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# Evaluation of stereopsis level and it's associated factors in patients with refractive accommodative esotropia

## Refraktif akomodatif ezotropya hastalarında stereopsis düzeyi ve ilişkili faktörler

**Bengi Demirayak** [1]

**Abstract**

Aim: To investigate the level of stereopsis in patients with refractive accommodative esotropia and to find out associated factors with good stereopsis.

Methods: The hospital records of patients with refractive accommodative esotropia from January 2010 to June 2020 were retrospectively reviewed. Age, cycloplegic refractive error, the difference of refractive error between two eyes, angle of deviation at near and distance fixation, fusional ability and stereoacuity were evaluated. Patients were divided into two groups according to the stereopsis level: good stereopsis (40-100 arcsec) and poor stereopsis (>100 arcsec).

Results: A total of 62 patients were inclueded. Of them, 14 patients (22.5 %) were in good stereopsis group. The mean age was 5.35 years (range 3-11 years). The mean age at first visit, refractive differences between eyes and final deviation angle with spectacle were smaller in the good stereopsis group than in the poor stereopsis group. But, only the mean deviation angle at distance was found significant statistically between two groups. (p=0.038)

Conclusion: Residual esodeviation at distance fixation was significantly lower in patients with refractive accommodative esotropia who have good stereopsis.

Key words: Accommodative esotropia, binocular vision, fusion, stereopsis.

**Öz**

Amaç: Refraktif akomodatif ezotropyası olan hastalarda stereopsis düzeyini değerlendirmek ve stereopsise etki eden faktörleri ortaya çıkarmak.

Yöntemler: Ocak 2010- Haziran 2020 yılları arasındaki refraktif akomodatif ezotropya ile takip edilen hastaların kayıtları retrospektif olarak gözden geçirildi. Yaş, sikloplejik kırma kusuru, iki göz arasındaki kırma kusuru farkı, yakın ve uzak fiksasyondaki kayma açısı, füzyon kabiliyeti ve stereo keskinlik değerlendirildi. Hastalar stereopsis düzeyine göre 2 gruba ayrıldı: iyi stereopsis (40-100 sn ark), zayıf stereopsis (>100 sn ark).

Bulgular: Toplam 62 hasta dahil edildi. Bunlardan 14 hasta (22,5%) iyi stereopsis grubundaydı. İlk vizitteki yaş ortalaması 5,35 idi (3-11 yaş aralığı). İlk vizitteki ortalama yaş, iki göz arasındaki kırma kusuru farkı, son vizitteki gözlükle kayma açısı, iyi stereopsis grubunda, zayıf stereopsis grubuna göre düşük bulundu. Ancak, yalnızca uzak fiksasyondaki ortalama kayma açısındaki düşüklük istatistiksel olarak anlamlı olarak bulundu. (p=0,038)

Sonuç: İyi stereopsise sahip olan refraktif akomodatif ezotropya hastalarında uzak fiksasyondaki artık kayma miktarı anlamlı derecede düşüktür.

Anahtar kelimeler: Akomodatif ezotropya, binoküler görme, füzyon, stereopsis.

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## Introduction

Accommodative esotropia can be defined as convergent deviation of the eyes related with an abnormal activation of the accommodation reflex. Refractive accommodative esotropia (RAE) includes accommodative convergence, uncorrected hyperopia and inadequate fusional divergence [1].

Stereopsis is the highest form of binocular vision and it is binocular perception of depth [2]. Possible factors affecting stereopsis in patients with esotropia were investigated by some authors. The presence of amblyopia or anisometropia, residual esodeviation, longer duration of esodeviation have been reported to be associated with poor stereopsis [3,4]. However, the factors influencing stereopsis in RAE are still unclear.

In this study, we aim to investigate the stereopsis level and factors associated with stereopsis in patients with RAE.

## Material and methods

This study included patients with refractive accommodative esotropia seen at Dr. Sadi Konuk Education and Research Hospital, Pediatric Ophthalmology department from January 2010 to June 2020. The study protocol followed the tenets of the Declaration of Helsinki. Approval from the local ethics committee was obtained and written informed consent from the parents of participants was taken (2021-04-19).

The records of patients with RAE were retrospectively reviewed. The patients whose examinations were performed properly and data kept regularly and follow up time upper from a year were included. Patients with neurological and mental disorders, systemic disease, history of previous eye surgery and history of prematurity were excluded.

Full ophthalmologic examination was performed in all patients. Children over four years of age had evaluated by ARK-700 (Nidek Co. Ltd, Japan) auto refractometer for refractive errors after using 3 drops of cyclopentolate 1%, and retinoscopy was accomplished after administering 3 drops of cyclopentolate 1% to children under 4 years of age. Best corrected visual acuity was measured with Snellen chart and E chart was used to test small children. Full hyperopic correction was prescribed for treatment. Deviation at distance and near determined by Krimsky test in small children and prism cover test in older patients.

Stereoacuity was examined using the Titmus test (Stereo Optical, Chicago, IL). Children looked at the stereogram at distance of 40 cm while wearing polarizing glasses. The subject was asked to grab the wings of the fly and touch to the animal and circle that seemed to 'jump off the page'. The last correct target identified was used as the subject's stereopsis level. Stereoacuity was recorded as nil if the largest disparity could not be identified, and a score for nil stereopsis was 6000 arcsec fort he purpose of statistical analysis.

Fusion was measured using the Worth-4-Dot test at distance fixation and final examination was analyzed.

We defined refractive accommodative esotropia as a residual esotropia under 10 prism D after full hyperopic correction at both near and distance.

The following parameters were reviewed: age, cycloplegic refractive error, the difference of spherical error between two eyes, deviation at near and distance fixation, stereoacuity and fusion ability.

### Statistical Analysis

All statistical analyses were performed using Number Cruncher Statistical System (NCSS Statistical Software, Kaysville, UT, USA). Shapiro-Wilk's test were used to assess the assumption of normality. Numeric variables were presented with mean±standard deviatiation. Categorical variables were summarized as counts (percentages). The Mann-Whitney *U* test was used the comparison of two independent groups with respect to quantitative data as the continuous variables were not normally distributed. Student-t test was used the continuous variables were normally distributed and $p<0.05$ was considered as statistically significant.

## Results

A total of 62 children with RAE were included. The mean age of the subjects at the final visit was 9.5 years (range 5-16 years). The mean age of patients at first visit was 5.35 years (range 3-11 years). Demograhical and clinical datas of patients were summarized in Table 1.

Table 1. Demographics and characteristics of patients with RAE.

| | | |
|---|---|---|
| Age (years) | Mean±SD | 9.55±2.5 |
| | Range | 5-16 |
| Cycloplegic refractive error. SE (D) | Mean±SD | 4.65±1.74 |
| | Range | 2-10 |
| Interocular difference (D) | Mean±SD | 0.46±0.69 |
| | Range | 0-4 |
| Near deviation (PD) | Mean±SD | 5.19±3.44 |
| | Range | 0-10 |
| Distance deviation (PD) | Mean±SD | 2.03±3.04 |
| | Range | 0-10 |
| Age at first visit (years) | Mean±SD | 5.35±2.00 |
| | Range | 3-11 |
| Worth 4 dot test; n(%) | Fusion | 44 (71) |
| | Supression | 18 (29) |
| | Range | 40-6000 |
| | ≤100 sn/arc | 14 (22.6) |
| Stereoacuities n(%) | >100 sn/arc | 48 (77.4) |

RAE: Refractive accommodative esotropia, D: diopter; PD: prism diopter, SE: spherical equivalent, SD: standard deviation.

Firstly, patients were divided into groups according to the degree of final stereopsis: good (40-100 arcsec) and poor (>100 arcsec). There were 14 patients in Group 1 and 48 patients in Group 2. Age, cycloplegic refractive error, the difference of spherical error between two eyes, deviation at near and distance fixation were compared between groups. Results were summarized in Table 2. Only the measurement of distance deviation was found significant between groups ($p=0.038$).

Table 2. Comparison of patients with RAE according to level of stereoacuities at the final follow-up.

| Clinical variable | | Good stereopsis (n=14) | Poor stereopsis (n=48) | *p* |
|---|---|---|---|---|
| Age (years) | Mean±SD | 10.07±1.49 | 9.40±2.72 | [a]0.135 |
| Cycloplegic refractive error SE (D) | Mean±SD | 5.03±1.50 | 4.54±1.81 | [b]0.354 |
| Interocular difference (D) | Mean±SD | 0.39±0.43 | 0.78±0.76 | [a]0.696 |
| Near deviation (PD) | Mean±SD | 4.14±4.11 | 5.5±3.21 | [a]0.303 |
| Distance deviation (PD) | Mean±SD | 0.57±1.65 | 2.46±3.23 | [a]0.038* |
| Age at first visit (years) | Mean±SD | 4.86±0.95 | 5.50±2.21 | [a]0.711 |

RAE: Refractive accommodative esotropia, D: diopter; PD: prism diopter, SE: spherical equivalent, SD: standard deviation.
*[a]Mann Whitney U Test, [b]Student T Test, *p<0.05*

Secondly, patients were divided into groups according to fusion ability. Forty-four patients were in fusion group, and 18 patients were in suppression group. The groups were compared

in terms of age, cycloplegic refractive error, the difference of spherical error between two eyes, deviation at near and distance fixation. Only the measurement of distance deviation was found significant between groups ($p$=0.001). Results were summarized in Table 3.

Table 3. Comparison of patients with RAE according to fusion ability at the final follow-up.

| Clinical variable | | Fusion (n=44) | Suppression (n=18) | p |
|---|---|---|---|---|
| Age (years) | Mean±SD | 9.59±2.53 | 9.44±2.50 | [a]0.778 |
| Cycloplegic refractive error. SE (D) | Mean±SD | 4.75±1.65 | 4.42±1.97 | [b]0.498 |
| Interocular difference (D) | Mean±SD | 0.39±0.53 | 0.61±0.99 | [a]0.707 |
| Near deviation (PD) | Mean±SD | 4.68±3.61 | 6.44±2.71 | [a]0.081 |
| Distance deviation (PD) | Mean±SD | 1.04±2.05 | 4.44±3.73 | [a]0.001* |
| Age at first visit (years) | Mean±SD | 5.30±1.96 | 5.50±2.18 | [a]0.769 |

RAE: Refractive accommodative esotropia, D: diopter; PD: prism diopter, SE: spherical equivalent, SD: standard deviation.
[a]*Mann Whitney U Test,* [b]*Student T Test, *p<0.05*

## Discussion

The present study was aimed to investigate the fusion ability, the degree of stereopsis and potential factors influencing stereopsis and fusion ability in patients with RAE. Of 62 patients, only 14 (22.5%) had 100 arcsec or better stereoacuity despite appropriate spectacle correction and well-aligned eyes. The mean age at first visit, the difference of refractive error between two eyes and final deviation angle with spectacle were smaller in the good stereopsis group than in the poor stereopsis group.

The onset of RAE usually occurs after two ages which significant maturation of stereopsis has completed. Therefore, some authors have suggested that most children with RAE should have a favorable prognosis for binocular vision [5, 6]. However, many children with accommodative esotropia have subnormal binocular single vision [6, 7]. There are two hypotheses evaluating the subnormal binocularity in accommodative esotropia: a congenital deficit infusion may predispose some children to accommodative esotropia, or brief periods of constant esotropia might disrupt stereopsis. In fact, neither hypotheses could be disproved [7]. Both the congenital deficits and the brief periods of misalignment causing abnormal visual experience could interrupt stereopsis in accommodative esotropia.

Of patients, 44 (70.9%) had fusion with Worth-4-dot test in our study. Berk et al. [8] reported 73.5% of the patients had fusion with the same test, similar with our study. Guclu et al. [3] found fusion was present 82.8% in their study. Although the results were similar about having fusion in RAE, stereopsis degrees were contradictory in the literature [9, 10]. Tomac et al. [11] reported 45% of patients had stereopsis. Berk et al. [8] demonstrated 24.2% of patients had 100 seconds of arc or better stereopsis. In our study, we found 22.5% of patients had good stereopsis. Lambert and Lynn [12] reported 30% of patients had good stereopsis and high levels of stereoacuity were found in patients whose esotropia occured at older age. It is known that if esotropia appears in first two years of life and stays uncorrected, binocular vision is broken down [13]. In our study, the mean age of patients found 5.3 years at first visit. The duration between first occuring of esotropia and accession for treatment might extended. That might be the reason of only 22.5% of our patients had good stereopsis. But we didn't find any relationship between age and high levels of stereopsis. Cakir et al. [14] did not find any significant correlation between mean onset age and stereopsis, too.

Although previous reports have defined accommodative esotropia as angle of deviation under 10 prism diopter after hyperopic correction, Wong and collegues who demonstrated the recent neuroanatomical findings reported that the true stereopsis might be possible only with a misalignment of ≤4 PD [15]. In our study, residual deviations after full hyperopia correction were 4.14 PD in good stereopsis group and 5.50 PD in poor stereopsis group at near fixation. That difference was not found significant. But at distance fixation, they were 0.57 PD in good stereopsis group and 2.46 PD in poor stereopsis group and the difference was found significant statistically. To minimize esodeviation may be important to achieve better stereopsis.

The relationship between refractive error and stereopsis was investigated previously in patients with RAE, and the authors didn't find any relation [7, 13]. In our study, we didn't find any relationship between these parameters, too. Lee et al. [4] suggested that anisometropia might cause abnormal binocular sensory function in patients with RAE [4]. There wasn't a relationship between interocular difference and stereopsis, in our study. This item must be investigated in further studies with larger sample size.

The present study has some limitations, too. Firstly, patients were reviewed retrospectively. Second, stereopsis was measured by using the Titmus stereotest which is prone to monocular clues and variability of the results [16]. The test was performed at least twice and the last measured results were analyzed to maximize the reliability.

As a conclusion, residual esodeviation at distance fixation was found significantly lower in patients with RAE who have good stereopsis.

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# The impact of prone position on optic nerve sheath diameter

## Prone pozisyonun optik sinir kılıfı çapına etkisi

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**Abstract**

Aim: The measurement of optic nerve sheath diameter is a reliable and non-invasive approach in determining increased intracranial pressure (ICP). We investigated the impact of prone position, a frequently used approach during surgery, on ICP in this present study.

Methods: Horizontal and vertical optic nerve sheath diameters for both eyes were measured by means of ultrasonography first immediately after intubation preoperatively and then when the patient has been placed in supine position prior to extubation postoperatively in a total of 60 patients scheduled for vertebral surgery in prone position in neurosurgical and orthopedic theatres. They were compared in terms of sex and duration as well as for left and right eye measurements.

Results: No significant differences were observed in terms of sex as well as right-left eye measurements in the patients. No changes were noted in optic nerve sheath diameter in the patients with shorter operative duration. However, there was a notable increase numerically in patients who had operation duration of over 240 minutes, although the difference was not established to be statistically significant.

Conclusion: Prone position was not associated with increased intracranial pressure in patients who undergo short or medium duration surgery. However, optic nerve sheath diameter is likely to increase in patients who undergo longer surgery. Therefore, care has to be taken in terms of elevated ICP in patients who remain in prone position for extended periods.

Keywords: Intracranial pressure increase, optic nerve sheath diameter, ultrasonography, prone position.

**Öz**

Amaç: Optik sinir kılıfı çapı ölçümü kafa içi basınç artışının tespitinde noninvaziv, güvenilir bir yöntemdir. Biz çalışmamızda, ameliyathane ortamında sık kullanılan prone pozisyonun kafa içi basıncına etkisini araştırdık.

Yöntemler: Prone pozisyonda vertebra operasyonu yapılacak toplam 60 beyin cerrahi ve ortopedi hastasının preoperatif indüksiyondan hemen önce ve postoperatif supine pozisyona alındığında ekstübasyondan önce ultrasonografi ile her iki gözde yatay ve dikey optik sinir kılıfı çapı ölçümlerini kaydettik. Cinsiyet, süre ve sağ göz- sol göz kıyaslamaları yapıldı.

Bulgular: Cinsiyet ve sağ göz- sol göz kıyaslamalarında fark gözlenmedi. Süre açısından kısa süren olgularda optik sinir kılıfı çapında değişiklik yoktu. 240 dakikadan uzun süren olgularda istatistiksel olarak anlamlı olmasa da nümerik olarak bariz artış gözlendi.

Sonuç: Prone pozisyonu kısa ve orta süreli operasyonlarda kafa içi basınç artışı yapmamaktadır. Ancak çok uzun süreli durumlarda optik sinir kılıfı çapı artma eğilimindedir. Bu nedenle çok uzun süre prone pozisyonda tutulan hastalarda kafa içi basınç artışı yönünden dikkatli olunmalıdır.

Anahtar Kelimeler: Kafa içi basınç artışı, optik sinir kılıfı çapı, ultrasonografi, prone pozisyon.

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## Introduction

Intracranial pressure (ICP) is the positive pressure created by the cranial cerebrospinal fluid, cerebral blood volume, and the brain itself. It was first described by Monro in 1783 [1]. It is normally 5-15 mmHg [2]. The components creating the pressure compensate the alterations for one another under normal conditions. However, when the compensatory mechanism has been impaired, it leads to raised intracranial pressure (rICP) syndrome. A value of 25-30 mmHg is fatal [2].

While haematoma, tumour, and elevated cerebrospinal fluid level (increased production or decreased absorption) are the leading reasons, increased cranial blood volume (such as venous sinus thrombosis, hypercarbia induced elevated blood flow etc.) is also a frequent cause [3]. Furthermore, certain patient positions used during administration of anaesthesia have been established to have an impact on ICP [4]. While rICP syndrome could manifest itself with symptoms such as postoperative nausea, vomiting, papilledema or headache [5], we might also observe delayed postoperative recovery from general anaesthesia, postoperative delirium, impaired cognitive reactions, or, albeit rarely, neurological deficit [4].

The measurement of ICP can be carried out by using invasive or non-invasive approaches. While external ventricular drain (EVD) has been established to be the golden standard for ICP measurement [5], it could lead to haemorrhage, and poses a risk in terms of infection in patients monitored for longer periods [6]. It is also possible to perform a reliable and quick ICP measurement by means of Computed tomography (CT), Magnetic resonance imaging (MRI) or Ultrasonography (USG) non-invasively. A number of studies demonstrated the reliability and efficiency of non-invasive measurement through ocular sonography [6, 7]. The measurement of optic nerve sheath diameter (ONSD) is a non-invasive reliable method well-correlated with ICP [4]. The retrobulbar segment of the optic nerve is surrounded by a subarachnoid space that can expand as a result of elevated cerebrospinal fluid pressure.

Although it was claimed that Trendelenburg position led to elevated ICP [7], we have come across few studies demonstrating that prone position singularly having an impact on the increase of the optic nerve sheath diameter.

Prone position is one of the most effective approaches utilized nowadays during the pandemic in management of COVID-19 patients. The increases in ICP and optic nerve sheath diameter have gained greater significance during the pandemic as patient monitoring time on average is 16-24 hours and following a short duration in supine position patients are returned to prone position.

In this study, we aimed to investigate the impact of prone position on ONSD and consequently on ICP.

## Material and methods

This prospective observational study was initiated following the approval of the local ethics committee of the University of Health Sciences, Prof.Dr. CemilTaşçıoğlu City Hospital (dated 17.12.2019 / protocol no.1518). A written informed consent was obtained from all patients. The study was conducted in accordance with the Helsinki declaration.

### Patient population

A total of 60 patients scheduled for elective vertebral surgery in prone position in neurosurgical and orthopedic theatres were enrolled in the study.

Inclusion Criteria: Aged 18-70 years, scheduled for elective surgery in prone position, surgery time of minimum 1 hour, American Society of Anesthesiologists (ASA)physical status classification I-II-III and consent to be enrolled in the study.

Exclusion Criteria: Patients with a history of ophthalmic surgery, glaucoma, diabetic retinopathy, cataract, retinal detachment, advanced-stage lung disease and advanced-stage cerebral disease as well as those not willing to be enrolled in the study and those who were established to be ASA IV or higher were excluded.

As intrathoracic pressure increase would lead to an increase in intracranial pressure[8], patients with elevated peak pressure intraoperatively were also planned to be excluded.

### Anaesthesia protocol

The patients were premedicated 30 minutes prior to surgery with 0.05 mg/kg i.m. midazolam. Following routine hemodynamic monitorization (Electrocardiography-ECG-, non-invasive blood pressure, pulse oximetry), bispectral index pads were placed in the right and left frontal region to record baseline values. Patients were administered with 2.5 mg/kg i.v. propofol and 0.6 mg/kg i.v. rocuronium to induce anaesthesia. Meanwhile,1 µg/kg i.v. fentanyl was administered. Orotracheal intubation was performed. During the anaesthetic procedure, bispectral index values were kept between 40 and 60 by administering sevoflurane (Minimum alveolar consentration= 0.8-1.0) and remifentanil infusion (0.03-1.0 µg/kg i.v.). To maintain myorelaxation, 0.1 mg/kg i.v. rocuronium was administered every half an hour. Volume-controlled ventilation was utilized during operation. Tidal volume was set at 8 ml/kg (ideal body weight) and I/E rate was 1/2. The frequency of ventilation was set at 10-15/min to keep end tidal carbon dioxide ($EtCO_2$) within normal range. Positive end expiratory pressure (PEEP) was set at 5 mmHg. Hypotonic and hypertonic liquids were avoided. In addition to demographical values, type and duration of surgery as well as values recorded during operation were noted for further assessment.

### Ocular sonography

Optic nerve sheath diameter (ONSD) was measured by means of ultrasonography by a trained researcher. After the patients were intubated in supine position and were stabilized, their eyes were closed, and a thin layer of gel was applied on their upper eye lids. A Mindray M5 system was used for the measurements. A linear probe at 7.5 MHz was placed on the eyelid without applying pressure. Optimal contrast between retrobulbar echogenic fat tissue and vertical hypoechoic band was achieved by adjusting the probe at an angle. The diameter of the hypoechoic structure was measured 3 mm posterior to optic disc in the image as shown in Figure 1. Vertical and horizontal measurements were performed in both eyes, and mean values were calculated for analyses. Although a cut-off value varying between 4.8 and 5.9 mm was used in different studies [9], a mean ONSD value of >5 mm was taken as positive in favor of ICP in our study[10,11]. Since it was not possible to carry out ONSD measurements when patients were in prone position during surgery, measurements were repeated and recorded at conclusion of surgery when patients were placed in supine position. It was planned that the patients with elevated values would be reassessed in the post-anaesthesia care unit at 30 minutes postoperatively. If the values were observed to be elevated again, they would be referred for further examination and consultation in terms of rICP.

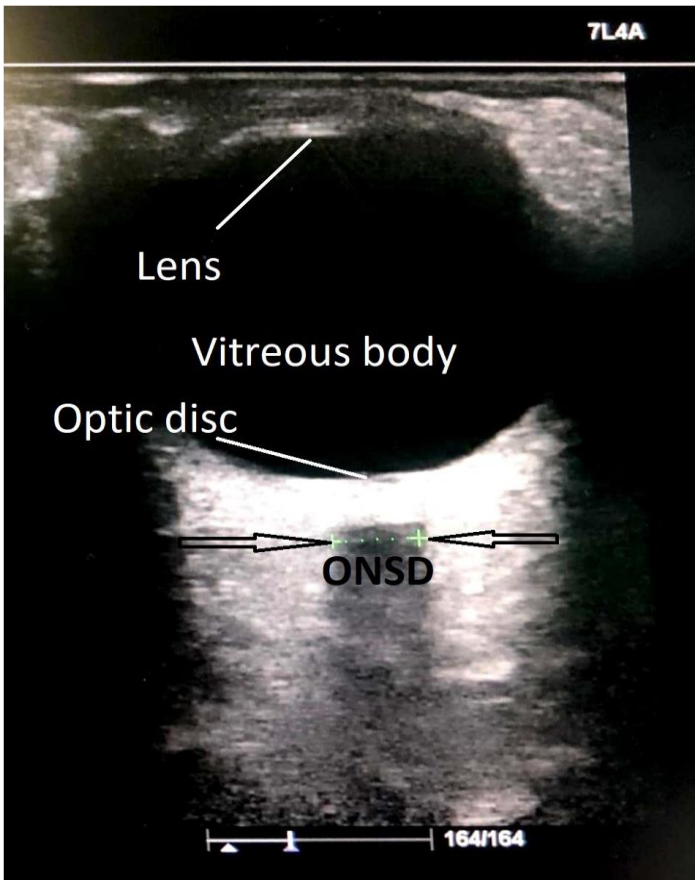

Figure1. Optic nerve sheath diameter measurement.

**Statistical analysis**

SPSS 20.0 was used for statistical analyses. Categorical variable frequencies of data were expressed numerically and in percentages, while continuous variables were given as mean and standard deviation. Kolmogorov-Smirnov test revealed that data distribution was not normal. As a result, a non-parametric test (Mann-Whitney U) was employed. Statistical significance was set at $p<0.05$. Comparisons were made in terms of sex, surgery duration (over or under 240 minutes) andright-left eye. Pearson Correlation as well as 2-tailed significance test were performed.

## Results

A total of 60 patients underwent measurements. As shown in Table 1, no statistically significant differences were observed in the group in terms of demographical data, including sex. Pulse, Oxygen saturation-SpO2-, mean arterial pressure and EtCO2 values were within normal range in the patients.

The difference in the numbers of male and female participants was not statistically significant in the group ($p=0.722$). Postoperative comparison of right and left eyes in terms of ONSD values independent of any variables (age, sex, duration) revealed a p value of 0.431, which was not statistically significant ($p>0.05$). Furthermore, as seen in Table 2, no significant differences were observed in terms of preoperative and postoperative ONSD values in either eye in the patients.

When evaluated in terms of surgery duration, postoperative measurements of the patients undergoing surgery for over 240 minutes revealed p values of 0.57 and 0.07 for the right and left eyes, respectively, in Table 3. The differences were not statistically significant. However, a numerical increase was noted in mean postoperative ONSD values. As seen in Figure 2, we observed that as the duration of surgery increased, ONSD values tended to increase as well.

Table 1. Demographic data.

| | Mean±SD |
|---|---|
| Age | 50.57±12.7 |
| Height (cm) | 168±8.3 |
| Weight (kg) | 74.6±11.2 |
| Pulse ( /min) | 75.8±9.6 |
| $SpO_2$ (%) | 99.0±0.5 |
| Mean Arterial Pressure (mmHg) | 77.68±5.1 |
| $EtCO_2$ (mmHg) | 31.48±1.2 |
| | n(%) |
| Gender | |
| • Male | 31 (51.7%) |
| • Female | 29 (48.3) |
| ASA | |
| I | 27(45%) |
| II | 26(43.3%) |
| III | 7(11.7%) |

$SpO_2$: Oxygen saturation, $EtCO_2$: end tidal carbon dioxide, ASA: American Society of Anesthesiologists, SD: standard deviation.

Table 2. Preoperative and postoperative ONSD values.

| | Preoperative | Postoperative | |
|---|---|---|---|
| n=60 | Mean±SD | Mean±SD | p values |
| Right eye (mm) | 4.34±0.49 | 4.38±0.48 | >0.05 |
| Left eye (mm) | 4.41±0.48 | 4.43±0.45 | >0.05 |

ONSD: optic nevre sheath diameter, SD: standard deviation.

Table 3. ONSD values for patients undergoing longer surgery and results of statistical analyses.

| | Preoperative | Postoperative | |
|---|---|---|---|
| n=16 | Mean±SD | Mean±SD | p values |
| Right eye (mm) | 4.22±0.42 | 4.42±0.50 | 0.56 |
| Left eye (mm) | 4.16±0.47 | 4.59±0.49 | 0.07 |

ONSD: optic nevre sheath diameter, SD: standard deviation.

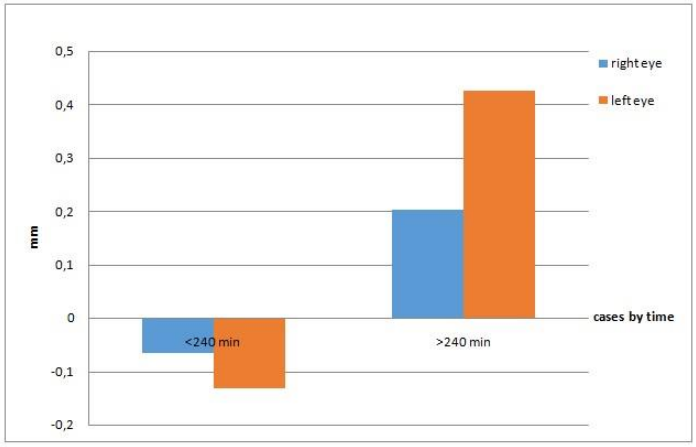

Figure 2. Time-related graph of the postoperative change of ONSD (Figures show the difference between postoperative and preoperative measurements)

## Discussion

Surgical prone position is an approach frequently used in various surgical operations. We observed that prone position did not have an impact on ONSD in surgical durations of 60 – 240 minutes in our study.

