safety incidents is difficult if the information is incomplete [16]. Globally, adverse event reporting has become a central element in effective patient safety systems, though their growth and implementation have been slow and

sluggish (Battles and Stevens 2009). The Sri Lankan adverse event reporting model also suffers from inadequate and incomplete documentation. The paper-based nature of reporting system in the country could have negatively contributed to the scenario.

Understanding the factors that determine the behavioral intention of healthcare professionals to comply with adverse event reporting is of utmost importance in the successful implementation of such a system. Some international researchers have noted that healthcare professionals were more likely to report a serious event [17] due to the better integrity of the reporting system, which was not seen in the current study. It was noted in the study that there was a tendency to better report adverse events with less gravity, such as falls, than those with more severity, which could be explained by fear of blame and shame associated with cultural issues [18].

The factors impeding the bringing of adverse events could be projected not only by professional, national, and organizational cultures but also by healthcare practice structural issues, including safety systems, rules, and regulations [19]. Knowledge, trust, and management support determine the healthcare workers' acceptance of adverse event reporting systems while minimizing negative organizational norms towards incident reporting.

Under-reporting of adverse events is a major concern in health care safety. The reasons for under-reporting include lack of awareness of reporting mechanism, poor leadership support, poor training and education regarding adverse event reporting, fear of punishment, and negative organizational culture. Adverse event reporting must become a culturally accepted activity within the healthcare community. An adverse event reporting system should be user-friendly and supported by leadership. Reporting becomes efficient when it is felt comfortable and assured to be free of negative consequences.

Health systems should be able to provide efficient technical support, training, and awareness programs for health workers involved and adequate resources to implement the adverse event reporting system. Ensuring a legal immunity against the possibility of using adverse event reporting data for disciplinary actions would remedy undue fear of punishment.

**Conclusion**

Although the state health sector of Sri Lanka has an established system of adverse event reporting, most reporting was limited to non-clinical events, such as falls. The staff seemed to have self-inhibitions in reporting relating to fear of blame and shame. The nature of the process of adverse event reporting being paper-based and cumbersome with repeated documentation has prevented it from being fully effective and popular.

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# Effects of polyacrylamide hydrogel used in the treatment of osteoarthritis on mesenchymal stem cells and human osteoblasts

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**Ethics Committee Approval**
The study was approved by the Istanbul Medipol University Noninterventional Clinical Research Ethics Committee (number: E-10840098-604.01.01-19391) Date: 03/07/2020.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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## Abstract

**Background/Aim:** Adipose-derived mesenchymal stem cells (AD-MSCs) have gained popularity for use in treating osteoarthritis (OA) although their long-term effects remain unsatisfactory for treating the end stages of OA. However, clinically injectable polyacrylamide hydrogels (PAHGs) remain in the joint indefinitely, which could make them ideal candidates as AD-MSC carriers. Our aim was to evaluate whether combinations of PAHG and AD-MSCs have positive effects on cell viability, thereby the potential of this combination prior to clinical use in OA treatment in addition to the effects of PAHG on human osteoblasts (HOBs).
**Methods**: Cell lines of AD-MSCs of canine origin and HOBs were culture-expanded and seeded in 96-well plates (0.5 x $10^5$ cells/well). The PAHG substrate at doses of 2, 6, 10, 20, or 40 µL per 200 µL of sample were added to the wells, and their effects were compared to the positive and negative control groups and among substrate doses for each cell line. The experiments were repeated three times, and cell viability was studied using tetrazolium (MTT) method.
**Results**: Cell viability in all dose groups was significantly greater than that of their negative control groups for both cell lines ($P < 0.001$). Among the different dose groups, significant dose-dependent viability increases were only observed for the HOB cell line ($P < 0.001$). The PAHG substrate was not lethal to AD-MSCs or HOBs up to the maximum assayed doses and had positive effects on the viability of these cell lines, including slight increases in proliferation.
**Conclusion**: Combination of PAHG with AD-MSCs may have positive long-term effects for OA treatment. However, further trials are needed.

**Keywords:** Adipose-derived mesenchymal stem cells, Cell viability, Human osteoblasts, Polyacrylamide hydrogels, Osteoarthritis

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## Introduction

Osteoarthritis (OA) is a highly prevalent joint disease worldwide [1]. OA is characterized by degeneration and loss of articular cartilage in association with changes in the underlying subchondral bone [2]. OA is not currently a curable disease because its pathophysiology and mechanism of progression remain incompletely understood. Therefore, the goal of OA treatment is simply to reduce pain and inflammation to allow a patient to maintain a better quality of life. The therapeutic spectrum ranges from conservative treatments (such as weight loss, physical therapy, biomechanical interventions, and intra-articular injections) to joint replacement surgery [1, 3].

Recently, adipose-derived mesenchymal stem cell (AD-MSC) therapy has increased in popularity for the treatment of OA and emerged as an intriguing therapy option because of their immunomodulatory behavior and potential to differentiate into chondrocytes and osteoblasts [4-6].

Polyacrylamide hydrogels (PAHGs) are fully synthetic, nontoxic, highly biocompatible, biostable filling materials that are commonly used in aesthetic and urologic interventions [7–9]. Because of their resistance to biodegradation, injectable PAHGs are used as long-term visco-supplementation agents in the treatment of OA, especially in Russia and Asian countries [10]. Furthermore, PAHGs have been widely researched *in vitro* as scaffolds of various stiffnesses to study the differentiation pathways of stem cells based on the mechano-transduction properties of the medium [11]. However, no *in vitro* or *in vivo* trials have been described in which the effects of clinically injectable PAHGs on mesenchymal stem cells (MSCs) and human osteoblasts (HOBs) have been evaluated together.

In the present study, we hypothesized that injectable PAHGs are good agents to promote AD-MSC survival and can facilitate the proliferation of AD-MSCs and human osteoblasts such that a PAHG and AD-MSC mixture appeared to be a good candidate for the treatment of OA. The study also aimed to establish the scientific basis of this treatment for further clinical trials.

## Materials and methods

### Ethics committee approval

Our experiments were approved by Istanbul Medipol University Non-Interventional Clinical Research Ethics Committee (number: E-10840098-604.01.01-19391) on 03/07/2020.

### Cell lines and cultivation

In our present study, canine AD-MSCs (cAD-MSCs) and a human osteoblast (HOB) cell line were used. HOB cells were obtained from PromoCell, Heidelberg, Germany (C-12720), while AD-MSCs of canine origin were purchased from Tekgen Health Services Company, İstanbul, Turkey (VetStem-D009/40) on January 24, 2020. Human Osteoblast Growth Medium (Merck- 417-500) supplemented with 10% fetal bovine serum (Sigma Aldrich-F7524) and a 1% antibiotic–antimycotic (Sigma Aldrich- A5955) solution was used to expand the HOB cell line. Low glucose Dulbecco's Modified Eagle's Medium (DMEM) from Biowest-L0060 supplemented with 15% fetal bovine serum (Sigma Aldrich-F7524), 2% L-glutamine (Sigma Aldrich- G6392), and 1% antibiotic–antimycotic solution (Sigma Aldrich A5955) was used to expand the cAD-MSCs. Frozen vials of cells were removed from a nitrogen tank and heated at 37 °C for 1 to 2 min after which the liquid suspensions of cells were transferred into a sterile tube and brought to a final volume of 5 ml with appropriate media. Subsequently, the cell suspensions were centrifuged at 1500 rpm for 5 min after which the supernatant was removed, and the cell pellet was suspended in 1 ml of medium before being transferred to T25 flasks containing 4 ml of medium. Subsequently, the cells in the flask were placed in a 37 °C incubator under an atmosphere with 5% $CO_2$ for cultivation.

### Characterization of AD-MSCs

Stem cells should highly express CD105, CD146, CD73 surface antigens and low levels of anti-HLA-DR, CD19, and CD25 surface antigens. The flow cytometry results were obtained from the company from which the cells were provided and is presented in the results section of the present study.

### Demonstration of AD-MSC nuclear morphology by DAPI staining

Cells cultured in T-25 flasks were removed by trypsinization and enumerated before being seeded in 8-well chambers (Ibidi- 80841**)** at 3,000 cells per well. The medium was aspirated the following day after the cells reached confluence and then washed once with phosphate-buffered saline ([PBS] Sigma Aldrich-BSS-1006). Subsequently, the cells were fixed in 10% neutral buffered formalin for 5 min and then rinsed briefly with PBS before undergoing permeabilization with 0.5% Triton X-100 for 10 min. After permeabilization, the cells were washed once with PBS for 5 mins, covered with a 300 nM 4'-6-diamidino-phenylindole (DAPI) staining solution and then incubated for 5 min while being protected from light. The staining solution was then removed, and the cells were washed three times with PBS. Images were recorded with a Zeiss Lsm 800 confocal microscope at a laser excitation of 405 nm.

### Preparation of synthetic implant doses based on polyacrylamide hydrogel

Noltrex™ (Bioform, Moskow, Russia) was selected as the PAHG substrate. Noltrex™ is a fully synthetic, three-dimensional (3D), cross-linked polyacrylamide hydrogel that is available in a volume of 2.5 ml. Furthermore, Noltrex™ has a high viscosity and consists of 4.0% ± 1.5% cross-linked polyacrylamide, 96.0% ± 1.5% purified water, and 0.001%–0.0025% silver ions. Stock concentrations were prepared in a 1:1 ratio by mixing 500 µL of Noltrex™ with 500 µL of the medium used for cell culture cells.

### Cell viability assays

Following aspiration of the cultured cell medium in T75 flasks, the cells were washed with sterile PBS after which 1 ml of trypsin/ethylenediaminetetraacetic acid (EDTA) from Sigma Aldrich (T4049) was added, and after 3 min in the incubator, the cells were removed. After a 5 min centrifugation step at 1500 rpm, the supernatant was discarded, and the cells were resuspended in medium. A Scepter Automated Cell Counter was used for cell counting. Cells were seeded into 96-well plates ($0.5 \times 10^5$ cells per well) and cultivated for 24 h until reaching confluence. Subsequently, the medium was aspirated from the wells.

Each dose of Noltrex™ (2, 6, 10, 20, 40, and µL/200 µL of sample) was added to the AD-MSC and HOBs with an equal amount of medium and incubated for 24 h. All samples were assayed in triplicate during the incubation period (24 h). Dose-free and Triton X-treated groups were used as the positive and negative control groups, respectively.

At the end of the incubation period, the medium-PAHG mixtures were aspirated away from the cells after which the tetrazolium (MTT) agent (Roche-11465007001) was added at a ratio of 1:20 (MTT agent: total medium) and incubated for 3 to 4 h (37 °C, 5% $CO_2$). Following the incubation period, the MTT was aspirated, the solvent dimethyl sulfoxide (DMSO) was added at a ratio of 1: 1 (medium: DMSO), and the samples were incubated in an orbital mixer for 1 h in darkness. Subsequently, the absorbance values of the samples were measured with a spectrophotometer (SpectramaxI3) at 570 nm to assess any resulting color changes.

**Statistical analysis**

Statistical analyses were performed using GraphPad Prism 6.0. One-way analysis of variance (ANOVA) was used to compare the assayed parameters among the dose groups (2, 6, 10, 20, and 40 µL/200 µL) and positive and negative control groups. An overall *P*-value of < 0.05 was considered to indicate a significant difference. When an overall significance was observed, a pairwise post hoc test was performed using Tukey's multiple comparisons test.

## Results

**Characterization of AD-MSCs**

The flow cytometry results showed that the surface markers CD105, CD146, and CD73 were highly expressed at rates of 73.46%, 82.62%, and 62.72%, respectively (Figure 1). In contrast, anti-HLA-DR, CD25, and CD19 showed significantly lower expression rates of 17.58%, 12.64%, and 19.43%, respectively (Figure 2).

Figure 1: Flow cytometry analysis of positive surface markers and percentage rates

| CD105-PE | | | | CD146-FITC | | | | CD73-PE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Quad | Events | %Gated | %Total | Quad | Events | %Gated | %Total | Quad | Events | %Gated | %Total |
| UL | 9 | 0.10 | 0.09 | UL | 131 | 1.45 | 1.31 | UL | 16 | 0.18 | 0.16 |
| UR | 7346 | 81.52 | 73.46 | UR | 8262 | 91.69 | 82.62 | UR | 6272 | 70.90 | 62.72 |
| LL | 152 | 1.63 | 1.52 | LL | 305 | 3.38 | 3.05 | LL | 354 | 4.00 | 3.54 |
| LR | 1504 | 1669 | 15.04 | LR | 313 | 3.47 | 3.13 | LR | 2204 | 24.92 | 22.04 |

PE: Phycoeryritrin, FITC: Fluorescein isothyocyanate

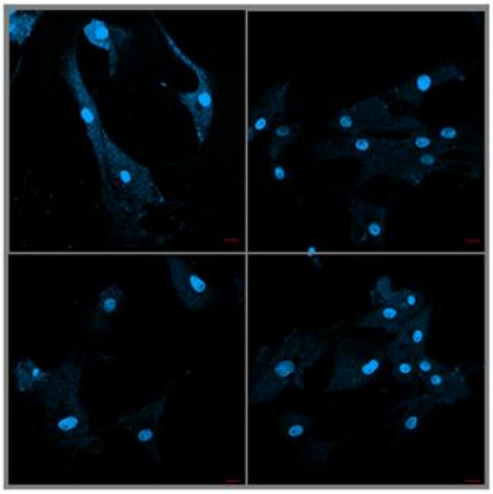
Figure 2: Flow cytometry analysis of negative surface markers and percentage rates.

| CD19-PerCP | | | | CD25-APC | | | | Anti-HLA-DR-PE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Quad | Events | %Gated | %Total | Quad | Events | %Gated | %Total | Quad | Events | %Gated | %Total |
| UL | 5518 | 62.38 | 55.18 | UL | 5946 | 67.22 | 59.46 | UL | 6007 | 67.24 | 60.07 |
| UR | 1943 | 21.96 | 19.43 | UR | 1264 | 14.29 | 12.64 | UR | 1758 | 19.68 | 17.58 |
| LL | 1373 | 15.52 | 13.73 | LL | 1588 | 17.95 | 15.88 | LL | 11.63 | 13.02 | 11.63 |
| LR | 12 | 0.14 | 0.12 | LR | 48 | 0.54 | 0.48 | LR | 6 | 0.07 | 0.06 |

APC: Allophycocyanin, PE: Phycoeryritrin, PerCP: Peridinin-Chlorophyll

Cell nuclei were stained with DAPI, and images from different areas in the cell were taken with a Zeiss Lsm 800 confocal microscope to assess cell and nuclear morphology (Figure 3).

Figure 3: Nuclear staining images of AD-MSCs (20× magnification)



**Cell viability assays**

Both cell lines were incubated for 24 h with PAHG at doses of 2, 6, 10, 20, or 40 µL/200 µL per sample. Wells containing only medium were used as the positive control group, whereas Triton X-treated wells served as the negative control group. The normalized values of the triplicate results are shown in Tables 1 and 2, and graphs created based on statistical results are shown in Figures 4 and 5.

Figure 4: Statistical graph of the absorbance values of adipose derived stem cells showing dose-dependent viability.

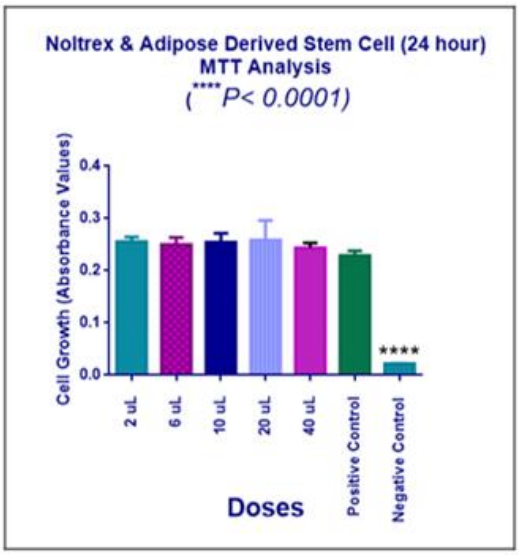
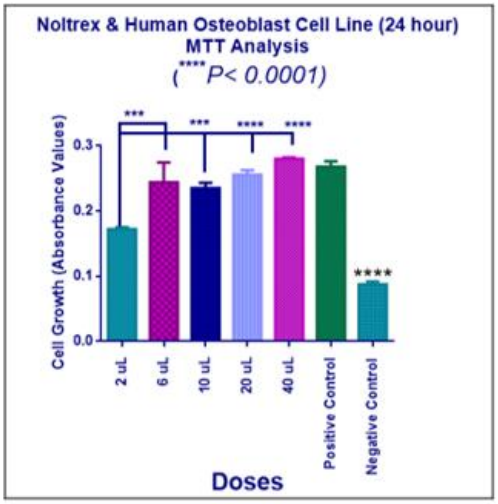



Figure 5: Statistical graph of absorbance values of human osteoblast cells showing dose-dependent viability



The MTT method, which is often the preferred technique, was used for viability analysis [12]. The MTT results for cAD-MSCs showed an obvious and significant difference when all dose groups were compared with the negative control group ($P < 0.001$). No significant differences were observed when the dose groups (2–40 µL/200 µL) were compared among themselves (Figure 4). In addition, the viability results of all dose groups were higher than that of the positive control group, even if no significant difference was observed. Therefore, the results clearly showed that the PAHG substrate Noltrex™ did not produce any negative effects on cAD-MSCs up to a dose of 40 µL / 200 µL of sample but did lead to an increase in cell proliferation (Table 1).

Table 1: Absorbance values shown as optical density (OD) unit of triplicate experiment showing the dose-dependent viability of adipose-derived stem cells and their mean values using one-way analysis of variance (ANOVA)0

| Dose | First Experiment | Second Experiment | Third Experiment | Mean Values (±SEM) |
|---|---|---|---|---|
| 2 µL/200 µL | 0.2667 | 0.2661 | 0.2389 | 0.2559±0.01[a] |
| 6 µL/200 µL | 0.2297 | 0.2748 | 0.2461 | 0.2502±0.01[a] |
| 10 µL/200 µL | 0.2214 | 0.2756 | 0.2678 | 0.2549±0.01[a] |
| 20 µL/200 µL | 0.2238 | 0.3338 | 0.2181 | 0.2585±0.01[a] |
| 40 µL/200 µL | 0.2603 | 0.2429 | 0.2274 | 0.2435±0.01[a] |
| Positive Control | 0.238 | 0.2381 | 0.2113 | 0.2291±0.01 |
| Negative Control | 0.0237 | 0.206 | 0.020 | 0.0221±0.01 |

a: $P < 0.001$ versus negative control group, SEM: Standard error of the mean

MTT analysis was also performed on HOBs using the same incubation time and the same doses. When the results of all doses were evaluated, significant differences were observed when compared with the negative control group ($P < 0.001$). No significant differences were observed when all dose groups were compared with the positive control group. When the 2 µL dose group was compared with the other dose groups, a significant difference in terms of increasing viability was observed (2 versus 6 µL/200 µL [$P < 0.001$], 2 versus 10 µL/200 µL [$P < 0.001$], 2 versus 20 µL/200 µL [$P < 0.001$], 2 versus 40 µL/200 µL [$P < 0.001$]), and significant differences at 6 and 10 µL versus 40 µL/200 µL ($P < 0.05$) comparisons ($P < 0.05$) were found (Figure 5). These results indicate that the PAHG substrate can cause an increase in cell viability to a certain extent. When the 40 µL dose was compared with the positive control group, it did not produce a negative effect on the viability of HOBs, similar to that observed for the cAD-MSCs although cell proliferation was increased even if it was not statistically significant (Table 2).

Table 2: Absorbance values as Optical Density (OD) unit of triplicate experiment showing the dose-dependent viability of human osteoblasts and their mean values using one-way ANOVA

| Dose | First Experiment | Second Experiment | Third Experiment | Mean Values (±SEM) |
|---|---|---|---|---|
| 2 µL/200 µL | 0.169 | 0.175 | 0.173 | 0.1723±0.00[a] |
| 6 µL/200 µL | 0.267 | 0.256 | 0.210 | 0.24443±0.02[a,b] |
| 10 µL/200 µL | 0.245 | 0.231 | 0.230 | 0.2353±0.00[a,b] |
| 20 µL/200 µL | 0.260 | 0.260 | 0.248 | 0.2560±0.00[a,c] |
| 40 µL/200 µL | 0.279 | 0.278 | 0.283 | 0.2800±0.00[a,c,d,e] |
| Positive Control | 0.271 | 0.275 | 0.259 | 0.2683±0.00 |
| Negative Control | 0.089 | 0.091 | 0.082 | 0.0873±0.00 |

a: $P<0.001$ versus Negative control group, b: $P < 0.001$ *versus* 2 µL/200 µL, c: $P < 0.001$ versus. 2 µL/200 µL, d: $P < 0.05$ versus 6 µL/200 µL. e: $P < 0.05$ versus 10 µL/200 µL, SEM: Standard error of the mean

## Discussion

In this experimental *in vitro* study, it was observed that the clinically injectable PAHG substrate Noltrex™ did not kill cAD-MSCs and HOBs up to the maximum assayed doses when dose groups for each cell line were compared with the corresponding Triton X-treated negative control group. In addition, the PAHG substrate led to an increase in the proliferation of cAD-MSCs at all doses but only at the 40 µL dose for HOBs although these differences were not significant when compared with the positive control group. No differences were observed among the different cAD-MSC cell line dose groups. However, significant dose-dependent viability increases in HOB cell line (2 versus 6 µL/200 µL, 2 versus 10 µL/200 µL, 2 versus 20 µL/200 µL, 2 versus 40 µL/200 µL, 6 versus 40 µL/200 µL, and 10 versus 40 µL/200 µL) were found. These findings show that the clinically injectable PAHG substrate Noltrex™ can be combined with MSCs to provide slightly increased proliferation rates.

Although PAHGs are thought to be nontoxic at the cellular level, clinical side effects of PAHGs other than Noltrex™, particularly after aesthetic surgeries, have been reported. These side effects have been described very rarely as transient surgical side pains, hematomas, irregularities, gel deposition, asymmetry, tissue reactions, including infection, foreign body granuloma, edema, inflammation, tenderness, and sensitivity, and adverse effects, including gel migration and induration [8, 9].