Increase in ICP in patients under anaesthesia is attributable to several factors such as anaesthetic agents, mean arterial pressure, elevated central venous pressure and blood CO2 level. The patients were administered the same anaesthetic agents by making adjustments depending on weight to ensure

standardization in our study. Remifentanil infusion was used to maintain mean arterial pressure within normal range. Fluid overload was avoided, and PEEP was set at 5 mmHg for all patients. EtCO2 values of the patients were maintained within normal range (30-45 mmHg). Eliminating the factors that might lead to increased ICP, we investigated the impact of prone position alone on ONSD and, consequently, on increased ICP. We established that prone position did not lead to increased ICP in operations with a duration of up to 4 hours, independent of sex and age.

Patients in prone position have inadequate pulmonary expansion, which results in increased intrathoracic pressure and consequently increased ICP. However, we did not observe high peak airway pressure in the patients during surgery. Therefore, no patients were excluded.

While day-surgery used to be limited to 90-minute operations, it has now risen to 3-4 hours [12]. Therefore, we considered cases that took longer than 240 minutes as long-duration surgery in our study and carried out statistical analyses accordingly. Although the change in ONSD was established to be more pronounced in the left eye compared with the right one in longer operations, and the p value was rather closer to 0.05, the difference between the right and the left eye was not statistically significant. In fact, significant contemporary changes observed in both eyes were interpreted in favour of rICP in the literature [10]. Therefore, the increase observed in the left eye was not considered to be a significant result.

We recorded the longest surgery duration to be 5.5 hours in our study. In this patient, we observed an increase in ONSD of 5.7 mm in the left eye and 6.1 mm in the right eye, indicating rICP. Although the mean numerical increase in patients undergoing longer surgery was not established to be statistically significant, we postulate that even longer durations in prone position would have a more significant impact since the patient with the longest surgery duration had an ONSD of >5 mm in both eyes, and it was interpreted in favour of raised ICP.

As patients were in prone position during the operation, the biggest limitation of our study was the fact that measurements could only be carried out at the beginning and later when they were in supine position at the conclusion of the operation. Therefore, the second-measurement point of the patients could not be standardized. Furthermore, while raised ICP is measured non-invasively by means of ONSD measurement, no consensus exists for the present moment regarding a reference value for a significant increase [7, 9].

No significant correlation was established between operation duration and ONSD increase in our study. However, fewer number of cases with longer operation duration is one of the limitations of this study.

In conclusion, the ultrasound measurement of ONSD in operations carried out in prone position is a reliable method well-correlated with raised ICP. We maintain that prone position does not lead to raised ICP in short and medium duration operations. However, care must be taken for longer operations.

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# Experience in surgical treatment of symptomatic hepatic hemangiomas

## Semptomatik hepatik hemanjiomlarda cerrahi tedavi deneyimi

**Oğuzhan Özsay [1], Mehmet Can Aydın [1]**

**Abstract**

Aim: Hemangioma is the most common benign tumor of the liver. They are rarely large, symptomatic, and show atypical imaging patterns. Surgical treatment indications are persistent symptoms, rapid size increase, life-threatening complications, and diagnostic uncertainty. In this study, we aimed to present the results of our patients who underwent surgical treatment for persistent symptomatic hepatic hemangiomas regardless of size.

Methods: We retrospectively evaluated the clinicodemographics, perioperative findings, and postoperative results of ten patients that we operated for symptomatic hepatic hemangiomas between 2017 and 2021. We made the diagnosis based on ultrasonography, computed tomography, and magnetic resonance imaging. Patients were evaluated in terms of age, gender, symptoms, tumor size and location, perioperative blood transfusion, operation time, postoperative complications, length of stay, and follow-up results.

Results: The median age was 51 (25-60) and all of them were female. Although the persistent symptom in all patients was abdominal pain, we also had patients with additional symptoms such as dyspepsia and nausea. We performed enucleation in two, left lateral segmentectomy in one, right hepatectomy in two patients and, non-anatomical segmental resection in the rest. Perioperative blood transfusion was median 1 (0-3) unit and required in seven. The median operation time was 170 (135-230) minutes, and the median postoperative stay was 8.5 (4-13) days. No serious complications developed in the postoperative period. The median follow-up time was 23.5 (9-40) months and, there was no recurrence or notable long-term complications.

Conclusion: Surgical treatment can be performed safely and effectively in experienced centers for patients with symptomatic hepatic hemangioma.

Keywords: Hepatic hemangioma, liver resection, enucleation.

**Öz**

Amaç: Hemanjiom, karaciğerin en sık görülen benign tümörüdür. Nadiren büyük, semptomatik ve atipik görüntüleme paterninde olabilirler. Cerrahi tedavi endikasyonları, persistan semptomlar, hızlı boyut artışı, yaşamı tehdit eden komplikasyonlar ve tanısal belirsizliktir. Bu çalışmada boyuttan bağımsız olarak persistan semptomatik karaciğer hemanjiomları nedeniyle cerrahi tedavi uygulanan hastalarımızın sonuçlarını sunmayı amaçladık.

Yöntemler: 2017-2021 yılları arasında semptomatik hepatik hemanjiyom nedeniyle opere ettiğimiz 10 hastanın klinikodemografik verilerini, perioperatif bulgularını ve postoperatif sonuçlarını retrospektif olarak değerlendirdik. Tanıyı ultrasonografi, bilgisayarlı tomografi ve manyetik rezonans görüntüleme ile koyduk. Hastalar yaş, cinsiyet, semptomlar, tümör boyutu ve yerleşimi, perioperatif kan transfüzyonu, operasyon süresi, postoperatif komplikasyonlar, hastanede kalış süresi ve takip sonuçları açısından değerlendirildi.

Bulgular: Hastaların ortanca yaşı 51 yıl (25-60) idi ve hepsi kadındı. Hastaların tamamında persistan semptom karın ağrısı olmakla birlikte, dispepsi ve bulantı gibi ek semptomu olan hastalarımız da mevcuttu. İki hastada enükleasyon, birinde sol lateral segmentektomi, iki hastada sağ hepatektomi ve diğerlerinde anatomik olmayan segmenter rezeksiyon yaptık. Perioperatif kan transfüzyonu medyan 1 (0-3) ünite idi ve yedi hastada transfüzyon ihtiyacı oldu. Ortalama ameliyat süresi 170 (135-230) dakika ve ameliyat sonrası ortalama hastanede kalış süresi 8,5 (4-13) gündü. Postoperatif dönemde ciddi bir komplikasyon gelişmedi. Medyan takip süresi 23,5 (9-40) aydı ve, nüks veya ciddi uzun dönem komplikasyon olmadı.

Sonuç: Semptomatik hepatik hemanjiyomlu hastalarda cerrahi tedavi, deneyimli merkezlerde güvenli ve etkin bir şekilde uygulanabilir.

Anahtar Kelimeler: Hepatik hemanjiom, karaciğer rezeksiyonu, enükleasyon.

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Ethics Committee Approval: This study was approved by Local Ethical Committee of Ondokuz Mayıs University School of Medicine (2012/461).
Etik Kurul Onayı: Bu çalışma Ondokuz Mayıs Üniversitesi Yerel Etik Kurulu tarafından onaylanmıştır (2012/461).

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## Introduction

Hepatic hemangiomas are the most common benign hepatic tumors with 1 to 20 % [1]. The incidence of hepatic hemangioma is highest in the third to fifth decade of life and is more common in women. Hemangiomas are classified as small (<4 cm), giant (>4 cm), and hypergiant (>10 cm) [2]. Although they are often solitary focal lesions, approximately 40 % of patients have multiple hemangiomas [3]. In most cases, hepatic hemangiomas are small, asymptomatic, and suitable for follow-up conservatively, but rarely, they may become symptomatic including persistent abdominal pain, especially due to capsular tension of the liver despite the use of analgesics. Persistent abdominal pain is the most often surgical treatment indication in the literature [3]. This study aimed to present the perioperative findings and postoperative results of the patients who underwent surgery for symptomatic hepatic hemangiomas in our center.

## Material and methods

The present study was approved by local ethical committee (2021/461). Ten cases of symptomatic hepatic hemangiomas were reviewed from 2017 to 2021. We made the diagnosis based on ultrasonography (USG) and computed tomography (CT). We performed magnetic resonance imaging (MRI) in two cases because CT findings did not provide sufficient data for the diagnosis. The informed consent was taken from all study group after the surgery decision. None of the cohort had interventional treatments before the surgery. Patients were evaluated in terms of age, gender, American Society of Anesthesiologists (ASA) score, symptoms, tumor size and location, perioperative blood transfusion, operation time, length of stay, and follow-up time. Indication for surgery was persistent abdominal pain in all. The patients underwent surgery in the supine position after urinary and central venous catheterization. We performed open surgery with Makuuchi incision in 7 cases and right subcostal incision in the rest. The type of liver resection was based on the size and location of the hemangioma. Liver resection or enucleation techniques were performed by using CUSA (Cavitron Ultrasonic Surgical Aspirator System), Aquamantys bipolar electrosurgical device, and wet bipolar forceps. We performed abdominal drains in all cohort. The drains were removed when their outputs decreased below 50 ml per day and all of the patients were discharged uneventfully after their drains removed. We followed the cases after discharge in the first, third and sixth months, and then annually. Statistical analyses were performed using SPSS software (version 21.0, IBM, Armonk, NY, USA). Data not showing normal distribution were reported as median (range).

## Results

Age, gender, symptoms, and performed surgery type of the patients are summarized in Table 1. The median age was 51 (25-60), and all study group were female. Three cases were ASA 2 status and the rest were ASA 1. The median size of hemangiomas was 9.5 (6-15) cm. The most seen symptoms were abdominal pain and dyspepsia. In one of the cases, the mass was pressing on the stomach (Figure 1). Five cases were giant, and five cases were hypergiant (Figure 2). Most of the lesions were located on the right lobe of the liver (7 in 10). Right hepatectomy was performed in two, left lateral segmentectomy in one, enucleation in two patients, and non-anatomical segmental resections in others. The median operation time was 170 (135-230) minutes, and the median postoperative stay was 8.5 (4-13) days. All of the patients had an uneventful postoperative recovery, and they were asymptomatic at a median follow-up of 23.5 (9-40) months (Table 2). No complications or recurrences were observed in the patients during the follow-up period.

Table 1. Clinicodemographics and performed surgical technique of the patients.

| No | Age | M/F | ASA | Symptom | Location of lesion | Size (cm) | Surgical technique |
|---|---|---|---|---|---|---|---|
| 1 | 34 | F | 1 | Abdominal pain+ dyspepsia | VI-VII | 13 | Right non-anatomical liver resection |
| 2 | 43 | F | 2 | Abdominal pain | V-VI-VII-VIII | 13 | Right hepatectomy |
| 3 | 42 | F | 2 | Abdominal pain | VII | 10 | Right non-anatomical liver resection |
| 4 | 48 | F | 1 | Abdominal pain | II-III-IV | 10 | Enucleation |
| 5 | 55 | F | 1 | Abdominal pain+ dyspepsia | V-VIII | 7.5 | Enucleation |
| 6 | 54 | F | 2 | Abdominal pain+ dyspepsia | IV | 5.5 | Right non-anatomical liver resection |
| 7 | 54 | F | 1 | Abdominal pain+ dyspepsia | VII-VIII | 7 | Right non-anatomical liver resection |
| 8 | 25 | F | 1 | Abdominal pain | VII | 15 | Right hepatectomy |
| 9 | 60 | F | 1 | Abdominal pain+ dyspepsia | VI | 6 | Right non-anatomical liver resection |
| 10 | 54 | F | 1 | Abdominal pain+ vomiting | II-III | 9 | Left lateral segmentectomy |

M: male, F: female, ASA: Americal Society of Anesthesiologists.

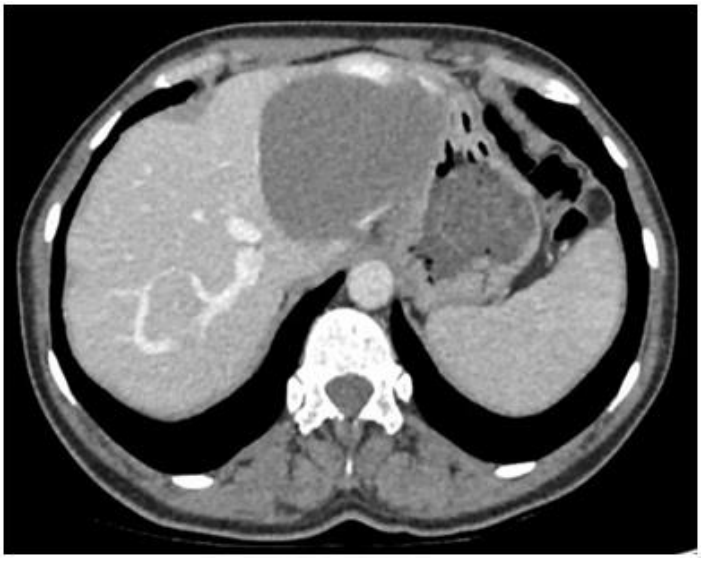

Figure 1. Computed tomography image of the case which hemangioma was pressing on the stomach.

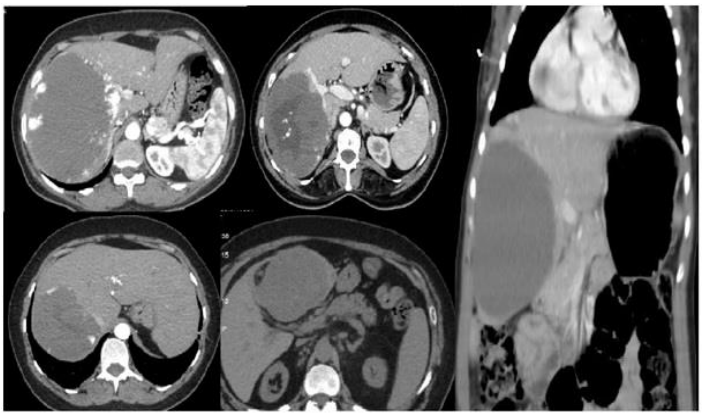

Figure 2. Computed tomography images of our five hypergiant cases.

Table 2. Perioperative findings and postoperative results of the patients.

| No | Perioperative blood transfusion (units) | Operation time (minutes) | Postoperative stay (days) | Follow-up time (months) |
|---|---|---|---|---|
| 1 | 3 | 150 | 9 | 10 |
| 2 | 3 | 180 | 7 | 12 |
| 3 | 2 | 160 | 8 | 9 |
| 4 | 0 | 230 | 9 | 18 |
| 5 | 0 | 225 | 13 | 40 |
| 6 | 2 | 155 | 10 | 30 |
| 7 | 0 | 215 | 9 | 25 |
| 8 | 1 | 218 | 7 | 28 |
| 9 | 1 | 150 | 8 | 22 |
| 10 | 1 | 135 | 4 | 25 |

## Discussion

In this study, we presented the results of our patients who underwent surgical treatment for persistent symptomatic hepatic hemangiomas. There were no serious complications in the postoperative period and, no symptoms, complications or recurrences were observed in the long-term follow-up.

Although they are usually asymptomatic and managed conservatively, hepatic hemangiomas may cause abdominal pain due to capsular distension, rarely [4]. Also, infarction and hemorrhage can cause pain too. Especially patients with giant or hypergiant hemangiomas are more likely to be symptomatic. However, the size is not the only reason for symptoms. While giant hemangiomas are related with abdominal pain more frequently, small hemangiomas may cause pain, too [3]. Like five of our cohort, small hemangiomas may cause abdominal pain, too. On the other hand, because of the localization, these lesions can press to other organs and cause symptoms due to compression, as one of our cohort presented with gastric outlet obstruction.

Because of their asymptomatic nature, hepatic hemangiomas are generally detected incidentally in radiological imaging procedures for other diseases. In clinical practice, USG, CT, and MRI are used for diagnosis. While all these methods have high sensitivity, MRI is the most reliable imaging with the characteristic 'right bulb' sign, and it is the gold standard non-invasive method for some authors [3, 4]. In 8 of our study group, we used USG and BT, but in two, we used MRI for diagnosis due to unclear definition of the imaging.

The surgical resection is the only curative treatment for hepatic hemangiomas and its indications are persistent symptoms, diagnostic uncertainty, enlargement, and rare complications of the lesion as compression to other organs, hemorrhage, rupture, or infarction [3-6]. There was not any rupture or infarction in our study group. While some authors recommend surgical treatment for the risk of rupture, especially in large hemangiomas [7-8] or high-risk lifestyle for hepatic trauma [9], these indications are controversial, and the general approach is conservative treatment in the literature [3, 5, 10].

The ideal surgical technique for hepatic hemangioma is debated. Although enucleation seems to be more beneficial, especially to avoid unnecessary removal of healthy parenchyma, resection may be a safer technique, especially in cases where the diagnosis is unclear. The most critical handicap for enucleation is that major perioperative bleeding can sometimes occur due to the lack of clear cleavage between hemangioma and liver parenchyma [3]. Singh et al. [11] showed that enucleation provides less bleeding, shorter operation time, fewer postoperative complications, and shorter hospital stay than resection. Similarly, studies that find better results of enucleation are in predominant in the literature [12-15]. Since enucleation may cause significant perioperative blood loss in large lesions, it may be more appropriate to prefer formal resections in experienced centers in this group [4]. Because of the benign nature of the tumor and its technical advantages, we recommend enucleation, if possible, for symptomatic hepatic hemangiomas.

We did not encounter any postoperative complications and mortality in our patients, similar to Hermann et al. [3] in terms of surgical treatment results. Conversely, surgical treatment has complications that can be serious for such a benign disease. Perioperative bleeding, postoperative biliary fistula or intra-abdominal abscess are some of these complications [3]. There are many studies reporting excellent surgical treatment results in the literature [12, 16 17]. However, on the contrary, unacceptable mortality rates of up to 2.4 % were observed in some studies prove that surgical treatment should be performed in experienced centers [9]. High surgical experience also minimizes the perioperative bleeding which is the biggest fear for surgeons. As a specialized center for hepatobiliary surgery and liver transplantation, we benefited from our current surgical experience in this patient group and all of the patients were discharged uneventfully.

Robotic and laparoscopic hepatic resections are associated with less intraoperative blood loss, better postoperative recovery, and lower pain scores in the literature [18]. They are alternative options to open surgery and can be performed in technically sufficient centers. Additionally, to minimally invasive surgery, interventional methods such as transarterial embolization and radiofrequency ablation are also effective in treatment of hepatic hemangiomas [19-21] and they may be alternative treatment modalities to surgery in the future. We think that, because hepatic hemangiomas are mostly seen in young women, using these minimally invasive surgical and interventional techniques may be a better option, especially for cosmetic expectancy.

The limitations of our study were the small number of patients and its retrospective nature.

For patients with persistent symptomatic hepatic hemangioma, surgical treatment can be performed safely and effectively in experienced centers. Enucleation may be a better technical choice from hepatic resection in selected patients.

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# Retrospective analysis of quality of life in patients with idiopathic sudden sensorineural hearing loss

## Ani idiopatik sensorinöral işitme kaybı olan hastalardaki yaşam kalitesinin retrospektif analizi

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Abstract

Aim: Idiopathic sudden sensorineural hearing loss (ISSNHL) is the unfortunate experience of rapid hearing loss and may affect patients' quality of life at different levels. The study's purpose is to measure how individuals were affected socially, psychologically, and mentally after they experienced ISSNHL.

Methods: This study was designed as a cross-sectional study with archive research and included patients who were diagnosed with ISSNHL between 2015 and 2020. Patients were asked to answer the Short Form 36 (SF-36) questionnaire form via tele-conversation. According to pure tone audiometry tests performed after treatment, patients were divided into two groups: "completely recovered" (<20 dB) and "partially recovered" (thresholds remained >20 dB). Mean scores of SF-36 quality of life subcategories were analyzed for each group

Results: The patients included 48 (58.5%) males and 34 (41.5%) females. As a result of the analyses, there were significant differences in physical functioning (p=0.046), vitality (p=0.049), and general health scores (p=0.038) between the two groups after the ISSNHL treatment. Although the mean scores of patients who were completely recovered were higher, no significant difference was found in the physical-role (p=0.125), emotional-role (p>0.05), mental health (p>0.05), bodily pain (p=0.48), and social role (p=0.713) subcategory scores between the two groups.

Conclusions: Our results showed that successful treatment and recovery prevented all kinds of the disease's potential physical and mental complications. Morbidity effects were felt by patients with somewhat more serious problems and could not make a full recovery.

Keywords: Idiopathic sudden sensorineural hearing loss, quality of life, general health, Short Form-36.

Öz

Amaç: Ani idiyopatik sensörinöral işitme kaybı (AİK), işitme kaybının hızla gerçekleştiği ve bireylerin yaşam kaliteleri üzerinde değişken seviyelerde etkisi olabilen talihsiz bir hastalık olarak karşımıza çıkmaktadır. Bu çalışma, kişilerin AİK geçirdikten sonra sosyal, psikolojik ve mental olarak nasıl ve ne düzeyde etkilendiklerini değerlendirmeyi amaçlamaktadır.

Yöntemler: Retrospektif arşiv taraması yapılarak kesitsel olarak planlanan çalışmaya 2015 ve 2020 yılları arasında AİK tanısı almış hastalar dahil edildi. Hastalara telefon görüşmesi yoluyla Short Form 36 (SF-36) yaşam kalitesi ölçeği anketi uygulandı. Tedavi sonrası saf ses odyometri test sonuçlarına göre hastalar 2 gruba ayrıldı: 'tam iyileşen' (<20 dB) ve 'tam iyileşmeyen' (eşikler >20 dB olarak devam eden). SF-36 yaşam kalitesi alt ölçekleri için ortalama skorlar iki grup için de hesaplandı.

Bulgular: Toplam 48 (58,5%) erkek ve 34 (41,5%) kadın hasta dahil edildi. Analizlerin sonucunda, AİK tedavisi sonrasında 2 grup arasında fiziksel fonksiyon (p=0,046), enerji-canlılık-vitalite (p=0,049) ve genel sağlık ölçütü (p=0,038) skorlarında anlamlı fark bulundu. Fiziksel rol güçlüğü (p=0,125), emosyonel rol güçlüğü (p>0,05), ruhsal sağlık (p>0,05), ağrı (p=0,48) ve sosyal rol güçlüğü (p=0,713) alt kategorileri skorlarında 2 grup arasında anlamlı fark olmasa da tam iyileşen hastaların ortalama skorları daha yüksek izlendi.

Sonuç: Sonuçlarımız, başarılı bir tedavi ve iyileşme sürecinin hastalığın potansiyel tüm fiziksel ve mental komplikasyonlarının önüne geçtiğini göstermiştir. Tam iyileşemeyen hastaların edindiği bu morbiditenin kişi üzerinde etkileri ise bir şekilde daha ciddi sorunlar ile ileride hissedilmeye başlanmaktadır.

Anahtar Kelimeler: Ani idiyopatik işitme kaybı, yaşam kalitesi, genel sağlık, Kısa Form-36.

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## Introduction

Idiopathic sudden sensorineural hearing loss (ISSNHL) is defined as a sensorineural hearing loss of 30 dB or more in at least three consecutive frequencies in pure tone audiometry and occurring over a period of 72 hours or less. Yearly incidences of ISSHL are reported as 5–30/100,000 [1]; however, the actual number of incidences is thought to be even more due to mildly affected individuals who rapidly recover and do not seek medical help [2]. The incidence peaks at 40–60 years of age, but it could be seen at any age and equally in males and females [3]. ISSNHL, which comprises only 1% of all sensorineural hearing losses [4], is an otologic emergency and requires early treatment as soon as diagnosed. While spontaneous recovery rate is reported as 32–65% (mean 46.7%) in different studies [5, 6], the onset of treatment is an important factor for a successful recovery in patients who do not recover spontaneously [7]. Some patients may not recover whether he or she had various treatments, including salvage therapy, or did not have any treatment at all; this status may affect their quality of life to different extents as seen in other hearing losses [8–10].

In this study, we aim to measure how and to what extent individuals were affected socially, psychologically, and mentally after they received ISSNHL treatment, to gain insight into their post-treatment states of health, and how they maintain their lives both physically and psychologically in a healthier manner.

## Material and methods

This study was designed as a cross-sectional study with a questionnaire and retrospective archival research in Medeniyet University, Faculty of Medicine, Department of Otorhinolaryngology. Istanbul Medeniyet University, Göztepe Prof. Dr. Süleyman Yalçın City Hospital, Clinical Investigation Ethics Committee approved the study (December 2, 2020 / Number: 2020/0716).

### Patient Selection

Individuals who were diagnosed with ISSNHL in our otorhinolaryngology department between January 1st, 2015-May 5th, 2020 and followed treatment for at least six months were chosen for this study. Patients <18 and >70 years old were excluded. Patients whom the department did not follow up with or with psychiatric disorders and those with severe hearing loss that prevented them from a phone conversation were also excluded from the study. Of the 299 patients admitted to our clinic during the given timeframe due to ISSNHL, 102 patients were reached and 82 of those (48 males and 34 females) consented to participate in the study.

### Quality of Life (SF-36) Questionnaire

Consenting patients were contacted over the telephone and interviewed using the Quality of Life Form 36 (SF-36) [11] questionnaire, which consists of 36 questions across seven general health subcategories (bodily pain, vitality, physical functioning, physical-role, general health, emotional-role, and mental health). With the answers obtained, information about patients' quality of life at least six months after the onset of the disease was gathered. The SF-36 health survey results of the patients were calculated by scoring each question in a range from 0 to 100, and their means were obtained. Scores closer to 0 indicated a worse health status, whereas those closer to 100 indicated a better state of health [12]. After scoring the 36 questions, each patient had a score in each subscale of health status.

### Archival research

We conducted archival research for the pure tone audiometry test results, which were performed at the time of diagnosis and a six-month follow-up after diagnosis, of the patients who answered the questionnaire. According to the six-month pure tone audiometry tests, patients were divided into two groups: "completely recovered" (air and bone conduction thresholds in 500, 1000, and 2000 Hz speech-frequencies were below 20 dB) and "partially recovered" (thresholds remained above 20 dB). It also noted the patients who received intra-tympanic steroid treatment.

### Statistical Analysis

The mean scores of SF-36 subscales were calculated for both groups. In SPSS® v20 for Mac (Statistical Package for Social Sciences, IBM, USA) statistical software, after seeing the normal distribution of the means in Skewness and Kurtosis tests, differences between the general health scores of the patients who were completely recovered and partially recovered were investigated for significance with an independent sample T-test.

## Results

Of the 82 patients, 34 (41.5%) of them had right-sided ISSNHL and 48 (58.5%) had left-sided ISSNHL. The number of totally recovered patients was 39 (47%) and 43 (53%) for partially recovered patients. The mean age of the total number of participants was found as 44,7 ±13,1 years (min:21, max:70). The mean age of the totally recovered patients was 43,4 ±13.3 years (min: 21, max:70), and the mean age of partially recovered patients was 45.9±13 years (min: 24, max: 69). The mean follow-up period was 36.5±15.9 months (min: 6, max: 60).

The initial and the last pure tone audiometry threshold means of completely recovered patients and partially recovered patients are given in Table 1. The comparison of the initial test and last test results were also found statistically significant for both groups.

Patient's scores for the SF-36 quality of life scale subcategories are compared. We found that totally recovered patients had better SF-36 quality of life scale results than the partially recovered group. These results were most evident in the vitality, general health, and the physical functioning subcategories (p= 0.049, 0.038, and 0.046 respectively; Table 2). The other subcategories, which are physical-role (p=0.125), emotional-role (p>0.05), mental health (p>0.05), bodily pain (p=0.48), and social role (p=0.713), did not show any significant differences (Table 2).

The number of patients who received the intratympanic steroid treatment was three for the recovered group (7.6%) and 21 for the partially recovered group (48.4%).

Table 1. Pure tone audiometric thresholds of the affected ears of ISSNHL patients, comparison of initial test and last test.

| | Completely recovered (n=39) | | Partially recovered (n=43) | |
|---|---|---|---|---|
| | Initial test | Last test | Initial test | Last test |
| Mean ± standard deviation (dB) | 33.4 ±11.9 | 10.7 ±4.1 | 48.4 ±16.4 | 40± 16.1 |
| Median (dB) | 32 | 10 | 47 | 38 |
| Minimum-maximum (dB) | 20-70 | 3-18 | 22-83 | 20-70 |
| p | <0.001 | | <0.001 | |

dB: decibel.

Table 2. ISSNHL patients' mean and standard deviation scores of SF-36 quality of life scale subcategories and comparative p values of the scores of completely recovered and partially recovered patients.

| | All patients n=82 | Completely recovered n=39 | Partially recovered n=43 | p |
|---|---|---|---|---|
| Bodily Pain | 76.1 ±31.2 | 78.8 ± 29.5 | 73.8 ± 32.8 | 0.480 |
| Vitality | 52.0 ±24.5 | 57.7 ± 23.5 | 47.0 ± 24.5 | 0.049 |
| Physical functioning | 80.7 ±23.6 | 86.3 ± 19.9 | 75.8 ± 25.7 | 0.046 |
| Physical-Role | 68.8 ±37.6 | 75.6 ± 33.6 | 63.3 ± 40.9 | 0.125 |
| General Health | 59.5 ±23.5 | 59.6 ± 23.8 | 54.4 ± 23.3 | 0.038 |
| Emotional-Role | 66.2 ±27.6 | 67.5 ± 22.5 | 65.1 ± 31.6 | >0.05 |
| Mental Health | 60.9 ±21.5 | 65.3 ± 20.1 | 57.0 ± 22.2 | >0.05 |
| Social-Role | 51.3 ± 17.0 | 50.6 ± 16.9 | 52.0 ± 17.2 | 0.713 |

All values are mean ± standard deviation.

## Discussion

How hearing loss impacts individuals' quality of life has been a research been a research subject for a long time; many studies have been conducted to reveal the possible effects. Zarenoe et al. discussed the negative effects of sensorineural hearing loss and tinnitus and found that hearing aids improve patients' quality of life [13]. Meyer et al. assessed youths with sensorineural hearing loss and also found that hearing aids or implants improve patients' quality of life [14].

Studies on the quality of life of patients with ISSNHL are limited in the literature. This can be attributed to factors such as the disease being infrequent in the general population, difficulties in long-term archival storage, changes in patients' contact information, and the lack of giving consent for such studies. In this regard, we believe that our study will contribute to the growing literature. Härkönen et al. studied the long-term results of sudden sensorineural hearing loss and discussed the poorer quality of life in patients who did not recover [15]. In our study, patients who recovered have mostly better results on quality-of-life questionnaires, which is compatible with the related literature. The mean results of completely recovered patients in our study clearly showed that successful treatment and recovery prevented several of the disease's potential physical and mental complications.

Our questionnaire has multiple subgroups that are related with different topics, with mental health being one of them. In our study, we revealed that recovered patients have better mental health results, and these are in a concordance with the current literature [13, 15]. The study also noticed that morbidity effects were felt by patients with somewhat more serious problems and could not make a full recovery.

When we examined patient answers to bodily pain questions, patients who were completely recovered felt less pain, although no significant difference was found in the pain scores between the two groups. In some of the patients who do not show a complete recovery, intratympanic steroid injections, which may cause a sharp pain during the penetration, are performed as salvage therapy, and it should be considered that pain due to this intervention might last for some time [16]. We think that those patients might be making connections to this treatment pain, resulting in slightly different pain scores.

In terms of energy levels, fully-recovered patients felt significantly more energetic than partially-recovered patients based on vitality scores. However, the overall energy levels of both groups were generally less than the Turkish population average [17], and they stated their energy levels were very low due to the COVID-19 pandemic. The literature supports our data, and it was reported that unrecovered ISSNHL patients, especially with tinnitus, were significantly more depressive in major life and physical activities [18]. When limitations in their physical functioning were questioned, patients who were completely recovered had significantly better physical activity compared to those who were partially recovered. However, we noted that some completely recovered patients experienced physical disabilities more than some partially recovered patients. When we examined how they were limited in fulfilling their physical roles (such as carrying items, kneeling or stooping, and climbing stairs), no significant difference was seen between the two groups. Although not significant, it was seen that partially recovered patients were less able to perform these physical activities than those who were completely recovered. When interpreting our results, it should be considered that physical conditions and disabilities could be affected by many factors, such as age, gender, comorbidities, and lifestyle.

The difference between the social role and emotional role functioning scores was minimal and not significant. It was concluded that any kind of recovery did not have a significant effect on the patients' emotional well-being. However, the standard deviations of the two groups (22.5 in completely recovered patients and 31.6 in the partially recovered group) showed that huge differences existed in the emotional role functioning among patients who only partially recovered. It could be said that some of those patients were more emotionally positive, whereas others mental state was much lower. In a previous study, including 198 ISSNHL patients, Zhao et al. [17] revealed that in patients with higher anxiety and depression scores usually suffered from other physical illnesses. In addition, Chen et al. [20] reported that patients with persistent tinnitus showed significantly higher emotional stress and depressive symptoms, and Zarenoe et al. discussed that the control of tinnitus may improve patients' quality of life [13]. In the light of these data, it would not be incorrect to say that emotional status and mental health are affected mainly by the presence of other symptoms rather than the degree of hearing loss.

In this study, we encountered some limitations. First, conducting interviews over the phone was challenging with some patients with severe hearing loss. Second, the limited number of patients who consented to this study underrepresents the population of people affected by ISSNHL. The third limiting factor is that the time between the six-month audiometry test and the survey was highly variable. Due to the nature of this study, we were not able to analyze the effect of the interval between the last follow-up test and questionnaire. In our study, we could not assess the presence of tinnitus, so ignorance of any existing tinnitus effect may be another limitation of this study. Our last limitation was failing to calculate the correlated physical and mental health scores. The related scores require an advanced calculation method, so we could not apply this calculation to this study.

At the end of the 5-year data analyses, among the quality-of-life subcategories, there was a significant difference in physical functioning, vitality, and general health scores between patients who were completely recovered and who were not after ISSNHL treatment. Although the mean scores of patients who were completely recovered were higher, no significant difference was found in the physical role, emotional role, mental health, and bodily pain subcategory scores between the patient groups. Our findings showed that it could be helpful to remark on the changes in the quality of life of patients who experienced ISSNHL in clinical practice and could be a guide for further studies.

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# Heart rate variability and analysis of rhythm in patients with restless legs syndrome: A prospective case-control study

## Huzursuz bacaklar sendromlu hastalarda kalp hızı değişkenliği ve ritim analizi: Bir prospektif olgu-kontrol çalışması

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**Abstract**

Aim: To investigate the involvement of cardiac autonomy by heart rate variability (HRV) analysis in patients with restless legs syndrome (RLS).

Methods: Patients with RLS and age/sex matched healthy controls were included in this prospective case-control study. The diagnosis of RLS was made according to the criteria determined by the International Restless Legs Syndrome Study Group. Demographic data and severity of disease were recorded. The HRV analysis was performed with a 24-hour electrocardiographic Holter monitoring in all patients and the control group.

Results: Sixty-five (of them, 50 female) patients and 58 (of them, 45 female) healthy individuals were enrolled into the study. Patients and control groups were similar for age and gender. No difference was found in the HRV analysis, except that the standard deviation of NN intervals (SDNN) at night was lower when RLS patients and the control group were compared (p=0.03). The HRV analyzes were compared according to disease severity, SDNN at night was significantly different between groups (p=0.03). In the pairwise comparison, the difference between the severe and very severe groups was significant (p=0.01) and severe group was lower than very severe group.

Conclusions: In the present study which evaluate the cardiac autonomic system with HRV in patients with RLS, a decrease in parasympathetic nervous system was found at night time.

Keywords: Restless legs syndrome, heart rate variability, autonomic dysfunction.

**Öz**

Amaç: Kalp hızı değişkenliği (KHD) analizi ile huzursuz bacaklar sendromu (HBS) hastalarında kardiyak otonomik tutulumu araştırmak.

Yöntemler: Prospektif olgu-kontrol çalışması tipindeki bu çalışmaya HBS hastaları ve yaş/cinsiyet uyumlu sağlıklı kontroller dahil edildi. HBS tanısı Uluslararası Huzursuz Bacaklar Sendromu Çalışma Grubu tarafından belirlenen kriterlere göre konuldu. Demografik veriler ve hastalığın şiddeti kayıt edildi. Tüm hasta ve kontrol gruplarında 24 saatlik elektrokardiyografik Holter monitörizasyonu ile HRV analizi yapıldı.

Bulgular: Çalışma grubunda 65 (50'si kadın), kontrol grubunda 58 (45'i kadın) hasta mevcuttu. Hastalar ve kontrol grupları yaş ve cinsiyet açısından benzerdi. HRV analizinde HBS hastaları ve kontrol grubu karşılaştırıldığında SDNN gece değerinin düşük olması dışında fark bulunmadı (p=0,03). KHD analizleri hastalık şiddetine göre karşılaştırıldığında SDNN gece değeri gruplar arasında anlamlı farklılık gösterdi (p=0,03). İkili karşılaştırmada şiddetli ve çok şiddetli gruplar arasındaki fark anlamlıydı (p=0,01) ve şiddetli grup, çok şiddetli gruba göre daha düşüktü.

Sonuç: HBS'li hastalarda HRV ile kardiyak otonom sistemi değerlendiren bu çalışmada, gece saatlerinde parasempatik sinir sisteminde azalma saptanmıştır.

Anahtar Kelimeler: Huzursuz bacaklar sendromu, kalp hızı değişkenliği, otonomik disfonksiyon.

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## Introduction

The restless legs syndrome (RLS) is a movement disorder characterized by an abnormal sensation on the extremities, especially on legs. Complaints occur especially at rest or at night and they are relieved by movement [1]. There are two clinical forms of RLS including primary RLS and secondary RLS. Primary RLS appear in young adults with family history and secondary RLS appear in older adults with comorbidities such as iron deficiency, chronic renal failure or neurological diseases [2]. The prevalence of RLS varies between 5% and 10% [3]. The prevalence of RLS increases with age and predominance in female gender [4].

Although the pathophysiology of RLS is not fully explained, radiological examinations have revealed the dopaminergic hypofunction [5]. Spectroscopy studies suggested the presence of a decrease in postsynaptic D2 receptor number and affinity [6]. In PET studies; significant reductions in dopamine uptake have been demonstrated in the caudate nucleus and putamen [7].

Clinical studies suggested that RLS may also be a risk factor for cardiovascular diseases [8]. Many studies have reported a significant relationship between increased heart rate and cardiovascular mortality in RLS [9]. Increased nocturnal blood pressure and heart rate, hypertension, cardiovascular disease, and cerebrovascular disease frequency in RLS patients suggest autonomic dysfunction in RLS patients [10]. The sympathetic and parasympathetic nervous systems of the autonomic nervous system modulate the heart rate, and the changes in the heart rate over time provide information about the functioning of the autonomic nervous system [11]. Heart rate variability (HRV) is one of the noninvasive methods used to identify disorders affecting the autonomous system by quantitatively measuring the sympathovagal balance and the change between two heart beats [12, 13]. The aim of this study was to examine cardiac autonomic involvement by HRV analysis in RLS patients.

## Material and methods

After local Institutional Review Board approval (2011/29), this prospective case-control was designed as a monocentric study and followed the principles of the Declaration of Helsinki. The study was performed between 01.03.2011-30.01.2012 in Karadeniz Technical University Sleep Polyclinic. The RLS patients and age/sex matched healthy controls were included in the study. Written informed consent were taken from the patients.

Inclusion criteria for the study group; regardless of etiology diagnosis of RLS and patients diagnosed with RLS should not have received any medical treatment for this disease or they should have had a break from their treatment for at least 3 months. Exclusion criteria for study group were; (1) age below 18 years, (2) presence of any sleep disorder (i.e. narcolepsy, sleep apnea); (3) any previously known cardiac disease (i.e. previous myocardial infarction, cardiac failure, cardiac arrhythmia); (4) use of medications that may affect the cardiac rhythm (i.e. anti-arrhythmic or anti-epileptic); (5) mental limitation to perceive the study; (6) disease duration of less than 1 year.

Inclusion criteria for control group were: (1) age above 18 years, (2) being healthy (3) having no exclusion criteria for study group.

Neurological examination and nerve conduction studies of the patients were performed. The diagnosis of RLS was made according to the criteria determined by the International Restless Legs Syndrome Study Group (IRLSSG) [14]. Demographic data of patients such as age, gender, concomitant diseases (hypertension, diabetes, hyperlipidemia, thyroid disease), and duration of the disease are recorded. The severity of RLS symptoms was assessed by using the IRLSSG rating scale (IRLS) with a sum of scores as mild (0–10), moderate (11–20), severe (21– 30), and very severe (31–40) [15].

The HRV analysis was performed with 24-hour electrocardiographic Holter monitoring in the patient and control groups of the study. Normal daily activities were monitored during Holter monitoring; the patient was instructed to avoid wetting the device and passing from X-ray devices. The whole day (24 hours) and night (6:00 PM-6:00 AM)/daytime (06:00 AM-6:00 PM) Holter recording of the patients were reviewed under following parameters; time domain parameters used the standard deviation (SD) of all normal to normal (NN) intervals (SDNN), the mean of the deviation of the 5-minute NN intervals over the entire recording (SDNN index), the square root of the mean squared differences of successive NN intervals (rMMSD), the proportion of adjacent RR intervals differing longer than 50 milliseconds in the 24-hour recording (pNN50) while frequency domain parameters used high frequency (HF) component (0.15–0.40 Hz), a low frequency (LF) component (0.04–0.15 Hz), very low frequency (VLF) component (0–0.4 Hz), Total Power (TP) (0–0.4 Hz) and LF/HF ratio [16]. Time domain parameters reflect both parasympathetic and sympathetic activities and are usually calculated as 24 hours. Frequency domain parameters usually provide information about the data in five-minute recordings. The TP is strongly associated with the risks of sudden cardiac death and all-cause death after myocardial infarction; VLF is an additional indicator of sympathetic function; LF reflects sympathetic and parasympathetic activities in short-term measurements and sympathetic activities in long-term measurements, HF reflects the parasympathetic or vagal activity of the autonomic nervous system. A higher LF/HF ratio means increased sympathetic activity or decreased parasympathetic activity [17].

### Statistical Analysis

The statistical analysis was performed through SPSS for Windows (version22.0). Descriptive analyses were presented by mean ± standard deviation. Quantitative data distribution was tested with the Shapiro Wilk test and data distribution was proper for non-parametric test. The Mann-Whitney U test was used for quantitative comparison of two independent groups. The Kruskal Wallis test was used for three independent groups. Bonferroni correction was used for pairwise comparisons. Chi square test was used to compare the qualitative data. Spearman's correlation analysis was used to examine the association of two quantitative data. Any p-value below 0.05 ($p<0.05$) was considered as statistically significant.

## Results

Totally 123 participants were included in the study. There were 65 (50 females, 15 males) patients in the study group and 58 (45 females, 13 males) individuals in the control group ($p=0.932$). The mean age was 49±12.2 years in the study group and 46±10.7 years in the control group ($p=0.173$). The concomitant diseases were hypertension in 17 (26.4%) patients, thyroid disease in 9 (9.2%) patients, diabetes mellitus in 5 (7.7%) patients, and hyperlipidemia in 4 (6.2%) patients. The mean disease duration was 7.12±7.24 (1-44) years. Neurological examination and neural conduction studies of the patients included in the study were within normal limits.

Table 1. Heart rate variability parameters in restless legs syndrome and control group.

| Parameter | RLS severity groups | | | | RLS Groups (n=65) | Control group (n=58) | p |
|---|---|---|---|---|---|---|---|
| | Moderate RLS (n=18) | Severe RLS (n=36) | Very-severe RLS (n=11) | p | | | |
| SDNN (total), ms | 151.0±42.8 | 134.7±35.4 | 150.8±24.6 | 0.102 | 141.9±36.5 | 144.8±43.9 | 0.275 |
| SDNN (daytime), ms | 129.0±35.1 | 130.7±36.0 | 133.1±23.2 | 0.681 | 130.6±33.5 | 131.3±37.5 | 0.773 |
| SDNN (night), ms | 135.2±46.4 | 115.4±26.8 | 144.7±31.5 | 0.037 | 125.8±35.6 | 137.5±41.2 | 0.037 |
| rMSSD (total), ms | 31.5±10.3 | 29.4±12.8 | 34.0±12.6 | 0.260 | 30.8±12.1 | 32.9±12.8 | 0.360 |
| rMSSD daytime),ms | 28.0±8.6 | 27.7±12.7 | 30.2±13.0 | 0.534 | 28.2±11.6 | 30.5±13.7 | 0.448 |
| rMSSD (night), ms | 34.8±13.8 | 30.9±14.1 | 37.3±13.3 | 0.172 | 33.1±13.9 | 35.2±14.9 | 0.390 |
| pNN50 (total), % | 8.9±6.4 | 7.5±8.4 | 11.6±8.5 | 0.191 | 8.6±8.0 | 9.5±8.8 | 0.651 |
| pNN50 (daytime), % | 6.1±5.0 | 6.2±7.3 | 8.2±8.6 | 0.498 | 6.5±6.9 | 7.3±7.3 | 0.618 |
| pNN50 (night), % | 11.8±9.7 | 10.1±12.9 | 15.3±9.9 | 0.161 | 11.4±11.6 | 12.2±11.5 | 0.612 |
| SDANN Index total) | 139.6±41.6 | 123.7±34.4 | 141.0±28.1 | 0.154 | 131.0±36.0 | 136.2±41.3 | 0.265 |
| SDNN Index (total) | 58.3±18.0 | 50.0±12.2 | 56.5±11.4 | 0.133 | 53.4±14.3 | 57.1±16.4 | 0.133 |
| HF (total), ms2 | 229.6±149.3 | 229.6±244.5 | 312.6±234.3 | 0.292 | 243.6±219.5 | 257.4±232.3 | 0.871 |
| HF (daytime), ms2 | 163.5±93.1 | 167.9±156.6 | 231.2±195.4 | 0.573 | 254.4±214.4 | 209.1±208.3 | 0.750 |
| HF (night), ms2 | 286.4±211.3 | 262.0±283.8 | 384.9±279.8 | 0.234 | 289.6±264.9 | 300.0±269.3 | 0.911 |
| LF (total), ms2 | 732.2±469.4 | 473.7±224.5 | 718.7±376.9 | 0.061 | 586.7±352.7 | 660.4±403.8 | 0.246 |
| LF (daytime), ms2 | 682.9±495.0 | 444.0±227.4 | 611.9±312.9 | 0.123 | 538.6±347.1 | 622.0±394.8 | 0.165 |
| LF (night), ms2 | 773.0±457.8 | 502.0±253.7 | 819.1±559.1 | 0.041 | 630.7±380.7 | 688.8±443.0 | 0.469 |
| LF/HF (total) | 3.4±1.1 | 3.0±1.3 | 3.0±1.7 | 0.371 | 0.1±1.3 | 0.64±2.0 | 0.891 |
| LF/HF (total) | 4.2±1.9 | 3.5±1.7 | 3.6±1.8 | 0.380 | 0.0±1.8 | 0.71±3.9 | 0.696 |
| LF/HF (night) | 3.2±1.1 | 2.8±1.2 | 2.9±1.8 | 0.491 | 0.1±1.3 | 0.57±3.2 | 0.746 |
| VLF (total), ms2 | 2655.6±1655.3 | 1794.0±908.8 | 2258.5±853.1 | 0.072 | 2111.2±1198.7 | 2406.79±1409.3 | 0.191 |
| VLF (daytime), ms2 | 2505.3±1652.5 | 1835.1±902.6 | 2063.6±725.7 | 0.171 | 2309.4±2296.3 | 2396.35±1473.9 | 0.232 |
| VLF (night), ms2 | 2794.0±1737.3 | 1784.7±1034.9 | 2449.6±1122.1 | 0.038 | 2176.7±1338.1 | 2368.75±1458.9 | 0.386 |
| Total power (total) | 3648.3±2219.1 | 2514.7±1166.8 | 3318.5±1308.1 | 0.061 | 2964.6±1608.4 | 3348.64±1937.0 | 0.248 |
| Total power (daytime) | 3374.9±2187.6 | 2489.5±1156.1 | 2930.6±1128.5 | 0.362 | 2809.3±1533.3 | 3300.31±2053.4 | 0.191 |
| Total power (night) | 3916.4±2401.9 | 2538.9±1298.2 | 3419.1±1486.3 | 0.041 | 3032.63±1748.2 | 3387.63±2020.4 | 0.291 |

Values are provided as Mean ± Standard deviation.

SDNN: Standard deviation (SD) of all normal to normal (NN) intervals, SDNN index: The mean of the deviation of the 5-minute NN intervals over the entire recording, rMMSD: The square root of the mean squared differences of successive NN intervals, pNN50: The proportion of adjacent RR intervals differing by >50 milliseconds in the 24-hour recording, HF: High frequency, LF: Low frequency, VLF: Very low frequency, TP: Total power.

No statistical difference was found in the HRV analysis, except that the SDNN night value was lower when RLS patients and the control group were compared (p=0.037) (Table 1). When patients were divided according to disease severity, 18 patients had mild, 36 patients had severe, and 11 patients had very severe disease; however, there was not any patient with mild disease. Since HRV analyzes were compared according to disease severity, SDNN night was significantly different between groups (p=0.037). In the pairwise comparison, the difference between the severe and very severe groups was significant (p=0.013), and severe group was lower than very severe group. LF night (p=0.041) and VLF night (p=0.03) values were significantly different between three groups but no difference was found in terms of in pairwise comparisons (p>0.016). In addition, there was no correlation between disease duration and HRV parameters (p>0.05).

## Discussion

The aim of the present study was to show the effect of autonomic dysfunction through comparison of HRV parameters in RLS patients with healthy controls. It was found that SDNN night value, which is a time domain parameter as an indicator of the cardiac effects of parasympathetic modulation and circulation dynamics, was significantly lower in RLS patients when compared to the control group.

Contradictory results were detected in manuscripts on HRV findings in the literature. Yıldız et al. [18] found in their study that SDNN, SDANN, and SDNN indexes were significantly lower and LF/HF ratio, which is an indicator of sympathetic nerve activity, was significantly higher in RLS patients when compared to the control group. Barone et al. [19] and Cikrikci et al. [20] detected no difference in HRV analysis. In these studies, the changes in the disease severity and duration in the HRV analysis data were not examined. It was found in our study that the disease duration and severity had no effect [18-20]. We have detected a significant decrease in the value of SDNN night. The circadian rhythm of the symptoms in RLS and the increase at night time may be associated with lower SDNN night value. This may be related to the fact that the pathogenesis is still not fully elucidated. We could not find a difference in the increased VLF, LF, LF/HF ratio parameters showing sympathetic activity in our study; however, the decrease in the SDNN night value associated with decrease parasympathetic activity indicates the need for further researched on this subject.

There have been studies in the literature investigating cardiac autonomic dysfunction in RLS patients with other techniques besides HRV. In a study evaluating cardiovascular

baroreflex gain with the Modified Oxford technique, increased sympathetic system activation was shown in RLS patients [21]. There are studies showing that, irritable bowel syndrome and other autonomic dysfunctions are seen more frequently in RLS patients besides cardiac autonomic dysfunction [22, 23]. Cardiac autonomic dysregulation with HRV may be an indicator of increased sympathetic system. Increased sympathetic activation can cause endothelial damage, atherosclerosis, and cardiac death [24]. It can be concluded that RLS disease, which increases the risk of cardiovascular disease, may have effects up to mortality.

Cardiac parasympathetic autonomic dysfunction was shown at night time; however, cardiac sympathetic autonomic dysfunction could not be demonstrated in HRV analyzes in this study.

As the limitations of the study, there was not any significant correlation between HRV analysis data for disease duration and severity. This may be caused by exclusion of the patients with a longer disease duration since they received treatment for RLS. Furthermore, the study group was relatively smaller. Comparison with HRV analyzes of the patients included in the study after treatment and follow-up periods may provide a better assessment of cardiac autonomic dysfunction in RLS patients.

In conclusion, in this study examining the cardiac autonomic system with HRV in patients with RLS, a decrease in parasympathetic nervous system was detected at night time. Therefore, there is a need for further studies with larger series and including control HRV analyses.

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# Prognostic value of metastatic lymph node ratio in patients undergoing D2 gastrectomy for gastric cancer

## Mide kanseri nedeniyle D2 lenf nodu diseksiyonu yapılan hastalarda metastatik lenf nodu oranının prognostik değeri

**Ali Alemdar [1], Selma Sengiz Erhan [2]**

**Abstract**

Aim: Aim of this study was to investigate the prognostic value of metastatic lymph node ratio (MLNR) staging in gastric cancer patients who underwent curative D2 lymph node dissection.

Methods: Place and Duration of Study: Department of General Surgery, University of Health Sciences Prof. Dr. Cemil Tascıoglu City Hospital, Istanbul, Turkey, from January 2012 to April 2021. Medical records of 171 patients undergoing D2 curative gastrectomy for locally advanced gastric cancer between January 2012 and April 2021 were reviewed retrospectively. Metastatic lymph node ratio (MLNR) and TNM staging system node ( Ns )staging was evaluated. Prognostic factors on overall and disease-free survival (DFS) were evaluated. MLNR was compared with number of lymph node metastasis.

Results: Mean age of patients included in present study was 60.7 (male: N = 118; female: N = 53). Based on TNM N staging, 62 patients N0, 25 patients N1, 35 patients N2, and 49 were N3 patients. According to classification based on metastatic lymph node ratio (MLNR), 62 patients MLNR 0, 35 patients MLNR 1, 35 patients MLNR 2 and 39 patients were in the MLNR 3 group. Stage migration were seen in 32 patients (% 18,7) in the classification of MLNR. Cox regression survival analysis showed MLNR is an independent prognostic factor.

(p =0.0001, 95% CI 81.31-101.04)

Conclusions: It has been found that using MLNR staging instead of number of positive lymph nodes in nodal evaluation can help to reduce the problem of stage migration. MLNR is an important prognostic factor for both OS and DFS.

Key words: Gastric cancer, lymph node metastasis, metastatic lymph node ratio, prognosis, stage migration.

**Öz**

Amaç: Bu çalışmanın amacı, küratif D2 lenf nodu diseksiyonu uygulanan mide kanseri hastalarında metastatik lenf nodu oranı (MLNR) evrelemesinin prognostik değerini araştırmaktır.

Yöntemler: Ocak 2012 ile Nisan 2021 arasında klinik olarak lokal ileri mide kanseri nedeniyle küratif gastrektomi ve D2 lenf nodu diseksiyonu uygulanan 171 hastanın kayıtları geriye dönük olarak incelendi. Metastatik lenf nodu oranı (MLNR) ve Tümör Lenf Nodu Metastazı (TNM) evreleme sistemine göre lenf nodu (N) evrelemesi değerlendirildi. Genel sağ kalım (OS), hastalıksız sağkalım (DFS), klinikopatolojik özellikler ve prognostik faktörler değerlendirildi. MLNR, lenf nodu metastazı sayısı ile karşılaştırıldı.

Bulgular: Çalışmaya dahil edilen hastaların ortalama yaşı 60.7 idi (erkek: N = 118; kadın: N = 53). TNM, N evrelemesine göre, 62 hasta N0, 25 hasta N1, 35 hasta N2 ve 49 hasta N3 idi. MLNR evrelemesine göre MLNR 0 62 hasta, MLNR 1 35 hasta, MLNR 2 35 hasta ve MLNR 3 grubunda 39 hasta vardı. MLNR evrelemesine göre 32 hastada (% 18,7) evre kayması görüldü. Cox regresyon sağkalım analizi, MLNR'nin bağımsız bir prognostik faktör olduğunu gösterdi. (p =0,0001, 95% CI 81.31-101.04)

Sonuç: Nodal değerlendirmede pozitif lenf nodu sayısı yerine MLNR evrelemesinin kullanılması, evre kayması sorununu azaltmaya yardımcı olabileceği bulunmuştur. MLNR, hem OS hem de DFS için önemli bir prognostik faktördür.

Anahtar kelimeler: Mide kanseri, lenf nodu metastazı, metastatik lenf nodu oranı, prognoz, evre kayması.

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## Introduction

Gastric cancer is the fifth most common diagnosed malignancy and the third leading cancer related deaths in the world among the cancer patients [1]. Besides, gastric cancer is a loco-regional disease with high incidence of lymph node metastasis. Lymph node dissection is an important part of the surgical treatment. In Asian countries D2 lymphadenectomy is a standard surgical procedure for clinically node-positive (cN+) or local advanced ( ≥ cT2) gastric cancer [2]. In Western countries, D2 lymphadenectomy is carried out in specialised, high-volume centers with appropriate surgical expertise and postoperative care [3].