However, the clinical side effects of PAHGs toward avascular joint tissues are limited. In a study involving 527 patients, Zar et al. [10] reported a post-Noltrex™ injection adverse effect profile as inflammable pain in 6.8% of patients at the injection area, joint pain in 9.3% of patients, and joint effusion in 0.05% of patients. They concluded that these adverse effects were resolved within three days using local cold compresses and acetaminophen treatments within, and joint aspiration or debridement was not required in any of the patients.

In contrast, Tonbul et al. [13] reported severe foreign body reactions in a 64-year-old female patient after injection with Noltrex™, which eventually required surgical interventions to drain out and debride the hydrogel in both knees. They also concluded that they altered the course of her osteoarthritis by biologically causing intra- and extra-articular fibrosis, which may have subsequently altered the potential available treatments, such as arthroplasties and post-operative rehabilitation. In their case report, they applied intra-articular Noltrex™ immediately after arthroscopic intervention of the right knee. In our opinion, this intervention may have subsequently triggered an overactive immune response and led to serious foreign body reactions in both knees. Although this case was a clinical case report regarding Noltrex™, any safety concern must be taken seriously. Therefore, we evaluated the *in vitro* effects of the PAHG substrate Noltrex™ on cAD-MSCs and HOB cells before designing a clinical trial in combination with MSCs for use in the treatment of advanced stage human osteoarthritis in which subchondral bone is also impaired.

The exact mechanism of OA is still not fully understood due to the complex series of events leading to articular cartilage degeneration. Whether events in articular cartilage precede those in subchondral bone, are concomitant with them, or whether subchondral bone changes can actually lead to early cartilage damage remains unclear [14–16]. Therefore, simultaneous healing of both articular cartilage and subchondral bone is required for ideal regeneration in advanced OA. The best candidate should be the agent that can facilitate healing or regeneration of both tissues together.

Recently, AD-MSC injections have become popular for OA treatment due to the immunomodulatory behavior and regenerative capabilities of these cells [4]. However, findings from clinical trials show that stem cell injections alone do not appear to have a lasting effect on end-stage osteoarthritis [17–19]. These results suggest that the stem cells require a suitable matrix in which to reside, propagate, and effectively differentiate for a desirable recovery in cases of severe OA. In the search for such a matrix, the *in vitro* effects of the PAHG derivative Noltrex™ were evaluated before using it in combination with AD-MSCs in human clinical trials and assessing its effect on HOBs based on the resulting pathogenesis. To the best of our knowledge, no previous study has evaluated the effects of PAHGs on both cell lines together in this way.

On the clinicaltrials.gov website, searches using the terms “osteoarthritis” and “polyacrylamide hydrogel” together resulted in the identification of five trials registered as NCT03897686, NCT04045431, NCT04179552, NCT03067090, and NCT03060421. Trials NCT03897686, NCT04045431, and NCT03067090 appear to still be recruiting. However, five trials study polyacrylamide hydrogels alone.

In future trials, the *in vivo* effects of injectable PAHGs combined with MSCs on cell differentiation and tissue reactions as foreign body reactions should be investigated.

Although PAHGs have high biocompatibility, they interact with their surrounding host tissue and allow for gradual vessel ingrowth, which is followed by a modest macrophage reaction at the host tissue-gel border. Once the macrophages enter the gel, they transform into fibroblasts that connect and eventually form a vascular fibrous network. Consequently, PAHGs, remain in the tissue indefinitely as a viscoelastic deposit traversed by a network of fibrous strands [20].

However, in the presence of mesenchymal stem cells in a hydrogel, the immunoregulatory properties of PAHGs can cause macrophages to remain as anti-inflammatory M2 type cells, which can prevent or reduce foreign body reactions and result in a decrease in fibrous strands [21]. These properties could positively affect articular and subchondral bone tissue regeneration, which should be elucidated in future trials.

Last, in our experimental study, the effects of the clinically injectable PAHG substrate Noltrex™ on stem cell differentiation were not evaluated. Furthermore, commercially available AD-MSCs of canine rather than human origin were used. In addition, another PAHG agent clinically used in the treatment of OA is Arthrosamid™ (Contura International A/S, Søborg, Denmark) [22, 23]. However, as Arthrosamid™ was not commercially available in our country when this experiment was performed, it was not possible to study or compare both agents together. These points are viewed as the weaknesses of our present study.

**Conclusion**

OA is primarily a disease of articular cartilage and subchondral bone. Therefore, the regeneration of both tissues would ideally be performed simultaneously. MSCs are considered suitable candidates for use in the treatment of OA due to their regenerative potential. However, in the late stages of OA, these cells provide unsatisfactory long-term effectiveness. However, PAHGs remain in the joint indefinitely. In our present study, Noltrex™ were not lethal to cAD-MSCs or HOBs and rather led to induction of slightly increased proliferation rates, suggesting that their use in combination with AD-MSCs may have positive long-term effects in the treatment of OA. However, this possibility should be evaluated in further animal and human trials.

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# New generation genome sequencing methods

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## Abstract

Sequencing procedures, which present information related to events in the living metabolism, are performed by genetic methods. Gene structure and genetic control mechanisms can be determined by sequencing methods, as they represent significant contributions to transcriptomic, ecological, and epidemiological studies. Thus, chromosomal abnormalities in living things with a whole genome analysis of different organisms (e.g., plants, bacteria, yeast, fungi, and viruses) are detected quickly, so that clinical disease can be diagnosed with reliable data from biomedical research. The nucleic acid sequencing processes have a history of about fifty years. With the technology developed in recent years, second and third-generation methods, known as next-generation sequencing (NGS), found their rightful place in the sector approximately 15 years ago. New-generation sequencing promotes inexpensive, routine, and comprehensive analyses of the living genome. In general, sequencing methods are separated as first-, second-, and third-generation sequencing methods, with the latter called 'new-generation sequencing:' our review focuses on these specific sequencing methods.

**Keywords:** DNA**,** Genome, New-generation sequencing, Sequencing methods

## Introduction

Nucleic acids are the main building blocks controlling metabolic events, heredity, and disease in living cells, while nucleic acids consist of simpler structures called nucleotides; the arrangement that comprise them is known as sequencing [1]: this contains important information about hereditary properties. In addition, determining nucleotide sequencing is necessary for biological and genetic research [2].

With the development of technology in recent years, sequencing and new-generation methods provide convenience in scientific research, medicine, and health fields. Although DNA and RNA sequencing date back nearly fifty years, large-scale mass parallel sequencing or next-generation sequencing have been studied for about 15 years [3]. NGS allows for a comprehensive analysis of the living genome, which is both inexpensive and widespread [4].

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Sequencing research emerged with an RNA molecule, as researchers looked for new methods for DNA to be sequenced as well [1]. The best-known method since the first appearance of sequencing is the Sanger method. In 1977, Frederick Sanger developed a technology based on chain termination. Thus, the first human genome project was carried out with this method [5], also known as Dideoxy or chain termination reactions. In the same year, Maxam and Gilbert developed another method based on chemical modification of DNA [3,6], known as the multidirectional sequence: DNA is cut from specific base sequences using chemicals and different length fragments and is then 'harvested.' In this method, forty clones can be analyzed from one sequencing gel. Nevertheless, it is the less preferred method than that of Sanger, since dangerous chemicals may be used. The Gilbert and Maxam-Sanger sequencing methods are defined as first-generation sequencing: they have been used in successful research involving human, animal, and plant genome work for the past 20 years. However, they are laborious, which led to next-generation sequencing methods; the ideal DNA sequencing technology must be accurate, easy, and inexpensive [5]. The genetic technology of next-generation sequencing is based on simultaneous processing of each part of a DNA molecule from a single living cell (consisting of millions of harmonious fragments) [7]. The technology has become an indispensable part of biological research in many fields with obvious advantages, such as obtaining data in a short time, which are richer and more reliable [1, 4]. At present, a variety of new-generation sequencing technologies are available, creating a library with many DNA fragments by segmenting the selected target area of genomic DNA from the biological material to be assessed via enzymes. Reproduction of the DNA fragments makes up the library, with an analysis of raw data by sequenced replicated fragments [8]. The next-generation methods are generally divided into second- and third-generation [9,10]. Roche 454 Genome Analyzer, Illumina, Inc, San Diego, CA, USA, SOLID, and IonTorrent (Thermo Fisher Scientific, Waltham, MA, USA) use second-generation sequencing for the formation of mRNA, a small RNA profile, with a genome length characterization, chromatin structure, ancestral DNA microbiology, and similar molecular characterization, as well as microbial communities [11].

**Emulsion PCR**

Mold DNA amplification in next-generation sequencing is performed with emulsion PCR, a template amplification cation in multiple platforms. The basic principle of EmPCR is dilution and division of template molecules in water-oil emulsion and droplets [11]. EmPCR allows for the amplification of DNA molecules in droplets of physically separated picoliter volumetric water, preventing formation of inefficient chimera and other artifacts in DNA sequences. Droplets are used in analytical applications, e.g., number determination with digital PCR and preparative applications: molecular evolution and genome-scale DNA [12].

**Second-Generation Sequencing Methods**

Given that time is valuable in diagnostic research, it was inevitable to develop the first sequencing technologies. SGSs on the market were more advantageous than first-generation technologies, a result of millions of parallel short readings, accelerating the sequencing process, a low sequencing cost, and direct detection without any need for electrophoresis [9].

**Roche 454 Sequencing Technology**

The importance of microbial communities for human and environmental health led researchers to develop methods for these communities. The most common and cost-effective is sequencing targeted genetic elements. The amplicon sequence of taxonomic marker genes, such as the 16s rRNA gene in bacteria contains fungi and the 18S rRNA gene in eukaryotes. This provides a community population count: researching functional diversity is accomplished through targeting functional genes: one method is the Roche 454 sequencing technology [13].

The technology was first patented by Melamede in 1989 [10, 14]. Moreover, 454 Life Sciences, the first NGS technology on the market, became Roche 454 (Branford, CT, USA) based on the pyrosequencing method, or that of detecting pyrophosphate released from the new synthetic DNA chain (formed after each nucleotide combination) [9, 10].

Although this method of pyrosequencing has some disadvantages, it has been accepted in metagenomic studies on complex microbial communities. Among its disadvantages are high cost, errors in reading complex sequences, inefficiency in loading limitations due to bead-based DNA molecules, and a limited number of reads [15,16].

**Illumina Genome Analyzer**

The Illuminia NGS technology, first introduced by Solexa and later purchased by Illumina, is a system based on the use of synthesis with reversible stain terminators [9, 10].

As with the 454 system, adapters are placed at the ends of the DNA sequence. However, the prior system uses beads and EmPCR, while Illumina uses planar solid glass support. During the first step of Illumina technology, DNA samples are broken down into random sequences, and adapters are connected to both ends of each array. These adapters connect to the corresponding complementary sequence. In the second stage, each sequence fixed to the solid plate is strengthened with PCR bridge amplification. A series of sequences formed from the same original is called a cluster, and each cluster contains approximately a million copies of the same original series. In the final stage, Illumina covers the synthesis approach, using reversible terminators to determine each nucleotide in the sequence [9, 10, 17].

Illumina is a commonly used NGS method worldwide [18]. Its technology evolved rapidly, providing high efficiency at low cost with improved read lengths and true paired end reads. One drawback is that it has extra requirements for sample load control, with a surfeit in these clusters that overlap and create incorrect sequencing. Overall, the error rate is about 1% [13, 18].

**Ion Torrent**

With the commercialization of the Ion Torrent Personal Genome Machine by Life Technologies (Carlsbad, CA, USA), this new-generation sequencing method simulates 454 pyrosequencing technology. However, Ion Torrent does not use fluorescent-labeled nucleotides as other technologies do; it is based on the hydrogen ion released during sequencing [9]. Accordingly, when new nucleotides are attached after pyrophosphate division, a proton is released. The well plate ensures that proton release is localized and maintained. The

signal is proportional to the number of free protons, enabling the sequence of homopolymeric regions of template DNA. Data collection uses a complementary semiconductor metal oxide sensor chip (CMOS), which can measure millions of sequence reactions at once [10].

The most important aspect of the Ion Torrent Personal Genome Machine (PGM), Ohio University, Athens, OH, USA, is that it uses synthesis sequencing strategy as an alternative approach, is low-cost, high-speed technology, with a medium-length read time [19-21].

**SOLiD**

SOLiD (Supported Oligonucleotide Ligation and Detection, Carlsbad, CA, USA) is a technology based on ligation biochemistry, and on the hybridization of 16 different props, the first two nucleotides which are known and capable of creating four different radiations on the template DNA chain, given the first two nucleotides, and the connection of probes to each other (with ligation) [5]. This system was introduced in 2006 by Agencourt, which later changed to Applied Biosystems, Bedford, MA, USA. The DNA fragments to be sequenced are connected to adaptor molecules and attached to the beads. Cloning is carried out with EmPCR [10, 22].

The long read times in SOLiD chemistry are a distinct disadvantage of this technology, with short read times and inaccuracy. However, in the latest releases, this has been improved [22].

**Third-Generation Sequencing Methods**

Second-generation sequencing technologies are often-used compared to first-generation sequencing. Yet, second-generation technologies may require PCR, so they become disadvantageous in time and cost [10]. As such, third-generation sequencing technologies (TGSs) were developed. Long-range DNA sequencing and mapping technologies created a new era in high-quality genome sequencing. Unlike second-generation sequencing, short readings with a length of several hundred base pairs, third-generation single-molecule technologies produce more than 10,000 bp of reading and map more than 100,000 bp of molecules [23, 24].

**Helicos Genetic Analysis Systems**

The commercial application of the single-molecule sequencing method, first introduced by Helicos Genetic Analysis Systems, Cambridge, MA, USA, made it possible to sequence DNA directly without creating DNA amplification [25]. Although it has similarities to Illumina technology in its fluorescence detection method, there is no need for the DNA amplification stage [2]. It is based on SBS, unlike many second-generation methods based on cloning [26].

The Helicos library is easier to prepare than sequencing technologies, as preparation for ligation and amplification is not required to form the library. Helicos systems are commercial TGSs based on single-molecule fluorescent sequencing: it determines quantities of RNA molecules without converting them to cDNA [22, 27].

**Pacific BioSciences Single-Molecule Real-Time Sequencing**

Single-molecule real-time (SMRT) sequencing was first developed by Pacific Biosciences, Menlo Park, CA, USA, based on real-time monitoring of modified enzymes, unlike the Helicos single-molecule sequencing method [6].

SMRT sequencing is now widespread in basic sciences and in applied realms, with field research involving agriculture, environment, and medicine. SMRT sequencing offers significant advantages over current short-read DNA technologies and for sequencing non-oxidized DNA molecules [28]. Its advantages are that it facilitates the sequencing of long DNA molecules with high accuracy. The SMRT sequence is a new resolution-level technology that examines the molecular mechanisms of living cells [29]. SMRT sequencing being error-prone is incorrect, as many studies show evidence that it is diagnostically useful, with advantages over short-read sequencers. The SMRT sequencing technology was applied in breast cancer cell models to identify new gene fusion events, with known oncogenes. It has been used in research, and will be used in the diagnosis of related diseases in the future [26].

**Nanopore Sequencing**

The sequencing method of Oxford Nanopore Technologies™ (ONT) Oxford, UK determines the order of nucleotides in a DNA sequence. Launched in 2014 by Oxford Nanopore Technologies MinION, the device produces longer readings with better performance [9]. The device is four inches long and connects to a USB 3.0 port of any PC [30]. Protein channels with nano-sized diameter dimensions (nanopores) are embedded in the lipid barrier of the sequencing system. Their biological task is to facilitate ion transitions, as nanopore sequencing is based on measuring voltage generated by these ions, as they pass through the pores [2, 6].

In this sequencing technology, the first strand of a DNA molecule is connected to the complementary strand via a molecule. The DNA fragment goes through a protein nanopore, producing a variation of ionic current, based on differences in moving nucleotides; this occupies pores when DNA is transformed through them. The change of the ionic current is gradually recorded in the graphic model and interpreted to describe the sequence [31, 32].

Nanopore sequencing is advantageous, as it is a low-cost and small-size device; data are displayed on screen and created without completing the work. The lysate can be sequenced without PCR amplification or a chemical labeling step. This minimized workforce cost in sequencing technologies until recently. However, the % error rate must also be improved [33].

**Conclusion**

Since the invention of the sequencing technologies, many developments were made in advancing science and technology. The Maxam and Gilbert method was behind the Sanger method, in terms of use of dangerous chemicals: the Sanger sequencing method has become a movement over time. Second-generation sequencing was created to reduce some of the cost-, labor-, time-related losses, and obtaining more data in a shorter time. Second-generation sequencing methods provide convenience in diagnosis and scientific research. The short read time of the SGS technologies, the need for bioinformatics equipment, and the clonal amplification processes of PCR has led to development of these technologies and third-generation sequencing; they have found their place in scientific research.

Instead of cloned sequencing, the TGS technologies sequence a single template DNA molecule and yield faster results in a shorter time, using fewer biochemicals. In this sense, it is more economical.

The NGS technologies are now the starting point for some research areas, as well as studying and analyzing biological sequences. However, they must be improved, in terms of reducing the margin of error, and as such, used more frequently.

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# Physiopathological effects of noise: Recent approaches to the treatment of hearing loss

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## Abstract

A significant problem can be exposure to noise, which is one of the negative effects of growing industry and production facilities around the world. Many individuals are exposed to high levels of noise at work, which causes problems. One of the most affected systems of exposure to noise is the auditory system. Hearing is damaged from exposure, such that individuals lose their sense of hearing. In the last 10 years, research has been done globally to prevent and treat noise-related hearing loss. When the data are examined, it can be seen there is protection and treatment for noise-induced hearing losses. The physiopathological effects of noise and new approaches are currently being examined.

**Keywords:** Noise, Physiopathological effects of noise, Treatment of noise-induced hearing loss

## Introduction

Many people are exposed to high levels of noise in daily life, damaging physiological mechanisms in the body and creating problems. The most affected system is undoubtedly the hearing system: it becomes damaged and individuals will lose their hearing over time. When studies are considered, it is clear that noise-induced hearing loss is possible to treat and should no longer be a problem.

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**Holistic approach to treat noise-induced hearing loss: Sound and noise**

According to the World Health Organization, sound is "…the name given to the energy that emerges as a result of the effect of vibrations radiating from an energy source." [1]. Noise is defined as unwanted sound signals in the environment [2].

**Hearing physiology**

The hearing system includes factors related to hearing, as well as factors that process sensory information for the balance system. The structures that maintain balance are part of the vestibular system, which process information about spatial orientation, balance, and stabilization. To hear, air and sound must be present, as well as sound waves transmitted to the ear. The auricle, acting as a funnel when sound reaches it, collects sound waves. It sends them towards the outer ear, tympanic membrane, middle ear cavity, malleus, incus, stapes, inner ear, cochlea, vestibulocochlear nerve, and auditory cortex: hearing occurs as processed signals reach these areas.

**Physiopathological damage caused by noise**

Noise creates physiopathological damage to the body, both indirectly and directly; it can disrupt the physiological and psychological order, with negative effects on general health [3]. Physiopathological damage caused by exposure to noise has been identified. It was reported that the risk of cardiovascular disease increases in those who are constantly exposed to noise, i.e., high blood pressure, while success rates at work decrease [4]. Individuals exposed to intermittent noise for long periods have more attention deficits than those who are constantly exposed to it; thus, their job success decreases at a higher rate [5]. It has been found that individuals' cortisol levels increase with exposure to noise, as they experience tachycardia, plus increased mental fatigue, and communication problems [4]. Although problems caused by exposure to noise at a cellular level are not fully understood, it was observed that it creates reactive oxygen uptake in the cell, with necrotic cell death. The hearing threshold gradually deteriorates as a function of prolonged exposure, loss of cochlear nerve cells, and delayed negative effects on the auditory nerve. It was also reported that when individuals are exposed to low-intensity noise for extended periods, the reactive oxygen balance of hairy cells slowly deteriorates: with this issue, metabolic damage occurs within the cell. Tinnitus and sensorineural hearing loss are reported in those exposed to noise, as it damages vestibular organs – which in turn undermines the balance system. With exposure to noise in pregnancy, the numerical density of neurons in the medial geniculate bodies of the fetus is lower than normal, as their numerical density in the fourth and sixth layers of the auditory cortex also decreases [6]. It was found that pregnant women, fetuses, and newborns are the most vulnerable group to noise-related problems, and must be protected [7].

**Safety devices for noise hearing loss**

Precautions against hearing loss are designed to prevent noise-induced loss, subject to implementation with laws and regulations of the states, using international standards - that is, the use of ear protectors, protection training, noise insulation, engineering services, occupational health and safety inspections, monitoring hearing health, and regulating work hours from ambient noise.

**Research: Protection from physical noise**

Personal ear protectors protect from noise; they are a type of protector attached to the ears to protect individuals who work in noisy workplaces. Considering studies on auditory ear protection systems, they reduce noise exposure by about 20 decibels on average. No significant difference could be detected between noise protection with any device: muffs (noise-protecting earplugs) and earplugs where the noise is over 89 decibels [8]. Noise-induced hearing loss can be prevented with equipment and engineering controls [9]. Noise exposure in most occupations is less than 95 decibels; but use of such devices is meager, unless stipulated [10].

**Research: Noise protection within the scope of occupational health and safety**

Within the scope of occupational health and safety, both employers and workers must comply in our country. Protection is determined through laws and regulations, and subject to inspection by institutions, workplace physicians, and occupational inspectors. We must assess studies investigating their effectiveness, along with a comparison of legislation for occupational noise in 22 countries (Latin America, Canada, and the United States). Most countries use a noise limit of 85 decibels, while some countries limit exposure to 140 decibels. This still leaves millions of workers unprotected from occupational noise, as devices are insufficient [11]. More than 13% of the working population in the United States are adversely affected by work noise, but hearing loss cannot be prevented. This loss can damage the country's economy, when it cannot be treated. If 20% of noise-induced hearing loss was prevented, it would contribute to between 58 billion to 152 billion dollars annually to the national economy [12].