Adequate staging is essential in predicting prognosis. Lymph node metastasis is the most important prognostic factor in gastric cancer [4, 5]. But the classification of lymph node status (N categories) is still controversial. Currently, American Joint Committee on Cancer (AJCC) classification system is widely used, based on the number of metastatic lymph nodes [6]. This classification system is simple. However, it has been reported that there were some problems. It may lead to stage migration and inadequate prediction of prognosis in gastric cancer surgery with extended lymphadenectomy [7, 8]. Particularly, the small number of dissected lymph nodes may be insufficient to determine prognosis [9]. However, the optimal number of dissected lymph node is still uncertain in accurate staging [5].

Metastatic Lymph node ratio (MLNR) is defined as the ratio of the number of the metastatic lymph nodes to the total number of dissected lymph nodes. MLNR has been recommended as a new staging system for gastric cancer in recent studies [10–12]. This suggested classification system may reduce stage migration and have more accurate prediction of long-term survival results [4, 13, 14]. MLNR is also used as a staging system in different types of cancers [15–17].

The aim of the present study was to evaluate prognostic value of MLNR in gastric cancer patients who underwent curative D2 gastrectomy and to determine stage migration, prediction of overall and disease free survival.

## Material and methods

Medical records of totally 200 patients underwent gastrectomy for gastric cancer were reviewed retrospectively. One hundred and seventy-seven patients were enrolled in the analysis based on the inclusion and exclusion criteria in Department of General Surgery, University of Health Sciences Prof Dr Cemil Tascıoglu City Hospital, between January 2012 and April 2021. Clinicopathological characteristics such as age; sex; tumor location; preoperative chemotherapy; type of surgery; tumor size; depth of tumor invasion; tumor differentiation; vascular, lymphatic, and perineural invasion; total number of harvested lymph nodes; TNM stage; follow-up data and time of death were retrieved from medical records.

Preoperatively 96 patients had chemotheraphy. Standart FLOT (5-fluorouracil, Leucovorin, Oxaliplatin, Docetaxel) chemotheraphy was given to most of the patients for four cycles.

The inclusion criteria were the patients with preoperative histologically proven primary gastric adenocarcinoma, clinically locally advanced cancer and the patients who underwent curative D2 open gastric surgery. Presence of distant metastases, postoperative pathology confirmed as non-gastric adenocarcinoma, detection of synchronous tumors, detection of positive microscopic resection margin, patients' mortality within the first 30 days and patients' incomplete data were defined as exclusion criteria.

Disease-free survival (DFS) was defined as the duration from the operation date to the first date of recurrence or last follow-up date. Overall survival ( OS ) were calculated from the date of surgery to the time of death from any cause. 171 patients were included in this study. Stages were determined according to the 8$^{th}$ edition of the AJCC Cancer Staging Manual [6]. MLNR, which is defined as the ratio of the number of metastatic lymph nodes to the number of harvested lymph nodes. MLNR grouping was based on definition of the Italian Research Group for Gastric Cancer Study (GIRCG) [18]. MLNR categories were defined as MLNR 0, (0%); MLNR 1, (1-9%); MLNR 2, (10-25%); MLNR 3, (>25%).

This retrospective study was approved by Ethics Committee of University of Health Sciences Prof. Dr. Cemil Tascıoglu City Hospital (2021/211).

### Statistical analysis

Data obtained in the present study were analyzed using v.22.0 of SPSS software. In evaluating data, definitive statistical methods (mean, standard deviation, frequency) were used as well as Kaplan-Meier analysis in analyzing the survival and significance level (Log Rank). Mann Whitney-U, Kruskal Wallis were used for analysis of difference between groups. In the multivariate analysis, independent factors predicting survival were analyzed by using Cox regression analysis. The results were evaluated with 95 % confidence intervals (CIs) and level of significance was determined as $p < 0.05$.

## Results

Of the patients, 118 were male and 53 were female. Mean age was 61 (29-89) years. Subtotal gastrectomy was performed in 62 patients (36.3 %). The correlations between lymph node metastasis and clinicopathological parameters of patients are summarized in Table 1.

With regard to tumor location, there were more patients who have tumors located on the proximal site. Number of patients with tumor diameter more than 6 cm were 35 of 171 (20.6%). The number of patients receiving neoadjuvant chemotherapy were 96 (56.1 %).

MLNR groups were shown according to N staging in Figure 1. When MLNR groups were examined, MLNR 0 was the same as N 0. MLNR 3 was relatively homogenous and mostly consisted of N3 patients. However, MLNR 1 and MLNR2 were heterogeneous groups. MLNR 2 included almost half of different N stages. MLNR 3 was consisted of 36 N3 patients (92,3%).

MLNR staging were migrated in total of 32 patients (18.7 %). Comparing N staging, 26 patients were understaged one level and 6 patients were overstaged one level. In understaged patients mean number of harvested lymph nodes were 51.3 (32-87). In all overstaged patients, the mean number of harvested lymph nodes were less than 15. In these patients with dissected lymph node below 15 all lymph node stations were dissected. The comparison of the clinicopathological characteristics of stage migrated patients are shown in Table 2.

Univariate regression analysis was performed to determine the predictors of OS and DFS. Univariate analysis factors affecting DFS were given in Table 3 and OS in Table 4. Cox regression analysis was performed to determine the independent predictors of OS and DFS. Cox regression test and the correlation between MLNR and clinicopathological parameters are analyzed. Lymphatic invasion, and MLNR were determined as independent prognostic factors in Cox regression analysis (p=0.0001 and p=0.0001 95% CI 47.79-67.64; 95% CI 81.31-101.04) respectively.

Table 1. Clinicopathological characteristics of patients.

| Patient characteristics | Lymph Node Metastasis Absent n=62 | Lymph Node Metastasis Present n=109 | *P* value |
|---|---|---|---|
| Age (mean ± SD, years) | 62.97 (35-78) | 59.45 (29-89) | - |
| Sex | | | |
| Male | 41 (66.12) | 77 (70.6) | 0.330 |
| Female | 21 (33.87) | 32 (29.4) | |
| Preoperative chemotherapy | | | |
| Not-received | | | |
| Received | 34 (54.8) | 41 (37.6) | 0.020 |
| | 28 (45.2) | 68 (62.4) | |
| Tumor differentiation | | | |
| Well or Moderate | 46 (74.2) | 44 (40.4) | 0.0001 |
| Poorly | 16 (25.8) | 65 (59.6) | |
| Tumor location | | | |
| Cardia-Corpus | 40 (64.5) | 63 (57.8) | 0.240 |
| Antrum | 22 (35.5) | 46 (42.2) | |
| Type of gastrectomy | | | |
| Total gastrectomy | 43 (69.4) | 66 (60.6) | 0.160 |
| Subtotal gastrectomy | 19 (30.6) | 43 (39.4) | |
| Lymphatic invasion | | | |
| Present | 16 (25.8) | 33 (30.3) | 0.0001 |
| Absent | 46 (74.2) | 26 (23.9) | |
| Vascular invasion | | | |
| Present | 16 (25.8) | 77 (70.6) | 0.0001 |
| Absent | 46 (74.2) | 32 (29.4) | |
| Perineural invasion | | | |
| Present | 12 (19.4) | 74 (67.9) | 0.0001 |
| Absent | 50 (80.6) | 35 (32.1) | |
| y/pTstage | | | |
| pT1-2 | 43 (69.4) | 21 (19.3) | 0.0001 |
| pT3-4 | 19 (30.6) | 88 (80.7) | |
| y/pTNMstage | | | |
| I-II | 62 (100) | 35 (32.1) | 0.0001 |
| III-IV | 0 (0) | 74 (67.9) | |
| Number of harvested lymph node | | | |
| ≤ 15 | 0 (0) | 4 (3.7) | 0.160 |
| > 15 | 62 (100) | 105 (96.3) | |
| Tumor maximum diameter | | | |
| ≤ 6 | 58 (93.5) | 78 (71.6) | |
| > 6 | 4 (6.5) | 31 (28.4) | 0.0001 |

Data presented as mean ± Standard deviation (SD), median( 1st-3rdquartiles ) or number in parantheses represent percentage ( % ).
*: malignant cells in peritoneal washings.

Table 2. Clinicopathological characteristics of stage migrated patients.

| | Overstaged n=6 | Understaged n=26 |
|---|---|---|
| Number of harvested lymph node | | |
| ≤ 15 | 4 (66.7) | 0 (0) |
| > 15 | 2 (33.3) | 26 (100) |
| Number of harvested lymph node (mean ± SD) | 14.3 (10-17) | 51.3 (32-87) |

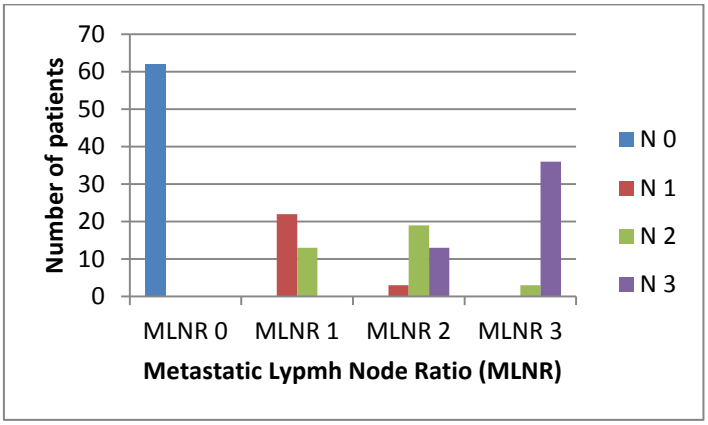


Figure 1. Metastatic lymph node ratio grouping.

Table 3. Disease-free survival analysis.

| Patient characteristics | | Median DFS (Month) | 95 % confidence interval (CI) | *P* value |
|---|---|---|---|---|
| Sex | | | | |
| Male | 118 (69.0) | 30.18 | 91.45-109.92 | 1.28 |
| Female | 53 (31.0) | 34.72 | 64.82-91.12 | |
| Age (mean ± SD, years) | | | | |
| ≤ 60 | 75 (43.9) | 35.37 | 83.38-107.49 | 0.86 |
| > 60 | 96 (56.1) | 28.63 | 83.09-104.86 | |
| MLNR | | | | |
| ≤ 0.25 | 132 (77.2) | 33.44 | 93.73-110.66 | 0.003 |
| > 0.25 | 39 (22.8) | 25.30 | 47.62-80.76 | |
| Lymphatic invasion | | | | |
| Present | 99 (57.9) | 26.74 | 61.58-81.61 | |
| Absent | 72 (42.1) | 38.6 | 104.81-121.03 | 0.0001 |
| Node metastasis | | | | |
| ≤ 2 | 25 (23.0) | 38.38 | 85.80-123.26 | 0.049 |
| > 2 | 84 (77.1) | 27.0 | 62.23-88.54 | |
| Tumor differentiation | | | | |
| Well or Moderate | 81 (47.4) | 31.27 | 67.62-89.47 | 0.058 |
| Poorly | 90 (52.6) | 31.87 | 93.85-113.46 | |
| pTstage | | | | |
| pT1 (early) | 37 (21.6) | 40.17 | 100.29-123.35 | 0.053 |
| pT 2-4 (advanced) | 134 (78.4) | 29.22 | 79.71-98.68 | |

MLNR: Metastatic lymph node ratio, DFS: Disease-free survival.

## Discussion

Lymph node metastasis is the most important prognostic factor in gastric cancer which is associated with a poor prognosis [5]. Classification of lymph nodes is controversial. Japanese Gastric Cancer Association (JGCA) first classified the lymph nodes based on anatomic location of the metastatic lymph nodes and then revised to the just number of the metastatic lymph nodes removed [2]. Union of International Cancer Control (UICC) and AJCC published a classification system based on number of the metastatic lymph nodes [6].

General approach for R0 resection and widespread lymph node dissection is to remove lymph nodes as many as possible in relation to tumor location. The more the depth of tumor, the more is the lymph node metastasis. Number of the removed lymph nodes may vary, even though the same technique is always used. Due to the factors such as surgical experience, surgical technique and biological factors. High body mass index (BMI) leads to decreased lymph node harvesting. Excessively adipose tissue, anatomical variations, insufficient surgical experience, and the pathologist's being less attentive to counting lymph nodes may lead to low number of lymph nodes examined following dissection [19]. The studies have shown that low

number of lymph nodes might lead to wrong decision in staging gastric cancer. For example, the tumor that should be stage 2 in fact, may be misdiagnosed as stage 3 (it may be overstaged). Stage migration will be more common especially in the patients with less lymph nodes removed [20].

Table 4. Overall survival analysis.

| Patient characteristics | | Median OS | 95 % confidence interval (CI) | *P* value |
|---|---|---|---|---|
| Sex | | | | |
| Male | 118 (69) | 31.17 | 68.18-90.66 | 0.378 |
| Female | 53 (31) | 37.83 | 66.01-92.03 | |
| Age (mean ± SD, years) | | | | |
| ≤ 60 | 75 (43.9) | 37.2 | 75.98-101.43 | 0.275 |
| > 60 | 96 (56.1) | 30.13 | 62.82-88.68 | |
| MLNR | | | | |
| ≤ 0.25 | 132 (77.2) | 34.76 | 81.31-101.04 | 0.0001 |
| > 0.25 | 39 (22.8) | 28.05 | 34.33-63.51 | |
| Lymphatic invasion | | | | |
| Present | 99 (57.9) | 28.86 | 47.79-67.64 | 0.0001 |
| Absent | 72 (42.1) | 39.51 | 96.75-116.75 | |
| Node metastasis | | | | |
| ≤ 2 | 25 (23) | 39.81 | 70.60-112.8 | 0.054 |
| > 2 | 84 (77.1) | 29.54 | 47.79-73.08 | |
| Tumor differentiation | | | | |
| Well or Moderate | 81 (47.4) | 33.26 | 57.07-79.60 | 0.084 |
| Poorly | 90 (52.6) | 33.2 | 76.07-100.87 | |
| pTstage | | | | |
| pT1(early) | 37 (21.6) | 41.00 | 82.46-115.98 | 0.045 |
| pT2-4(advanced) | 134 (78.4) | 31.09 | 66.06-86.78 | |

MLNR: Metastatic lymph node ratio, OS: Overall survival.

In the present study, we performed total gastrectomy for proximally located tumors and subtotal gastrectomy for distal site. A standard D2 lymph node dissection was performed in all patients.

There is no consensus on the cut-off value of MLNR. Several cut-off categories of MLNR were used in other studies [21–23]. In this study we used GIRCG cut-off value for determined MLNR [18]. In multivariate analysis, MLNR (>0.25) was poor prognostic factor for OS. In terms of DFS, MLNR (>0.25) was found to be as an independent prognostic factor. In this study, MLNR was as an important prognostic factor for both OS and DFS.

MLNR might be reduced and thus the prognosis would be improved by increasing the number of lymph nodes harvested. It was also noted that staging wouldn't change in patients with involvement of metastatic lymph nodes following adequate number of the lymph node dissection but prognosis would be improved by increasing the number of removed lymph nodes. Cancer prognosis would be improved with more adequate surgery. It was specified that the lymph nodes containing micrometastatic cells which are undetectable pathologically could be removed by D2 dissection as mean wide surgical excision [24].

In this study, we have limitations like the small number of patients. The study should be repeated in in high-volume setting. The number of harvested lymph nodes in four patients was less than fifteen. This is a strength of the study. The stage migration was more in patients with low number of lymph nodes dissected. The results we obtained in patients staged according to MLNR was similar to the literature, showing a more homogeneous distribution in accordance with patients' survival. Compatibility with the present literature is one of the strength of the study. The reasonably even distribution of patients through tumor stage is another strength of the study.

Stage migration may be seen due to inadequate lymph node dissection. Mean survival extends in the group of patients with higher N stage as a consequence of stage-migration. MLNR can prevent stage migration. Thus, it may be recommended in nodal staging of gastric cancer.

As the results of the present study, it was seen that MLNR staging may be used especially in the patients with low number of removed lymph nodes. Thus, predicting the prognosis may be clarified. It is possible to prevent heterogeneous survival and stage-migration.

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# Effect of probiotic supplementation after laparoscopic sleeve gastrectomy on constipation and gastrointestinal quality of life

## Laparoskopik sleeve gastrektomi sonrası probiyotik takviyesinin konstipasyon ve gastrointestinal yaşam kalitesi üzerine etkisi

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**Abstract**

Aim: In this study, we aimed to investigate the early effect of probiotic supplementation after Laparoscopic Sleeve Gastrectomy (LSG) on constipation and gastrointestinal quality of life compared to control group.

Methods: This study was a prospective, randomized clinical trial. Participants were recruited to Bariatriklab Obesity and Metabolic Surgery Center for LSG. All patients were divided into 2 groups as probiotic and control by using simple randomization. The probiotic group consumed Bifidobacterium animalis lactis BB-12 strain as a probiotic supplement during 6 weeks after LSG. Gastrointestinal Symptom Rating Scale (GSRS), Constipation Severity Instrument (CSI), Patient Assessment of Constipation Quality of Life Scale (PAC-QOL), Bristol Stool Form Scale (BSFS), Gastrointestinal Quality of Life Index (GIQLI) of the patients were recorded before LSG and at the 2nd, 4th, 6th weeks after LSG.

Results: The probiotic group had an average age of 37.00±8.92 years (18 female, 12 male), the control group had an average age of 41.03±11.29 years (23 female, 7 male). CSI (16.50 ± 14.76 vs. 31.37 ± 15.34), PAC-QOL (58.53 ± 12.59 vs 72.30 ± 19.70), GSRS (26.83 ± 9.14 vs. 37.93 ± 16.59) and total score mean were lower compared to the control group, GIQLI total score average (147.50 ± 11.79 vs 136.87 ± 18.98) was found higher ($p < 0.05$) in probiotic group.

Conclusions: Probiotic supplementationimproved the constipation and gastrointestinal quality of life in the early post LSG-period in the brobiotic group compared to the control group.

Keywords: Bariatric surgery, gastrointestinal quality of life, constipation, sleeve gastrectomy, probiotics.

**Öz**

Amaç: Bu çalışmada, Laparoskopik Sleeve Gastrektomi (LSG) sonrası probiyotik takviyesinin, konstipasyon ve gastrointestinal yaşam kalitesi üzerine erken dönem etkisinin araştırılması amaçlanmıştır.

Yöntemler: Bu çalışma, prospektif, randomize klinik çalışmadır. Katılımcıları Bariatriklab Obezite ve Metabolik Cerrahi Merkezi'ne LSG için başvuran bireyler oluşturmaktadır. Tüm hastalar, basit randomizasyon kullanılarak randomize örnekleme ile probiyotik ve kontrol olmak üzere 2 gruba ayrıldı. Probiyotik grubu, LSG sonrası 6 hafta boyunca probiyotik takviyesi olarak Bifidobacterium animalis lactis BB-12 suşunu kullandı. Hastaların, LSG öncesi ve sonrası 2.hafta, 4.hafta, 6.haftanın sonunda Gastrointestinal Semptom Derecelendirme Ölçeği (GSRS), Konstipasyon Ciddiyet Ölçeği (CSI), Konstipasyon Yaşam Kalitesi Ölçeği (PAC-QOL), Bristol Dışkı Formu Skalası (BSFS), Gastrointestinal Yaşam Kalitesi İndeksi (GIQLI) kaydedildi.

Bulgular: Probiyotik grubun yaş ortalaması 37,00±8,92 (18 kadın, 12 erkek), kontrol grubunun yaş ortalaması 41,03±11,29 (23 kadın, 7 erkek) idi. CSI (16,50 ± 14,76 ile 31,37 ± 15,34), PAC-QOL (58,53 ± 12,59 ile 72,30 ± 19,70), GSRS (26,83 ± 9,14 ile 37,93 ± 16,59) ve toplam puan ortalaması kontrol grubuna göre daha düşük, GIQLI toplam puan ortalaması (147,50 ± 11,79 ile 136,87 ± 18,98) daha yüksek bulundu ($p < 0,05$).

Sonuç: LSG sonrası erken dönemde probiyotik takviyesi alan grupta, kontrol grubuna kıyasla konstipasyon ve gastrointestinal yaşam kalitesinde iyileşmeler gözlenmiştir.

Anahtar Kelimeler: Bariatrik cerrahi, gastrointestinal yaşam kalitesi, konstipasyon, sleeve gastrektomi, probiyotik.

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## Introduction

In recent studies, Laparoscopic sleeve gastrectomy (LSG) is recognized as one of the standard approach of bariatric surgery [1]. LSG surgery aims to restrict the volume of food taken without affecting nutrient absorption and reduces the feeling of hunger [2]. LSG has become more preferred surgical treatment of obesity alone or combination with other bariatric surgical methods [3]. Gastrointestinal (GI) symptoms might be seen after bariatric surgery, and these symptoms are associated with impaired quality of life [4]. In addition, these symptoms may be observed by physiological differences in the digestive system due to anatomical changes after surgery [5]. Furthermore, GI quality of life score was lower in morbidly obese individuals than non-obese individuals [6]. For instance, one of the symptoms is the constipation which was seen after bariatric surgery ranges between 7-39%. The constipation is observed after bariatric surgery due to the taken of post-operative dehydration, calcium, iron, vitamin and mineral supplements [7].

In addition to that, the probiotics are used as a treatment method which provides benefits in patients with constipation. The probiotics are considered that as a treatment of constipation and previous study showed the differences in gut microbiota between healthy individuals and patients with chronic constipation [8]. "Bifidobacterium animalis subsp. lactis BB-12" is the broadest investigated probiotics on the market. Some human studies show that Bifidobacterium animalis subsp. lactis BB-12 is efficient in improving constipation, modulating intestinal flora. For example, it was invetigated that Bifidobacterium animalis subsp. lactis BB-12 is demonstrated a significant improvement in young adults and the elderly population compared to placebo treatment on the frequency of defecation [9]. Few studies investigated the effectiveness of probiotic supplementation after bariatric surgery in decreasing lactose intolerance, better digestion of proteins, increase in vitamin and mineral bioavailability, but studies on the effectiveness of probiotics in GI symptoms after LSG are not sufficient and require more research [10]. The purpose of our study is to investigate the early effect of probiotic supplementation after LSG on constipation and gastrointestinal quality of life compared to control group.

## Material and methods

### Study population

This study is a prospective clinical trial. Power analysis was conducted and satisfied (80%). 80% power analysis, 30 patients in probiotic and 30 patients in control group, 60 patients in total, were participated the study from Bariatriklab Obesity and Metabolic Surgery Center. The remaining 60 patients were divided into 2 groups by random sampling using simple randomization. Participants were randomly allocated to the probiotic group or the control group as 30 participants for each (simple allocation using www.random.org). The inclusion criteria were the age of 18-65, BMI $\geq$40 kg/m$^2$ or BMI $\geq$35 kg/m$^2$ and having at least one comorbid disease related to obesity, following a regular dietitian control for at least 6 weeks after LSG were included the study.

The exclusion criteria were; under the age of 18 and over the age of 65, pregnant women or who are in breastfeeding, not have constipation treatment at least a month, LSG pre-with a history of antibiotic use over the last six months, at least three months use of laxatives probiotic, prebiotic supplements, patients are not eligible for surgery by the surgeon or patients who undergone different bariatric surgical procedures, patients who are not on regular dietitian follow-up for at least 6 weeks after LSG and individuals with BMI <35 kg/m$^2$. This study was endorsed suitable for medical ethics with the 2017/13/51 decision no and date 03.08.2017 by Acibadem Mehmet Ali Aydinlar University and Acibadem Health Organizations Medical Research Ethics Board (ATADEK). All participants provided written consent in accordance with the declaration of Helsinki. The principal researcher received the consent forms from the volunteers who agreed to participate in the study.

### Patients' characteristics

Before LSG procedure, patient's demographic information was recorded such as age, gender, number of births, educational status, employment status and exercise habits.

### Data collection and questionnaires

In this study, the scales to evaluate patients' status were used; Gastrointestinal Symptom Rating Scale (GSRS), Constipation Severity Instrument (CSI), Constipation Quality of Life Scale (PAC-QOL), Bristol Stool Form Scale (BSFS), Gastrointestinal Quality of Life Index (GIQLI). In addition, participants' information forms were applied, 24-hour dietary recall and anthropometric measurements were taken. All scales and measurements were applied face-to-face by the researcher at pre-op and the end of the 2nd, 4th, 6th week after the surgery.

### Gastrointestinal Symptom Rating Scale (GSRS):

It is a Likert scale consisting of 15 items to evaluate the symptoms frequently observed in GI symptoms developed by Revicki et al. (1998) and validity and reliability in Turkish version was performed by Turan and Asti (2011) [11]. The scale has five sub-dimensions for abdominal pain, reflux, diarrhea, indigestion, constipation. Higher scores from the scale indicate that symptoms are more severe, Cronbach alfa was found 0.82 for all items [12].

### Constipation Severity Instrument (CSI):

This is a scale of 16 questions developed by Varma at al (2008) [13], conducted by Turkish validity and reliability Kaya and Turan (2011), used to determine the frequency, intensity and difficulty in defecation of individuals and to evaluate symptoms of constipation. It has three lower dimensions: obstructive defecation, colonic internia, pain. A high score from the scale indicates that the symptoms are serious, Cronbach alpha was determined between 0.92 and 0.93 [14].

### Constipation Quality of Life Scale (PAC-QOL):

This is a scale consisting of 28 questions developed by Marquis et al. (2005), whose validity and reliability [15] in Turkish was carried out by Dedeli et al. (2007), including physical discomfort, psychosocial discomfort, worries and discomfort, satisfaction subscales in order to evaluate the impact of constipation on quality of life. It is considered that the quality of life of individuals who received high scores was negatively affected, and Cronbach alpha was defined 0.91 for all items [16].

### Bristol Stool Form Scale (BSFS):

It divides fecal shapes into seven fecal types. Confirmed as a measure of bowel passage. According to BSFS, Type 1 and Type 2 express constipation, Type 3 and Type 4 express normal stool, Type 5, Type 6 and Type 7 express diarrhea [17].

**Gastrointestinal Quality of Life Index (GIQLI):**

GIQLI, developed by Eypasch et al. (1995), explores the patient's self-assessment over the last 2-week period. It contains 36 questions each with five response categories such core symptoms, physical items, psychological items, social items, disease-specific items [18]. Each question is scored from zero to four (0: worst, 4: best). The maximum score is shown 144 and the normal score range is shown between 118-126 and Cronbach alpha was figured out as between 0.76 and 0.86 [19].

**Anthropometric Measurements**

The Tanita Body Composition Analyser model SC-330 (Tanita Corp Tokyo, Japan) was used to measure the body composition (body fat ratio, body fat mass, and body muscle mass) of the patients [20].

**The determination of the nutritional consumption status of patients**

24-hour dietary recall was taken to evaluate the nutritional status of the patients before and 2nd week, 4th week and 6th week after LSG. The energy and nutrients taken from the daily diet were analyzed using the Computer-Assisted Nutrition Program developed for Turkey, the Nutrition Information Systems package program (BEBIS) (Version 7.1)" [21]. In the evaluation of the amount of fluid intake of the patients other than water, the liquids they specified in the nutrient consumption records (tea, coffee, protein shake etc.) as a consideration.

**Probiotic Supplement**

The probiotic group used live freeze-dried Bifidobacterium animalis lactis (BB-12) $1x10^9$ CFU as a probiotic supplement. Patients used this probiotic supplement for the first 6 weeks after discharging from LSG under the control of the dietitian. The supplement is in the form of a sachet, and it is recommended to be mixed with 1 cup of warm water twice a day in the morning and evening.

**Statistical analysis**

The SPSS 24.0 was used for statistical analysis. In the questionnaire applied, qualitative data were evaluated as number (S) and percentage (%). The arithmetic mean (x), standard deviation (SD), median, lower, and upper values were found in the data obtained from patients. Descriptive statistics for the data were given. Pearson Chi-square test and Fisher Exact test were used to compare qualitative data. Independent samples t-test and one-way ANOVA were used for group comparisons. The statistical p value was set at $p<0.05$ in the 95% of confidence interval.

## Results

The average age of the probiotic group participating in the study was 37.00 ± 8.92 years, and the average age of the control group was 41.03 ± 11.29 years. There are 18 (60.0%) women and 12 (40%) men in the probiotic group, and there are 23 (76.7%) women and 7 (23.3%) men in the control group. There were no statistically significant differences in age, gender, number of births, marital status, educational status, labor status, and constipation problem parameters between probiotic and control groups before LSG ($P<0.05$) (Table 1).