**Current medical approaches**

Those exposed to noise at work may experience hearing loss, as it is impossible to eliminate noise completely. Given legislation in many countries, ear plugs and noise isolation systems are available, but these systems cannot fully protect hearing. Research is being conducted to prevent hearing loss from deficiencies: medical research is comprised of 3 groups: pharmacological research, traditional complementary medical research, and genetic therapy research.

**Pharmacological research**

In pharmacological studies, substances are tested on those exposed to daily noise. Promising results have been achieved to prevent hearing loss. Examining the curative effect of oleuropein, an antioxidant for noise-induced hearing loss, it is beneficial if used with noise-induced hearing loss [13]. In one study, examining the protective effect of alpha lipoic acid against hearing loss, it showed therapeutic efficacy against noise-induced ototoxicity [14]. A large-scale study with various types of antioxidants for hearing loss reported that glutathione, D-methionine, ebselen, resveratrol, ascorbic acid, and water-soluble coenzyme Q10 antioxidants have a positive effect on noise-induced hearing loss if used before exposure [15]. In another study, in which glutathione (GSH), a powerful intracellular antioxidant, and reactive oxygen species (ROS), a cleansing anti-apoptotic agent were applied together - their application protects the cochlea from negative effects of noise and possible ototoxic damage [16]. It was reported that a decrease in noise-induced

hearing loss was seen in cases where adenosine amine congener (ADAC), a selective A1 adenosine receptor agonist, was administered regularly in acute noise-induced hearing loss [17]. With the combined use of vitamins and minerals for hearing loss, an injection of beta carotene, vitamin C, vitamin E, and magnesium, the damage in auditory sensory cells was prevented [18].

**Traditional and complementary medical studies**

Traditional complementary medical studies are carried out to treat hearing loss. If we examine traditional studies, in which the effect of propolis is assessed, it shows a significant protective effect [19]. In one study on curcuma longa (curcumin), the roots of the turmeric plant, it was shown to be effective against noise-induced hearing loss with antioxidant activity [20]. Korean Red Ginseng was also investigated, and found to be a strong antioxidant [21].

**Genetic therapy studies**

In recent genome studies, it was shown that those with a genetic susceptibility are more likely to experience loss when exposed to noise [22]. Genetic research on C57BL/6J mice found that compared to other mouse species, they were more likely to experience noise-induced hearing loss [23]. Genetic factors play a key role in the formation of noise-induced hearing loss. The medical realm must detect related genes and develop therapies to correct them. In the future, this therapy will yield treatment for noise-induced hearing loss.

If we assess recent studies, genes encoding procadherin and myosin carry risk factors for noise-induced hearing loss, with gene mutations that cause hearing impairment and tinnitus [23]. Research on the Vglut3 gene found that existing hearing loss was treated and improved with inner ear gene therapy [24]. It is clear that some types of hearing loss can be fully prevented by rapid genetic screening studies in childhood, so that the public health is protected [25]. Given the genome study using the Beethoven model, engineered genome editing agents that disrupt preference for the dominant deafness-associated allele were applied. The next step involved Cas9-guide RNA lipid complexes from the neonatal tmc1bth/+ strain (targeting the Tmc1bth allele gene) injected into the cochlea: a significant reduction in hearing loss was seen with this study [26]. With similar studies, there is great promise for progressive noise-induced hearing loss.

## Discussion

Those with noise-induced hearing loss is increasing. When studies in the last 10 years are assessed, we show that existing noise protection and controls are inadequate. With economy-based studies, individuals with hearing loss increase, dragging economies into financial loss. Pharmacological agents and gene therapies will be of great importance.

**Conclusion**

The measures implemented to prevent noise-induced hearing loss within the scope of occupational health and safety assists sound insulation services, personal protective equipment, and noise protection training, as the occurrence of hearing loss is reduced. It is estimated that the incidence of noise-induced hearing loss will decrease more with the development of preventive methods. As studies on the formation and treatment of hearing loss are better evaluated, there are some genes more affected by noise, such that hearing loss can be prevented and treated with new gene therapy. Hearing loss can be eliminated with the development of pharmacological agents and complementary medical products when exposed to noise. In cases of prevention or treatment, the existing economic damage around the world can be prevented. It was also observed that the added value of new studies in this field is extremely high, with greater reductions.

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# A rare complication of COVID-19 infection: bilateral spontaneous pneumothorax and pneumomediastinum

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**Informed Consent**
The authors stated that they reached the relatives of the patient, whose data they shared in the article. A signed consent was obtained from his wife and father.

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## Abstract

Coronavirus 2019 (COVID-19) infection has affected the whole world since the end of 2019. Patients with the disease may present with various symptoms and develop various associated complications. Spontaneous bilateral pneumothorax and pneumomediastinum can also be detected in patients with COVID-19 who do not receive positive pressure ventilation support and do present with an intense cough. A case of bilateral spontaneous pneumothorax and pneumomediastinum over the course of the COVID-19 infection in a young male patient is reported.

**Keywords:** COVID-19, Spontaneous pneumothorax, Pneumomediastinum

## Introduction

In late 2019, coronavirus disease (COVID-19) caused by acute severe respiratory syndrome coronavirus 2 (SARS-CoV-2) started in China and rapidly spread worldwide, causing a pandemic. Although most patients with COVID-19 show a course of viral pneumonia with good prognosis, in some cases, the disease progresses rapidly to acute respiratory distress syndrome (ARDS), a severe form of acute lung injury [1]. The first COVID-19 case in Turkey was observed on November 3, 2020; in the following eighteen months, the number of cases exceeded six million, and the number of deaths exceeded 50,000.

The occurrence of bilateral spontaneous pneumothorax (BSP) in COVID-19-associated pneumonia has not been widely reported in the literature so far, except for a few cases [2]. Therefore, our case was documented because BPS is a rare complication that may be encountered in COVID-19 patients.

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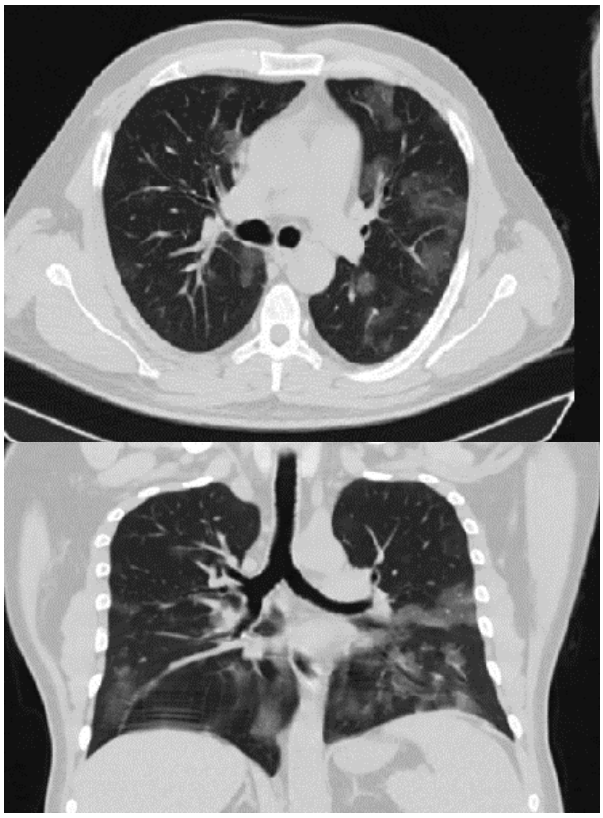
## Case presentation

This case study presents a 40-year-old male patient with a history of mild creatinine elevation and who was not undergoing any medical treatments. He was admitted to the emergency department with fever (> 39° C) and diffuse muscle–joint pain. He was a non-smoker. On physical examination, his general condition was good. His blood pressure was 120/70 mmHg, pulse 110, respiratory rate 16, oxygen saturation 94, and fever 36.5 °C. No pathology was found in the respiratory system examination. No shortness of breath or cough was reported. Upon observing patchy ground-glass opacities in both lungs on thorax computed tomography (CT), he was considered at high risk for COVID-19 and then hospitalized (Figure 1). Based on blood test results, fibrinogen was 559 mg/dl, white blood cell (WBC) count was 21.7 K/uL, creatinine 2.84 mg/dl, potassium 5.1 mmol/L, procalcitonin 0.9 ug/L, and C-reactive protein (CRP) 112 mg/L. COVID-19 real-time polymerase chain reaction assay was positive. Azithromycin, hydroxychloroquine, and favipiravir were used for treatment. Anticoagulation was administered with enoxaparin sodium. Positive pressure oxygen support was not given to the patient. Oxygen support was provided with a nasal cannula.

Figure 1: Thoracic CT image at the time of hospitalization. Bilateral diffuse ground-glass opacities were seen.



On the second day, thorax CT was performed again because of respiratory distress and subcutaneous emphysema in the neck. Bilateral pneumothorax, pneumomediastinum, and increased pulmonary infiltrate were observed (Figure 2). A bilateral thorax tube was inserted, and he was taken to the intensive care unit (Figure 3). Because of the worsening respiratory distress, he was intubated, and mechanical ventilator support was provided. The patient, whose general condition worsened gradually and did not respond to further treatment, died on the 27th day due to multi-organ failure.

Figure 2: An increase in ground-glass opacities, bilateral pneumothorax and pneumomediastinum are observed in thoracic CT taken on the second day of the treatment.

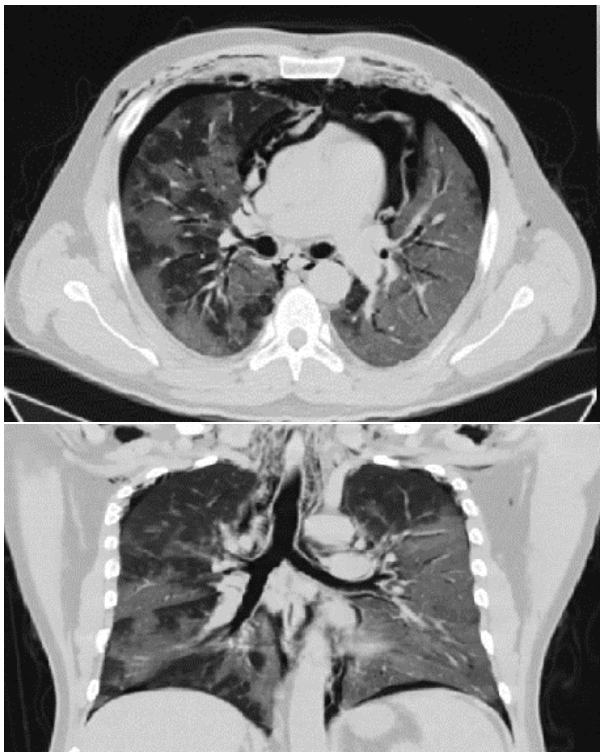
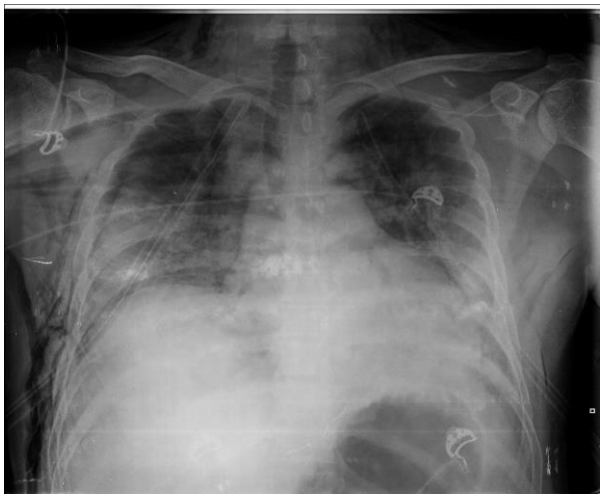


Figure 3. X-ray imaging after bilateral tube thoracostomy.



## Discussion

Many pulmonary pathologies have been postulated as causes of spontaneous pneumothorax. Although the exact component of spontaneous pneumothorax appearance in COVID-19 is obscure, it may be connected to several processes. Infiltrated areas caused by COVID-19 infection in the lung may facilitate the development of pneumothorax. As a result of widespread alveolar damage caused by severe COVID-19 pneumonia, the alveoli may be susceptible to rupture. Pneumothorax may develop in these patients without non-invasive or invasive respiratory support. In addition, patients have a pronounced cough that may induce alveolar rupture. Rafiee et al. [3] reported that strong cough and pre-existing chronic obstructive pulmonary disease (COPD) were possible risk factors for the patients in their report. In our case, bilateral spontaneous pneumothorax and pneumomediastinum developed in a patient without either one of these conditions.

Mediastinal emphysema is caused by a sudden increase in alveolar pressure, causing an air leak with alveolar rupture and

interstitial emphysema and can be seen in ARDS cases [1]. Bilateral spontaneous pneumothorax and pneumomediastinum developed in our patient who did not present excessive coughing and did not receive positive pressure ventilation support. We think that the dissection of the broncho-vascular structures with air (Macklin's effect) caused this situation [4] due to the deterioration of the wall structure of the alveoli, which developed intense infiltration caused by the COVID-19 infection.

Radiological imaging is used in patients who develop sudden respiratory distress. As an imaging method, an X-ray can be used primarily because of its easy and fast accessibility. On the other hand, we think that thoracic CT imaging can provide more detailed information regarding pulmonary infiltration and mediastinal evaluation. Among the most characteristic CT findings in COVID-19 pneumonia are ground-glass and consolidated opacities and septal thickening [2]. In some cases, these lesions can progress despite treatment and may lead to complications.

**Conclusion**

In case of sudden worsening of dyspnea and decrease in respiratory sounds in unilateral or bilateral hemithorax, a pneumothorax should be considered and treated with appropriate methods. When bilateral pneumothorax is detected in patients diagnosed with COVID-19, we recommend performing a bilateral tube thoracostomy and immediately draining intrathoracic air. After drainage of intrathoracic air, regression of the pneumomediastinum is expected. However, it should not be overlooked that COVID-19 infection may affect multiple systems, and patients may die due to multi-organ failure.

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Journal of Surgery and Medicine

# Iliopsoas abscess: A clinical dilemma — case report

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**Abstract**

Iliopsoas abscess (IPA) is the accumulation of pus inside the iliopsoas muscle compartment. The early stages of its clinical presentation are often non-specific and therefore incidentally diagnosed with computed tomography. We describe the case of a 27-year-old man with a history of Crohn's disease presenting with right-sided lower back pain radiating downwards to the lateral part of his thigh and exacerbated with hip movement. Examination of the patient showed a cachectic physique with a fixed flexion deformity at the right hip with a positive psoas sign. We further report the clinical dilemma on the diagnosis between Crohn's disease and intestinal tuberculosis and the subsequent management of IPA secondary to Crohn's disease. Our patient was managed with a loop ileostomy for bowel rest with continuous abscess draining and discharged after 3 months. After the reversal of ileostomy, the patient was satisfied with the overall outcomes. The clinical dilemma stems from the rising incidence of Crohn's disease in Malaysia, as the clinical presentation of intestinal tuberculosis and Crohn's disease is similar. Therefore, it is important for countries transitioning to higher income groups to be able to suspect and treat the condition accordingly.

**Keywords:** Psoas abscess, Crohn Disease, Tuberculosis, Gastrointestinal

## Introduction

Iliopsoas abscess (IPA) accumulates pus inside the iliopsoas muscle compartment, usually by the direct extension from adjacent anatomical structures from a secondary cause. The abscess is detected only in the late stage when it compresses on the iliopsoas muscle presenting as lower back pain exacerbated by hip movement radiating downwards to the lateral thigh and resulting in a fixed flexion deformity [1]. IPA can be diagnosed using computed tomography (CT) [2] and rarely occurs in patients with CD (0.4%–4.3%) [1]; however, the underlying cause for IPA can be difficult to identify in countries where there is an increased incidence of CD with a concurrently high incidence of tuberculosis. We describe a 27-year-old Malay man diagnosed with IPA secondary to CD and how to further differentiate CD and intestinal tuberculosis in countries with increasing incidences of both.

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## Case presentation

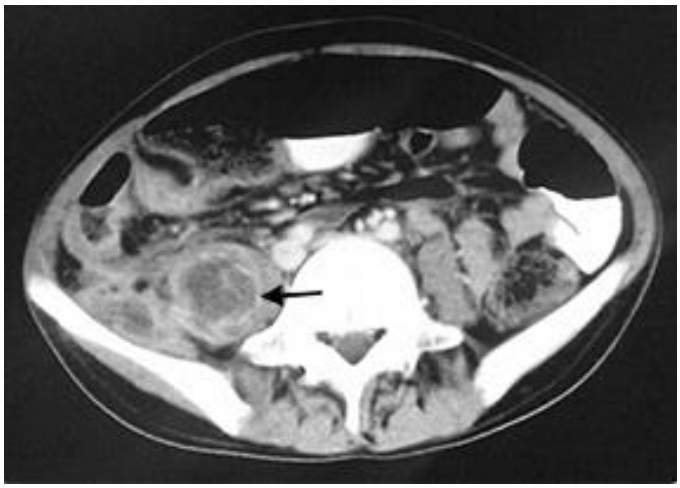
A 27-year-old Malay man presented to us with a 1-week-old right-sided lower back pain radiating down his right thigh, exacerbating with movement forcing him to keep the hip in flexion. Other symptoms included chronic weight loss and repeated hospital admissions for fever of unknown origin associated with diarrhea. During previous admissions, he was suspected of having CD and was started on oral sulfasalazine. He had a history of appendicectomy 4 years ago and denied any record of smoking or other illicit intravenous drug use; his family history was also unremarkable.

Physical examination revealed a young cachectic man lying uncomfortably on his left side in obvious pain with a fixed flexion deformity at the right hip. His abdomen was soft and non-tender with no palpable masses or pain over the inguinal ligament, but the psoas sign was positive. There was no fever, and all his vital signs were stable.

Regarding the laboratory findings, his white cell count was $12.3 \times 10^9$/l. Chronic changes were seen, such as microcytic hypochromic anemia with a hemoglobin count of 5.6 g/dL. His erythrocyte sedimentation rate was above 90 mm/h, C-reactive protein > 50 mg/L, and faecal calprotectin level was 4652 µg/mg. Lowenstein-Jensen (LJ) medium culture and GeneXpert polymerase chain reaction test from the pigtail drain was negative for *Mycobacterium tuberculosis*.

His chest X-Ray was clear; the CT abdomen revealed a multiloculated abscess collection in the right iliopsoas measuring 2.7 × 3.0 × 16.0 cm, with a fistulous connection to the caecum (Figure 1). Transmural discontinuous lesions were found in his colonoscopy, and the ileocecal valve was covered with slough, so the ileum could not be accessed; a biopsy was taken from the discontinuous lesions. Histopathology of the intestinal wall demonstrated a noncaseating granuloma; crypt abscess and basal plasmacytosis were not seen. However, there was evidence of moderate neutrophilic infiltration, consistent with an acute inflammation suggestive of inflammatory bowel disease. Consequently, his Crohn's Disease Activity Index was calculated as 332, indicating active CD. Accordingly, he received aggressive medical treatment with oral sulfasalazine (500 mg) twice daily.

Figure 1: Black arrow shows the right large Iliopsoas multiloculated abscess measuring 2.7 × 3.0 × 16.0 cm having a fistulous communication with the caecum.



**Management**

An ultrasound-guided pigtail catheter was inserted to drain the abscess beginning with 400 cc/24 h. The initial pus culture collected from the drain was positive for extended-spectrum beta-lactamase *Escherichia coli*. One month after inserting the pigtail catheter, a mixed growth of gut microflora was cultured from the purulent collection and a repeat CT scan month after pigtail insertion showed a smaller right iliopsoas collection with persisting fistulous communication with the cecum.

Intravenous antibiotics — meropenem (500 g thrice a day), cefoperazone (1 g twice a day) and metronidazole (300 mg four times a day) — were given for 10 days during his admission. Five packed cell units were transfused until his target hemoglobin count was above 10 g/dL, which stabilized during his admission, and he received total parenteral nutrition throughout the hospital stay to manage his weight loss.

We decided to perform a loop ileostomy to rest the bowel after the second CT scan and allow the abscess to be drained since the purulent collection did not resolve completely. Intraoperative findings showed dense adhesions between the caecum, terminal ileum, and retroperitoneum; accordingly, a peritoneal washout, adhesiolysis, and a diverting-loop ileostomy were done.

Postoperatively, discharge from the pigtail drain was reduced to < 50 cc/24 h, and the patient was finally discharged. Three months later, the pigtail drain discharge dropped to 0 cc/24 h. A repeat abdominal CT scan showed complete resolution of the right psoas collection; the pigtail catheter was removed, and the ileostomy was reversed. The repeat colonoscopy findings showed disuse colitis. No further intervention was done for the IPA after ileostomy reversal, and the patient continued to follow up at the surgical clinic for his CD monitoring. The patient has consented to publish this case report.

## Discussion

Our patient presented with the classical signs and symptoms of IPA. Pott's disease is described as the most common cause of IPA [1]; however, in countries where the incidence of tuberculosis is low, IPA is usually diagnosed as secondary to gastrointestinal tract fistulae, which is a common finding in CD [3].

Malaysia has a high incidence of tuberculosis; therefore, the most common cause of IPA in Malaysia is Pott's Disease. However, CD cannot be ruled out owing to the low sensitivity of laboratory tests for tuberculosis [4, 5, 6]. In our patient, CD was diagnosed after excluding a tuberculous disease by negative results of repeated Mantoux test, acid-fast bacilli test, LJ-medium culture, and GeneXpert. The colonoscopic biopsy further confirmed this, which showed no granulomatous inflammation in the transmural lesions taken from the ascending and descending colon.

Tuberculosis has been a major public health concern in Malaysia, with an estimated incidence of 93 new cases per 100,000 people annually in 2017. However, the incidence is lower when compared with other neighboring countries: (per 100,000) Indonesia (319), Thailand (156), Philippines (554), and Myanmar (358) [7]. Although Malaysia is a high-middle-income

country, tuberculosis is still common because of the rapid influx of foreign workers.