There were no statistically significant differences between probiotic and control groups in terms of body weight, BMI, body fat weight, body fat percentage and body muscle mass before and after LSG ($p>0.05$) (Table 2).

When the distribution of stool characteristics of patients in the probiotic and control groups compared to BSFS was examined, the rate of constipation was observed lower in patients in the probiotic group compared to the control group, and the rate of normal defecation and diarrhea was observed higher. These results were statistically significant ($p <0.05$) (Figure 1 and 2).
GSRS diarrhea subscale mean score of patients in the probiotic group at post-op 4th week, GSRS indigestion subscale mean post-op at 6th week, GSRS constipation sub-mean score post-op at 2nd week, GSRS total score average post-op 2nd week and post-op 6th week were observed statistically significantly lower compared to the control group ($p <0.05$) (Table 3).

Table 1. Distribution of patients by pre-LSG socio-demographic characteristics.

| Parameter | | Probiotic Group (n=30) | | Control Group (n=30) | | |
|---|---|---|---|---|---|---|
| | | n | % | n | % | p |
| Age (year) | 9-18 | 1 | 3.3 | 7 | 11.7 | $\chi^2$ =5.281 |
| | 0-39 | 17 | 28.3 | 14 | 23.3 | p=0.152 |
| | 40-49 | 6 | 10 | 5 | 8.3 | |
| | 50 and above | 6 | 10 | 4 | 6.7 | |
| Gender | Female | 18 | 60.0 | 23 | 76.7 | $\chi^2$=1.926 |
| | Male | 12 | 40.0 | 7 | 23.3 | p=0.133 |
| Number of births | 0 | 7 | 38.9 | 6 | 26.1 | $\chi^2$=7.309 |
| | 1 | 8 | 44.4 | 4 | 17.4 | p=0.120 |
| | 2 | 2 | 11.1 | 8 | 34.8 | |
| | 3 | 1 | 5.6 | 4 | 17.4 | |
| | 4 | 0 | 0.0 | 1 | 4.3 | |
| Marital status | Single | 12 | 40.0 | 6 | 20.0 | $\chi^2$=2.857 |
| | Married | 18 | 60.0 | 24 | 80.0 | p=0.079 |
| Education level | Education | 1 | 3.3 | 0 | 0.0 | $\chi^2$=6.687 |
| | Primary school graduate | 0 | 0.0 | 4 | 13.3 | p=0.153 |
| | Secondary school graduate | 0 | 0.0 | 1 | 3.3 | |
| | High school graduate | 6 | 20.0 | 7 | 23.3 | |
| | College/University graduate | 23 | 76.7 | 18 | 60.0 | |
| Employment status | Employed | 24 | 80.0 | 22 | 73.3 | $\chi^2$=0.373 |
| | Unemployed | 6 | 20.0 | 8 | 26.7 | p=0.381 |
| Smoking | Yes | 12 | 40.0 | 9 | 30.0 | $\chi^2$=0.659 |
| | No | 18 | 60.0 | 21 | 70.0 | p=0.294 |
| Alcohol consumption | Yes | 21 | 70.0 | 12 | 40.0 | $\chi^2$=5.455 |
| | No | 9 | 30.0 | 18 | 60.0 | p=0.018 |
| Constipation problems | Yes | 16 | 53.3 | 18 | 60.0 | $\chi^2$=0.271 |
| | | | 4 | | | p=0.397 |
| | No | 14 | 46.7 | 12 | 40.0 | |
| Regular exercise activity | Yes | 2 | 6.7 | 1 | 3.3 | NA |
| | No | 28 | 93.3 | 29 | 96.7 | |

NA: not applicable.

The CSI obstructive defecation subscale mean score of patients in the probiotic group were post-op 2nd week and post-op 6th week, the CSI colonic inertia dimension mean scores were post-op 2nd week, post-op 4th week and post-op at 6th week, CSI pain subscale mean scores pre-op, post-op at 2nd week and post-op at 6th week, CSI total score averages pre-op, post-op 2nd week and post-op 6th week were found significantly lower compared to the control group ($p <0.05$) (Table 4). PAC-QOL physical discomfort subscale mean scores of patients in the probiotic group pre-op, post-op 2nd week and post-op 6th week, PAC-QOL psychosocial discomfort subscale mean scores pre-op, post-op 4th week, post-op 6th week, PAC-QOL worries, and discomfort subscale mean scores pre-op, post-op 2nd week, post-op 4th week, post-op 6th week, PAC-QOL total score averages pre-op, post-op 4th week, post-op 6th week were observed lower than the control group ($p <0.05$) (Table 5).

GIQLI core symptoms sub-mean score average of the probiotic group was post-op at 6th week, GIQLI disease-specific items sub-dimension mean scores pre-op, post-op at 2nd week and post-op at 6th week and GIQLI total score average post-op 6th week compared to the control group were observed statistically significantly higher ($p <0.05$) (Table 6).

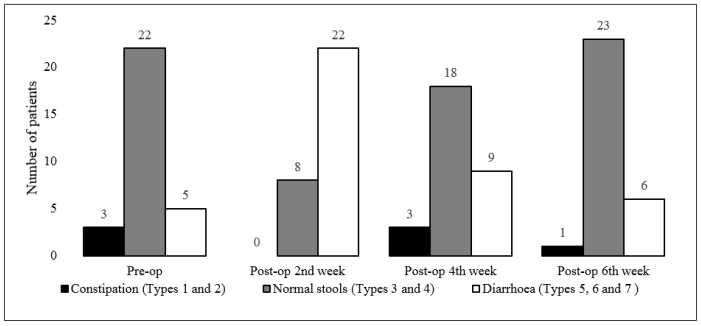


Figure 1. Distribution of fecal characteristics of probiotic group according to Bristol Stool Form Scale.

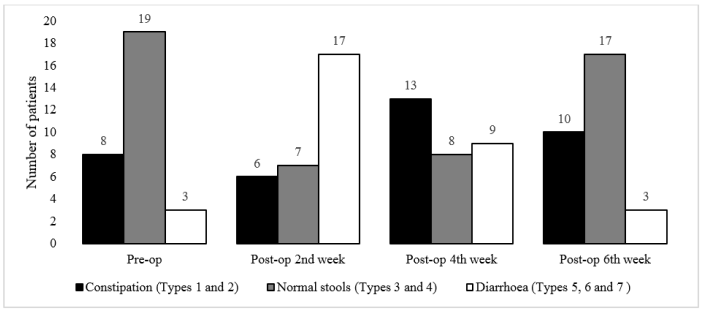


Figure 2. Distribution of fecal characteristics of control group according to Bristol Stool Form Scale.

## Discussion

Our study is one of the first examining the effect of probiotic supplementation after LSG on constipation and GI quality of life. Disordered bowel habit is observed in patients after bariatric surgery and this may affect the quality of life of the patients negatively. Obesity is associated with disordered bowel habit but the effects of bariatric surgery on bowel habits are not well studied and unclear. In the previous study conducted by Afshar et al., more than a quarter of patients who were on 6-month follow-up after bariatric surgery had constipation [22]. Meanwhile, the study performed by Menenakos et al. [23] 1-year follow-up after LSG for the treatment of morbid obese patients, 15% of patients developed constipation and 3% anal fistula. Moreover, a study showed that a significant increase in GI symptoms such as swelling, diarrhea, increased feeling of satiety and constipation after bariatric surgery were observed [24].

Table 2. Anthropometric measurements of patients before and after LSG.

| | | Probiotic Group (n=30) | Control Group (n=30) | p |
|---|---|---|---|---|
| Weight (kg) | pre-op | 121.44± 27.64 | 114.18 ± 21.31 | 0.250 |
| | post-op 2nd week | 111.63 ± 29.00 | 106.19 ± 20.58 | 0.400 |
| | post-op 4th week | 109.31 ± 24.82 | 101.85 ± 20.08 | 0.200 |
| | post-op 6th week | 105.27 ± 24.40 | 97.12 ± 20.73 | 0.160 |
| BMI (kg/m$^2$) | pre-op | 42.10 ± 6.62 | 41.27 ± 5.64 | 0.600 |
| | post-op 2nd week | 38.89 ± 4.68 | 38.36 ± 5.31 | 0.680 |
| | post-op 4th week | 37.41 ± 4.70 | 36.92 ± 5.61 | 0.710 |
| | post-op 6th week | 36.03 ± 4.70 | 35.12 ± 5.67 | 0.500 |
| Body fat mass (kg) | pre-op | 54.22 ± 16.73 | 53.07 ± 15.22 | 0.780 |
| | post-op 2nd week | 50.45 ± 13.82 | 48.75 ± 14.99 | 0.650 |
| | post-op 4th week | 45.10 ± 12.87 | 44.39 ± 15.78 | 0.850 |
| | post-op 6th week | 42.09 ± 12.19 | 40.58 ± 14.70 | 0.660 |
| Body fat ratio (%) | pre-op | 44.64 ± 7.60 | 46.08 ± 5.20 | 0.390 |
| | post-op 2nd week | 44.19 ± 6.43 | 45.41 ± 5.98 | 0.440 |
| | post-op 4th week | 41.21 ± 7.57 | 42.81 ± 7.22 | 0.400 |
| | post-op 6th week | 39.96 ± 7.77 | 41.05 ± 7.41 | 0.570 |
| Body muscle mass (kg) | pre-op | 62.91 ± 16.62 | 58.05 ± 9.17 | 0.160 |
| | post-op 2nd week | 60.06 ± 14.76 | 54.61 ± 9.08 | 0.090 |
| | post-op 4th week | 60.99 ± 16.36 | 54.84 ± 9.42 | 0.080 |
| | post-op 6th week | 59.97 ± 16.68 | 53.68 ± 9.60 | 0.080 |

All values are mean ± standard deviation

Bariatric surgery has a significant impact on food intake, defecation stereotypes, and metabolism. It was reported that no changes were found on constipation and prevalence of anal incontinence after 6-month of LSG. In addition, it was emphasized the weight loss after bariatric surgery could be associated with recovery of the constipation symptoms [25].

It is thought that probiotics can alter the changing intestinal microbiota and increase intestinal motility in patients with constipation, as well as regulate the lumen environment by increasing the end products of bacterial fermentation and reducing the luminal ph [26]. Probiotics are increasingly used in the treatment of constipation, and Bifidobacterium is among the most widely used probiotic strains [8]. For instance, In a meta-analysis performed by Dimidi et al. [27], it was demonstrated that Bifidobacterium lactis strains, microbiological microorganisms, improved the intestinal transit time, stool frequency and consistency. In addition, performed by Chen et al. [5] reported that probiotics may improve GI symptoms and quality of life after bariatric surgery. In our study, PAC-QOL total score averages of the control group were statistically higher than probiotic group in the 4th week of post-op and 6th week of post-op. Likewise, In the randomized placebo control study performed by Kommers et al. [28], it was reported that the PAC-QOL satisfaction subscale score of the experimental group who received 15 days of probiotic supplementation was higher than the control group. In our study, we reported that the PAC-QOL satisfaction subscale score of the probiotic group was higher than the control group, but there was no statistically significant difference.

Table 3. Comparison of GSRS scores before and after LSG of patients in probiotic and control groups.

| | | Probiotic Group (n=30) | Control Group(n=30) | p |
|---|---|---|---|---|
| Diarrhea | pre-op | 6.73 ± 4.62 | 6.77 ± 4.02 | 0.970 |
| | post-op 2nd week | 6.17 ± 3.15 | 6.93 ± 3.29 | 0.360 |
| | post-op 4th week | 4.17 ± 1.93 | 5.53 ± 2.90 | 0.030 |
| | post-op 6th week | 4.37 ± 2.28 | 5.10 ± 2.91 | 0.280 |
| Indigestion | pre-op | 9.53 ± 5.63 | 12.03±6.74 | 0.120 |
| | post-op 2nd week | 7.47 ± 3.73 | 8.90 ± 5.42 | 0.230 |
| | post-op 4th week | 7.57 ± 3.13 | 9.47 ± 5.67 | 0.110 |
| | post-op 6th week | 7.20 ± 3.76 | 10.03 ± 6.00 | 0.030 |
| Constipation | pre-op | 6.87 ± 4.79 | 9.53 ± 6.53 | 0.070 |
| | post-op 2nd week | 5.73 ± 2.65 | 8.47 ± 4.97 | 0.010 |
| | post-op 4th week | 8.37 ± 5.12 | 10.10 ± 5.60 | 0.210 |
| | post-op 6th week | 6.80 ± 3.60 | 11.77 ± 6.02 | <0.001 |
| Abdominal pain | pre-op | 6.20 ± 3.00 | 7.87 ± 3.64 | 0.050 |
| | post-op 2nd week | 5.20 ± 1.67 | 6.30 ± 4.33 | 0.200 |
| | post-op 4th week | 5.63 ± 2.77 | 6.10 ± 4.37 | 0.620 |
| | post-op 6th week | 5.30 ± 2.34 | 6.77 ± 4.89 | 0.140 |
| Reflux | pre-op | 4.73 ± 3.45 | 5.27 ± 3.46 | 0.550 |
| | post-op 2nd week | 3.13 ± 1.68 | 4.13 ± 2.98 | 0.110 |
| | post-op 4th week | 3.27 ± 1.91 | 3.77 ± 2.81 | 0.420 |
| | post-op 6th week | 3.17 ± 1.91 | 4.27 ± 2.94 | 0.090 |
| GSRS total score | pre-op | 34.07±15.89 | 41.47±19.11 | 0.100 |
| | post-op 2nd week | 27.70 ± 8.84 | 34.73 ± 15.32 | 0.030 |
| | post-op 4th week | 29.00±9.37 | 34.97±14.54 | 0.060 |
| | post-op 6th week | 26.83± 9.14 | 37.93 ± 16.59 | <0.001 |

All values are mean ± standard deviation.
GSRS: Gastrointestinal Symptom Rating Scale, GSRS Cronbach Alpha (pre-op, post-op 2$^{nd}$ week, post-op 4$^{th}$ week, post-op 6$^{th}$ week) = 0.903-0.858-0.831-0.872.

Obesity is associated with impaired gut microbiota and lack of micro nutrition. The weight loss after the bariatric surgery is associated with recovery of the comorbidity of obesity and changes of the function of the microbiota in the gut. The balance in the gut microbiota after bariatric surgery could not be remained. The imbalance of gut microbiota could be due to the adverse consequences of the weight loss and lack of nutrition. Therefore, it is considered that the probiotics could be used as tool to provide improved balance for gut microbiota [29]. In a

study performed by Wildt et al. [30] patients with chronic constipation were given probiotics, a mixture of Lactobacillus acidophilus strain LA-5 and Bifidobacterium animalis subsp lactis BB-12, as probiotics for 12 weeks. They found differences in the probiotic group with improved symptoms and stool consistency. This suggests that probiotic supplementation may have a therapeutic effect on constipation. Also, in the study conducted by Eskesen et al. [9], the mean defecation frequency of patients who used B. lactis BB-12 for 4 weeks for constipation treatment was significantly higher than placebo for all weeks. Our results also indicated that CSI total score were found significantly lower than the control group in the post-op 2nd week and in post-op 6th week of the probiotic group.

Table 4. Comparison of CSI scores before and after LSG of patients in probiotic and control groups.

| | | Probiotic Group (n=30) | Control Group (n=30) | p |
|---|---|---|---|---|
| Obstructive defecation | pre-op | 9.60 ± 7.93 | 14.10 ± 9.60 | 0.053 |
| | post-op 2nd week | 8.20 ± 6.39 | 13.83 ± 8.73 | 0.006 |
| | post-op 4th week | 11.73 ± 7.83 | 13.43 ± 8.45 | 0.422 |
| | post-op 6th week | 9.07 ± 7.51 | 15.97 ± 7.70 | 0.001 |
| Colonic inertia | pre-op | 8.40 ± 7.38 | 10.67 ± 7.71 | 0.250 |
| | post-op 2nd week | 5.90 ± 5.60 | 11.10 ± 6.45 | 0.001 |
| | post-op 4th week | 7.67 ± 6.24 | 11.37 ± 5.39 | 0.017 |
| | post-op 6th week | 6.00 ± 6.07 | 11.80 ± 5.79 | <0.001 |
| Pain | pre-op | 0.83 ± 1.76 | 3.67 ± 4.69 | 0.004 |
| | post-op 2nd week | 1.17 ± 2.09 | 3.63 ± 4.51 | 0.010 |
| | post-op 4th week | 2.23 ± 2.80 | 3.43 ± 4.67 | 0.233 |
| | post-op 6th week | 1.43 ± 2.62 | 3.60 ± 4.50 | 0.027 |
| CSI total score | pre-op | 18.83 ± 14.54 | 28.43 ± 20.43 | 0.041 |
| | post-op 2nd week | 15.27 ± 11.84 | 28.57 ± 18.30 | 0.002 |
| | post-op 4th week | 21.63 ± 14.75 | 28.23 ± 15.47 | 0.096 |
| | post-op 6th week | 16.50 ± 14.76 | 31.37 ± 15.34 | <0.001 |

All values are mean ± standard deviation.
CSI: Constipation Severity Instrument, CSI Cronbach Alpha (pre-op, post-op 2nd week, post-op 4th week, post-op 6th week) = 0.934-0.937-0.874-0.879

GI complaints are common in morbidly obese individuals and this may affect GI quality of life. A significant decrease in GIQLI scale can be observed after bariatric surgery. This is thought to be caused by changes in the intestinal microbiota of obese individuals and the development of GI symptoms. In a study performed by Yu et al. [31], a significant deterioration in GI symptoms was detected in the seriously obese patient group using the GIQLI scale. Also, in a study performed by Ignat et al. [32] found that GIQLI score after LSG as 90, 113, 114, 113 in the pre-op and post-op 1st, 2nd, 3rd and 5th years, respectively. To compare with our results, GIQLI total score mean was observed statistically lower in probiotic group in post-op 6th week compared to control group score mean. However, no study was observed with GIQLI in the early post-LSG period in the literature.

There are several limitations in our study. First, our study was placebo-controlled and not double-blind. Also, another limitation for our study is small sample size. The duration of the follow up of the study is short compared to other studies.

Table 5. Comparison of PAC-QOL scores before and after LSG of patients in probiotic and control groups.

| | | Probiotic Group (n=30) | Control Group (n=30) | p |
|---|---|---|---|---|
| Physical discomfort | pre-op | 7.96 ± 3.65 | 10.70 ± 4.48 | 0.012 |
| | post-op 2nd week | 6.40 ± 2.32 | 9.00 ± 4.57 | 0.008 |
| | post-op 4th week | 7.83 ± 3.29 | 9.30 ± 4.15 | 0.135 |
| | post-op 6th week | 7.80 ± 3.07 | 10.53 ± 4.69 | 0.010 |
| Psychosocial discomfort | pre-op | 13.13 ± 5.15 | 16.46± 6.99 | 0.040 |
| | post-op 2nd week | 14.66 ± 6.40 | 15.50 ± 7.62 | 0.648 |
| | post-op 4th week | 12.76 ± 4.41 | 17.00 ± 7.12 | 0.008 |
| | post-op 6th week | 12.63 ± 3.96 | 17.96 ± 7.58 | 0.001 |
| Worries and discomfort | pre-op | 21.70 ± 8.89 | 27.46 ± 12.24 | 0.041 |
| | post-op 2nd week | 18.13 ± 6.43 | 25.53 ± 10.41 | 0.019 |
| | post-op 4th week | 20.43 ± 6.97 | 25.13 ± 10.54 | 0.046 |
| | post-op 6th week | 19.67 ± 8.20 | 26.63 ± 10.74 | 0.006 |
| Satisfaction | pre-op | 16.43 ± 3.61 | 17.33 ± 3.46 | 0.329 |
| | post-op 2nd week | 17.06 ± 3.87 | 16.40 ± 4.14 | 0.522 |
| | post-op 4th week | 18.26 ± 2.84 | 17.43 ± 3.80 | 0.341 |
| | post-op 6th week | 18.43 ± 2.29 | 17.16 ± 3.36 | 0.095 |
| PAC-QOL total score | pre-op | 59.24±14.58 | 71.96 ± 22.54 | 0.014 |
| | post-op 2nd week | 56.27 ± 15.37 | 64.43 ± 21.39 | 0.089 |
| | post-op 4th week | 59.30 ± 11.38 | 68.86 ± 19.37 | 0.024 |
| | post-op 6th week | 58.53 ± 12.59 | 72.30 ± 19.70 | 0.002 |

All values are mean ± standard deviation.
PAC-QOL: Patient Asssessment of Constipation Quality of Life Scale, PAC-QOL Cronbach Alpha (pre-op, post-op 2nd week, post-op 4th week, post-op 6th week) = 0.919-0.915-0.889-0.901.

Table 6. Comparison of GIQLI scores before and after LSG of patients in probiotic and control groups.

| | | Probiotic Group (n=30) | Control Group (n=30) | p |
|---|---|---|---|---|
| Core symptoms | pre-op | 35.93±8.77 | 33.00 ± 7.99 | 0.180 |
| | post-op 2nd week | 37.67 ± 6.64 | 35.03 ± 7.04 | 0.140 |
| | post-op 4th week | 39.23 ± 5.31 | 37.00 ± 8.24 | 0.210 |
| | post-op 6th week | 40.77 ± 4.75 | 36.50 ± 8.12 | 0.010 |
| Physical items | pre-op | 17.57 ± 7.97 | 18.30 ± 6.75 | 0.700 |
| | post-op 2nd week | 23.57 ± 5.22 | 22.37 ± 4.78 | 0.350 |
| | post-op 4th week | 24.67 ± 4.44 | 23.43 ± 4.52 | 0.290 |
| | post-op 6th week | 25.37 ± 3.98 | 24.50 ± 4.58 | 0.430 |
| Psychological items | pre-op | 24.36 ± 6.44 | 22.56 ± 5.07 | 0.240 |
| | post-op 2nd week | 26.90 ± 4.21 | 27.03 ± 3.05 | 0.890 |
| | post-op 4th week | 26.63 ± 3.28 | 26.33 ± 3.44 | 0.730 |
| | post-op 6th week | 26.83 ± 3.58 | 26.53 ± 4.18 | 0.760 |
| Social items | pre-op | 12.26 ± 3.72 | 13.13 ± 3.09 | 0.330 |
| | post-op 2nd week | 12.53 ± 3.18 | 13.23 ± 21.69 | 0.290 |
| | post-op 4th week | 12.86 ± 2.41 | 12.67 ± 1.91 | 0.720 |
| | post-op 6th week | 13.60 ± 2.60 | 13.16 ± 1.96 | 0.470 |
| Disease-specific items | pre-op | 41.20 ± 4.01 | 37.26 ± 6.45 | 0.000 |
| | post-op 2nd week | 39.96 ± 3.73 | 36.60 ± 6.80 | 0.020 |
| | post-op 4th week | 39.76 ± 4.12 | 36.93 ± 7.01 | 0.060 |
| | post-op 6th week | 41.00 ± 3.85 | 36.73 ± 7.49 | 0.000 |
| GIQLI total score | pre-op | 131.86± 22.49 | 124.47 ± 20.10 | 0.180 |
| | post-op 2nd week | 140.27 ± 18.51 | 133.63 ± 16.58 | 0.140 |
| | post-op 4th week | 140.30 ± 13.54 | 1036.07 ± 18.38 | 0.080 |
| | post-op 6th week | 147.50 ± 11.79 | 136.87 ± 18.98 | 0.010 |

All values are mean ± standard deviation.
GIQLI: Gastrointestinal Quality of Life Index, GIQLI Cronbach Alpha (pre-op, post-op 2nd week, post-op 4th week, post-op 6th week) = 0.894-0.870-0.872-0.871.

As a conclusion, probiotic supplementation improved the constipation and gastrointestinal quality of life in the early post-LSG period compared to the control group. However, the effect of probiotic supplementation after LSG on constipation and GI quality of life are limited in the literature. Probiotics may be preferred for the purpose of improving post-LSG constipation, GI symptoms and GI quality of life. Since GI symptoms may

vary according to the bariatric surgical procedure, the selection of probiotics should be symptom-specific and Bifidobacterium animalis lactis BB-12 strain may be considered as an alternative probiotic supplement for common constipation after early period of LSG. The outcome of the study provides a foundation for future studies focusing on the understanding of the effect of the probiotic supplements on constipation and GI quality of life after bariatric surgery.

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# Should histopathological evaluation of all appendectomy specimens essential?

## Tüm apendektomi örneklerinin histopatolojik olarak değerlendirilmesi gerekli midir?

**Mustafa Dönmez [1], Mustafa Ömer Yazıcıoğlu [2]**

**Abstract**

Aim: Acute appendicitis is a surgical emergency. While fecaliths and lymphoid hyperplasia are the most common etiological factors, some unexpected reasons can also be encountered. In order to evaluate the cause, all appendectomy specimens are routinely sent for pathological examination in our hospital. However, some studies question the routine sending of appendectomy specimens for pathological examination. The aim of this study is to appreciate whether routine pathological evaluation is necessary.

Methods: The histopathological reports of 1,358 patients who underwent appendectomy between February 2019 and July 2020 in Ankara City Hospital, were retrospectively evaluated. The rate of unestimated or unexpected findings was detected. It was evaluated whether unexpected findings were suspected clinically, radiologically and macroscopically. In addition, the effects of unexpected results on the treatment of the patient were identified.

Results: 811 male and 547 female were included in the study. Unexpected pathological findings were detected in 57 patients. Of the 14 patients suspected of having unexpected findings, six were confirmed pathologically, of which one was suspected by preoperative imaging methods and five were macroscopically suspected during surgery. While the presence of unexpected pathological findings caused additional medical treatment in two patients and additional surgical treatment in three patients, seven patients are still being followed up in different clinics.

Conclusion: We recommend that appendectomy specimens be routinely sent for pathological examination, as unexpected results that may affect the patient's health, may be missed without histopathological examination.

Key words: Acute appendicitis, appendectomy, appendectomy specimen, histopathologic evaluation, histopathologic diagnose, unexpected findings.

**Öz**

Amaç: Akut apandisit cerrahi acil bir durumdur. Fekalitler ve lenfoid hiperplazi en sık karşılaşılan etiyolojik nedenler olmakla birlikte bazı beklenmedik faktörlerle de karşılaşılmaktadır. Bunu tespit etmek amacıyla hastanemizde rutin olarak tüm apandektomi örnekleri patolojik incelemeye gönderilmektedir. Ancak bazı çalışmalar apandektomi örneklerinin rutin olarak patolojik incelemeye gönderilmesini sorgulamaktadırlar. Bu çalışmanın amacı, apandektomi örnekleri için rutin patolojik incelemenin gerekli olup olmadığını değerlendirmektir.

Yöntemler: Ankara Şehir Hastanesinde Şubat 2019-Temmuz 2020 tarihleri arasında apandektomi yapılan 1358 hastanın histopatolojik raporları retrospektif olarak değerlendirildi. Tahmin edilemeyen ya da beklenmedik histopatolojik sonuçların oranı, klinik, radyolojik ve makroskopik olarak şüphelenilip şüphelenilmediği ve ayrıca hastanın tedavisine etkileri tespit edilerek değerlendirme yapıldı.

Bulgular: Çalışmaya 811 erkek ve 547 kadın hasta dahil edildi. 57 hastada beklenmeyen patolojik sonuçlar tespit edildi. Beklenmedik sonuç çıkabileceğinden şüphelenilen 14 hastanın 6'sı patolojik olarak doğrulandı. Bunlardan 1'inden ameliyat öncesi görüntüleme yöntemleriyle ve 5'inden ameliyat sırasında makroskopik olarak şüphelenildi. Beklenmedik patolojik bulguların varlığı 2 hastada ilave medikal tedaviye, 3 hastada ise ek cerrahi tedaviye neden olurken, 7 hastanın farklı kliniklerde takibi halen daha devam etmektedir.

Sonuç: Histopatolojik inceleme yapılmadan hastanın sağlığını etkileyebilecek beklenmedik sonuçların gözden kaçabilme ihtimalinden dolayı, apandektomi örneklerinin rutin olarak patolojik incelemeye gönderilmesini öneriyoruz.

Anahtar Kelimeler: Akut apandisit, apandektomi, apandektomi materyali, histopatolojik değerlendirme, histopatolojik tanı, beklenmeyen bulgular.

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## Introduction

Acute appendicitis is one of the most common surgical conditions of the abdomen in general surgery. It has disclosed in the studies that the incidence of acute appendicitis increases with the development of lymphoid tissue and it is more common in males, especially between the ages of 10 and 30 years. Luminal obstruction is the most important reason for ocuring accute appendicitis. While fecaliths are the most common etiological factors, some unexpected cases such as benign or malign tumors, intestinal worms, polyps, diverticulitis, endometriosis can also be included among causative factors [1-5].

Although sending appendectomy specimens for routine histopathological examination varies in different centers [6,7], it is accepted to sent routinely in our hospital. To our knowledge, there are scant number of articles evaluating the presence of unexpected findings by comparing age, gender and especially the diameter of appendix.

The aim of this study is to evaluate the unexpected findings in appendectomy specimens and identify whether routine histopathologic examination is needed or not.

## Material and methods

All appendectomy specimens that underwent open or laparoscopic surgery with the suspicion of acute appendicitis between February 2019 and July 2020 in Ankara City Hospital, were retrospectively evaluated. The study protocol was approved (Ethics No: E1-21-1515) by the hospital ethics committee and the research was conducted according to the Declaration of Helsinki.

The patient's complaints, physical examination, laboratory results and imaging methods were evaluated together and decided to perform appendectomy. Ultrasound (US) was the first choice as the imaging modality, but computed tomography (CT) scan was considered in case of suspicion clinically or radiologically.

The demographic, operative and pathologic records such as gender, age, date of surgery, additional disease history, preoperative imaging studies, macroscobic and microscobic characteristics of appendix, primary or coexisting findings while surgery and need for additional postoperative treatment were analyzed for each patient. Patients under the age of 18, had incidental appendectomy while other surgeries, those with familial or chronic bowel disease such as crohn's, ulcerative colitis, familial adenomatous polyposis and additionally those with known metastatic or nonmetastatic malignancies were excluded from the study.

Appendectomy specimens were fixing in formalin before transporting to the pathology laboratory. A form containing the estimated diagnosis and suspicious findings stated in imaging methods and during surgery was also sent with the sample.

Those whose pathology results were reported as appendix vermiformis, lymphoid hyperplasia, fibrous obliteration were accepted as negative appendectomy, and those with acute appendicitis, phlegmonous appendicitis, gangrenous appendicitis, necrotizing appendicitis and perforated appendicitis were accepted as positive appendectomy. These patients were accepted as Group 1, other than these, such as the presence of polyp, parasite, cyst, diverticulum, endometriosis, granulomatous appendicitis, adenoma, mucinous neoplasm, neuroendocrine tumor, adenocarcinoma were evaluated as unexpected findings and accepted as Group 2.

### Statistical Analysis

Statistical analyses were performed using SPSS version 25.0 (SPSS, Inc, Chicago, IL). Demographic, perioperative, and follow-up datas were analyzed with Mann-Whitney U test, Chi Square ($\chi 2$) test and Receiver Operating Characteristic (ROC) Curve analysis as appropriate. Continuous variables were presented as mean ± standard deviation. A *p* value of less than 0.05 was considered statistically signifcant.

## Results

A total of 1478 patients who underwent appendectomy were screened, of whom totally 1358 patients, 811 (59.7 %) male and 547 (40.3 %) female, who met the criteria were included in the study. The ages of the patients ranged from 18 to 89 years with the mean age 34.9 ± 14.7 years old in men and 36.5 ± 15 years old in women.

After the histopathological examination of surgical specimens, 1183 (87.1 %) patients were diagnosed as appendicitis, and 118 (8.7 %) as negative appendectomy. Unexpected findings were detected in 57 (4.2%) patients. The patients with unexpected pathology, 28 (3.5%) were male and 29 (5.3%) were female, and no statistically significant difference could be detected. (p=0.096) The mean age in Group 1 was 35.1 ± 14.5 years old but it was 45.7 ± 18.6 years old in Group 2 and there was a statistically significant difference. (p<0.0001) In the evaluation with the regarding of the diameters of appendix stated in CT or US reports, the mean diameter was 13.3 ± 7 mm in the patients with unexpected pathological findings, while it was 10.3 ± 2.7 mm in the other group and was statistically significant. (p=0.002) When the effect of patient's age, gender, and appendix diameter on unexpected findings were evaluated with univariate and multivariate analyzes, and the age of patient and the diameter of appendix were found to be significantly effective in both univariate and multivariate analyses. (Tables 1-3)

Table 1: Characteristics of patients

| Parameters | Group 1 (n=1,302) | Group 2 (n=57) | *p* value |
|---|---|---|---|
| Age. mean ± SD | 35.1 ± 14.5 | 45.7 ± 18.6 | <0.0001 |
| Gender, n (%) | | | 0.096 |
| Male | 783 (96.5) | 28 (3.5) | |
| Female | 518 (94.7) | 29 (5.3) | |
| Diameter of appendix, cm, mean ± SD | 10.3 ± 2.7 | 13.3 ± 6.9 | 0.002 |

SD: standard deviation

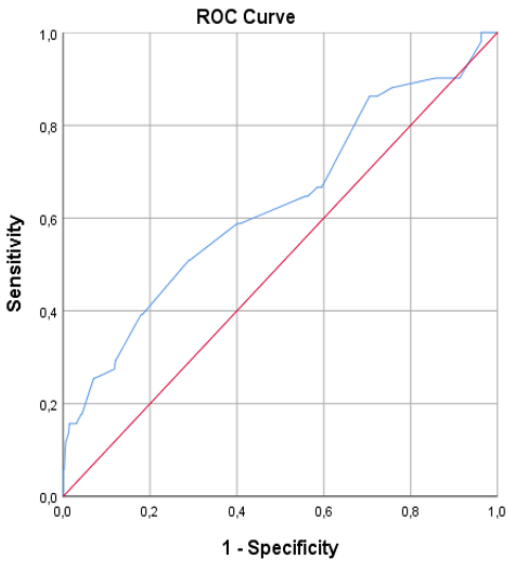

Figure 1. Graph showing the relationship between the diameter of appendix and unexpected results.

Table 2. Histopathological diagnosis of appendectomy specimens.

| Histopathological Diagnosis | n (%) |
|---|---|
| Appendicitis | 1,183 (87.1) |
| Acute appendicitis | 731 (53.8) |
| Phlegmenous appendicitis | 320 (23.6) |
| Gangrenous appendicitis | 88 (6.5) |
| Necrotizing appendicitis | 9 (0.7) |
| Perforated appendicitis | 35 (2.6) |
| Normal appearing appendix vermiformis | 118 (8.7) |
| Appendix vermiformis | 22 (1.6) |
| Lymphoid hyperplasia | 85 (6.3) |
| Fibrous obliteration | 11 (0.8) |
| Unusual findings | 57 (4.2) |
| Polyp | 5 (0.4) |
| Parasites | 3 (0.2) |
| Cyst | 1 (0.1) |
| Diverticulosis | 9 (0.7) |
| Endosalpingiosis | 1 (0.1) |
| Endometriosis | 2 (0.1) |
| Granulomatous appendicitis | 5 (0.4) |
| Adenoma | 8 (0.6) |
| Mucinous neoplasm | 11 (0.8) |
| Neuroendocrine cell hyperplasia | 1 (0.1) |
| Neuroendocrine tumor | 10 (0.7) |
| Adenocarsinoma | 1 (0.1) |

Table 3. Evaluation of the factors affecting the unexpected findings.

| Parameters | Univariate Analysis | Multivariate Analysis |
|---|---|---|
| | p value | p value |
| Gender (Male/female) | 0.096 | |
| Age | <0.001 | <0.001 |
| Diameter of appendix | <0.001 | 0.046 |

The ROC analysis shows the relationship between appendix diameters and age of patients in group 1 and group 2. As the cut-off values, the diameter of the appendix was 10.15 mm and the age of patient was 36.5 years, were detected. (AUC: 0.630, Sensitivity: 0.588, Specificity: 0.592, AUC: 0.670, Sensitivity: 0.614, Specificity: 0.644, respectively) According to this, the number of patients under the age of 37 with unexpected findings was 22 (2.6 %), while it was 35 (7 %) over the age of 37, and it was statistically significant. (p<0.0001) Similarly, the number of unexpected findings in patients with appendiceal diameter less than 10.15 mm was 21 (2.9 %), while it was 35 (5.8 %) for the patients with an appendiceal diameter greater than 10.15 mm and this was also statistically significant. (p=0.01) (Figure 1, Figure 2)

There were arosed a suspicion for unexpected findings in a total of 14 (1 %) patients, of whom seven were on preoperative imaging methods and seven were intraoperatively. The pathology results of six (42.9 %) of them were compatible with unexpected findings. It was stated that 5 (83.3 %) of these six patients were suspected intraoperatively. While it was decided that appendectomy was sufficient in 40 (70.2%) of 57 patients and that no additional treatment was needed, seven (17.5 %) of these patients are still being followed by gastroenterology, medical oncology and general surgery.

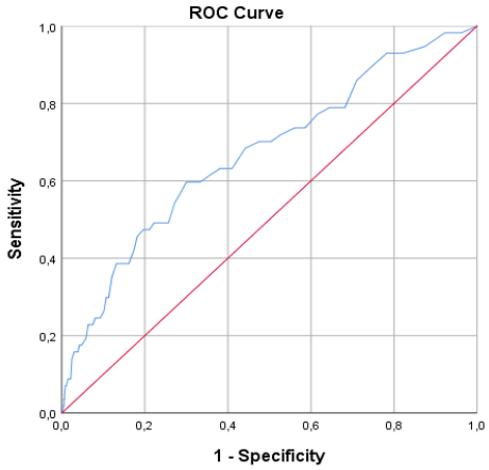


Figure 2. Graph showing the relationship between the age of patients and unexpected results

Additional treatments (E. vermicularis medical teratment in one patient, right hemicolectomy in three patients, Crohn's disease treatment in one patient) were applied to five (29.8 %) of the remaining 17 patients but other 12 patients were lost to follow-up.

## Discussion

Appendectomy is one of the most common acute surgical procedure. Luminal obstruction of the appendix, especially with lymphoid hyperplasia or fecaliths, are the most common etiological factors for acute appendicitis mentioned in literatures and text books. But some unusual factors such as benign or malign tumors, parasites, foreign bodies, endometriosis, granulomatous pathologies could be detected [1, 5, 8]. In our study, we have defined 1,183 (87.1 %) acute appendicitis cases and 118 (8.7 %) negative appendectomy cases. In previous studies, unexpected results were found to vary between 0.7% and 8.3% [2, 5]. In this study, this rate was 4.2%, and was consistent with them.

The necessity of routine histopathological evaluation of the specimen after appendectomy is still confusing. The reason for this confusion is the contradiction between the thought that the presence of unexpected findings may change our treatment method and will create an economic burden due to low incidence [1, 2, 4, 6, 9]. In our center, we routinely send all resected appendectomy specimens for histopathological evaluation but some centers prefer to send if they are suspected clinically, radiologically or macroscopically [4, 6, 9, 10]. It was demonstrated in our study that there were a suspicion for unusual finding in only 14 (1%) patients, of whom 6 of them had pathology results consistent with suspicion. In other words, if we had sent only suspicious samples to pathological examination, we would have missed the results of the other 51 patients.

There are few studies reporting the association of unexpected results with age and gender. Akbulut et al. [8] defined in their study that most of the patients with unexpected findings were male (55.5 %) and the median age was 32.2 ± 15.1 years (15-84 years old). Ma et al. [11] reported that neoplastic lesions were mostly seen in men and over 50 years of age. [11] Elfaedy et al. [3] stated in their study that unusual finding was higher in > 30 year age group but unlike the previous two

studies, unexpected results were found to be more in female (7.1 %) gender. In our study, unexpected findings were found in 28 (3.5%) male and 29 (5.3%) female patients, but no statistically significant difference was detected. While the mean age of the group with unexpected findings was 45.7 ±18.6 years old, it was 35.1 ±14.5 years old in the other group, and a statistically significant difference was found. ($p<0.0001$) In addition to other studies, we have evaluated in our report whether there was a relationship between the diameters detected in preoperative imaging methods and unexpected results. The mean diameter detected in Group 1 was 10.3 ±2 .7 mm and it was 13.3 ±6 mm in Group 2 and this was statistically significant. ($p=0.002$) Moreover, we also calculated the cut-off values for both age (36.5 years) and appendix diameter (10.15 mm) in our study and above these values, unexpected results were found to be increased significantly.

Some studies have indicated that unexpected findings of the appendix can be suspected macroscopically, clinically or radiologically, while others have shown that they may be missed if pathological examination is not performed [6, 7, 9, 10]. In our study, as mentioned above that there were suspicion in only 14 (1%) patients, and only 6 of them were compatible with suspicion. Five (83.3 %) of these 6 patients were macroscopically suspected during surgery. As these results show, unexpected findings were not encountered in most of the suspected cases. In addition, when evaluation is made only macroscopically or with clinical or radiological data without pathological examination, the probability of detecting unexpected results will low.

In our study, histopathological examination directly affected the treatment process of 5 (8.8 %) patients (medical treatment, surgical treatment), while 7 patients were still being followed up by different clinics. Additionally, considering that 12 patients did not come to postoperative follow-up, the importance of histopathological evaluation would be understood more clearly.

The retrospective design of the study and the small sample size were considered as our limits. In addition, being covered a relatively short period of approximately 2 years and was not including the pediatric age group can be counted among our limits. Despite our limitations, we were able to reach some outcomes as a result of our work.

In conclusion, we think that the diagnosis of unexpected results without histopathological evaluation is challenging because of not having a typical clinical findings different from acute appendicitis, not making a definite diagnosis with preoperative imaging methods and not detecting macroscopically. Thus, considering the previous studies and the data we obtained from our own study, it was concluded that it would be appropriate to send all appendectomy specimens for histopathological evaluation, as it may directly affect the treatment protocol and, of course, the health status of the patient.

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# The effect of vitamin D deficiency and 1,25(OH)$_2$D$_3$ treatment on oxidative stress and Nrf2-antioxidant signaling in ethanol-induced hepatotoxicity

## D vitamini eksikliği ve 1,25(OH)$_2$D$_3$ uygulamasının etanole bağlı karaciğer hasarında oksidatif stres ve Nrf2-antioksidan sinyal sistemi üzerine etkisi

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**Abstract**

Aim: Vitamin D deficiency (VDD) is suggested to enhance hepatotoxicity in chronic liver diseases. However, there is limited knowledge about the association between VDD and alcoholic liver damage. Therefore, the effect of VDD on ethanol (EtOH)-induced hepatotoxicity was investigated in this study. Moreover, the role of the Nrf2-antioxidant signaling pathway in the hepatoprotective potential of 1,25(OH)$_2$D$_3$ was also searched in EtOH-treated rats.

Methods: Male Wistar rats were fed on VDD-diet for 12 weeks. EtOH (5-20%) was applied in drinking water in increasing concentrations for the last 8 weeks. In addition, one group of rats were injected with 1,25(OH)2D3 (5µg/kg; twice a week; i.p.) during this period. Hepatic triglyceride and hydroxyproline levels, inflammation markers, lipid peroxides, protein carbonyls, mRNA expressions of Nrf2, superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), SOD and GSH-Px activities, glutathione levels and histopathology were examined.

Results: EtOH application caused steatosis and fibrosis, elevated hepatic TG, lipid peroxide, protein carbonyls and hydroxyproline levels and inflammation markers. VDD did not aggravate EtOH-induced liver damage, steatosis and inflammation, but reactive oxygen species and lipid peroxide levels were slightly increased in VDD+EtOH group. Gene expressions of Nrf2-SOD-GSH-Px, enzyme activities and glutathione levels were also higher in VDD+EtOH group than EtOH group. Additionally, 1,25(OH)$_2$D$_3$ elevated mRNA expressions and activities of SOD and GSH-Px in EtOH-treated rats.

Conclusion: Our results indicate that VDD diet did not cause an additive effect on EtOH-induced hepatotoxicity. Moreover, it was detected that the activation of Nrf2-antioxidant signaling pathway may play a role in the protective effect of 1,25(OH)$_2$D$_3$ against EtOH-induced hepatotoxicity.

Keywords: Vitamin D deficiency, 1,25(OH)$_2$D$_3$, ethanol, liver damage, oxidative stress, Nrf2.

**Öz**

Amaç: D vitamini eksikliğinin (VDE) kronik karaciğer hastalıklarında karaciğer hasarını arttırdığı ileri sürülmektedir. Bununla birlikte, VDE ile alkole bağlı karaciğer hasarı arasındaki ilişkiye ait bilgiler sınırlıdır. Bu nedenle, bu çalışmada VDE'nin etanol (EtOH) ile indüklenen karaciğer hasarı üzerindeki etkisi araştırıldı. Ayrıca, EtOH uygulanan sıçanlarda 1,25(OH)$_2$D$_3$'ün karaciğeri koruyucu potansiyelinde Nrf2-antioksidan sinyal yolunun rolü de araştırıldı.

Yöntemler: Erkek Wistar sıçanlar 12 hafta süreyle VDE diyet ile beslendi. Son 8 hafta içme suyunda artan konsantrasyonlarda EtOH (% 5-20) verildi. Ayrıca, bir grup sıçana bu süreçte 11,25(OH)$_2$D$_3$ (5µg/kg; haftada iki kez; i.p.) uygulandı. Karaciğerde trigliserit (TG) ve hidroksiprolin düzeyleri, inflamasyon göstergeleri, lipid peroksitler, protein karboniller, Nrf2, süperoksit dismutaz (SOD) ve glutatyon peroksidaz (GSH-Px)'ın mRNA ekspresyonları, SOD ve GSH-Px aktiviteleri, glutatyon düzeyleri ve histopatolojik incelemeler yapıldı.

Bulgular: EtOH uygulaması steatoz ve fibroza, karaciğerde TG, lipit peroksit, protein karbonil ve hidroksiprolin düzeyleri ile inflamasyon göstergelerinde artışa neden oldu. VDE, EtOH'a bağlı karaciğer hasarını, steatoz ve fibrozu yoğunlaştırmadı. Ancak, reaktif oksijen türleri ve lipit peroksit düzeylerinde VDE+EtOH grubunda ılımlı artış bulundu. Nrf2-SOD-GSH-Px'in gen ekspresyonları, enzim aktiviteleri ve glutatyon düzeyleri de VDE+EtOH grubunda EtOH grubundan daha yüksekti. Ayrıca, 1,25(OH)$_2$D$_3$, EtOH uygulanan sıçanlarda karaciğerde SOD ve GSH-Px'ın mRNA ekspresyonlarını ve aktivitelerini arttırdı.

Sonuç: Bulgularımız VDE diyetin EtOH ile indüklenen karaciğer hasarı üzerinde ek bir etki oluşturmadığını gösterdi. Ayrıca EtOH'a bağlı karaciğer hasarında 11,25(OH)$_2$D$_3$'ün koruyucu etkisinde Nrf2-antioksidan sinyal yolunun aktivasyonunun rol oynayabileceği saptandı.

Anahtar Kelimeler: D vitamini eksikliği, 1,25(OH)$_2$D$_3$, etanol, karaciğer hasarı, oksidatif stres, Nrf2.

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## Introduction

Vitamin D (Vit D), a hormone precursor, is present in two forms, vit D2 (ergocalciferol) and vit D3 (cholecalciferol). Dietary and synthesized Vit D undergo hydroxylations to 25-hydroxyvitamin D [25(OH)D] in the liver and then to 1,25-dihydroxyvitamin D [1,25(OH)2D], the active form of Vit D, in the kidney. Serum 25(OH)D levels are used as an indicator of Vit D stores [1].

Vitamin D deficiency (VDD) is an important public health problem in the world. VDD may induce insulin resistance (IR), oxidative stress, inflammation, and mitochondrial dysfunction [2] and it is accepted as a risk factor for chronic diseases such as bone diseases, immune disorders, cardiovascular diseases, diabetes mellitus [1,2], although there are some different opinions on this issue [1]. VDD is also common in chronic liver diseases such as viral hepatitis, cirrhosis, and fatty liver diseases and may induce fat accumulation in hepatocytes, as well as inflammation and fibrosis in the liver [3].

Fatty liver diseases are classified as alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) [4]. Vit $D_3$ or $1,25(OH)_2D_3$ treatments were detected to be effective in the amelioration of hepatic lesions in dietary models of NAFLD in rodents [5-7]. Moreover, some studies have shown that VDD may be an aggravating factor in the formation and progression of hepatic lesions in NAFLD [8,9], but others did not confirm this effect of VDD [6,10].

On the other hand, the relationship between ALD and vit D has generally been investigated in patients with ALD and the results are inconsistent. [11,12]. In vitro [13,14] and experimental [15] studies investigating the effect of Vit $D_3$ or $1,25(OH)_2D_3$ on ethanol (EtOH)-induced hepatotoxicity are also quite limited. Moreover, there is only one experimental study on the relationship between VDD and ALD [16]. Since we have recently reported that $1,25(OH)_2D_3$ treatment alleviated hepatic lesions by decreasing ROS formation, lipid and protein oxidation products in the liver of EtOH-treated rats [7], the current study was primarily aimed to investigate whether the VDD diet could affect EtOH-induced hepatotoxicity and oxidative stress in the liver of rats.

Vit $D_3$ and $11,25(OH)_2D_3$ are not only direct-acting antioxidants, but they are also effective by increasing the gene expression of proteins/enzymes in the antioxidant system [17,18]. It has been suggested that the nuclear factor-erythroid-2-related factor 2 (Nrf2) transcription factor and downstream target genes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione (GSH) biosynthetic enzymes may play a role in the antioxidant potential of Vit $D_3$ and $1,25(OH)_2D_3$ in some oxidative stress-induced pathologies [17, 19, 20]. Since there is no experimental study in this subject, we also investigated the effect of both $1,25(OH)_2D_3$ treatment and VDD on Nrf2-SOD-GSH-Px system in EtOH-treated rats. Our results indicate that the VDD diet did not cause an additive effect on EtOH-induced hepatotoxicity and the induction of Nrf2-antioxidant signaling pathway were effective in the protective role of $1,25(OH)_2D_3$ in EtOH-induced hepatotoxicity.

## Material and methods

**Chemicals**

Chemicals were obtained from Sigma- Aldrich (Saint-Louise, MI, USA). $1,25(OH)_2D_3$ (Ostriol, 2µg/mL) was donated by VEM ILAC San. A.S. (Istanbul, Turkey).

**Animals**

Male Wistar rats (140-160 g) used in this study were obtained from Aziz Sancar Experimental Medical Research Institute of Istanbul University. The animals were provided with food and water *ad libitum.* They were housed in polypropylene cages (three to four per cage) at 22 $^0$C, with 12- h light and 12-h darkness without shielding from ultraviolet B radiation (290-320 mn). All procedures were carried out in accordance with the protocols of the Animal Care and Use Committee of the University of Istanbul (Approval number: March 29, 2018-2018/28)

**Diets and experimental design**

Rats were divided into five groups as follows: Control (n=6), VDD (n=8), EtOH (n=7), VDD+EtOH (n=8) and EtOH+$1,25(OH)_2D_3$ (n=7) groups. Control, EtOH and EtOH +$1,25(OH)_2D_3$ groups were fed on PicoLab Rodent diet 5053 (2300 IU Vit D3/kg added), however, VDD and VDD+EtOH groups were fed on Modified LabDiet 5053 (Vit D3 not added) for 12 weeks as previously reported [21]. These diets were supplied from LabDiet (St. Louis-Missouri, USA).

EtOH (5-20%; v/v) was applied in drinking water in increasing concentrations for the last 8 weeks to EtOH, VDD+EtOH and EtOH+$1,25(OH)_2D_3$ groups. These groups were treated with 5% (v/v) and 10 % (v/v) EtOH for the first and second weeks, respectively. For the last 6 weeks, 20% (v/v) EtOH was administered. However, in EtOH+1,25 $(OH)_2D_3$ group, rats were injected with 1,25 $(OH)_2D_3$ (5µg/kg; twice a week) intraperitoneally [5, 7] for the last 8 weeks along with EtOH.

**Blood and tissue samples**

The animals were fasted overnight and anesthetized with ketamine (35 mg/kg, i.p., Pfizer, USA) and xylazine HCl (15 mg/kg, i.p., Bioveta, Czech Republic). Blood samples were taken into dry tubes by cardiac puncture and centrifuged at 1500xg for 10 min to separate the sera. Liver tissues were removed, washed with chilled 0.9% NaCl and kept in ice. The liver index was calculated as liver weight /body weightx100.

Liver tissues were homogenized in ice-cold phosphate-buffered saline (PBS; 0.01M, pH:7.4). Homogenates were centrifuged at 600x g for 10 min at 4°C to remove unbroken tissue samples and nuclear fraction. Post-nuclear supernatant (PNS) was used for biochemical analyses in the liver. A part of PNS was centrifuged at 10000xg for 20 min at 4$^o$C to obtain post-mitochondrial fraction (PMF). This fraction was used for the determination of activities of antioxidant enzymes. All materials were stored at -80$^o$C until analyzed.

**Determinations in serum**

Serum fasting glucose, triglyceride (TG), calcium and inorganic phosphorus levels, and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured using a Cobas Integra 800 autoanalyzer (Roche Diagnostics, Mannheim, Germany). Serum $25(OH)D_3$ and insulin levels were determined by ELISA kits (Abbkine, Wuhan-China). The homeostasis model the assessment (HOMA) index was used to evaluate insulin resistance (IR) and calculated using the formula: fasting insulin concentration (pmol/L) x fasting glucose concentration (mmol/L)/135. High HOMA scores indicate IR (low insulin sensitivity).

**Determinations in the liver**

**Triglyceride (TG) levels**

Hepatic lipids were extracted with a mixture of chloroform: methanol (2:1) [22]. After the evaporation process, the extracts were dissolved in alcohol: ether (3:1). TG levels were measured by the kits provided from Biolabo Biochemistry and Coagulation (Maizy, France).

**Tumor necrosis factor-alpha (TNF-α) and hydroxyproline (Hyp) levels**

The liver tissue was homogenized in PBS (0.01M, pH: 7.4). The homogenates (10%; w/v) were sonicated for 30 seconds and then centrifuged at 5000xg for 5 minutes at +4°C. Supernatants were used to measure levels of TNF-α (Abbkine, Wuhan, China) and Hyp (Bioassay Technology Laboratory, Shanghai, China) by sandwich ELISA method.

**Myeloperoxidase (MPO) activity**

Liver tissue was homogenized in chilled phosphate buffer (50 mM; pH: 6.0) containing hexadecyltrimethylammonium bromide (HTAB). After freezed-thawed three times, and centrifuged at 15000xg. The supernatant was used for the measurement of MPO enzyme activity [23].

**Reactive oxygen species (ROS), lipid and protein oxidation products**

The formation of 2′,7′-dichlorofluorescein was determined in the liver homogenate incubated with 1 mM 2′,7′-dichlorodihydrofluorescein diacetate at 37 °C for 30 min in a fluorometric microplate reader (Fluoroskan Ascent FL., Thermo Scientific Inc, USA) with an excitation of 485 nm and emission of 538 nm [24].

Hepatic lipid peroxidation was evaluated by determining thiobarbituric acid reactive substances (TBARS) and diene conjugate (DC) levels. TBARS levels were determined according to Buege and Aust [25]. The mixture of liver homogenates and Buege-Aust reagent incubated in a boiling water bath for 15 min were cooled and then centrifuged at 1000xg for 10 min. The absorbances of supernatants were measured at 532 nm. To determine DC levels, liver lipids were extracted in chloroform/methanol (2:1) and then redissolved in cyclohexane, and absorbances at 233 nm were recorded [25].

Protein carbonyl (PC) levels were evaluated according to the method of Reznick and Packer [26] which is based on the measurement of protein hydrazones. Results were calculated from the maximum absorbance (360 nm) using a molar extinction coefficient of 22,000 $M^{-1}$ $cm^{-1}$.

**Antioxidant system parameters**

Hepatic ferric reducing anti- oxidant power (FRAP) levels were determined spectrophotometrically to evaluate antioxidant power in the liver. The electron donating antioxidants found in liver tissue reduce a ferric-tripyridyltriazine complex to the ferrous form. The reaction is observed by measuring the change in absorption at 593 nm [27]. Hepatic glutathione (GSH) levels were determined by using 5,5-dithiobis-(2-nitrobenzoate) at 412 nm [28]. Superoxide dismutase (SOD) activity was determined according to Mylorie et. al. [29] and calculated using a standard curve prepared by bovine SOD. Glutathione peroxidase (GSH-Px) activity was measured using cumene hydroperoxide as a substrate [30]. The reaction was followed spectrophotometrically (340 nm) at 37°C, and activity was calculated using the extinction coefficient of NADPH ($6.22x10^3$ $M^{-1}cm^{-1}$).