CD is rare in Asia, especially in Malaysia, where the prevalence is 2.17 per 100,000 population, and the incidence rate is 0.18 per 100,000 population [8]. However, the incidence rate of CD in Malaysia is lower than in Singapore (0.39) and Indonesia (0.27) [9]. Likewise, within the Malaysian demographics, Malays had a lesser CD occurrence than the Indians (14.7% vs. 76.5%) [10]. This shows that our patient had a rare IPA presentation secondary to CD.

The gold standard for making a definitive IPA diagnosis is by CT scan with a sensitivity of 100% [2]; however, it is difficult to determine the underlying etiology, and tuberculosis is a significant differential diagnosis because of its prevalence in the region. Given the low sensitivity of serologic testing and biopsies, a definitive diagnosis is also challenging. Testing for inflammatory bowel disease can be done with fecal calprotectin, which shows high sensitivity (93%) and specificity (96%) for diagnosing inflammatory bowel disease [1, 11, 15]. However, fecal calprotectin levels cannot exclude other inflammatory bowel diseases, such as intestinal tuberculous disease [12]. Therefore, a diagnosis of CD can be confirmed by ruling out ulcerating colitis on colonoscopy – discontinuous lesions from the anus and slough found at the ileocecal junction in our patient are the commonly found, but skipped, lesions reported in CD [1, 15]. On the other hand, granulomatous inflammation is commonly found in intestinal tuberculosis, besides the presence of a caseating granuloma, which is pathognomonic for intestinal tuberculosis, whereas this presentation is never found in CD [13]. A misdiagnosis between CD and gastrointestinal tuberculosis could cause a rapid and severe deterioration of the patient's health [14]; therefore, understanding the different laboratory testing is crucial [15].

In our case, performing a loop ileostomy for bowel rest allowed the abscess to be adequately drained before reversal; however, there is no consensus regarding reversal timing. Our patient was reportedly upset over his prolonged hospital stay during the disease management process, continuous monitoring of the ileostomy, and reducing weight. However, after the ileostomy reversal, he was happy with the outcome while continuing care for his CD.

To summarize, IPA secondary to CD is rarely seen in Malaysia; however, with the increasing incidence of CD, it is crucial to recognize it as a potential cause of IPA. Patients diagnosed with IPA should be investigated promptly to optimize treatment outcomes as a delayed diagnosis can reduce the patient's quality of life and increase morbidity. Further, extrapulmonary tuberculosis must always be suspected in countries where tuberculosis is still rampant. Based on our report, it can be concluded that loop ileostomy for bowel rest and continuous drainage of the abscess is a feasible option for patients diagnosed with IPA secondary to CD with intestinal fistula.

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# Undifferentiated pleomorphic sarcoma with focal myogenic differentiation mimicking left atrial myxoma

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## Abstract

Primary cardiac tumors are very rare. Cardiac sarcomas can be detected at any age, regardless of gender. At diagnosis, these are mostly symptomatic and one-third metastatic. Undifferentiated pleomorphic sarcomas account for 1.7% of all cardiac tumors, are typically localized in the left atrium, and involve the mitral valve. Here we present a case of left atrial undifferentiated pleomorphic sarcoma with myogenic differentiation that clinically and radiologically mimicked myxoma.

**Keywords:** Left atrium, Myogenic differentiation, Myxoma, Sarcoma

## Introduction

Primary cardiac tumors are very rare. Their incidence in the autopsy series has been reported as 0.001-0.28%. Undifferentiated pleomorphic sarcomas (UPSs) account for 1.7% of all cardiac tumors [1]. UPSs are typically localized in the left atrium and tend to involve the mitral valve [2]. Here we present a case of left atrial undifferentiated pleomorphic sarcoma with myogenic differentiation that clinically and radiologically mimicked myxoma.

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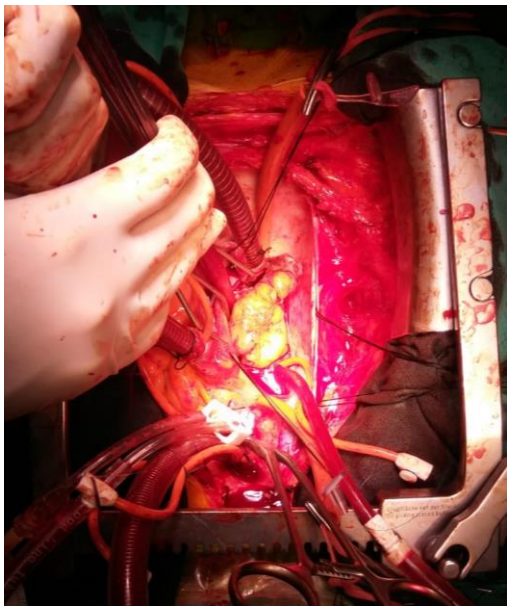
## Case presentation

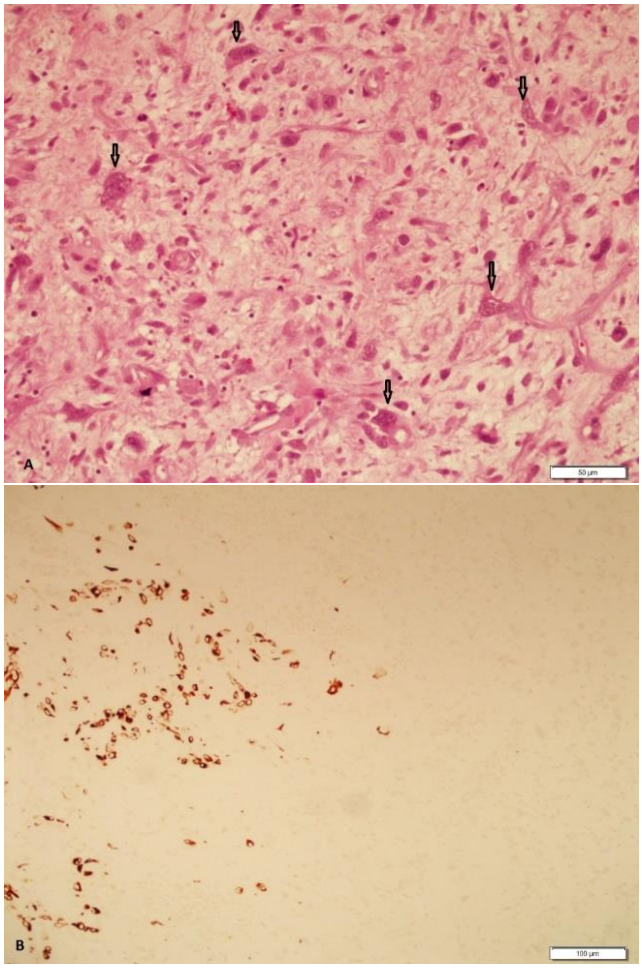
A 37-year-old male patient was admitted due to the complaints of chest pain, palpitation, syncope attacks, and dyspnea that had continued for approximately the past 2 months. His physical examination revealed a 6/5 murmur in the mitral focus. Echocardiography identified a mass (prediagnosed with myxoma) associated with a mitral valve in the left atrium, with a size of 41 × 28 mm, grade 2 mitral regurgitation, and advanced tricuspid regurgitation. The pulmonary arterial pressure was 90 mmHg, and the ejection fraction was 58%. After performing preoperative preparations of the patient and providing required pre-surgical patient instructions, he was submitted to median sternotomy under general anesthesia and right atriotomy under cardiopulmonary bypass following implementation of aorto bicaval cannulation. Left atriotomy was performed because the neighborhood of the mass could not be clearly differentiated when resection of the mass was attempted, and it was observed that the mass elongated from the subendocardial region to the anterolateral commissure of the mitral valve and obstructed mitral orifice (Figure 1). The tumor was subtotally resected. The patient who was postoperatively taken to the CVC intensive care unit was weaned from mechanical ventilation at the postoperative 4 h and externed 6 days later.

Figure 1: Subendocardial cream yellowish mass seen during left atriotomy



Pathological examination, macroscopically, indicated a dirty-cream colored mass with a size of 6 × 4 × 2.5 cm, regular contour, and its crosssection surface with cream-brown color and heterogeneous appearance. Microscopic examination encountered a tumor characterized by diffuse infiltration of pleomorphic, partly multinuclear atypical cells with a large polygonal shape, a large eosinophilic cytoplasm, and intense and atypical mitotic figures in the myxoid stroma (Figure 2A). There were foci of necrosis, and some areas were more cellular. The tumor cells were diffusely immunoreactive with vimentin and focally immunoreactive with SMA and desmin (Figure 2B). Pancytokeratin, S-100, CD34, CD31, myogenin, and myoD1 were non-immunoreactive. The Ki-67 proliferative index was 40%. The patient was diagnosed with undifferentiated pleomorphic sarcoma with myogenic differentiation.

Figure 2: A. Diffuse infiltration of atypical cells (arrows) with hyperchromatic nuclei, a large polygonal shape, large eosinophilic cytoplasm, atypical mitotic figures in the myxoid stroma (Hematoxylin-eosin ×200). B. Focal immunoreactivity in tumor cells with desmin (Desminx100)




No new mass was encountered by echocardiography imaging of the patient at the seventh postoperative month. No residual valvular leak or organic complication was detected by the echocardiographic examination.

## Discussion

Cardiac sarcomas can be detected at any age, regardless of gender. At diagnosis, they are mostly symptomatic and one-third metastatic [3]. Dyspnea, pulmonary venous hypertension, and pulmonary edema are the most commonly seen symptoms [4]. Other symptoms are fever, arrhythmia, chest pain, pericardial tamponade, and congestive heart failure [3]. Also, in our case, dyspnea, tachycardia, and pulmonary hypertension were present consistently with the literature. However, no metastasis was detected.

Left atrial primary sarcomas constitute at least 50% of cardiac sarcomas. UPS is the most frequently type seen among those. A small part of UPS may be seen in the other cardiac sites. These tumors can be easily differentiated from myxomas with characteristics of infiltrative growth in the arterial wall and the absence of a connection with atrial septum by cardiac MRI [5, 6]. Cardiac sarcomas are mostly diagnosed by transthoracic echocardiography. The combination of echocardiography, CT, and MRI guides the preoperative evaluation of the tumor in terms of size, localization, its anatomical relationship with cardiac structures and valves, and differentiation between benign and malignant tumors [3]. This differentiation has importance in the determination of the most appropriate surgical procedure.

The presence of the mass can be detected using only echocardiography, whereas its use alone may be deceptive in identifying the real nature of the atrial masses [7]. UPS may be confused with myxoma because of its endocardial growth pattern in echocardiography [6]. Also, in our case, only echocardiography was implemented as the preoperative imaging technique. However, MRI or CT was not performed. Consequently, the tumor with left atrial localization that presented endocardial growth was erroneously prediagnosed with myxoma in the preoperative period.

The definite diagnosis of the cardiac tumors requires histopathological examination. Almost all soft tissue sarcomas may be seen in the heart. However, consideration of the same histological classification is difficult because there is a limited site where they may reveal morphologically specific differentiation. Besides, even detailed immunohistochemical assessment may be inadequate for differential diagnosis due to the frequent lack of tissue-specific antigens [3]. Microscopically, the important diagnostic parameters are structural, cellular, and microvascular patterns with stromal reaction, hemorrhage, and necrosis [3]. The diagnosis of UPS is reached as an elimination diagnosis. Therefore, a detailed immunohistochemical panel is crucial for differential diagnosis. The differential diagnosis involves other pleomorphic sarcomas, melanoma, anaplastic large cell lymphoma, and pleomorphic and metastatic carcinomas. In our case, anaplastic large cell lymphoma, melanoma, pleomorphic and metastatic carcinomas, and rhabdomyosarcoma were eliminated by the negativity of LCA, S-100, pan-cytokeratin, and myogenin, respectively. UPSs may rarely indicate myogenic differentiation, and this entity has been reported as a poor prognostic factor [8]. Cipriani et al. [8] have detected myogenic differentiation in 15 of 38 UPS cases in their study. Focal expression of desmin and SMA was present in our case. Thus, our patient was diagnosed with undifferentiated pleomorphic sarcoma with myogenic differentiation based on the absence of diffuse expression for these two muscle markers (desmin and SMA), myxoid background, and pleomorphic cells.

The treatment of cardiac sarcomas is complete surgical resection. However, it is usually unavailable. Palliative surgery may be performed to relieve valvular or vascular obstruction symptoms in cases where complete surgical resection cannot be performed. However, local recurrence or metastasis is frequently seen in the first year for the cases who underwent palliative surgery [9]. Although additional adjuvant treatment with chemotherapy or radiotherapy has been defined after surgical treatment, they can provide a limited treatment benefit, and there is still no evidence-based recommendation yet [9, 10]. Complete surgical resection could not be performed. Also, in our case, the mass that elongated from the subendocardial field to the mitral valve and caused an obstruction could be subtotally resected. No recurrence and/or metastasis was detected in the patient at the seventh postoperative month.

**Conclusion**

Primary cardiac sarcomas are rarely seen, presenting a poor prognosis despite surgical and adjuvant treatments. Even though concurrent use of multiple imaging techniques is useful at the stage of prediagnosis to create an appropriate surgical strategy in the establishment of the accurate diagnosis, definite diagnosis requires histopathological examination. It should be kept in mind that, particularly as an elimination diagnosis, that UPS may present myogenic differentiation. The inclusion of such rarely seen subtypes of cardiac sarcoma in the literature will help raise physicians' awareness of this possibility.

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# SARS-CoV-2 and community-acquired pneumonia leading to euglycemic diabetic ketoacidosis in two patients with type-1 diabetes mellitus who were not using SGLT2 inhibitors

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**Abstract**

Diabetic patients are at high risk for mortality after contracting several infections. Additionally, diabetes has been mentioned as an independent factor for progression to severe disease in seasonal influenza and in-hospital deaths due to coronavirus 2019 (COVID-19). Diabetic ketoacidosis is a well-known complication of type-1 diabetes and is characterized by hyperglycemia, metabolic acidosis, and high ketone levels. Euglycemic diabetic ketoacidosis (EDK) is a rare variant of diabetic ketoacidosis in which the blood glucose levels remain within normal limits, but ketoacidosis develops. Although EDK has often been associated with the use of sodium–glucose transport-2 (SGLT-2) inhibitors, it can be induced by several factors, including infectious diseases. EDK may present during the course of an infection, and it also can be a manifestation indicating infection. In this report, two cases of EDK due to pneumonia caused by two different pathogens are presented. Moreover, it is important to emphasize that EDK can occur in type-1 diabetic patients who are not using SGLT-2 inhibitors. Additionally, EDK can be a manifestation of infection and a possible marker of progression to severe disease in patients with type 1 diabetes.

**Keywords:** Acidosis, COVID-19, Diabetic ketoacidosis, Euglycemia, Ketosis, Pneumonia

## Introduction

Impaired immunity due to chronic hyperglycemia leading to dysfunctional lymphocyte and macrophage functions causes diabetic patients to become more susceptible to bacterial and viral infections [1, 2]. Previous studies indicate that diabetic patients are at high risk for mortality in influenza A (H1N1), severe acute respiratory syndrome coronavirus and Middle Easter respiratory syndrome infections (SARS-CoV, and MERS-CoV, respectively) [3, 4]. Furthermore, diabetes was found to be an independent risk factor for progression to severe disease in seasonal influenza [5]. Also, it was shown that types-1 and -2 diabetes are associated with in-hospital deaths due to COVID-19 [6].

Diabetic ketoacidosis is a well-known complication of type-1 diabetes, which is characterized by hyperglycemia, metabolic acidosis, and high ketone levels [7]. Euglycemic diabetic ketoacidosis (EDK) is a rare variant of diabetic ketoacidosis in which the blood glucose levels remain within normal limits. Although EDK has often been associated with the use of sodium-glucose transport-2 (SGLT-2) inhibitors, it can be induced by several factors, including those related to infections. EDK may also occur over the course of infections; furthermore, it may be a manifestation of infections, including COVID-19 [8].

In this report, two cases of EDK due to pneumonia caused by two different pathogens are presented. Thus, it must be emphasized that EDK can be seen in type-1 diabetic patients who are not using SGLT-2 inhibitors. Additionally, EDK can be a manifestation of infection and maybe a marker of progression to severe disease in patients with type 1 diabetes.

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## Case presentation

### Case 1

The first case involved a 24-year-old female patient with type-1 diabetes who was admitted to the emergency department for vomiting and confusion. Based on blood gas analysis, blood pH was 6.80 (normal range: 7.35 - 7.45), bicarbonate 7 mmol/L, oxygen saturation 94%, and carbon dioxide pressure was 10 mmHg. The urinary analysis was positive for 3+ ketonuria. Her blood glucose was 198 mg/dL, and her glycosylated hemoglobin (HbA1c) level was 15. Her parents stated that her oral intake has decreased for the past few days, but she has continued to inject insulin at the same dosage. The patient deteriorated shortly and was intubated and then transferred to the intensive care unit. The leucocyte level was $21\times10^9$ /L, the C-reactive protein level was 23 mg/dL, and the patient was diagnosed with community-acquired pneumonia. Although her blood pH normalized within days, the infection led to a fatal septic shock three weeks later.

### Case 2

The second case was a 37-year-old male with type-1 diabetes who was admitted to the hospital with the symptoms of confusion and vomiting. Blood gas analysis revealed metabolic acidosis with pH 6.90 and bicarbonate 9 mmol/L. Ketonuria (3+) appeared in the urine analysis, and the blood glucose level was 187 mg/dL. Three days before, the patient's polymerase chain reaction (PCR) analysis was positive for SARS-CoV-2. The chest computed tomography (CT) scan showed bilateral infiltrations indicative of COVID-19 pneumonia after which the patient then was transferred to the intensive care unit (ICU). During the follow-up in the ICU, mechanical ventilation was not needed. After medical treatment of ketoacidosis, the patient was discharged from the ICU to the ward.

## Discussion

Diabetic ketoacidosis can be the diagnostic symptom of diabetes mellitus or the hallmark of an underlying condition, such as an infection in a patient with type-1 diabetes. On the other hand, in EDK cases, patients usually present with ketonemia and metabolic acidosis, but marked hyperglycemia is absent [7]. In these cases, diagnosis is often delayed leading to a more perilous condition due to its unique presentation.

Cocaine use, pancreatitis, pregnancy, and Ramadan fasting had previously been suspected as contributing to EDK etiology [9–12]. Recently, data concerning SGLT-2 inhibitors suggest EDK occurs in both type-1 and type-2 diabetes patients with the concomitant use of these drugs. Additionally, after the beginning of the outbreak, several cases of COVID-19-associated EDK in both types of diabetes in the setting of SGLT-2 inhibitor use were presented [13, 14]. Furthermore, it has been suggested that EDK may be one of the endocrinological manifestations of COVID-19 [8, 15]. However, the feature common to all of these COVID-19-induced EDK cases was the use of SGLT-2 inhibitors during the infection. In contrast to the previous cases, none of the patients presented in this report were using any blood glucose-lowering medication other than insulin, and EDK was caused by pneumonia in both patients.

### Conclusion

In conclusion, bacterial and viral pneumonia, including COVID-19 infection, may cause EDK in type-1 diabetes mellitus patients who are not using SGLT-2 inhibitors.

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Journal of Surgery and Medicine

# Cranial air embolism after transthoracic lung biopsy: A case report of a rare complication

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## Abstract

Cerebral air embolism is a rare and fatal complication of computed tomography-guided transthoracic lung biopsy. Lesions with pulmonary vein involvement—especially cavitary lesions—require particular care during procedures performed with a guided needle. Only 2 ml of an air embolism reaching the cerebral arteries is fatal, and a 1-ml cardiac air embolism can be fatal. Hyperbaric oxygen therapy should be started immediately to reduce mortality and ensure recovery among patients who develop unconsciousness and extremity paralysis during or after the procedure including when diagnosed with cranial CT imaging. Hyperbaric oxygen therapy has been reported to reduce mortality by up to 7% and reduces neurological deficits after 48 hours—even in delayed cases. Thus, transthoracic lung biopsy is important for the diagnosis of peripheral lung masses. Here, we present a rare complication after this procedure. Our goal here was to contribute to early diagnosis and treatment by creating awareness.

**Keywords:** Lung biopsy, CT, Air embolism

## Introduction

Cerebral air embolism usually develops after invasive medical procedures and presents with clinical findings such as loss of strength in the extremities, confusion, and speech difficulties. It is a rare cause of cerebral infarction caused by air bubbles blocking the cerebral vessels [1]. Its etiology has been associated with a number of medically invasive procedures such as central venous catheter intervention, laparoscopic/endoscopic procedures, bronchoscopy, dialysis, and lung biopsy [2]. Computed tomography-guided lung biopsy is frequently preferred—especially in peripheral lung masses. There are complications that require conservative or invasive procedures such as hemorrhage and pneumothorax. However, as in the case presented here, cerebral air embolism is a very rare and fatal complication (0.02% to 0.07% of cases in the literature): This rate is estimated to be higher in asymptomatic patients because it cannot be diagnosed [3, 4]. In this respect, we think that our case will contribute to the literature and raise awareness.

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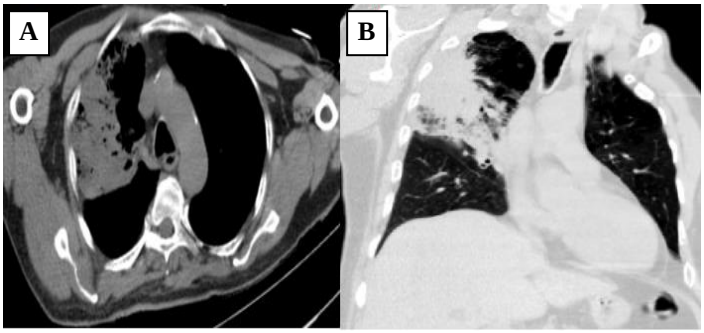
## Case presentation

Here, a 75-year-old male patient presented to the emergency department with complaints of dyspnea, persistent cough, and hemoptysis. Laboratory tests showed that sedimentation and C-reactive protein values were high. Thorax CT showed a mass lesion was observed in the upper lobe of the right lung. A lesion with high-density ground-glass areas was detected extending from the apical part of the upper lobe of the right lung to the posterior (Figure 1 A-B). There was no shrinkage in the lesion after antibiotic therapy.