**mRNA expressions of Nrf2, SOD and GSH-Px**

The homogenate of liver tissue was performed using a handheld homogenizer (SCILOGEX D160, USA). Total RNA was isolated (NucleoSpin RNA Isolation Kit, #740955, Macherey-Nagel, Germany) and then cDNA was synthesized (SCRIPT cDNA Synthesis Kit, Jena Bioscience, GmbH, Jena, Germany) using 5 ng RNA. Nrf2 (F: 5'-GTGGATCTGTCAGCTACTCCC-3'; R: 5'-CTGGGAATATCCAGGGCAAGC-3'), SOD (F: 5'-GGTCCAGCGGATGAAGAG-3'; R: 5'-GGACACATTGGCCACACC-3'), GSH-Px (F: 5'-CGACATCGAACCCGATATAGA-3'; R: 5'-ATGCCTTAGGGGTTGCTAGG-3') and the housekeeping gene GAPDH (F: 5'-CAGGGCTGCCTTCTCTTGTG-3'; R: 5'-AACTTGCCGTGGGTAGAGTC-3') primers were obtained from LGC Biosearch Technologies (Denmark). Quantitative real-time polymerase chain reaction (qPCR) was carried out using qPCR Green Master with UNG (Jena Bioscience, GmbH, Jena, Germany) in a real-time PCR system (Bio-Rad CFX Connect, California, USA). The $2^{-\Delta\Delta Ct}$ method were used for quantifying the expression levels of mRNA.

**Protein levels**

Protein levels of the liver tissue were measured spectrophotometrically using bicinchoninic acid [31].

**Histopathologic examination**

Livers were fixed in 10% buffered formalin solution for histopathological examinations. Tissues were embedded in paraffin and sectioned and stained with hematoxylin and eosin (H&E) and Masson's trichrome (MTC). Fibrosis scores were evaluated according to the protocol described in our previous study [32].

**Statistical analysis**

Statistical analysis was evaluated by using the Statistical Package for The Social Sciences program (21.0; SPSS Inc., Chicago, IL, USA) program. All variables were expressed as mean ± standard deviation (SD). Data distributions and test of normality were investigated by the Kolmogorov-Smirnov test. One-way ANOVA test (post-hoc Tukey's test) was used to assess the parameters with normal distribution. The homogeneity of variances was evaluated with the Levene test. Kruskal-Wallis test (post-hoc Mann Whitney-U test) was used to compare the parameters without normal distribution. In all cases, a difference was considered significant when $p < 0.05$.

## Results

**Final body and liver weights and liver index**

Final body weight, liver weight and liver index remained unchanged in EtOH and VDD+EtOH groups as compared to the control group (data not shown).

**Effect of VDD feeding on serum and hepatic parameters in rats**

In the VDD group, there were significant decreases in serum $25(OH)D_3$ and significant increases in serum glucose, HOMA, TG and TC levels were detected as compared to controls. In addition, hepatic TG and TNF-α levels and MPO activity also increased. However, there were no changes in hepatic Hyp levels, prooxidant and antioxidant parameters and histopathological findings (Tables 1-3 and Figures 1-3).

**Effect of VDD feeding on serum parameters in EtOH-treated rats**

Serum 25(OH)D levels remained unchanged in the EtOH group, but it diminished significantly (47.5 %; $p<0.01$ and 45.2%; $p<0.001$) in the VDD+EtOH group in comparison with control and EtOH groups, respectively. Calcium and phosphorus levels did not alter in both groups.

Serum glucose, insulin, HOMA, and TC levels did not alter, but only TG levels increased in EtOH and VDD+EtOH groups in comparison with controls. While insulin, HOMA and TG levels did not change, glucose and TC levels were higher in the VDD +EtOH group than the EtOH group.

ALT and AST activities were found to be increased in EtOH and VDD+EtOH in comparison with control. However, there were no differences in ALT and AST activities between the two groups (Table 1).

**Effect of VDD feeding on hepatic histology in EtOH-treated rats**

In hematoxylin and eosin (H&E) and Masson's trichrome (MTC) staining of liver sections, the EtOH and VDD+EtOH groups exhibited microvesicular steatosis not exceeding 5%. In the EtOH group, 4/7 of the cases showed mild portal and periportal

Table 1. Effect of VDD on serum parameters in EtOH-treated rats (Mean±SD)

| Variable | Control (n=6) | VDD (n=8) | EtOH (n=7) | VDD+EtOH (n=8) |
|---|---|---|---|---|
| 25(OH)D (ng/mL) | 28.4±3.09 | 15.7±1.07$^{a2}$ | 27.2±6.58 | 14.9±2.38$^{a2,b1}$ |
| Calcium (mmol/L) | 2.42±0.16 | 2.50±0.18 | 2.60±0.19 | 2.65±0.34 |
| Phosphorus (mmol/L) | 2.55±0.18 | 2.58±0.26 | 2.47±0.24 | 2.61±0.75 |
| Glucose (mmol/L) | 7.53±1.44 | 9.76±1.04$^{a3}$ | 6.50±1.91 | 9.51±1.32$^{b2}$ |
| Insulin (pmol/L) | 29.2±4.37 | 29.3±2.98 | 24.5±11.5 | 27.2±3.62 |
| HOMA | 1.61±0.33 | 2.10±0.21$^{a2}$ | 1.22±0.75 | 1.89±0.21 |
| TC (mmol/L) | 1.67±0.18 | 2.04±0.19$^{a3}$ | 1.62±0.28 | 1.98±0.25$^{b3}$ |
| TG (mmol/L) | 0.48±0.09 | 0.71±0.05$^{a2}$ | 0.89±0.26$^{a3}$ | 0.80±0.18$^{a2}$ |
| ALT (U/L) | 43.3±13.1 | 53.6±8.53 | 114.0±33.1$^{a2}$ | 99.1±27.2$^{a2}$ |
| AST (U/L) | 108.0±10.2 | 114.6±19.0 | 228.5±75.6$^{a2}$ | 194.5±49.6$^{a2}$ |

VDD: Vitamin D deficiency; EtOH: ethanol; 25(OH)D: 25-hydroxyvitamin D; HOMA: homeostasis model the assessment index; TC: total cholesterol; TG: triglyceride; ALT: alanine aminotransferase; AST: aspartate aminotransferase
a2 p<0.01; a3 p<0.05 as compared to controls
b1 p<0.001; b2 p<0.01; b3 p<0.05 as compared to EtOH group

fibrous expansion and 3/7 of the cases showed fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging. However, In the VDD+EtOH group, only 2/7 of the cases showed mild portal fibrous expansion (Figure 1). Fibrosis scores were calculated as 1.85±1.06 and 0.25±0.46 in EtOH and VDD+EtOH groups, respectively. According to this, the fibrosis score decreased significantly (p<0.01) in the VDD+EtOH group in comparison with the EtOH group.

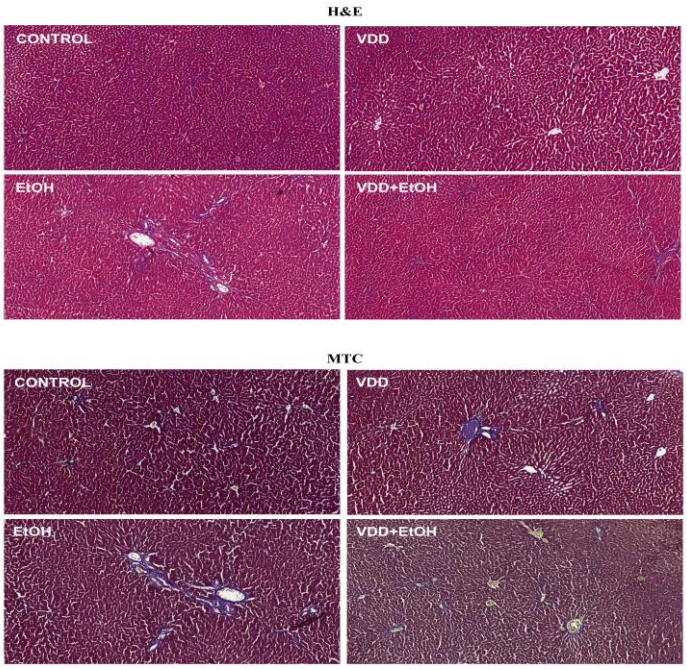


Figure 1. Effect of VDD on hepatic histology in EtOH-treated rats (Mean±SD). VDD: Vitamin D deficiency; EtOH: ethanol; H&E: hematoxylin and eosin; MTC: Masson's trichrome

**Effect of VDD feeding on hepatic parameters in EtOH-treated rats**

Hepatic TG, Hyp and TNF-α levels and MPO activity elevated significantly in EtOH and VDD+EtOH groups. However, there were no changes in these parameters between the two groups (Table 2).

Table 2. Effect of VDD on hepatic TG, Hyp and TNF-α levels and MPO activity in EtOH-treated rats (Mean±SD)

| Variable | Control (n=6) | VDD (n=8) | EtOH (n=7) | VDD+EtOH (n=8) |
|---|---|---|---|---|
| TG (µmol/g tissue) | 15.8±2.83 | 22.5±3.42$^{a1}$ | 22.8±1.77$^{a2}$ | 24.3±3.55$^{a1}$ |
| Hyp (ng/mL protein) | 2.02±0.27 | 2.27±0.28 | 2.78±0.25$^{a1}$ | 2.45±0.18$^{a3}$ |
| TNF-α (ng/mg protein) | 2.75±0.57 | 3.71±0.38$^{a3}$ | 6.33±0.89$^{a1}$ | 7.09±0.45$^{a1}$ |
| MPO (µmol/min/mg protein) | 5.17±0.96 | 7.21±0.72$^{a1}$ | 7.74±0.73$^{a1}$ | 8.56±0.81$^{a1}$ |

VDD: Vitamin D deficiency; EtOH: ethanol; Hyp: hydroxyproline; TNF-α: tumor necrosis factor-alpha; MPO: myeloperoxidase
a1 p<0.001; a2 p<0.01; a3 p<0.05 as compared to controls

Hepatic TBARS, DC, and PC levels elevated in both groups. However, ROS and TBARS levels, but not DC and PC levels were higher in the VDD+EtOH group than the EtOH group (Figure 2).

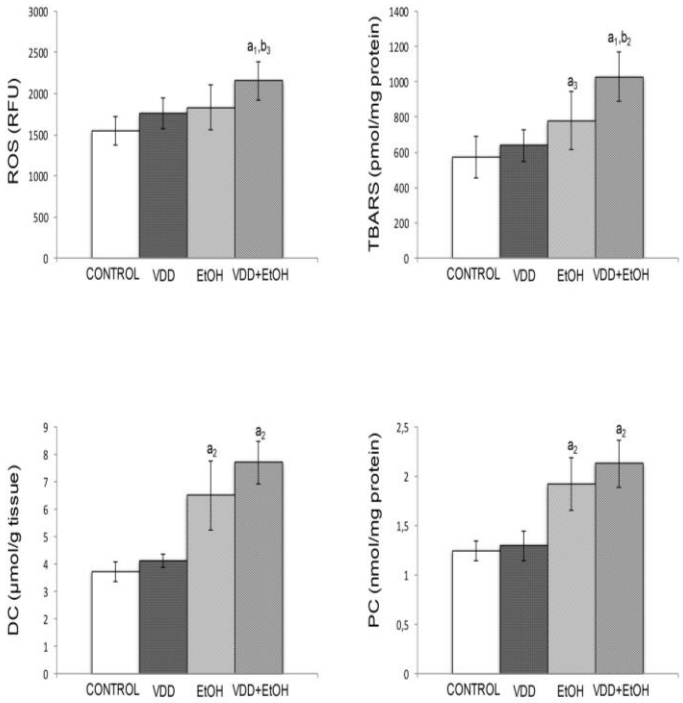


Figure 2. Effect of VDD on hepatic ROS, TBARS, DC and PC levels in EtOH-treated rats (Mean±SD).
VDD: Vitamin D deficiency; EtOH: ethanol; ROS: reactive oxygen species; TBARS: thiobarbituric acid reactive substances; DC: diene conjugate; PC: protein carbonyls
a1 p<0.001; a2 p<0.01; a3 p<0.05 as compared to controls
b2 p<0.01; b3 p<0.05 as compared to EtOH group

Hepatic Nrf2, SOD, and GSH-Px mRNA expressions and SOD and GSH-Px activities diminished significantly, but FRAP and GSH levels remained unchanged in EtOH group as compared to controls. These values were significantly higher in the VDD+ETOH group than the EtOH group (Figure 3 and Table 3).

**Effect of $1,25(OH)_2D_3$ on hepatic Nrf2, SOD and GSH-Px mRNA expressions in EtOH-treated rats**

$1,25(OH)_2D_3$ treatment did not alter EtOH-induced Nrf2 mRNA downregulation. However, this treatment restored EtOH-induced SOD and GSH-Px mRNA downregulations. In addition, SOD and GSH-Px activities were significantly higher in the EtOH+$1,25(OH)_2D_3$ group than EtOH group. However, there were no changes in GSH and FRAP levels in $1,25(OH)_2D_3$-treated ETOH rats (Figure 4 A/B).

Table 3. Effect of VDD on hepatic SOD and GSH-Px activities as well as FRAP and GSH levels in EtOH-treated rats. (Mean±SD)

| Variable | Control (n=6) | VDD (n=8) | EtOH (n=7) | VDD+EtOH (n=8) |
|---|---|---|---|---|
| SOD (U/mg protein) | 20.1±2.89 | 18.7±1.43 | 16.3±2.36$^{a3}$ | 22.4±2.56$^{b1}$ |
| GSH-Px (nmol/min/ mg protein) | 520.9±64.1 | 433.0±71.9 | 389.1±78.5$^{a2}$ | 497.9±35.9$^{b3}$ |
| FRAP (nmol/mg protein) | 80.3±11.9 | 76.9±15.8 | 68±13.3 | 87.4±6.75$^{b3}$ |
| GSH (nmol/mg protein) | 23.9±3.02 | 24.9±6.30 | 21.4±5.17 | 31.2±5.30$^{b2}$ |

VDD: Vitamin D deficiency; EtOH: ethanol; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; FRAP: ferric reducing antioxidant power; GSH: glutathione
a2 p<0.01; a3 p<0.05 as compared to controls
b1 p<0.001; b2 p<0.01; b3 p<0.05 as compared to EtOH group

## Discussion

Excessive alcohol drinking is a leading cause of chronic liver disease. The liver is the main organ where EtOH metabolism takes place. EtOH is metabolized to acetaldehyde (AA) by alcohol dehydrogenase and cytochrome P450 (CYP2E1), which is further metabolized by aldehyde dehydrogenase to acetate. Impaired lipid homeostasis, ROS-induced oxidative stress, increases in AA-protein adducts formation and inflammatory cytokine levels, endotoxemia, and mitochondrial damage were suggested to play an important role in the pathomechanism of EtOH-induced hepatotoxicity [33]. In our study, EtOH (5-20%, v/v) was applied in drinking water in increasing concentrations for 8 weeks as previously reported. Our results have shown that chronic EtOH application led to lipid accumulation, increases in inflammation (TNFα, MPO) and fibrosis (Hyp) markers in the liver of rats. Additionally, increases in lipid (TBARS, DC) and protein oxidation (PC) products and significant decreases Nrf2, SOD and GSH-Px mRNA expressions as well as SOD and GSH-Px activities were detected in the liver of EtOH-treated rats. Our findings are accordance with previous studies [7,34-37].

There is only one study about the effect of VDD on EtOH-induced hepatotoxicity. This study has reported that VDD aggravated the hepatic injury by increasing ROS-induced oxidative stress, pro-inflammatory cytokines and chemokines and decreasing GSH levels in the liver of mice fed with 4% (w/v) ethanol containing liquid diet for 6 weeks [16]. They have also detected that VDD attenuated EtOH-induced upregulations of hepatic SOD and GSH-Px. Therefore, we wanted to provide additional information to the topic by investigating the effect of VDD on EtOH-induced liver damage. Since 25(OH)D levels were reported to fall below 20 ng/ml in mice fed on VDD diet as early as 1 month [21], in this study, EtOH application started after 1 month of VDD diet administration and continued 8 weeks. When the findings obtained in EtOH and VDD+EtOH groups were compared, it was found that VDD did not have an augmenting effect on EtOH-induced increases in serum transaminase activities, hepatic TG, TNF-α and Hyp levels and MPO activity. Some factors such as use of the Lieber-De Carli ethanol liquid diet, the simultaneous application of the VDD diet and EtOH, and the use of mice as experimental animals may be responsible for the different findings between the two studies. In line with our findings in ALD, some authors have also detected that the VDD diet did not aggravate IR, hepatic lipid accumulation, and inflammation, even attenuated them, in rodents fed high fat- [10] and high fructose [6]-diets, which are dietary models of NAFLD.

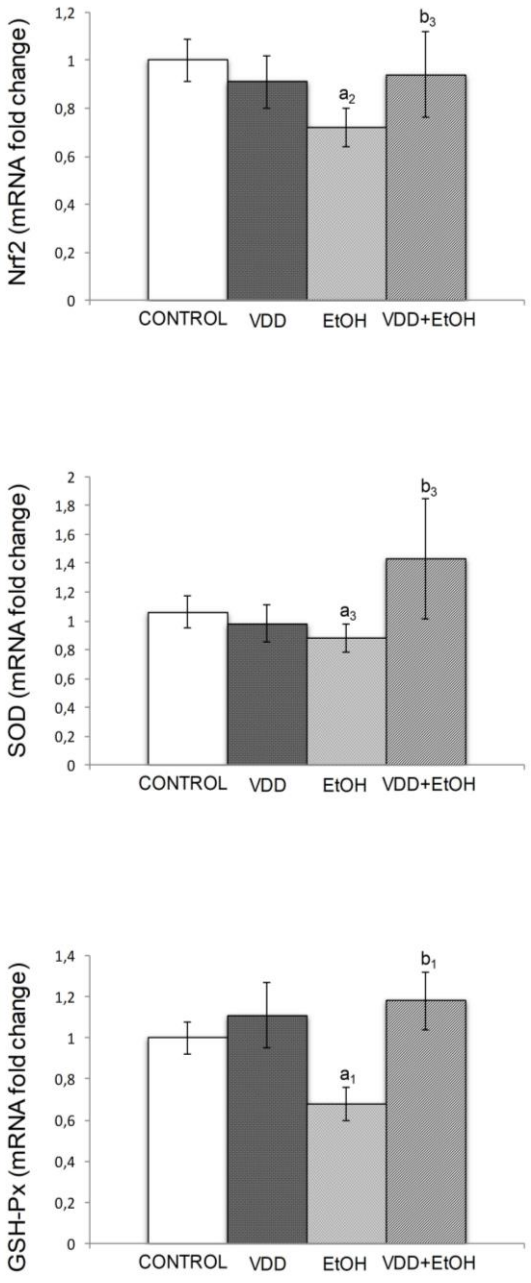


Figure 3. The effect of VDD on hepatic Nrf2, SOD and GSH-Px mRNA expressions in EtOH-treated rats (Mean±SD)
VDD: Vitamin D deficiency; EtOH: ethanol; Nrf2: nuclear factor-erythroid-2-related factor 2; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase
a1 p<0.001; a2 p<0.01; a3 p<0.05 as compared to controls
b1 p<0.001; b3 p<0.05 as compared to EtOH group

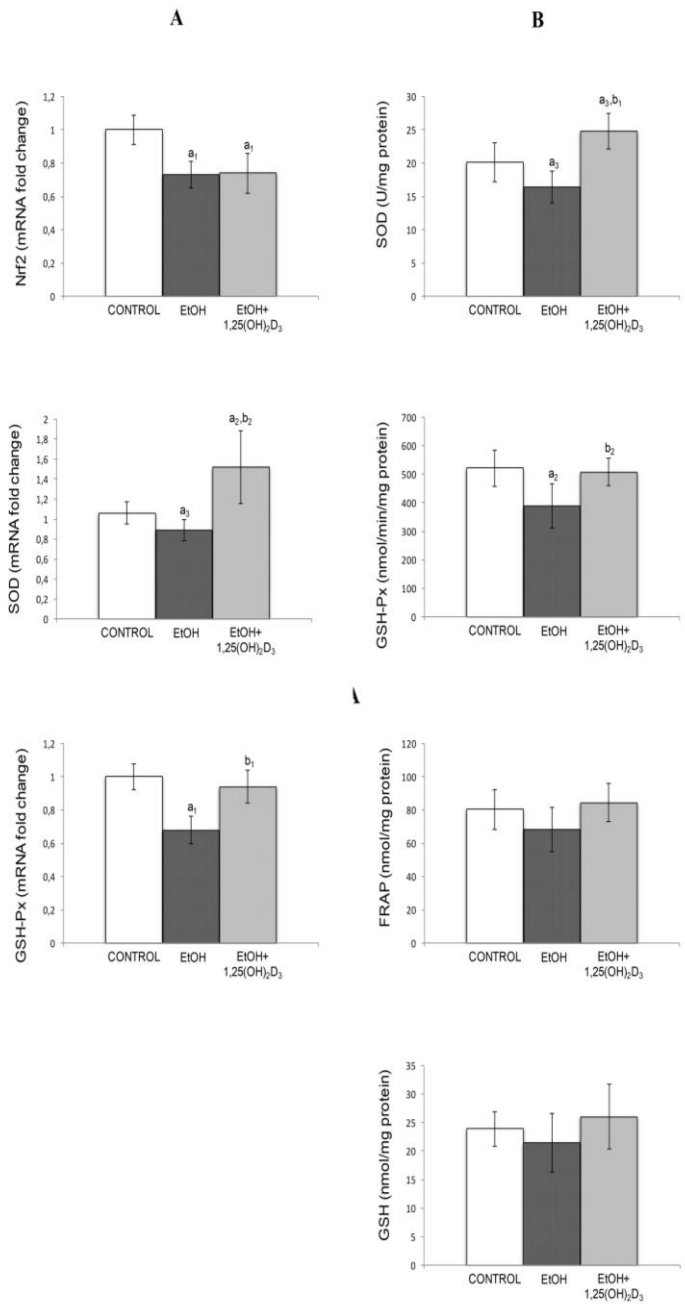


Figure 4. Effect of $1,25(OH)_2D_3$ on hepatic Nrf2, SOD and GSH-Px mRNA expressions (A) as well as SOD and GSH-PX activities (B) in EtOH-treated rats (Mean±SD).
$1,25(OH)_2D_3$: 1,25-dihydroxyvitamin D3; Nrf2: nuclear factor-erythroid-2-related
factor 2; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase
a1 $p<0.001$; a2 $p<0.01$; a3 $p<0.05$ as compared to controls
b1 $p<0.001$; b2 $p<0.01$ as compared to EtOH group

Nrf2 is a transcription factor located in the cytoplasm under normal conditions. Kelch- like ECH-associated protein 1 (Keap 1) is a sensor of oxidative stress and couples Nrf2. Mild oxidative stress could induce the uncoupling of Nrf2 and Keap 1 and then Nrf2 transfers into nucleus to play its role in the antioxidant protection. Thus, it provides the transcription of several antioxidant and cytoprotective genes through the antioxidant response element (ARE) by entering into the nucleus [38]. In this study, hepatic ROS and TBARS, but not DC and PC levels were significantly higher in VDD+EtOH group than EtOH group. However, a tendency for increased DC and PC levels was also detected. In these conditions, hepatic Nrf2, SOD and GSH-Px mRNA expressions, SOD and GSH-Px activities, and GSH and FRAP levels were also found to be higher in the VDD+EtOH group than EtOH group. Our results indicate that activation of Nrf2-antioxidant signaling may protect against further increases in EtOH-induced hepatotoxicity and inflammation and oxidative stress in rats fed on VDD.

It has been accepted that VDD may have a promoting role in chronic diseases such as metabolic syndrome, diabetes mellitus, cardiovascular and liver diseases [1,2]. However, there are contradictory findings in rodents fed on the VDD diet alone. Some studies have shown that VDD-diet did not affect IR, serum transaminase activities, hepatic lipid accumulation, histology, inflammation, and oxidative stress parameters in rodents [10,16]. However, others have detected that VDD-diet caused IR, fatty liver and mild fibrotic changes, inflammation and oxidative stress in the liver [6,9,17]. In our study, although significant increases in HOMA values, hepatic TG and inflammation parameters were detected, there were no changes in serum transaminase levels, hepatic histopathological findings, and pro-oxidant and antioxidant parameters in rats fed on VDD alone.

Vit D3 and 1,25(OH)2D3 are known to have antioxidant potential. This potential is related to their structural similarity to cholesterol and ergosterol [18]. However, Vit D and 1,25(OH)2D3 are also effective in the regulation of gene expression of proteins/enzymes in the antioxidant system by increasing Nrf2 transcription factor [17,18]. In vitro studies showed that 1,25(OH)2D3 increased the translocation of Nrf2 to the nucleus and induced the expression of several antioxidant enzymes in human endothelial [39] and epithelial [40] cells incubated by oxidative stressors. Similarly, 1,25(OH)2D3 treatment was also observed to increase Nrf2-antioxidant signaling in the liver of rats fed on a high fat diet [5], in the renal tissue of diabetic rats [19], and in the brain tissue of rats with experimental Alzheimer's disease [20]. However, there is a limited knowledge on the changes in Nrf2-antioxidant signaling pathway in EtOH-induced toxicity. Some in vitro studies have shown that 1,25 (OH)2D3 resulted in a protective effect on EtOH-induced cytotoxicity and oxidative stress by upregulating Nrf2 [13] or facilitating the translocation of Nrf2 into the nucleus [14], and thus improving endogenous antioxidant defense system. However, there is no experimental study on this issue. In this study, for the first time, 1,25(OH)2D3 treatment was found not to alter mRNA expressions of Nrf2, but it elevated SOD and GSH-Px mRNA expressions, as well as SOD and GSH-Px activities in the liver of EtOH-treated rats. Our results indicate that, besides direct antioxidant potential, 1,25(OH)2D3 may play a protective role against EtOH-induced oxidative stress probably by augmenting Nrf2 translocation into the nucleus. According to this, the activation of the Nrf2-antioxidant signaling pathway may also play an active role in the protective effect of 1,25(OH)2D3 against EtOH-induced hepatotoxicity and oxidative stress, which was detected in our previous study [7].

In conclusion, our results indicate that the VDD diet did not augment EtOH-induced hepatotoxicity. Moreover, for the first time 1,25(OH)2D3-induced Nrf2-antioxidant signaling pathway was detected to be effective in the protection against EtOH-induced hepatotoxicity in rats.

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# Classification of primary glenohumeral osteoarthritis in a Turkish population - morphological study

## Türk popülasyonunda primer glenohumeral osteoartrit sınıflaması – morfolojik çalışma

**Mustafa Özçamdallı[1], Sinan Oğuzkaya[2], Gökay Eken[3], Turan Bilge Kızkapan[4], Erdal Uzun[5]**

**Abstract**

Aim: This study aimed to investigate the distribution of modified Walch classification groups in a Turkish patient population. We think that there may be glenoid morphologies that do not fit the modified Walch classification due to ethnic differences.

Methods: Computed tomography images of 113 patients with primary glenohumeral osteoarthritis (GHOA) were evaluated. Version angle was measured according to the Friedman method, and classification was made by two blinded surgeons according to the modified Walch classification. Patients who did not meet the modified Walch groups were labeled as "Unclassifiable" and were analyzed as the 8th group.

Results: The mean age of the patients was 85.5 ± 7.5 years. Forty-eight patients were male (42.5%). Eighty-two patients (72.6%) belonged to Walch group A (60 A1 and 22 A2, respectively). The total percentage of group B was 16.7% (20 patients). Two patients were included in the "Unclassifiable" group. One of the patients had glenoid convexity due to advanced disease with 1° anteversion. The other patient in this group had anterior subluxation without underlying pathology.

Conclusion: The distribution of modified Walch groups in the Turkish population may be different from other populations. Posterior defective glenoid and excessive retroversion may concern less in the Turkish population with primary GHOA. Our results were obtained limited population and we think that further epidemiologic studies with larger sample size are needed.

Keywords: glenohumeral joint, osteoarthritis, morphology, Walch classification.

**Öz**

Amaç: Bu çalışmanın amacı, Türk popülasyonunda modifiye Walch sınıflamasına göre glenohumeral osteoartrit dağılımını belirlemektir. Etnik farklılıklar nedeniyle modifiye Walch sınıflamasına uymayan glenoid morfolojilerinin olabileceğini düşünmekteyiz.

Yöntemler: Primer glenohumeral osteoartrit (GHOA) tanısı almış olan 113 hastanın bilgisayarlı tomografi görüntüleri incelendi. Versiyon açıları Friedman metoduna göre ölçüldü. İki cerrah tarafından modifiye Walch sınıflamasına göre sınıflandırma yapıldı. Modifiye Walch sınıflamasındaki kriterlere uymayan hastalar "tanımlanmamış tip" olarak adlandırıldı ve 8. grup olarak belirlendi.