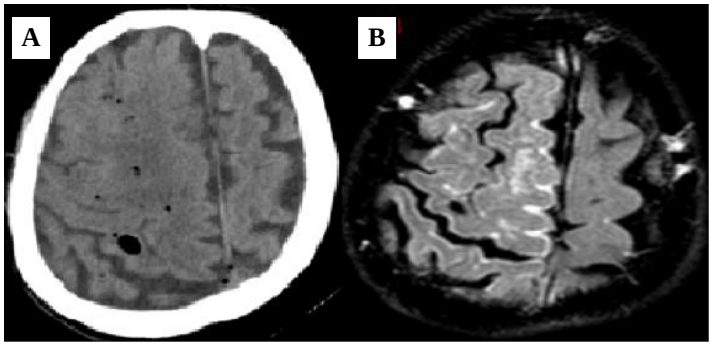
Figure 1: Thorax CT shows a mass lesion extending posteriorly from the upper lobe apical segment in the right lung in the mediastinum and parenchyma window (A-B)




Positron emission tomography (PET) indicated a SUV max value of 9.6. The patient was processed for thorax biopsy under the guidance of computed tomography from the lesion, which was found to be suspicious for malignancy. After cleaning the area, the patient was placed in a decubitus position, and a sample was taken under sterile conditions via a coaxial 17-gauge introduced via an 18-gauge core biopsy needle after local anesthesia. The process was then terminated. The patient was diagnosed with an adenocarcinoma and was biopsied again one week later for molecular testing. This process was repeated and three samples were taken. Control imaging was also performed, and no pneumothorax or hemothorax detected.

When the patient was starting to stand, there was a sudden loss of strength and a change in consciousness on the left side. Areas compatible with air embolism were then observed on cranial CT. Cranial MR showed curvilinear signal changes in the FLAIR sequence due to increased vascularity in the sulci (Figure 2 A-B). The patient had decreased saturation and a general worsening condition; they were intubated and died shortly thereafter. Permission was obtained from the patient's daughter to present the case and share the CT and MRI images.

Figure 2: Cranial tomography image shows millimeter-scale air densities in the parietal lobe on the right (A). Cranial MR image shows curvilinear signal changes in FLAIR imaging due to increased vascularity in the sulci (B).




## Discussion

Air embolisms can be evaluated in two categories. Venous embolisms (as in this case) usually do not pass into the arterial circulation and do not cause cardiac or cerebral embolism. However, this assumes that the patient does not have congenital cardiac defects (ventricular, atrial, or septal defects). In contrast, arterial embolisms may reach the heart or brain with systemic circulation and cause systemic problems [5, 6]. In our case, an intracranial air embolism developed because of an arterial embolism. Only 2 ml of air reaching the cerebral arteries can be fatal; 0.5 or 1 ml of air reaching the coronary arteries can cause myocardial infarction [7].

Several factors have been suggested to increase the risk of air embolism: using a guide needle or a thick needle, coughing during the procedure, cavitary lesions, or involvement of the pulmonary vein. However, air embolisms are sometimes found with thinner needles without using a guide [8]. A guide needle was used in our case as well. Therefore, some air enters the parenchyma during each biopsy. However, no air enters in biopsies performed without using a guide, and thus the risk of air embolism is also reduced. In the treatment of cerebral air embolism, it is important to give hyperbaric oxygen at 2.5-3 atmospheres for four hours [9]. Emergency hyperbaric oxygen therapy can reduce mortality by up to 7% and even in delayed cases it provides a reduction in neurological deficits after 48 hours [10]. There is no hyperbaric oxygen center in our center, and thus this treatment could not be applied because patient transport was not possible. Thus, there are limitations in our case. The procedure was done carefully by experienced people knowing the risk factors, but there can be rare and fatal complications such as air embolisms. Emergency intervention should be performed in patients who develop confusion.

### Conclusion

Cerebral air embolism is a rare but fatal complication of lung biopsy. Although it is very rarely reported in the literature, the number is thought to be higher especially in asymptomatic cases. Cranial tomography plays an important role in the diagnosis. This case will hopefully raise awareness of this risk.

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# Acute severe headache: Association of herpes zoster meningitis and sinus vein thrombosis

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## Abstract

The development of neurological complications related to varicella zoster virus (VZV) is rare, especially in immunocompetent children. We report a case of a 14-year-old male patient who presented with severe headache and diagnosed with acute aseptic meningitis and sinus vein thrombosis caused by reactivated VZV infection. Magnetic resonance (MR) venography identified limited flow, consistent with a thrombus in the right sigmoid sinus. Pressure of cerebrospinal fluid (CSF) was measured as high, with the PCR meningitis panel positive for VZV. These results show an association of VZV meningitis and sinus vein thrombus. Pediatric cases associated with aseptic meningitis and cerebral venous sinus thrombosis to varicella zoster reactivation have not yet been reported in the literature.

**Keywords:** Children, Headache, Herpes zoster meningitis, Immunocompetent, Sinus vein thrombosis

## Introduction

Varicella zoster virus (VZV) is a virus of the herpes family, primarily causing chickenpox, which may remain latent in cranial nerves and dorsal root ganglia after primary infection and then lead to shingles [1]. One of the rarest neurological complications linked to VZV is VZV aseptic meningitis (5-10%), which is an infection of the brain membranes and clinically presents as a headache, vomiting, and high fever. Patients may have neurological symptoms like encephalopathy, convulsions, or motor deficit. A stiff neck and the presence of meningeal irritation (Kernig or Brudzinski signs or nuchal rigidity) on physical examination will direct clinicians to assess the patient in terms of meningitis [2].

We present a 14-year-old male with a diagnosis of acute aseptic meningitis and sinus vein thrombosis by reactivated VZV infection. The patient had a severe headache due to increased intracranial pressure, without other complaints, related to meningitis but no physical examination findings; he had previously been healthy and immunocompetent.

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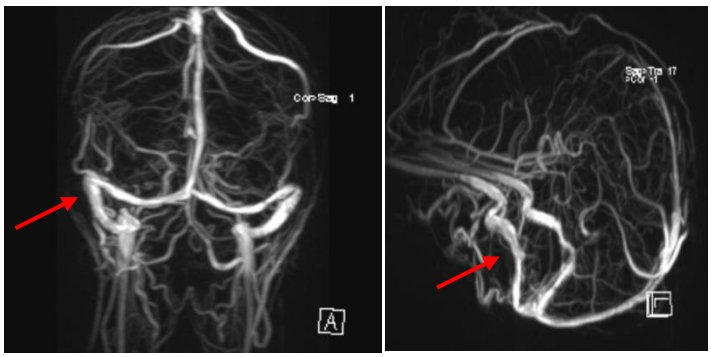
## Case presentation

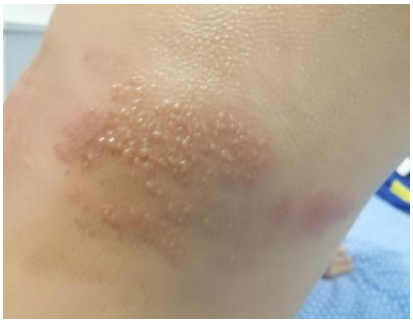
Verbal and written consent was obtained from the patient and family. A 14-year-old male patient attended the pediatric neurology clinic as an outpatient with a severe headache for four days. There were no other complaints such as fever, vomiting, blurred vision, double vision, etc. Headache was severe and continuous, without a special character; however, no factors intensified or lessened the headache. When its severity did not ameliorate after analgesia but continued for four days, an external center performed brain magnetic resonance (MR) imaging for the headache and administered antibiotic treatment with findings consistent with sinusitis. Given that the headache continued with a rash on the body after antibiotics, the patient was referred to our pediatric neurology clinic by the pediatrician at the external center. Physical examination there found a moderate general status, with a pale and anxious appearance. Fever was 37.2°C, respiratory count 17/min, peak heart rate 88/min, arterial pressure 118.75 mmHg, and oxygen saturation 98%. Apart from postnasal purulent discharge and swollen skin with a light pink color and a papule rash in the right lumbar region, there were no pathologic features. A full neurological examination was normal, including the ocular fundus. Due to missing sequences in imaging at the external center, contrast brain MR and MR venography tests were requested. The former identified widespread bifrontal and maxillary sinusitis, while the latter found limited flow, consistent with a thrombus in the right sigmoid sinus (Figure 1). A lumbar puncture was performed with a preliminary diagnosis of increased intracranial pressure (pseudotumor cerebri). Cerebrospinal fluid (CSF) pressure was measured at 27 mmHg. With an intracranial pressure increase, linked to sinus vein thrombosis, the patient consulted with pediatric hematology and was administered two doses per day of low molecular weight heparin. Hypercoagulability studies were normal. CSF glucose was 41 mg/dL (simultaneous blood glucose was 85 mg/dL), and CSF protein was 157 mg/dL. A direct microscopic investigation identified 400 leukocytes/mm3, so ceftriaxone+vancomycin treatment was started with a meningeal diagnosis. CSF included a multiplex polymerase chain reaction (PCR) for meningitis panel and culture.

Figure 1: Magnetic resonance venogram of the brain, showing loss of normal signal intensity in the right sigmoid sinus (red arrows, coronal view, and sagittal view)



On the second day of observation, the vesicular rash in the lumbar region, including dermatomal pain, were consistent with shingles (Figure 2); thus, acyclovir (10 mg/kg/dose, 3 doses) was added to the treatment. With no proliferation of the CSF culture, the multiplex PCR meningitis panel was positive for VZV. Human immunosuppressive virus (HIV) serology was negative. Lymphocyte subgroups, serum immunoglobulin, and complement (C3 and C4) values were normal. The headache gradually diminished and the skin rash ameliorated on the 5$^{th}$ to 6$^{th}$ day of treatment. Acyclovir treatment was administered intravenously for 14 days, but the patient was discharged without complaints by the end of the 14$^{th}$ day. Heparin treatment was continued for three more months. After a four-week follow-up, there were no sequelae and in addition, no comorbidity was identified.

Figure 2: Vesicular lesions with an erythematous background in the left lumbar region



## Discussion

Central nervous system (CNS) infections are significant clinical events with potential morbidity and mortality. Meningitis, an infection of the protective membranes of the CNS, plays a critical role in the spectrum of these infections. The use of conjugated vaccinations reduces the incidence of meningitis from bacterial agents, with viral meningitis as one of the most common types. Enteroviruses, herpes simplex virus (HSV), and VZV are the main etiologic agents of viral meningitis, with other viral vectors such as mumps orthorubulavirus, measles morbillivirus, influenza type A and B, arboviruses (e.g., West Nile virus), and lymphocytic choriomeningitis mammarenavirus leading to meningitis [3].

Herpes zoster is a function of the reactivation of VZV in the sensory ganglion cells after chickenpox or vaccination, with peaks in the 6$^{th}$ to 7$^{th}$ decades of life [4]. In children, recent publications find that it generally peaks from 10 to 14 years of age [5]. In the elderly or immunosuppressed, reduction in cell-mediated immunity to VZV is thought to trigger varicella zoster reactivation [6].

Neurological complications may develop following primary infection and reactivation of VZV, such as encephalitis and cerebellitis. Complications observed with lower frequency include Guillain-Barré syndrome, meningoencephalitis, transverse myelitis, aseptic meningitis, optic neuritis, postherpetic neuralgia, herpes zoster ophthalmicus, peripheral motor neuropathy, and rarely stroke and cerebral venous thrombosis (CVT) [7].

Development of neurological complications like meningitis linked to VZV reactivation is believed to be rare, especially in an immunocompetent child [1]. A study with 92 pediatric patients with herpes zoster reported meningitis in only 5.4% of cases [6]. For this reason, herpes zoster aseptic meningitis is also rare in children with resilient immune systems. Yet, in our patient, there were no results considered to be a specific immune failure in either interrogation about history, family history, or via blood tests.

It is known that neurological complications with VZV infection emerge with immune suppression and vesicular rash [8]. In the literature, pediatric cases are discussed in conjunction with the diagnosis of meningitis with atypical shingles rash in those who are immunocompetent but do not have a skin rash, those with erythema, and papules but no vesicles [4]. Localized pain and paresthesia are common before the occurrence of a rash in herpes zoster. Typical rashes in shingles present in the form of vesicles with dermatomal distribution [9]. In the literature, there are cases with a VZV meningeal diagnosis without a rash and/or irritation findings [8].

A study by Mehta et al. [10] discussed the rare neurological complication of CVT in two cases with chickenpox infection. In the literature, there are adult cases that develop thrombosis of primary infection with VZV [11]. However, a single case associated with acute herpes zoster and cerebral venous sinus thrombosis was found in a 73-year-old male patient [12]. In our literature screening, we did not encounter other pediatric cases associated with aseptic meningitis or cerebral venous sinus thrombosis, nor was they linked to varicella zoster reactivation.

VZV is the only virus replicating in the veins and has been shown to cause vasculopathy in humans. The mechanism for cerebrovascular events following VZV infection may be vasculitis; however, the pathogenesis of a vascular disease is still controversial [10]. A variety of histopathological studies of patients with chickenpox vasculitis show the virus to be in the vein wall causing a non-cytolytic infection in smooth muscle cells, which may cause functional injury to vascular endothelium. These thromboses and smooth muscle cells may lead to subendothelial proliferation of fibroblasts and collagen, causing stenosis [13]. Formation of a thrombus in the venous sinuses is a result of stasis and hypercoagulopathy status due to local trauma in the vein wall, known as the Virchow triplet [14].

VZV meningitis diagnosis is linked to clinical findings with an observation of VZV DNA in CSF with PCR. These patients require antiviral, as well as symptomatic treatment with

IV heparin and oral anticoagulants [11]. Early initiation of antivirals is required, since delayed or inadequate treatment may cause serious neurological complications or even death. As such, IV acyclovir of 10 mg/kg is recommended three times a day for 10-14 days for VZV meningitis [15]. Our patient was discharged in good health after 14 days of IV acyclovir treatment.

**Conclusion**

Children with acute severe headache require a CSF investigation, including CSF pressure measurements even without meningeal findings, although vascular complications must be considered, while venographic and arteriographic assessments should also be planned.

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Journal of Surgery and Medicine

# A case of giant lipoma of parietal peritoneum with literature review

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## Abstract

Lipomas are the most typically diagnosed soft tissue tumors. A lipoma is composed of adipose tissue, may vary in size, and is usually seen in the subcutaneous region, most commonly over the extremities followed by trunk and neck. In this case report, a rare case of giant lipoma that was found in the preperitoneal space arising from the parietal peritoneum measuring 40 x 36 x 5 cm and weighing 3.7 kg is described. This lipoma was successfully excised without any immediate or late post-operative complications and no recurrence for 24 months post-operatively.

**Keywords:** Giant lipoma, Parietal peritoneum, Mass, Surgical excision

## Introduction

A lipoma is a common and asymptomatic soft tissue tumor which is a benign encapsulated fatty tumor, sometimes admixed with other elements (such as fibrous tissue, blood vessels, cartilage) universally can occur anywhere in the human body and does differ in size. The incidences of simple solitary and multiple lipomas are reported to be higher in males than females. Most lipomas are solitary, but multiple lipomas associated with several syndromes have been described. Small lipomas do not require intervention unless the patient requests it. Symptomatic large or deep lesions may be removed by excision. Recurrence rates after excision of a simple superficial lipoma are 1%–2%, whereas rates are significantly higher for other lipoma types [1]. A case of a giant lipoma arising from parietal peritoneum, which presented as an abdominal mass, is described.

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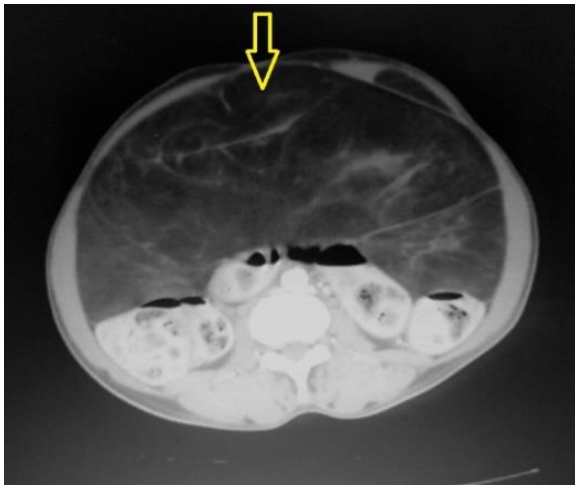
## Case presentation

A 50-year-old woman presented with complaints of a vague non-radiating lower abdominal pain, nausea, reduced appetite, and constipation. No significant bladder disturbances were present, and her vital signs and general physical examination were normal. On examination of the abdomen, lower abdominal distension and a soft, vague mass occupying umbilical, lumbar, iliac fossa, and hypogastric regions were detected. The swelling was intraabdominal with a dull note on percussion. Bowel sounds were present, and no hepatosplenomegaly was noted. No specific laboratory investigation abnormalities were present; abdominal ultrasonography (USG) revealed a sizeable intraperitoneal mass, probably a lipomatous neoplasm with anterior intramural fibroid. The patient had an abdominal computed tomography (CT) scan, which showed a large mass of fat echogenicity measuring approximately 25 x 22 cm within the peritoneal cavity extending from epigastrium to hypogastrium (Figure 1). The lesion created an indentation in the superior surface of the bladder and caused displacement of adjacent small and large bowel loops with anterior intramural uterine fibroid measuring approximately 2 cm.

Figure 1: Computed tomography (CT) scan shows a mass of fat echogenicity measuring approximately 25 × 22cm noted within the peritoneal cavity extending from epigastrium to hypogastrium; the lesion is noted to cause indentation of the superior bladder surface and cause displacement of adjacent small and large bowel loops.



The patient underwent laparotomy. Intra-operatively, a giant lipoma measuring 40 cm vertically and 36 cm horizontally arising from the parietal peritoneum in the preperitoneal space posterior to the anterior abdominal wall was noted. Excision of the tumor was done after ligating the feeding vessels (Figure 2). The parietal peritoneum was preserved since no infiltration to the surrounding structures had occurred. The specimen weighed 3.7 kg and was sent for histopathological analysis, which showed a well-encapsulated tumor with lobules of fat separated by fibrous septa with no obvious lipoblast, suggestive of a benign lipoma (Figure 3). The post-operative period was uneventful, and the patient was discharged on the 8th post-operative day. She remained asymptomatic for 24 months post-operatively.

Figure 2: Intra-operative images of the parietal peritoneal lipoma

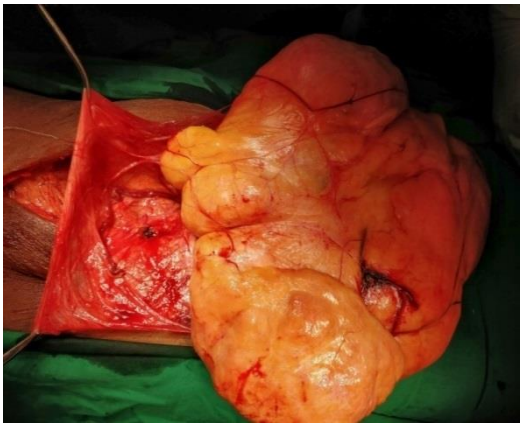
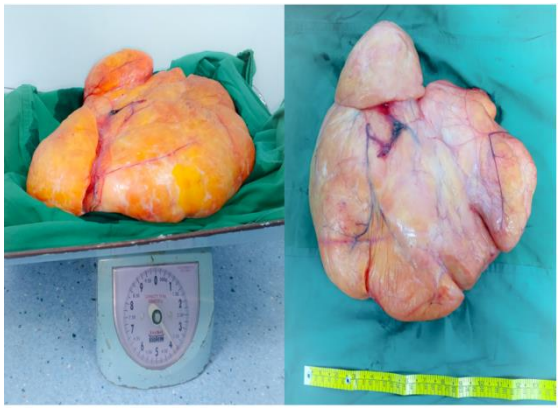


Figure 3: Resected specimen of parietal peritoneal lipoma showing the weight and measurements



## Discussion

Lipomas in the abdominal cavity can present themselves in the mesentery, omentum, and retroperitoneum, but a lipoma of the parietal peritoneum is extremely rare. A deep lipoma usually presents itself when the tumor grows in size or when the patient becomes symptomatic. A giant lipoma can cause symptoms of indigestion, abdominal pain, diarrhea, constipation, ulcer, intestinal obstruction, and even intussusceptions that require surgical or endoscopic treatment [2]. The pathogenesis of a lipoma in the parietal peritoneum is obscure and debatable as it has been classified as a primary peritoneal tumor. Some reports have loosely classified sub-peritoneal lipomas as primary peritoneal tumors, whereas other studies did not [3]. A giant lipoma is defined by Sanchez et al. as a lesion that measures at least 10 cm in one dimension or weighs a minimum of 1000 g [4]. Seven cases of parietal peritoneal lipoma were reported in 2016 (Table 1). The first case of lipoma of the parietal peritoneum was reported by Barut et al. [5] in 2016. Three patients presented to the emergency department with symptoms mimicking appendicitis. These patients presented with abdominal pain, either diffuse or localized to the right iliac fossa. One patient reported by Barut et al. presented with nausea, loss of appetite, and constipation, which were complaints similar to our case. The largest lipoma up to this point had a diameter of 22 cm as reported by Hanlim Choi et al. and weighed 942 grams [5–11]. CT and magnetic resonance imaging (MRI) scans have a significant role in diagnosing a giant intra-abdominal lipoma. Features associated with liposarcoma are lipoma with a size > 10 cm, presence of thick (> 2 mm) septa, presence of non-adipose areas, and lesions that are < 75% adipose tissue [12]. Lipomas

are best treated by a simple excision beyond the tumor capsule, whereas liposarcoma treatment involves a more complex resection with attention to adequate margins. Other differential diagnoses to be considered while diagnosing peritoneal lipoma are lipoblastoma, lymphangioma, and liposarcoma while lymphangiolipoma should also be considered when the tumor size is > 10 cm in size.

Table 1: Cases reported in literature with diagnosis of lipoma of parietal peritoneum and treatment

| No | Reference | Year | Age (years) | Sex | Surgical procedure | Maximum diameter (cm) | Weight (kg) | Presentation |
|---|---|---|---|---|---|---|---|---|
| 1 | Barut et al. [5] | 2006 | 67 | F | Open | 6 | - | Abd pain, nausea vomiting |
| 2 | Bunker et al. [6] | 2013 | 34 | F | Laparoscopy | - | - | Abd pain |
| 3 | Bang et al. [7] | 2014 | 75 | M | Open | 4.5 | - | Abd pain, palpable mass |
| 4 | Shrestha et al. [8] | 2014 | 32 | M | Laparoscopy | 3 | - | Abd pain, loss of appetite |
| 5 | Sathyakrishna et al. [9] | 2014 | 21 | F | Laparoscopy | - | - | Abd pain |
| 6 | Salgaonkar et al. [10] | 2016 | 79 | M | Laparoscopy | 6.3 | - | Abd pain |
| 7 | Hanlim Choi et al.[11] | 2018 | 36 | M | Laparoscopy | 22 | 0.94 | Urinary frequency |
| 8 | Present case | 2020 | 50 | F | Open | 40 | 3.7 | Abd pain, loss of appetite |

### Conclusion

This case is the rarest presentation of a lipoma and highlights the clinical manifestation based on the location and massive size of the tumor. The case highlights the need for consideration as one of the differential diagnoses for pain and mass abdomen. Surgical treatment is based on tumor size and tumor association with the surrounding structure.

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Case report

Journal of Surgery and Medicine

# Gastrointestinal fistula due to multiple neodymium magnet ingestions

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## Abstract

Foreign body ingestion (FBI) is one of the critical conditions encountered in pediatric emergency services. FBI can lead to high mortality in children. Neodymium magnets are increasingly popular due to children's great interest in them. In cases of multiple magnet ingestion, the risk of mucosal perforation is higher when compared with other types of FBI. With this case, an attempt to raise awareness of this medical problem was made by sharing the case of a neodymium magnet body in a 24-month-old patient. The magnet was detected incidentally during our fever etiology examination although possible ingestion of a foreign body had previously been reported in her medical history. Our patient presented with abdominal tenderness, agitation, and other symptoms. An abdominal X-ray was taken. A foreign body that resembled a neodymium magnet sphere was observed. During surgery, a gastrocolic fistula in the antrum was observed. Multiple magnets are foreign bodies that present a higher risk of causing intestinal perforation compared to other foreign bodies. It is known that multiple magnet ingestions may cause mucosal ulceration, and it should be emphasized that such ingestion may cause gastrointestinal fistulas. Since pediatric patients in a certain age group tend to put objects in their mouths, each patient with an unclear cause of fever and abdominal sensitivity should be questioned with respect to FBI and then tested radiologically.

**Keywords:** Foreign body ingestion, Gastrointestinal fistula, Magnets, Pediatric

## Introduction

Foreign body ingestion (FBI) is one of the most critical conditions encountered in pediatric emergency services and general outpatient clinics. FBI can lead to high mortality in children. Neodymium magnets are increasingly popular due to children's great interest in them. In cases of multiple magnet ingestion, the risk of perforation is higher when compared with other types of FBI. The incidence of FBI as reported by the American Association of Poison Control Center's National Poison Data System in 2016 is 1,810,030 patients per year [1]. FBI occurs at a rate of 80% in children, especially in children under three years of age [2]. With this case, an attempt was made to raise awareness of this medical problem by sharing the case of a neodymium magnet body in a 24-month-old patient. The magnet was detected incidentally during our fever etiology examination although possible ingestion of a foreign body had previously been reported in her medical history.

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The authors stated that the written consent was obtained from the parents of the patient presented with images in the study.

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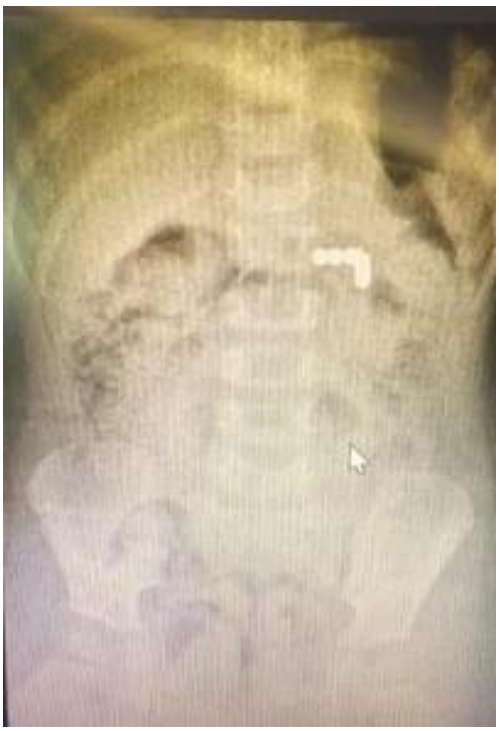
## Case presentation

A 24-month-old girl presented to the pediatric emergency service with a fever of 38.5 ºC. Physical examination revealed abdominal tenderness and agitation. Laboratory tests were unremarkable. Complete urinalysis was normal. Due to the sensitivity of the abdominal examination, an abdominal X-ray was taken (Figure 1). A foreign body that resembled a beaded necklace was noted. When the medical history was taken from the family and they were questioned about the foreign body, the mother stated that her daughter had played with her beaded necklace 20 days ago. The appearance on the X-ray was compatible with the metallic object in the foreground.

Figure 1: Metallic foreign body seen in the stomach on the patient's first abdominal X ray



The metallic opacity of the foreign body in the stomach of the patient could be observed, and due to the fact that it was swallowed 20 days ago, the operating room was prepared and gastroscopy was planned. During gastroscopy, a foreign body fragment that had stuck to the gastric antrum mucosa and caused an ulceration underneath was seen (Figure 2). The piece that was removed with the help of a basket was in the form of three small metallic spheres. On the X-ray taken during the procedure, it could be seen that three pieces were still stuck in the mucosa (Figure 3).

Figure 2: The appearance of an ulcerated foreign body stuck in the gastric antrum mucosa during gastroscopy

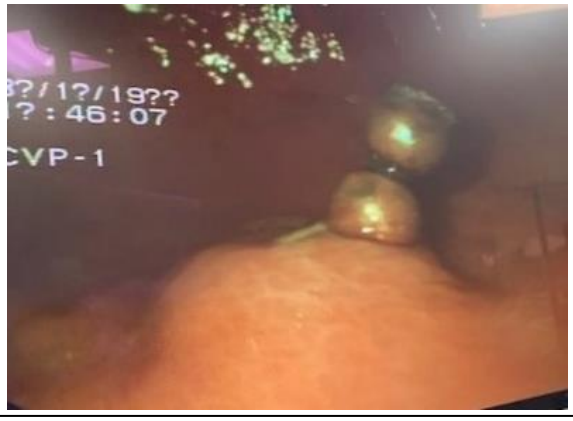



Figure 3: Remaining pieces of magnet causing fistula in the mucosa after gastroscopically removing the piece

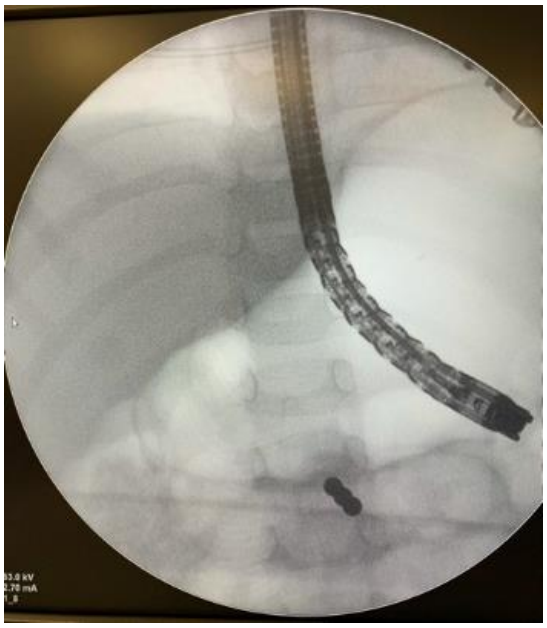
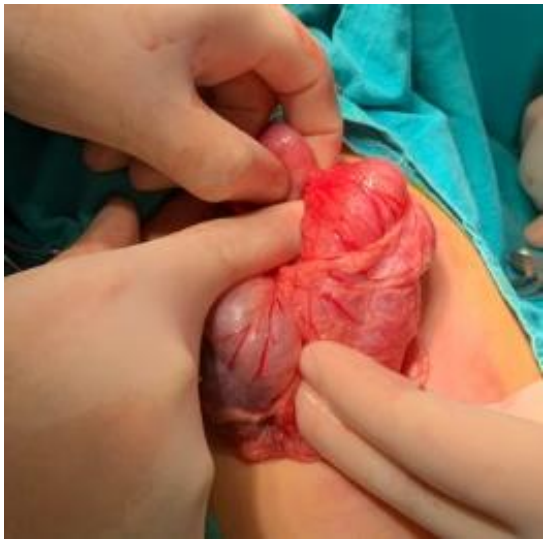


Figure 4: Fistula formed between stomach antrum and transverse colon



It was found that the swallowed object was a neodymium magnet sphere. Surgery with laparotomy was initiated. A gastrocolic fistula between the antrum and the transverse colon was found, and a second fistula was found between the parts beyond the intestine, jejunum, and ileum. (Figure 4). The fistula between the parts of the intestine was repaired, and continuity of the gastrointestinal passage was provided. No complications in the post-operative follow-up of the patient occurred. When she came for the routine control checkup, the patient's examination was normal, and she had no complaints. Informed consent was obtained from the parents.

## Discussion

FBI is an important problem encountered frequently in pediatric emergency departments. While 80% of the swallowed bodies can travel through and be eliminated by the gastrointestinal system without any problem, 10%–20% must be removed endoscopically, and generally, no serious complications are encountered during this process [3]. However, in some conditions, such as button battery ingestion, serious complications can occur even though some children are asymptomatic, whereas others present symptoms. Complications

are most severe in children under four years of age [4]. Symptoms after FBI are nonspecific and can also be seen in other diseases as FBI can mimic a viral infection or cause an infection-related complication [5].

Surgical intervention is performed in 1% of these cases due to obstruction, fistula, and perforation. Multiple magnets are foreign bodies that have a higher risk of intestinal perforation compared to other foreign bodies. Multiple magnet ingestions may cause mucosal ulcerations, and as in our case, it must be emphasized that such ingestion may cause gastrointestinal fistulas. During the procedure, the transition to surgical intervention should be planned, and if possible, removal under operating room conditions should be considered. Perforations have frequently been reported in case reports [6, 7].

**Conclusion**

Since pediatric patients in a certain age group tend to put objects in their mouths, each patient with an unclear cause of fever and abdominal sensitivity should be questioned while recording the medical history and should then be tested radiologically if necessary. It is difficult to recognize the correct diagnosis when the ingestion of the foreign body is not witnessed and therefore, not mentioned in the history by the parents. A delay in diagnosis can cause an increase in serious complications.

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Journal of Surgery and Medicine

# Placement of lumboperitoneal shunt: Etiology of iatrogenic gastric perforation

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## Abstract

Iatrogenic perforation can cause gastric perforation; among the complications of lumboperitoneal shunt placement, intestinal perforation is extremely rare vs. infectious complications; perforation may occur in the ascending colon with projection of the incision site. This case involves a 41-year-old patient, with gastric perforation from pseudotumor cerebri during incision in the abdominal right quadrant to insert a lumboperitoneal shunt.

**Keywords:** Iatrogenic gastric perforation, Lumboperitoneal shunt complications, Pseudotumor cerebri, Obesity

## Introduction

Gastric perforation is a surgical emergency, with high mortality and morbidity. One common cause is peptic ulcer, which can randomly occur, or be due to endoscopic interventions [1, 2].

Pseudotumor cerebri manifests with increased intracranial pressure, although its etiology is not clear; it is known to be more common in women and obese women of childbearing age compared to the general population, with the primary goal of treatment to reduce intracranial pressure. Secondary causes and weight loss in obese patients must be recognized [3]. A lumboperitoneal shunt was applied, while inserting a catheter for cerebrospinal fluid drainage at the appropriate intervertebral distance; it is a surgical procedure in which the distal end of the catheter is advanced under the skin and delivered to the incision from the abdomen; this end is inserted into the peritoneum [4]. Iatrogenic intestinal perforation, though rare, is a possible complication with the incision in the right abdominal quadrant for lumboperitoneal shunt insertion, more likely in the ascending colon. The aim of this report is to discuss the development of gastric perforation, and the approach to lumboperitoneal shunt insertion.

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## Case presentation

We present a 41-year-old female patient with no known issues other than 2 lumboperitoneal shunt insertions from pseudotumor cerebri and a history of shunt dysfunction: the neurosurgery team planned to reinsert a lumboperitoneal shunt. The proximal end of the shunt was placed appropriately in the lumbar region, with the distal end advanced through subcutaneous tunneling to the minimal incision area from the right quadrant of the abdomen; the incision in the right quadrant was deepened, so this tip can be placed on the peritoneum. A general surgery team is there for repair of the ascending colon; this resembled a gastric structure vs. the distal intestines, so its contents were clear with minimal bile, and there was no digested intestinal content. Her incision was widened a few cm and the anterior surface of the stomach antrum was observed (Figure 1). The perforated area was repaired with a double layer of primary suture. It was observed that there was no leakage by administering methylene blue with the inserted nasogastric tube, so the operative area was irrigated with saline, and the fascia was closed without the distal end of the shunt placed in the abdomen, given the possibility of infection. The distal end was placed in the subcutaneous region of the peritoneum in a second surgery, with precautions to prevent drainage. In the postoperative period, oral intake was started by removing the nasogastric tube, which was accomplished after control with a fluoroscopic upper gastrointestinal system passage graph. The patient was discharged without any abnormality in the graph. After ~1 month, gastroscopy was performed. There was no abnormality in the repair area with other gastroscopic findings. The distal end in the right quadrant of the abdomen was reoperated after the shunt was active and cerebrospinal fluid drainage was ensured; it was advanced to the abdomen and left on the peritoneum after a controlled, gradual incision (Figure 2). There was no pathological finding at discharge with routine controls.

Figure 1: Supramuscular placement of the distal end of the lumboperitoneal shunt after primary repair and abdominal closure of the gastric perforation site

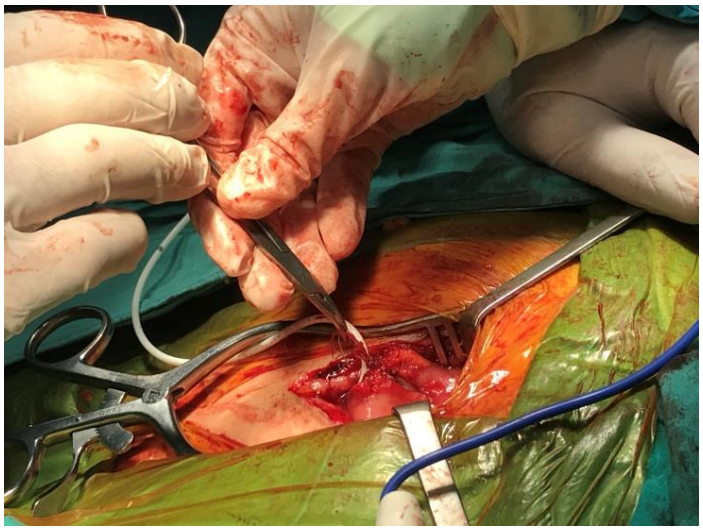
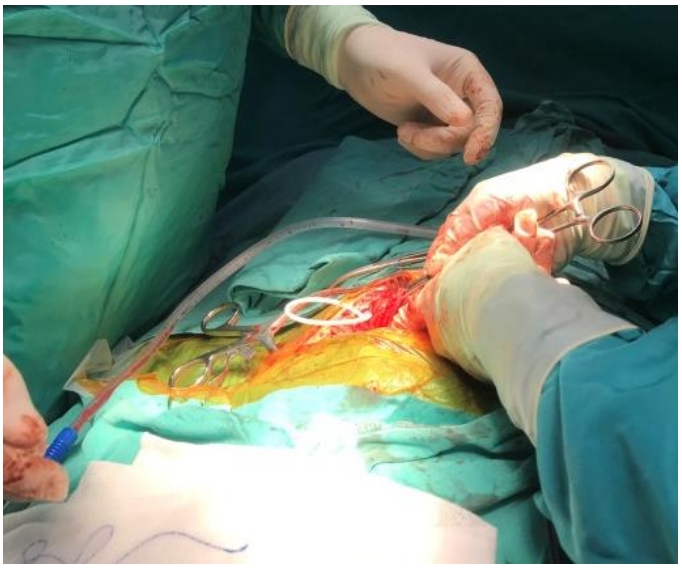


Figure 2: Placement of the distal end of the lumboperitoneal shunt in the peritoneum following surgery



## Discussion

The most common complication with lumboperitoneal shunts is obstruction, and the second complication is intracranial hypotension, while infectious complications are among them [5]. In addition, studies report that the mortality rate can reach 2% in patients who have undergone shunt placement in the past [6], as a perioperative complication had developed. The gastric perforation was noticed in time, and brought under control with follow-up and control imaging in the postoperative period.

Rather than an iatrogenic intestinal injury, we had an extremely wide, elongated gastric structure that allows complications, extending to the right quadrant. It is clear that a full stomach can expand by liters, so preoperative fasting precluded pathology after gastric emptying, which occupies a large anatomical space. In addition, perforations are observed more frequently in patients with gastric pathology, which prompted us to question this situation [7]. Obesity, which is occasionally present due to pseudotumor cerebri, emphasizes caution for nutritional problems and gastric pathology, but there was no obvious abnormality in this patient.

### Conclusion

Frequent complications with lumboperitoneal shunts are obstruction and intracranial hypotension, with infections also occurring. Intestinal injury is rare in this case. Obesity, which may be present with pseudotumor cerebri saw no obvious abnormality in this patient. The team must be cautious with nutritional problems and gastric pathologies in such patients.

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Journal of Surgery and Medicine

# A case of multiple sclerosis diagnosed with tuberculosis during teriflunomide therapy

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**Informed Consent**
The authors stated that the written consent was obtained from the patient presented with images in the study.

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

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Previous Presentation
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## Abstract

A case of pulmonary tuberculosis (TB) in a patient with multiple sclerosis (MS) receiving teriflunomide is described. A 59-year-old woman with MS started interferon beta-1a treatment in 2009. Due to side effects, her physician switched her to glatiramer acetate. Over the last three years, she opted not to continue the glatiramer acetate. At the end of 2017, her symptoms reappeared, and her radiological and clinical examination showed disease progression. Teriflunomide (14 mg/day) treatment was started, and in the 15th month of teriflunomide use, a rash on the skin, coughing, night sweating fits, weakness and back pain developed. She was diagnosed with pulmonary TB based on pulmonary examination. Her purified protein derivative (PPD) score was 22. Anti-TB therapy was initiated immediately. At last visit in January 2020, TB was discovered. Her blood tests were within normal limits. Her expanded disability status score (EDSS) score was 2.5. Subcutaneous glatiramer acetate was re-initiated. A rare case of pulmonary TB under teriflunomide use is described in this case. Teriflunomide therapy should be considered in cases in which latent TB may be re-activated.

**Keywords:** Pulmonary tuberculosis, Multiple sclerosis, Teriflunomide

## Introduction

Tuberculosis (TB) is an infectious disease that is triggered by respiratory transmission of the bacterium, *Mycobacterium tuberculosis*, which may spread via the lympho-hematogenic pathway into all organs. While TB's incidence is decreasing, it can still be fatal due to latent infection in immunocompromised people. Teriflunomide is an anti-inflammatory immunomodulatory/immunosuppressive medication that is classified as safe and effective in multiple sclerosis (MS) patients [1]. The case of pulmonary TB in a patient with MS receiving teriflunomide is described.

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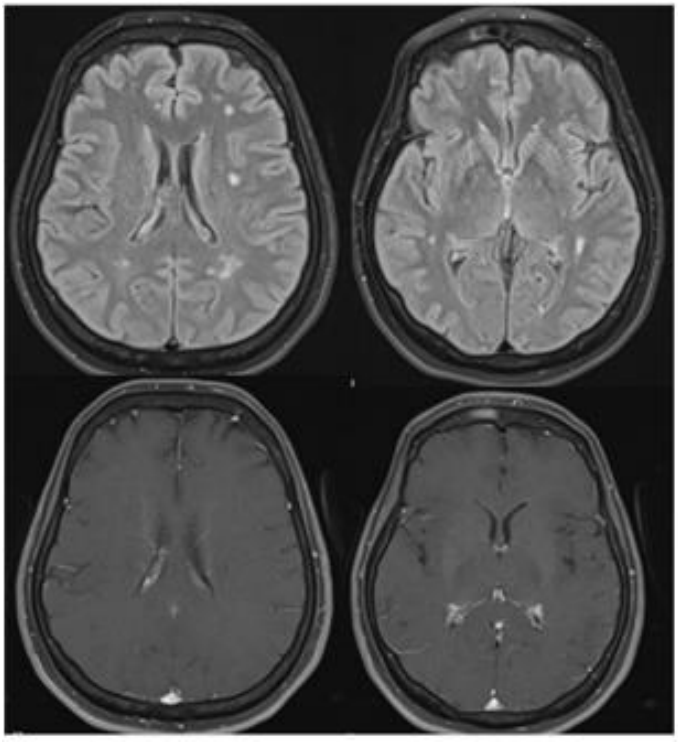
## Case presentation

The necessary permission was obtained from the patient to share her information.

A 59-year-old female patient presented to our hospital for the first time in 2009, and interferon beta 1a treatment was started after a diagnosis of MS was obtained. Due to allergic responses, treatment-related issues, and flu-like effects, her drugs were changed more regularly without any causing any problems. However, the patient had not received medical care and had not received glatiramer acetate for almost three years at her own discretion. At the end of 2017, the patient approached our clinic for a short walking distance test. Radiological and clinical analyses revealed disease progression (Figure 1). Her expanded disability status score (EDSS) score was 2.5. Teriflunomide (14 mg/day) was ordered for the patient since she requested oral medication. A skin rash, coughing, night sweats, fatigue, and back pain during the 15$^{th}$ month of teriflunomide treatment developed. Thorax computed tomography (CT) results in combination with a pulmonary examination identified pulmonary TB. Her purified protein derivative (PPD) score was 22 (normal range: 0-15). Her blood tests were within normal limits. No additional risk factors for tuberculosis were found. She immediately began therapy with ethambutol 1000 mg/day, pyrazinamide 1000 mg/day, isoniazid 300 mg/day, and rifampicin 600 mg/day. Those treatments ended after the recommended time. She was free of TB at her last visit in January 2020, and her blood tests were within the normal limits. Her EDSS score was 2.5. Subcutaneous glatiramer acetate was then reinitiated.

Figure 1: Multiple demyelinating lesions of the patient. The first row shows axial FLAIR, and the second row shows the corresponding contrast-enhanced axial T1-weighted images



## Discussion

Teriflunomide presents clear, reliable, and achievable protection and tolerability profiles as reported in the literature, and no evidence of an elevated risk of any infection in placebo-controlled trials has been found (Teriflunomide versus placebo: 52.7% versus 53.4% or serious infection 2.7% versus 2.2%). This drug also presents a risk for causing severe opportunistic infections in 0.2% of patients. Lymphopenia and neutropenia were confirmed to occur in the first three months of diagnosis, but this prevalence did not reach 15% [1].

Teriflunomide causes a decrease in T-cell activation by altering integrin activity and intracellular calcium signals leading to a shift in the immune cytokine profile to anti-inflammatory T-helper 2. It may also affect components of the innate immune system by modifying the role of adhesion molecules, neutrophils, and macrophages and causing an increase in interleukin-10 (IL-10) secretion by macrophages and microglia [2]. The IL-10 cytokine family has a significant function in TB. IL-10 and -26 suppress antimycobacterial immunity, creating a suitable environment for TB survival [3].

As a treatment for multiple sclerosis (MS), immunomodulators and immunosuppressive therapies are commonly used, but long-term follow-up is important, particularly in terms of side effects, such as activation or reactivation of latent infection. TB cases under interferon-beta-1a among other therapies have been reported in the literature [4]. Distinct mechanisms that control gene expression during TB infection have been found. However, the mechanism by which *Mycobacterium tuberculosis* causes development of interferon-stimulated genes in human macrophages is currently unknown [5]. A case of TB-related meningitis has also been documented under fingolimod therapy, which is one of the new oral treatments; however, the main mechanism for meningitis development needs to be clarified. The underlying mechanism can be explained by fingolimod-induced effects on the lymph nodes affecting T-and B-cells and by causing a reduction in the number of lymphocytes in the peripheral blood. The loss of peripheral lymphocytes impairs immune function and triggers serious infection, such as varicella-zoster virus [6].

### Conclusion

Immunosuppression primarily occurs due to lymphopenia/neutropenia-induced opportunistic infections. However, infection can also be caused by different pathways, such as interleukin pathways, without influencing the number of cells. Therefore, it is necessary to evaluate patients undergoing these treatments in detail, particularly for TB in clinically suspicious cases. It should be emphasized that patients who use different immunosuppressive–immunomodulatory therapies should be followed more closely in terms of opportunistic infection development.

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Journal of Surgery and Medicine

# A rare case of pseudoglandular schwannoma

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**Abstract**

A pseudoglandular schwannoma is a rare benign tumor. Controversy in the literature regarding the histogenesis of pseudoglandular schwannoma exists. Histological features of pseudoglandular schwannomas are different than those found in classical schwannomas. A patient with pseudoglandular schwannomas has a good prognosis, and no cases of recurrence have been reported in the literature.

**Keywords:** Pseudoglandular schwannoma, Pseudocyst, Schwannoma

## Introduction

Pseudoglandular schwannoma is a rare type of schwannoma first described by Ferry et al. [1] in 1988. Known variants of schwannoma are cellular, plexiform, ancient, melanocytic schwannoma, and glandular schwannomas [2]. Pseudoglandular schwannoma has not yet been included in the textbooks. Very few cases of this type of schwannoma have been reported as only 10 cases in the literature can be found. Pseudoglandular schwannoma is histologically similar to typical schwannoma findings accompanied by cystic spaces of various sizes. Although histogenesis is a controversial topic in the literature, two theories have been proposed [1, 2]. The first theory suggests that cystic cavities occur due to the degenerative change of schwannoid cells, and the second suggests that Schwann cells develop via “epithelial metaplasia”. In this study, a case of pseudoglandular schwannoma with a predominant pattern is presented. Pseudoglandular schwannoma is uncommon and can serve as a diversion when attempting to distinguish it from other tumors that are included in the differential diagnosis of schwannoma.

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**Informed Consent**
The authors stated that the written consent was obtained from the patients presented with images in the study.

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No conflict of interest was declared by the authors.

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## Case presentation

In our case, a 49-year-old female patient from whom informed consent was obtained to publish this case report was admitted to the orthopedic clinic with the complaint of a mass in the right shoulder. On a contrast-enhanced magnetic resonance (MR) image, a well-circumscribed, 4 x 4 x 3 cm, septated, heterogeneous mass was observed. The tumor did not invade surrounding muscle tissues and was reported to be compatible with synovial sarcoma.

Part of the material was excised for pathologic examination. The material consisted of small, brown tissue suggestive of the cyst wall on macroscopic examination. Microscopic examination under low magnification revealed tumoral tissue with an encapsulated appearance and small cystic cavities(Figure 1). When the cystic cavities were viewed under higher magnification, it was observed that 2–3 rows of epithelial-looking cells lined the lumen. These cells had large cytoplasmic areas and formed eosinophilic zones on the apical sides (Figure 2). In the lumen, eosinophilic secretions with bleeding, foamy, and hemosiderin-laden macrophages were observed in the cyst wall. It was observed that around the cysts, spindle-shaped, narrow eosinophilic cytoplasmic cells formed bundles. Peripheral nerve bundles and muscular tissues were observed within the soft tissue around the tumor. It was noticed that the peripheral nerve bundle was continuous with the tumor capsule.

Figure 1: A,B,C: Pseudoglandular areas at low magnifications. D: Foamy macrophages and hemosiderin-laden macrophages on the cyst wall (Hematoxylin and eosin [H&E] staining x40)

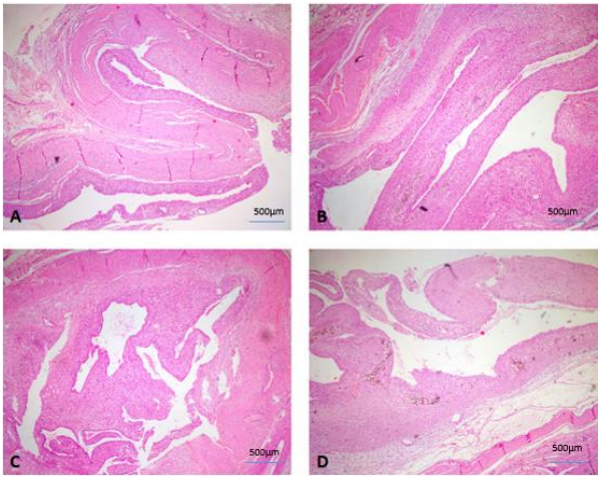
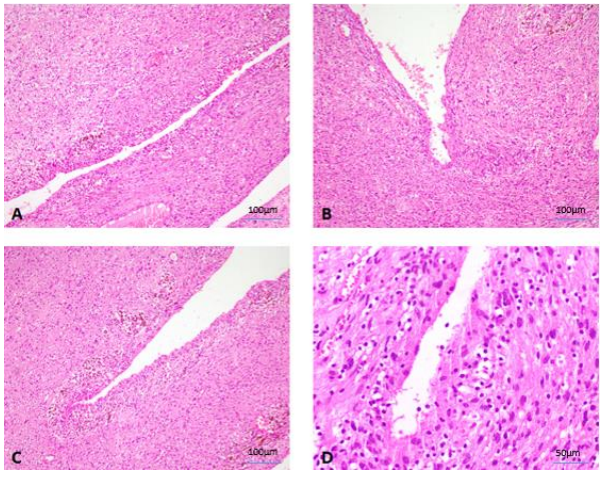
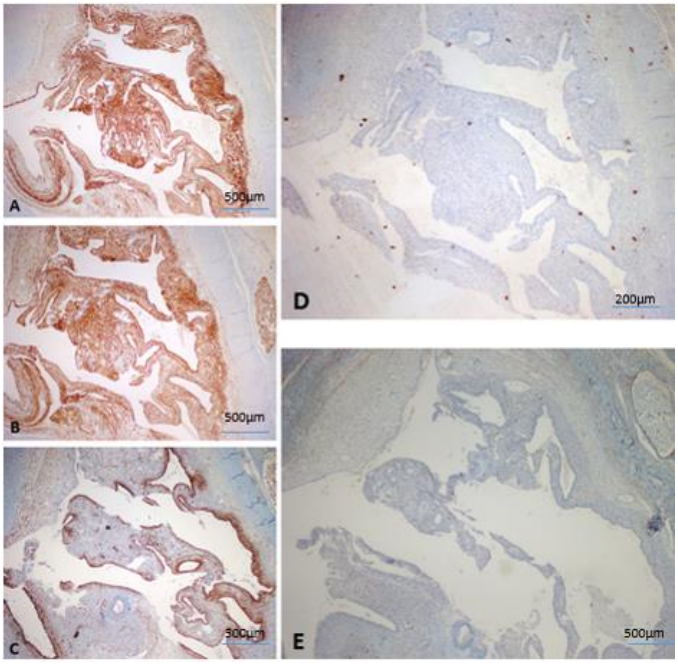



Figure 2: A, B, C, D: 2–3 rows of epithelial-looking cells lined the lumen, these cells had large cytoplasm and formed an eosinophilic zone on the apical side. (A,B,C: H&E x200 D: H&E x400)




In the immunohistochemical study, the pseudostratified epithelial cells exhibited diffuse and strong positivity for cytoplasmic S100, WT1, and CD56. Negative staining with pan-cytokeratin, TLE-1, and epithelial membrane antigen (EMA) was observed in both spindle areas and cystic cavities (Figure 3). The Ki67 proliferation index was found to be 3% in spindle cell areas of the tumor.

Figure 3: A: Strong and cytoplasmic staining with CD56 (x40) B: Strong and cytoplasmic staining with S100 (x40) C: Strong and cytoplasmic staining with WT1 (x40) D: Negativity staining with pan-cytokeratin (x100) E: Negativity staining with EMA (x40)




With the present findings, our case was diagnosed as a pseudoglandular schwannoma. After the diagnosis was made, total excision was of the mass was performed. No additional treatment was required. Complications and recurrence did not develop in the patient for whom regular follow-up was recommended.

## Discussion

A pseudoglandular schwannoma was first described in a mass with spinal canal localization by Ferry et al. [1] in 1988. In another article, a series of three cases with retrobulbar, submandibular, and shoulder locations was presented [2]. Two of these cases involved female patients, and one involved a male. Ages ranged between 24 and 37. A similar case with one shoulder location was found in the literature. Our case involved a middle-aged female patient and describes the second case of pseudoglandular schwannoma was located in the shoulder. In a retrospective study by Robinson et al. [3] in 2005, 202 schwannoma cases were examined, and 16 (7.9%) were found to contain pseudoglandular structures. While the frequency of pseudoglandular structures varies between cases, only one cystic cavity was observed in one case, and more than 80 cystic spaces were observed in another case. In a similar study with more cases, pseudoglandular structures were seen in 61 (6.3%) of 971 schwannoma cases [4]. In this study, it was reported that an average of seven cystic cavities were observed per case, and no correlation between the tumor size and the number of cystic spaces was found.

Controversy in the literature regarding the histogenesis of pseudoglandular schwannoma exists. The theory considers the capabilities of nervous system cells to differentiate into various cells. Cells that form pseudoglandular structures undergo "epithelial metaplasia". In the case report by Ruggeri [5], the findings of focal epithelial membrane antigen (EMA) and cytokeratin positivity in pseudoglandular areas support this theory. It has also been reported that pseudoglandular schwannoma may progress to glandular schwannoma [6, 7]. The second, more common theory states that pseudoglandular areas are formed by degeneration and cystic enlargement of the Verocay bodies [1, 2]. The observation of hemosiderin-laden macrophages accompanying pseudoglandular areas in most cases may also be evidence that these areas are degenerative [4]. In the case presented by Ferry et al. [1], the cells lining these cystic cavities proved to be of schwannoid origin based on results from both ultrastructural and immunohistochemical evaluations. In our case, the fact that the pseudoglandular areas did not express pancytokeratin and the diffuse and strong reaction observed with S100 supports the second theory.

When the histopathology of pseudoglandular schwannomas is examined, it can be seen that Antoni A and B areas, which cause the biphasic appearance observed in typical schwannoma morphology, are accompanied by various enlarged cystic cavities. In most cases of pseudoglandular schwannomas encountered in literature, the cystic cavities are observed as scattered foci within the tumor and do not form a dominant pattern. In the article of Deng et al. [8] in which pseudoglandular schwannoma with skin localization is presented, it was demonstrated that the dominant pattern was pseudoglandular structures. In our case, pseudoglandular areas constituted the dominant component of the tumor and created scarring of a small tumor.

In parallel with the idea that pseudoglandular areas are of schwannoid cell origin, it has been reported that these areas exhibited a diffuse and strong positivity for S100. In our case, a strong and diffuse reaction was obtained with CD56 and WT1 in addition to S100. It is known that schwannomas are frequently stained with CD56 [9, 10]. CD56 staining of a pseudoglandular schwannoma has been reported in one case in the literature [11]. However, to our knowledge, cytoplasmic staining with WT1 in pseudoglandular areas has not been previously reported. If supportive findings are obtained in future studies, WT1 may be a helpful marker in the diagnosis of pseudoglandular schwannomas.

In our case, a neurofibroma was excluded after observing strong and diffuse staining with S100. Diffuse staining with S100 is not expected in neurofibromas because neurofibromas contain a mixed cell population [11]. Another difference is that neurofibromas are infiltrative, whereas schwannomas are usually encapsulated. A malignant peripheral nerve sheath tumor was excluded based on the absence of pleomorphism and mitosis in the case and diffuse S100 staining. Contrary to what is expected in schwannomas, our case had dense cystic spaces that caused a heterogeneous appearance radiologically. In the adult patient, the diagnosis of the mass located in the vicinity of the large joint was synovial sarcoma. Synovial sarcoma was excluded because we observed TLE-1, pan-cytokeratin, and EMA negativity.

Patients with pseudoglandular schwannomas have a good prognosis, and no cases of recurrence have been reported in the literature. Removing schwannomas can cause some sensational deficit; however, this deficit is temporary [12].

**Conclusion**

A pseudoglandular schwannoma is a rare entity with controversial histogenesis. According to the common view in the literature, pseudoglandular areas are formed as a result of degenerative changes in schwannomas. The diagnostic criteria of this entity, which has not yet been found in textbooks, are not clear, and it may be possible to establish a diagnostic criterion by determining a cut-off value based on the number of pseudoglandular structures. Our case draws attention to schwannom variants and their histopathological features. The presentation of such cases can be useful for awareness of this rare structure, and pseudoglandular schwannoma cases can be considered as a different entity.

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Journal of Surgery and Medicine

# Rare nervous system involvement in an anti-myelin oligodendrocyte-positive case: spinal leptomeningeal involvement

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## Abstract

Anti-myelin oligodendrocyte (MOG)-positive cases have a widespread clinical presentation. MOG autoantibodies are associated with acute disseminated encephalomyelitis, multiple sclerosis, clinically isolated syndrome, neuromyelitis optica, isolated optic neuritis, and transverse myelitis. Our patient had bilateral optic neuritis with acute tetraparesis. Cranial magnetic resonance imaging (MRI) showed leptomeningeal contrast involvement and contrast enhancement in all cranial nerve nuclei. Spinal MRI revealed leptomeningeal contrast enhancement, and a contrast-enhancing lesion was found in the cervical spinal cord.
This case is worth presenting because spinal leptomeningeal involvement is rare in anti-MOG-positive patients.

**Keywords:** Anti-MOG, Tetraparesis, Spinal, Leptomeningeal enhancement

## Introduction

Anti-myelin oligodendrocyte (MOG) is found on the outer surface of the myelin sheath and on oligodendrocytes in the central nervous system [1-3]. Anti-MOG-positive cases have a widespread clinical presentation. MOG autoantibodies are associated with acute disseminated encephalomyelitis (ADEM), multiple sclerosis (MS), clinically isolated syndrome, neuromyelitis optica (NMO), isolated optic neuritis (ON), and transverse myelitis (TM) [2, 4-7]. Here, we report the case of a patient with spinal leptomeningeal contrast enhancement, which has rarely been reported.

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The authors stated that the written consent was obtained from the patient presented with images in the study.

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## Case presentation

Written informed consent was obtained from the patient to publish this study. An 18-year-old female patient was admitted with complaints of progressive blurred vision, arm weakness, and gait disturbance. An eye examination revealed a bilateral relative afferent pupillary defect, and she could see only bilateral hand motion. Bilateral papilledema was also observed during the fundus examination (Figure 1). Motor strength was bilaterally 4/5 in upper extremities and 2/5 bilaterally in lower extremities, deep tendon reflexes were globally hypoactive, and the plantar response was neutral. Urinary retention occurred the next day. Cranial magnetic resonance imaging (MRI) showed leptomeningeal contrast involvement and contrast enhancement in all cranial nerve nuclei (Figure 2). Spinal MRI revealed leptomeningeal contrast enhancement (Figure 3), and a contrast-enhancing lesion was found in the cervical spinal cord (Figure 4). A lumbar puncture was performed. Ten monocytes were detected in cerebrospinal fluid (CSF), CSF protein was 112 mg/dL, and CSF cytology was negative. Angiotensin-converting enzyme, tuberculosis-Polymerase Chain Reaction (PCR), salmonella, brucella, syphilis tests, CSF cytological examination, and aquaporin-4 antibody test results were negative. The IgG index was 0.79. The CSF oligoclonal band was type 2-positive.

Figure 1: Bilateral papilledema in the patient's fundus upon examination

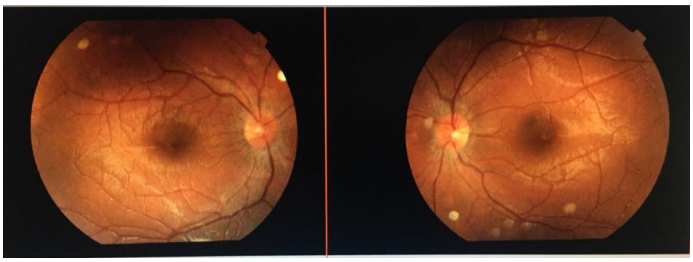


Figure 2: Brain MRI images showing linear contrast enhancement in axial (a) and sagittal (b) postcontrast series, which is inconsistent with leptomeningeal involvement.

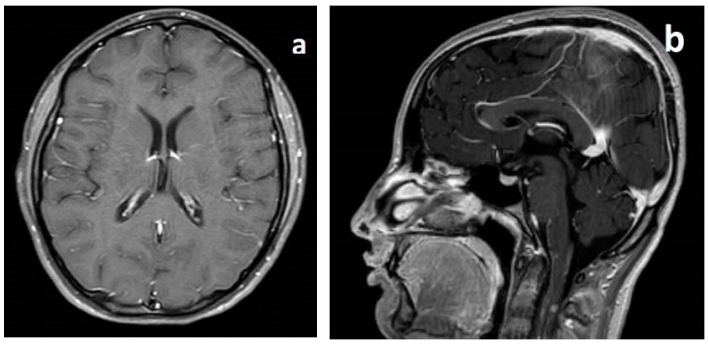



Figure 3: Linear contrast enhancement was consistent with leptomeningeal involvement in the patient's spinal lumbar (a) and cervical (b) postcontrast T1A images

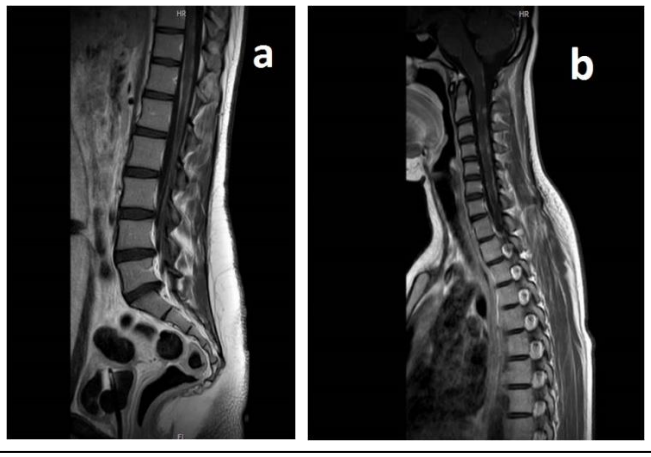
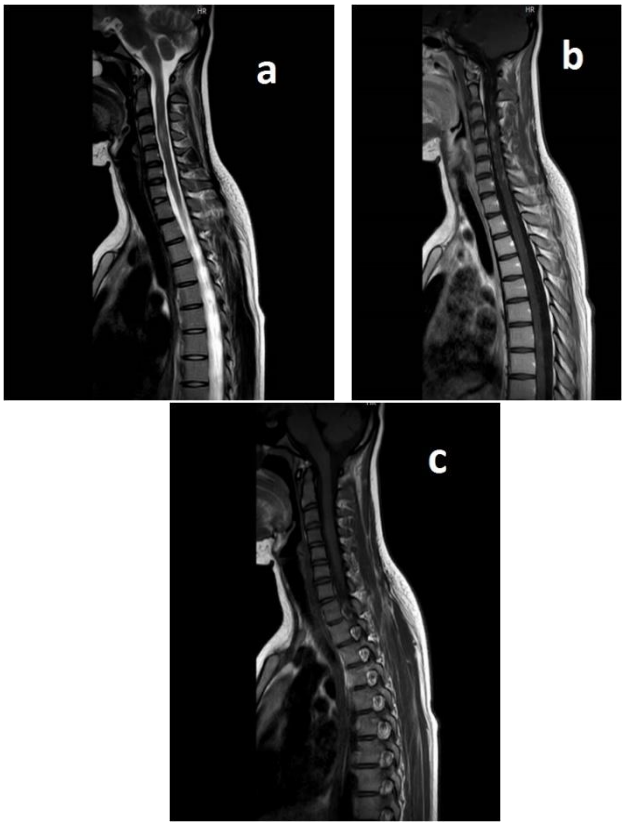



Figure 4: Cervical sagittal MRI sequences of T2A (a), post-contrast (b) and pre-contrast (c). T2A images showed a mild expansile lesion with contrast enhancement in the patient's cervical spinal cord.




The patient was started on 1000 mg/day of intravenous methylprednisolone, and after 10 days, treatment was continued with 1 mg/kg/day of oral methylprednisolone. Approximately 10 days after treatment initiation, the patient's visual acuity had improved to 0.5 in the right and left eyes, and the muscle strength was bilaterally 5/5 in the proximal upper extremities, 4/5 in the distal extremities, and 4/5 in the lower extremities. She was discharged on 1 mg/kg/day of oral methylprednisolone. During the outpatient clinic follow-up, the anti-MOG result was positive, and azathioprine was started. Our patient responded well to steroids and azathioprine, and her clinical symptoms completely resolved. We followed the patient for 2 years, and no new episodes developed.

## Discussion

Information on antibody-mediated diseases in the central nervous system has increased in the last decade. One of these antibodies is the MOG antibody. Anti-MOG has been identified in diseases with different clinical spectra, including seronegative neuromyelitis optica spectrum disease (NMOSH), ON, TM, ADEM, and encephalopathic diseases [8-11]. Our patient had bilateral ON and tetraparesis and responded well to steroid and azathioprine treatment, and her clinical symptoms subsequently resolved completely.

The interesting feature of this patient is the presence of spinal leptomeningeal involvement. Leptomeningeal involvement has recently been recognized as an important feature in MS and AQP4-positive NMOSH [12]. However, few anti-MOG-positive cases have been reported with leptomeningeal involvement as case reports in the literature. Reported rare cases with leptomeningeal involvement are associated with cranial leptomeningeal involvement [13-15].

There are only a few patients with spinal leptomeningeal involvement, such as in our case [16, 17]. The spinal cord was involved in two different ways on the MRI from patients with a myelitis episode; one is T2 signal hyperintensity exceeding three vertebral segments, and the other is involvement not exceeding two vertebral segments. Most lesions involved white matter, with more than 50% of the cord in the axial section, and this was accompanied by cord edema. The lesions involved the entire spinal cord, but conus involvement was specific. A patched, cloud-like enhancement with unclear borders was observed [18].

Inflammation in CNS demyelinating diseases is often associated with blood–brain barrier disruption. Leptomeningeal enhancement, which indicates an abnormally permeable leptomeningeal blood barrier, was accompanied by intraparenchymal blood–brain barrier breakdown during an acute myelitis episode, as visualized by contrast enhancement on T1-weighted imaging. This finding suggests that meningeal inflammation may have occurred as a bystander reaction following MOG–IgG-related parenchymal inflammation associated with subpial demyelination [18].

**Conclusion**

Our case shows that there might also be spinal leptomeningeal involvement in anti-MOG-positive myelitis patients. As the literature on these subjects increases, the clinical and radiological findings in anti-MOG patients will be more clearly defined.

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# Metachronous ovarian cancer metastasis of large bowel presenting similar imaging features of GIST

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## Abstract

Ovarian cancer most likely spreads into the peritoneal cavity or can be only superficially invasive. Although the most common metastatic pathway used by ovarian cancer cells involves peritoneal seeding, hematogenous and lymphatic dissemination can also occur. Metastatic colorectal cancers arising from the ovary are extremely rare. A mass arising from the colon wall at the level of the hepatic flexure was detected by tomography during routine follow-up of a patient who underwent abdominal hysterectomy + bilateral salpingo oophorectomy for ovarian cancer two years ago. Radiological findings revealed that the mass mimicked a gastrointestinal stromal tumor, and the patient underwent surgery based on a pre-diagnosis of gastrointestinal stromal tumor. Pathological examination of the specimen showed that the present mass was an ovarian tumor metastasis. Immunohistochemical staining of the sample was positive for CK7, PAX-8, WT-1, P16, and P53 and was negative for CK20 and CDX-2. A case of gastrointestinal metastasis of ovarian cancer is presented in which the imaging features mimicked a gastrointestinal stromal tumor that appeared two years after the first surgery. Although similar imaging characteristics of these two tumors were present, it was thought that gastrointestinal metastasis of ovarian cancer should be considered first in the differential diagnosis of patients who had history of previous ovarian cancer. The clinical presentation, management, and outcome in that case are discussed.

**Keywords:** Colon, Metastasis, Ovarian cancer

## Introduction

The most common cancers of gastrointestinal tract are colorectal. Colorectal cancer is the third most frequent malignancy worldwide. However, metastatic neoplasms in the colon are an uncommon finding. Only 1% of colorectal cancers are metastatic [1].

Ovarian cancers are the sixth most frequent malignancy in the worldwide, and ovarian cancer is the most common cause of cancer-related deaths from gynecological malignancies [2]. Ovarian cancer cells mainly metastasize to the peritoneal cavity, sigmoid colon, and reproductive organs (ovaries, uterus, and fallopian tubes) [3]. Hematogenous spread is less common. Distant metastases usually occur in the liver, brain, and/or lungs.

This case report aimed to determine clinical features and differential diagnosis of gastrointestinal metastasis of ovarian cancer and review the literature concerning this topic.

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## Case presentation

A 66-year-old patient with a previous history of total abdominal hysterectomy + bilateral salpingo-oophorectomy and chemotherapy due to ovarian carcinoma was found to have a colonic mass during her routine examination. Upper abdominal tomography revealed a large exophytic, heterogenous mass with a diameter of 28 x 14 mm that was projecting into the lumen and involved the hepatic flexure (Figure 1, 2). Laboratory findings indicated that the level of cancer antigen 125 (CA 125) has increased slightly (46.8 U/mL (reference range: 0-35)). The patient had no clinical complaints. Standard colonoscopy revealed no gastrointestinal mucosal lesions, and colonoscopic findings were thought to be the mass suggestive of a gastrointestinal stromal tumor (GIST) as shown in Figure 3. A biopsy was not taken from the patient with suspected GIST. Thoracic and lower abdominal tomography were normal.

Figure 1: Tomography image of the mass located intramurally in the hepatic flexure, in the transverse plane

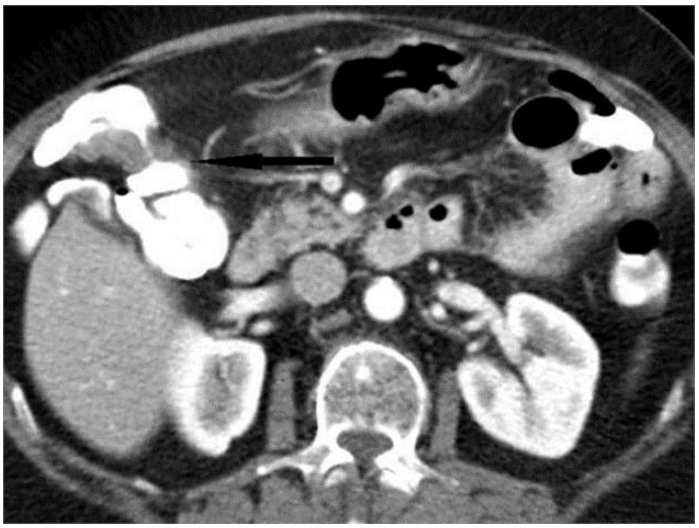


Figure 2: Tomography image of the mass located intramurally in the hepatic flexure, in the coronal plane

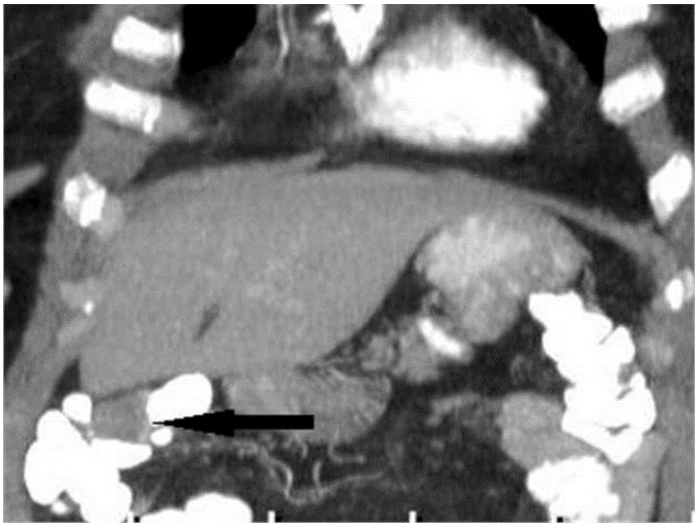
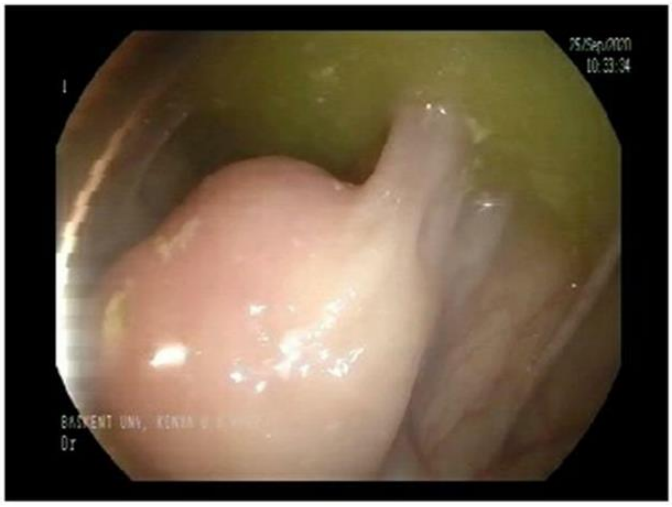


Figure 3: Colonoscopic view of the mass in the hepatic flexure



After considering endoscopic and radiological findings, the lesion was initially considered to be a GIST. During a laparotomy an intramural mass was observed, and the serosa was normal. Wedge resection was performed, and the specimen sent for frozen sectioning. Because the intra-operative frozen section confirmed the adenocarcinoma, right hemicolectomy was performed.

Microscopic examination revealed solid, isolated, or papillary clusters of neoplastic epithelial cells infiltrated within the lamina propria of the colon, but the mucosal surface was normal (Figure 4). Tumor cells have prominent cytoplasmic membranes, moderate amounts of eosinophilic cytoplasm, and intermediate- to high-grade nuclei, which are markedly atypical. Immunohistochemical staining of the tumor showed positivity for cytokeratin-7 (CK-7) as shown in Figure 5, Wilms tumor-1 (WT-1) protein and PAX-8 as shown in Figure 6, and P16 and P53 but was negative for CK-20 and cdx-2. These findings indicated that the tumor was consistent with high-grade serous ovarian carcinoma. No lympho-vascular invasion or regional lymph nodes metastasis was detected. Without development of any problems, the patient was discharged on the sixth post-operative day. No findings suggesting recurrence or metastasis were found on the first month follow-up tomography. Adjuvant chemotherapy treatment is ongoing after the post-operative medical oncology follow-up. Informed consent was obtained from the patient for this study.

Figure 4: Hematoxylin and eosin (H&E) staining shows solid and papillary cluster of neoplastic cells infiltrated within lamina of colon. (HEx10)

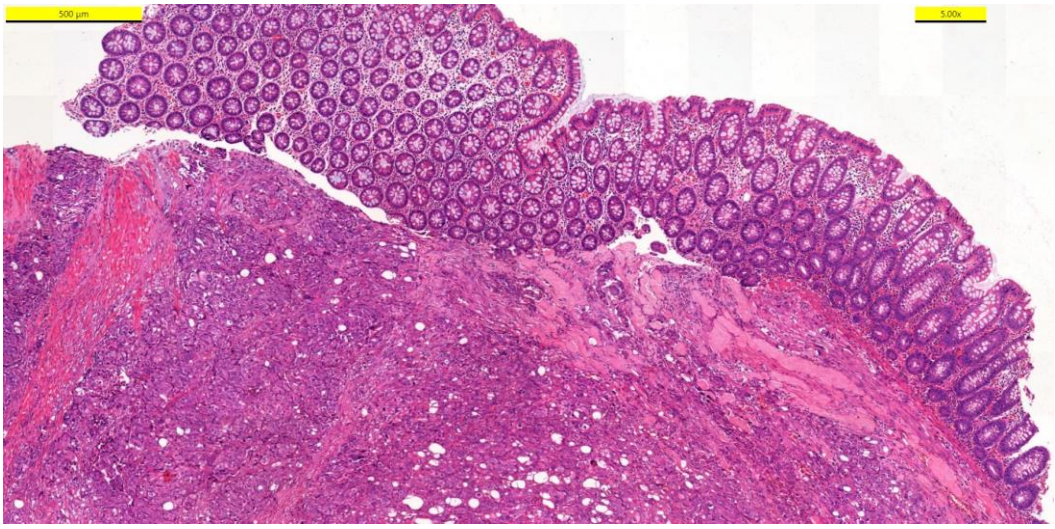



Figure 5: Figure shows positivity of CK-7 staining (CK7x20)

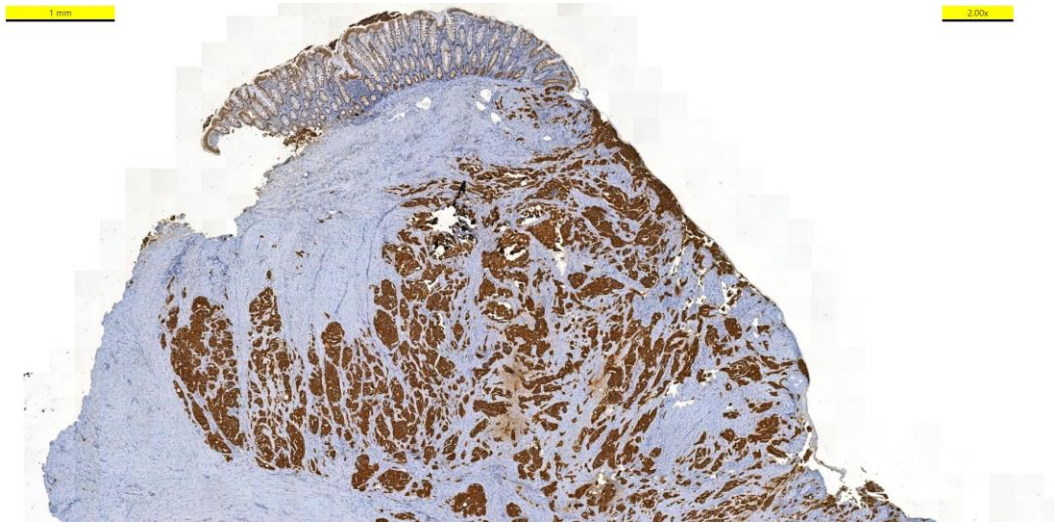
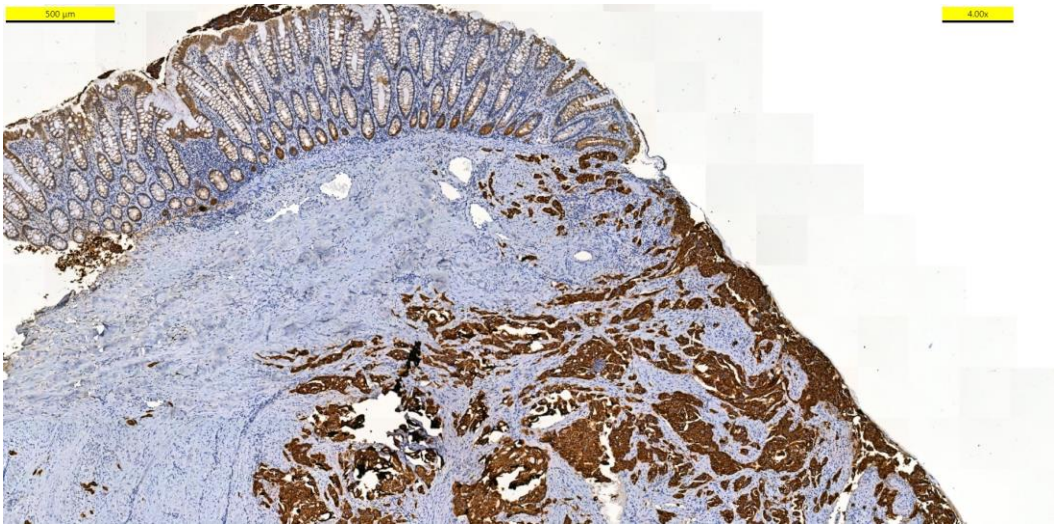



Figure 6: Figure shows positivity of PAX-8 staining (PAX8x20)




## Discussion

Ovarian cancer metastasis usually occurs in the peritoneal cavity or on the peritoneal surface. Although less common, hematogenous and lymphatic spread may also occur. Distant metastasis generally occurs in the pleura, lung, liver, and/or lymph nodes [4]. At the time of diagnosis, approximately 70% of ovarian cancer patients present with extensive disease, but distant metastasis is rare. The presence of synchronous colonic metastasis with ovarian carcinoma is also extremely rare.

Several authors have reported that colorectal metastases originating from ovarian cancer are very rare in Japan with ratio of 6.0% of cases while others have reported only 19 cases in Japan since 2005 [5, 6]. The study also showed that the median age of the patients was 58.8 (34–77) years. According to location, the number of patients presenting with colorectal metastases was equally distributed in rectum and sigmoid, descending transverse, and ascending colons [5]. Macroscopic appearance was variable, but most of the cases showed

protrusion into lumen; however, only a few cases were located in the submucosa. Therefore, the authors thought that based on the macroscopic appearance, distinguishing colorectal metastasis from ovarian carcinoma or primary colon cancer would be difficult. Our case was also localized in the submucosa.

Colorectal metastasis of ovarian cancer can be synchronous or metachronous. Metachronous tumors may occur over a period of time ranging from 1 to 22 years [5]. In the present case, colorectal metastasis from ovarian cancer was observed two years after the patient had undergone ovarian cancer surgery. Ovarian cancer generally metastasizes through the pelvic lymph nodes and within the peritoneal cavity. However, recent publications have shown that ovarian cancer can also metastasize via a hematogenous route. The most common pathway of metastatic spread to the bowel is through direct invasion to the serosa or hematogenous and lymphatic or peritoneal dissemination [7]. In most cases, the colonic serosa undergoes invasion first after which the tumor infiltrates the subserosa, muscularis propria, and mucosa [5]. Although rare, the mucosa may be infiltrated hematogenously via the submucosal capillary network [5]. In our case, no infiltration of the serosal surfaces or mesocolon occurred, and tumoral involvement was not detected in any lymph nodes. Therefore, it was thought that the patient's metastasis resulted from infiltration of the capillary network.

Patients may present with dyspepsia, intestinal obstruction, intussusception, palpable mass, or may not have any symptom [1]. Although the presence of high levels of CA 125 is used as a diagnostic marker of disease progression, it may also remain at normal levels in such cases. For diagnosis, tomography and ultrasonography may help detect pelvic masses, regional lymphadenopathy, a mass extending into the colon wall or lumen, and/or ascites [1]. Endoscopy or fine needle biopsy is helpful for making a definitive diagnosis [8].

It is sometimes difficult to distinguish ovarian from colon carcinomas, and immunohistochemical methods may be needed. Serous ovarian carcinomas are often CK20 negative and CK7 positive, but gastrointestinal carcinomas tend to be CK20 positive and CK7 negative [5]; however, colon and gastric adenocarcinomas can express CK7, and 33% of ovarian mucinous adenocarcinomas are CK20 positive, so this expression pattern is not always organ specific. To confirm that the cancer was of an ovarian origin immunohistochemical staining of CEA, PAX-8, and WT-1 protein was also performed. WT-1 is essential in the normal development of the genitourinary tract organs and mesothelium. Wilms tumors and most serous carcinomas of the peritoneum and ovary have been shown to express WT-1. PAX-8 is a transcription factor involved in the development of the Müllerian system, and it shows positive nuclear staining in most serous carcinomas.

Indeed, tumors are usually involved the seromuscular site of the intestinal wall or mesocolon in patients having colon metastasis from ovarian cancer [7]. In the present case, an intramural mass without infiltration of the serosal surface or any involvement in the mesocolon was found during surgery. The patient underwent a right hemicolectomy after the frozen examination was reported as malignant. Six days after surgery the patient was discharged without any complications. Up to the follow-up at six months, the patient received adjuvant chemotherapy.

The main treatment of metastasis includes surgery and adjuvant chemotherapy [7]. The efficacy of surgical options, such as metastasectomy or wide surgical resection, is not clearly defined. However, the general opinion is that bowel resection, including at least with paracolic lymph node excision, should be performed [9].

**Conclusion**

In conclusion, a successfully treated case of ovarian cancer with metachronous metastasis to the colon is presented. In evaluating our findings together with the literature, several conclusions were drawn:

- Metastatic ovarian carcinoma spread to the colon may occur as an intramural lesion with serosal sparing even in the absence of peritoneal disease.
- Radiologic appearance may mimic GIST.
- Despite spending two years disease-free after surgery, anamnesis of the patient's ovarian cancer history should be kept in mind.
- Because the treatment and prognosis of metastatic colorectal cancer originating from ovary and primary colorectal cancer are similar, histopathological diagnosis is important.
- Immunohistochemistry for CK7, CK20, WT1, and PAX8 can be helpful for making a differential diagnosis of colonic metastasis originating from ovarian cancer.

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