Bulgular: Hastaların ortalama yaşı 85.5 (±7.5) ve 48' i erkek (%42,5) idi. 82 hasta (%72,6) Walch grup A (60 A1 ve 22 A2)'ya dahil idi. Grup B' de 20 (%16,7) hasta var idi. 2 hasta tanımlanmamış grupta değerlendirildi. Bunlardan birinde 1 derecelik bir anteversiyonla ileri bir osteoartrit ve glenoid konveksitesi mevcut idi. Diğer hastada ise alta yatan başka bir patoloji olmaksızın anterior subluksasyon saptandı.

Sonuç: Türk popülasyonundaki modifiye Walch gruplarının dağılımı diğer toplumlardan farklı olabilir. Primer GHOA olan hastalarda posterior defektif glenoid ve aşırı retroversiyonun daha az görüldüğü saptandı. Çalışmamız kısıtlı popülasyonda yapılmış olup daha geniş hasta grubunda daha net sonuçlar elde edilebileceğini düşünmekteyiz.

Anahtar Kelimeler: Glenohumeral eklem, osteoartrit, morfoloji, Walch sınıflaması.

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## Introduction

Primary glenohumeral osteoarthritis (GHOA) is a well-defined cause of pain and disability in the elderly population. Degeneration of articular cartilage and subchondral bone with narrowing of the glenohumeral joint are the main characteristics of GHOA. Posterior glenoid is usually first affected region of the shoulder joint during osteoarthritis (OA) progression [1]. Normal shoulder anatomy can show variations among different populations. East Asian individuals have smaller glenohumeral dimensions than North American individuals except acromial length [2,3]. Moreover, there are many significant differences between the Chinese population and the Western population regarding proximal humeral anatomy [4].

Glenoid morphology is not also homogeneous among patients with GHOA. Walch et al. [5, 16] described five glenoid types according to the glenoid version and relation to the humeral head. Interobserver reliability of the original Walch classification has been questioned by many studies [6-8]. Bercik et al. [9] modified the Walch classification and added two more groups to the original Walch classification, which improved interobserver and intraobserver reliabilities with a modified Walch score. The specific outcomes are also supported by other studies [10].

Although the modified Walch classification suggested increased reliability, some points still exist for improvement. The modified version uses the scapular axis method (SM) to calculate humeral head subluxation, rather than the mediatrice method (MM) used in the original Walch classification. Although the SM has better interobserver and intraobserver reliabilities than the MM, Kidder et al had demonstrated that the SM notably increases the measurement for posterior humeral head subluxation [11]. Despite these known findings, the value for a centered humeral head remained unchanged at 45% to 55% [11]. Further evaluation by Jacxsens et al revealed that by using a 95% normal range to determine the cutoff value, displacement of the humeral head beyond 62% is considered posterior subluxation when using the SM, but this has yet to be incorporated into the modified Walch classification [12].

The Turkish gene pool contains a mixture of Asian, Middle Eastern, and European characteristics. Through studying the existing literature, to our knowledge, there have been no studies investigating primary GHOA morphology in the Turkish population. The primary aim of this study was to describe glenoid morphologic characteristics in patients with primary GHOA in the Turkish population.

## Material and methods

Ethical approval was obtained for this morphologic type of study from institutional review board (Ordu University Clinical Research Ethics Committee, Approval date-number: 09/05/2019-2019/70) and medical records of the patients who were admitted to the outpatient clinic with shoulder pain between January 2016 and June 2019 at the Ordu University Hospital, Kırsehir Ahi Evran University Hospital, and Bursa Cekirge State Hospital were evaluated. This study has been performed in accordance with the 1964 Declaration of Helsinki and its later updates. Patients who have international classification of disease (ICD) codes related with shoulder diseases were searched and the files of patients with new or old diagnoses of glenohumeral osteoarthritis were selected. The inclusion criteria for the study were primary glenohumeral osteoarthritis with adequate computed tomography (CT) scan images to measure the glenoid version. In our clinic, CT is performed to patients who have been diagnosed with GHOA using X-ray radiography and require a treatment plan (surgery or conservative), trauma patients with suspected shoulder fracture or dislocation on X-ray images, patients with shoulder problems who cannot undergo an MRI for various reasons, patients with Bankart lesions to assess for bone pathology, and patients who undergo CT for shoulder problems. Exclusion criteria were having a history of previous rotator cuff disease, posttraumatic arthritis, avascular necrosis, and other secondary causes of GHOA. A total of 723 patient medical records were evaluated. Overall, 556 patients had underlying shoulder pathology (previous trauma, rotator cuff tear, avascular necrosis, etc.), and CT scans in 54 patients were not considered appropriate for glenoid version measurement. After excluding non-suitable participants, CT images of 113 patients were evaluated (Figure 1). The demographic data of the patients were recorded. Glenoid version was measured according to Friedman's [13] method by two blinded examiners twice 30 days apart. Glenohumeral interrelation was categorized according to the modified Walch classification system [9]. Patients who did not meet the criteria for any of the modified Walch groups were marked as "Unclassifiable." Those patients were analyzed together as a separate group. All measurements were performed by an orthopedic surgeon and a musculoskeletal radiologist. Gender and side differences were evaluated.

The modified Walch classification of primary glenohumeral osteoarthritis includes the following types. Type A1, minor erosion of glenoid with centered humeral head. Type A2, major central glenoid erosion with centered humeral head. Type B1, humeral head posterior subluxation without bone defect. Type B2, posterior erosion of glenoid cavity with biconcavity of the glenoid and posterior subluxated head. Type B3, mono concave and posteriorly defective glenoid with more than 15° retroversion and/or posterior humeral head subluxation of more than 70%. Type C, dysplastic glenoid with more than 25° retroversion. Type D, anteverted glenoid or subluxated humeral head of less than 40% [9].

### Statistical analysis

The mean, standard deviation, frequency, and ratio were used in the descriptive statistics. The intraclass correlation coefficient was used in the evaluation of agreement between individual measurements. We defined 0–0.2 as slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, and 0.61–0.8 as substantial agreement, and values >0.81 as perfect agreement in the intraclass correlation (ICC) evaluations according to Landis and Koch [14]. The significance level was set at $p<0.05$. IBM SPSS for Windows, version 22 (IBM corp., Armonk, NY, USA) was used in the statistical analyses.

## Results

The mean age in the study population was 85.5 (SD: 7.5) years. Forty-eight patients were male (42.5%) and 65 (57.5%) were female. Eighty-five patients had right (75.2%) side OA and 28 patients had left (24.8%) side OA. Two patients (1.8%) did not meet any of the criteria of the modified Walch classification and these patients were grouped as unclassified as less frequent type. One of the patients had 1° of anteversion and the glenoid cavity lost its concavity because of advanced GHOA (Fig. 2). This patient had symmetrical bone erosion but belonged neither to the A1 nor to the A2 group. Another patient had asymmetrical OA with anterior subluxation without anterior erosion of the glenoid and without any history of previous trauma, rotator cuff disorder,

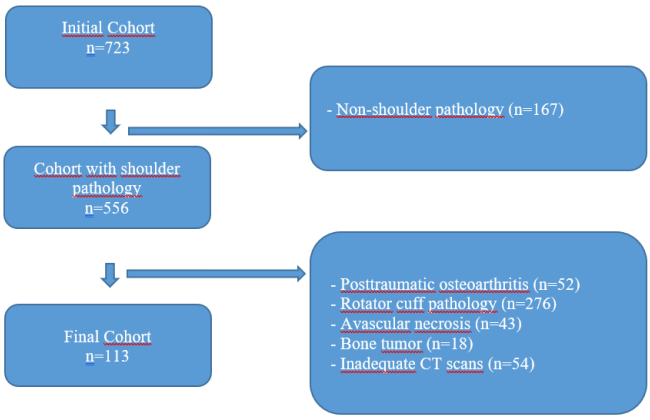


Figure 1. Flowchart of the study. CT: computed tomography

or anterior glenohumeral joint instability. This patient also did not fit any of the seven modified Walch groups and was marked as "Unclassifiable" (Fig. 3).

The frequencies of the modified Walch groups are shown in Table 1. Most frequent types were; 60 patients had type A1 (53.1%), and 22 patients had type A2 (19.5%) glenoid morphology. The mean retroversion of the modified Walch groups is shown in Table 2. Perfect intra- (ICC: 0.825 [0.819 - 0.831]) and interobserver (ICC: 0.846 [0.836 - 0.856]) agreement were achieved regarding all measurement parameters.

Table 1. Distribution of modified Walch classification groups in the study population.

| Modified Walch Classification | Frequency n (%) |
|---|---|
| A1 | 60 (53.1) |
| A2 | 22 (19.5) |
| B1 | 10 (8.8) |
| B2 | 7 (6.2) |
| B3 | 3 (2.7) |
| C | 6 (5.3) |
| D | 3 (2.7) |
| Unclassifiable | 2 (1.8) |
| Total | 113 (100) |

Table 2. Glenoid version of the modified Walch classification groups.

| Modified Walch Classification | Mean Version (°) | Standard deviation |
|---|---|---|
| A1 | -9.0 | 7.7 |
| A2 | -8.3 | 5.4 |
| B1 | -13.0 | 6.8 |
| B2 | -15.7 | 8.8 |
| B3 | -23.0 | 10.8 |
| C | -24.3 | 8.1 |
| D | -0.3 | 5.8 |
| Unclassifiable | 0.5 | 0.7 |
| Total | -9.7 | 8.1 |

## Discussion

This is the first study evaluating the glenohumeral morphology in the Turkish population with primary GHOA. Our findings may be helpful for surgeons in patient selection and preoperative planning. To the best of our knowledge no study previously examined "Unclassifiable" Walch groups in GHOA. From our study, it was derived that even a small number of two patients with "Unclassifiable" morphology can guide future studies to define new categories of Walch groups.

The modified Walch classification of primary glenohumeral osteoarthritis distribution in the population reported at many previous studies. Neyton et al. [15] evaluated the CT images of 611 patients with primary GHOA, and their study population consisted of 30.6% type A, 62.4% type B, 4% Type C, and 3% type D glenoid. At another morphologic study related with shoulder osteoarthritis, based on an American population, showed similar distribution in Walch groups [10]. In the present study, type A glenoid frequency was twice higher than that previously reported. However, type B glenoid frequency was lower than previous studies. Our results were different from previous studies that included different ethnic populations. These differences may be due to ethnic differences. Besides, we did not include patients who had undergone shoulder arthroplasty for GHOA, which might have affected our results. Gender distribution was similar between previous studies and our study [5]. We think that the cause of our glenoid morphology distribution is related with ethnic differences.

GHOA can change the glenoid morphology. Moreover, the morphology can be changed during glenohumeral OA progression [5]. Walker et al. [5] evaluated the shoulders of 65 patients during a 24-month interval. According to their results, they concluded that asymmetrical bone loss is unlikely in type A1 glenoid. However, type B1 glenoid tend to progress to type B2 or B3 glenoid. Based on our findings, we can speculate that posterior defective glenoid incidence is of less concern in the Turkish population.

The main limitation of this study was the retrospective design and use of 2D images for defining glenoid morphology. Only retroversion of the glenoid was assessed; inclination and posterior humeral subluxation were not discussed in the present study. However, the use of 2D images for defining glenoid morphology is not necessarily an inferior method of measurement. Dominique et al. [17] reported that 3D CT showed no superiority in measuring the glenoid version compared to other methods. Moreover, our study was a single-center one, limited number of patients and our sample may not be considered representative compared to the entire population of Turkey. The interobserver reliability of the modified Walch classification has been discussed in a few studies in the existing literature and has been so far reported to be fair to moderate [9, 10]. However, perfect intra- and interobserver agreement was found in our study. Other limitation is that the progression of GHOA with time was not included in this study. GHOA may progress with time and Walch type of a patient can change. Further studies are needed which assess the progression of GHOA.

Preoperative planning for shoulder arthroplasty is a crucial step for to obtain satisfactory long-term results. For Turkish patients, it should be kept in mind that glenoid morphology may be different from general population, which was described by previous studies. During preoperative planning, surgeons should consider such ethnic differences in joint morphology, as it might affect bone cut and placement of shoulder prosthesis, which might lead to long-term complications.

In conclusion, the distribution of modified Walch groups in the Turkish population may differ from other populations that reported in the previous literature. According to our findings, most patients had type A glenoid, and type B glenoid was less frequently detected. Posterior defective glenoid and excessive retroversion may concern less in the Turkish population with primary GHOA. Also, two unidentified morphologies show us that modified Walch classification may not be enough for primary GHOA classification. Our results were obtained from limited population, and we think that further epidemiologic studies with larger sample size are needed for

more realistic conclusions and new classifications may include all the glenoid morphologies.

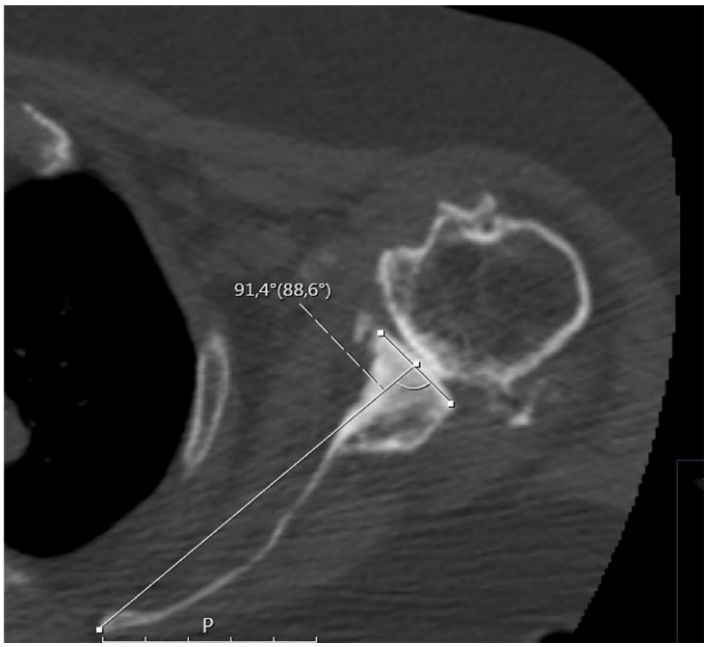


Figure 2. Unclassifiable patient 1. One degree of anteversion with the loss of glenoid cavity concavity.

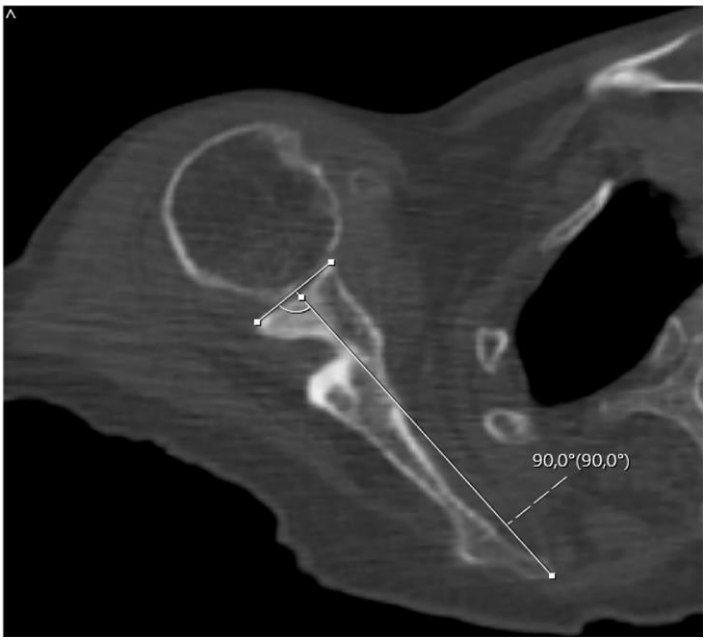


Figure 3. Unclassifiable patient 2. Asymmetrical OA with anterior subluxation of the humeral head without anterior erosion of the glenoid.

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# The short-term results of cytoreduction+hyperthermic intraperitoneal chemotherapy (HIPEC) in patients with grade IIIC and IV ovarian cancer

## Evre IIIC ve IV over kanserli hastalarda kısa dönem sitoredüksiyon + hipertermik intraperitoneal kemoterapi (HIPEC) sonuçlarımız

**Mehmet Esat Duymuş [1], Hülya Ayık Aydın [2]**

Aim: Cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) has been shown to prolong survival in patients with advanced-stage ovarian cancer. This study aimed to determine present the short-term outcomes of CRS +HIPEC in patients with primary and recurrent advanced-stage ovarian cance.
Methods: A retrospective study was performed in 18 patients with advanced stage ovarian cancer who received CRS+HIPEC at a single center between May 2019 and March 2020. The demographic data, CA-125 values, peritoneal carcinomatosis index (PCI), completeness of cytoreduction score (CC), surgical procedures, complications, disease-free survival (DFS) and overall survial (OS) data of each patient were recorded.
Results: In the group with PCI ≥10, DFS was 18 months, and OS was 19 months, and the group with PCI ≤9, the DFS and OS were 20 months. No significant difference was observed in the DFS and OS between the two groups in respect of DFS and OS (p = 1.000). In patients with CC0, DFS and OS were calculated as 20 months; in those with CC1+CC2, DFS was 12 months and OS was 13.2 months. A statistically significant difference was observed in the DFS and OS (p = 0.039) between the two groups.
Conclusion: Our results showed that optimal CRS with HIPEC prolonged the survival in patients with advanced-stage ovarian cancer.

Key Words: cytoreduction, HIPEC, hyperthermia, ovarian cancer, survival.

Öz
Amaç: İleri evre over kanserinde sitoredüktif cerrahi (SRC) ile birlikte yapılan Hipertermik İntraperitoneal Kemoterapi (HİPEK)'nin sağkalımı artırdığı gösterilmiştir. Bu çalışmada; tek merkezde primer ve rekürren ileri evre over kanseri tanısı ile SRC+HİPEK yapılan hastaların erken dönem sonuçlarını sunmayı amaçladık.
Yöntemler: Mayıs 2019- Mart 2020 tarihleri arasında tek merkezde ileri evre over kanseri tanısı ile SRC+HİPEK yapılan hastaların sonuçları retrospektif olarak incelendi. Hastaların; demografik verileri, preopereatif CA-125 değerleri, peritoneal karsinomatozis indexi (PKİ), sitoredüksiyon skoru (SS), cerrahi işlemler ve komplikasyonları, postoperatif takip süreleri, hastalıksız sağkalım HSK ve genel sağ kalım (GSK) süreleri kayıt altına alındı.
Bulgular: PKİ ≥ 10 olan grupta HSK 18 ay, OS 19 ay iken, PKİ ≤ 9 olan grupta HSK ve GSK 20 ay olarak bulunmuştur. İki grup arasında HSK ve GSK'de anlamlı fark yoktur (p=1.000). SS0 olanlarda HSK ve GSK 20 ay, SS1+SS2'de ise HSK 12 ay, GSK 13,2 ay olarak hesaplanmıştır. İki grup arasında HSK ve GSK'de istatistiksel anlamlı fark mevcuttur (p=0,039).
Sonuç: Sonuçlarımız; over kanserlerinde optimal SRC ile yapılan HİPEK'in sağkalımı uzattığını göstermiştir.

Anahtar Kelimeler: sitoredüksiyon, HİPEK, hipertermi, over kanseri, sağ kalım.

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## Introduction

According to the 2018 data of the Global Cancer Observatory (GLOBOCAN), approximately 295,000 patients are diagnosed with ovarian cancer per year worldwide, and ovarian cancer has a mortality rate of 185,000 [1]. Epithelial carcinoma is the most frequently observed type of ovarian cancer with an incidence of 95% [2]. Unfortunately, ovarian cancer patients are generally diagnosed at an advanced stage, and at least 75% of these patients have widespread peritoneal involvement and findings of obstruction [3].

Debulking surgery and chemotherapy regimens have shown good clinical outcomes in patients with advanced-stage ovarian cancer. To date, however none of the treatments have shown an improvement success rate of more than 30% in the 5-year survival rates [4]. Epithelial ovarian cancer (EOC) metastasizes via local, systemic or lymphatic routes. The most significant feature of EOC is peritoneal carcinomatosis (PC) that is caused by spread through implantation to the peritoneum. Sugarbaker recommended cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) for the first time for the treatment of PC and showed promising results [5]. The advantages of HIPEC are as follows: it exerts at direct effect on cancer cells, increases the cytotoxic effect of chemotherapy with an increase in temperature, and inhibits angiogenesis [6]. This study was aimed to investigate the short-term clinical outcomes of patients with a diagnosis of primary and recurrent ovarian cancer (International Federation of Gynecology and Obstetrics-FIGO IIIC, IV) receiving CRS+HIPEC.

## Material and methods

### Ethical approval

Approval for the study was granted by the Non-interventional Clinical Research Ethics Committee of Mustafa Kemal University (decision no: 10, session no:10, dated: 03/09/2020).

### Patient selection

The results of patients with advanced stage ovarian cancer (IIIC and IV) confirmed histologically who received CRS+HIPEC at Hatay Training and Research Hospital between May 2019 and March 2020 were retrospectively reviewed. For patients with primary EOC, six cycles of paclitaxel (175 mg/m2) and carboplatin (AUC 5-6) were administered after optimal CRS+HIPEC, and patients who underwent interval debulking surgery received three cycles of neoadjuvant chemotherapy (NACT) preoperatively. Patients with low performance and not suitable for optimal CRS with imaging methods were accepted as contra indicated for NACT. Patients showing recurrence were followed up with second-generation platin-based chemotherapy following CRS+HIPEC. Thoracic and abdominopelvic computed tomography (CT) images of all patients were acquired perioperatively. A diagnosis of recurrence was confirmed in patients with an increase in CA-125 levels or, when necessary, by peritoneal sampling (laparoscopic or Tru-cut biopsy). CRS+HIPEC was performed in all the patients by the same surgical team. Peritonectomy and diaphragm stripping was performed in all patients. When there was invasion of the diaphragm, resection was preferred to stripping. Resections were performed in solid organs (liver metastasectomy and splenectomy) and the gastrointestinal tract (gastrectomy, small intestine resection, colectomy) showing tumor invasion, and lymph node dissections (pelvic, para-aortic, hepatoduodenal) were performed in lymph node-positive patients. Immediately after the termination of the operation, a catheter was placed in the four quadrants of the abdomen and HIPEC was using with the closed method [7] using a perfusion pump (Stockert S5®, Germany, 2018) with a single agent at 41.5°C for 90 min. For patients sensitive to platin, 100 mg/m2 cisplatin [8] was administered, and for patients resistant to platin, 175 mg/m2 paclitaxel was administered [9].

### Inclusion and exclusion criteria

The patients included in the study were aged 18-70 years, had ovarian cancer (FIGO grade IIIC, IV), Eastern cooperative oncology group performance status (ECOG PS) of 0-2, and sufficient bone marrow function for performing HIPEC (neutrophil count ≥1000 mm3, platelet count ≥100,000/ mm3, hemoglobin level ≥8.5 g/dL, international normalized ratio (INR) ≤1.5, creatinine and bilirubin levels ≤1.5 fold of the upper limit, and alanine aminotransferase and aspartate aminotransferase levels ≤3-fold of the upper limit). Patients with an ECOG PS of ≥3, a history of cancer other than ovarian cancer, with >3 intestinal obstructions, unresectable liver metastasis, or involvement of the mesenteric root of the small intestine were excluded from the study.

### Data collection

The demographic information, CA-125 levels, the presence of ascites, ECOG PS, surgical procedures, operative time, bleeding amount, length of stay, pathology results, and surgical complications were recorded. The surgical complications were classified according to the National Cancer Institute, Common Terminology Criteria for Adverse Events version 3 (NCI, CTCAEv3). The criteria were defined as follows: G1, mild; G2, moderate; G3, severe; and G4, life-threatening [10]. The peritoneal cancer index (PCI), which reflects the intra-abdominal cancer burden and distribution, was calculated perioperatively for each patient. For calculating the PCI, the abdomen was separated into 13 anatomic regions. Each region was scored scoring according to the largest tumor diameter (TD) as follows: TD-0, no tumor; TD-1, ≤0.5 cm; TD-2, 0.5–5 cm; and TD-3, ≥ 5cm, with a maximum possible score of 39 [11]. Residual disease was classified according to the completeness of cytoreduction (CC) scoring system defined by Sugarbaker et al. [12] According to this system, CC0, no macroscopic tumor; CC1, the largest macroscopic tumor is ≤2.5 mm; CC2, ≥2.5 to ≤2.5 cm; and CC-3, ≥2.5 cm. The patients were separated into subgroups as those with PCI ≤9 and ≥10 and those with CC0 and CC1+CC2.

### Disease-free survival and overall survival

The follow-up period was accepted as the period from the date of the operation to the last date on which information was taken from the patient. Disease-free survival (DFS) was recorded as the time from the operation to recurrence, and overall survival (OS) was the time from the operation to death or the final follow-up examination.

### Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS version. 27.0 software (Chicago, IL, USA). Descriptive statistics were stated as mean ± standard deviation (SD), median, minimum, and maximum values, number (n), and percentage (%). Conformity of the variables to normal distribution was tested using the Kolmogorov–Smirnov test. The Mann Whitney U-test was used in the analysis of independent quantitative data. The independent qualitative data were analyzed using the chi-square test or the Fisher test, where appropriate. Survival analysis was applied in a 95% confidence interval using

the Kaplan–Meier (log-rank) method. A value of $p < 0.05$ was accepted as statistically significant.

## Results

### Clinical and pathological characteristics

Between May 2019 and March 2020, 23 consecutive patients underwent an operation for advanced-stage (FIGO IIIC and IV) ovarian cancer. Five patients did not meet the inclusion criteria; therefore, 18 patients were included in the evaluation. Cisplatin was administered to 17 patients (94.4%), and paclitaxel was administered to 1 patient (5.6%) because of platin resistance. The median age of the patients was 51.5 years (range, 22–69 years), and the median operating time was 350 mins (range, 250.0–590.0 mins). Primary cytoreduction (PC) was performed in 7 patients (38.9%), interval debulking cytoreduction (IDC) in 3 patients (16.7%), and secondary cytoreduction (SC) in 8 patients (44.4%) with recurrence. The CC evaluations were CC0 in 9 patients (50%), CC1 in 7 patients (38.9%), and CC2 in 2 patients (11.1%). The most common diagnosis was serous carcinoma in 13 patients (72.2%). Ten patients (55.6%) patients were classified as FIGO IIIC and 8 patients (44.4%) as grade IV. The demographic data, surgical data, and pathological results of all the patients are shown in Table 1. PCI was calculated as median 10 (range, 2–22); 7 patients with PCI ≤9 and 11 patients with PCI ≥10. Comparison according to the PCI showed that the operating time (CRS+HIPEC) was significantly longer in the PCI ≥10 group than in the PCI ≤9 group ($p = 0.006$). The ECOG PS was higher in the PCI ≥10 group ($p = 0.043$). No significant difference was observed in other results ($p > 0.05$, Table 2). The operating time was longer, the perioperative bleeding was higher, and the rate of splenectomy were statistically significantly higher in the CC1+CC2 patients than in the CC0 patients ($p = 0.042$, $p = 0.049$, and $p = 0.016$, respectively). The mortality rate was higher in the CC1+CC2 group ($p = 0.09$, Table 3).

### Survival

The median follow-up period of the patients was 13.5 months (range, 1–22 months), the median DFS was 18.8 months (95%CI: 15.69–21.97), and the median OS was 20 months (95% CI: 16.84-21.97). Five patients (27.8%) died during this period. In the group with PCI ≥10, the DFS was 18 months, and OS was 19 months, and in the group with PCI ≤9, the DFS and OS were 20 months. No significant difference was observed in the DFS and OS ($p = 1.000$) between the two groups (Figure 1). The DFS and OS in the CC0 group were 20 months, and in the CC1+CC2 group, the DFS was 12 months and the OS was 13.2 months. A statistically significant difference was observed between in the DFS and OS ($p = 0.039$) between the two groups (Figure 2).

**Table 1.** Characteristics of patients (n = 18).

| | Min - Max | Median | Mean ± SD |
|---|---|---|---|
| Age (years) | 22.0-69.0 | 51.5 | 51.7±12.7 |
| CA-125 | 8.7-6742.1 | 274.0 | 985.0±1661.9 |
| PCI | 2.0-22.0 | 10.0 | 10.9±5.2 |
| Number of positive LN | 0.0-32.0 | 2.0 | 9.3±11.5 |
| Total LN | 10.0-74.0 | 32.5 | 36.1±18.9 |
| Duration of CRS+HIPEC (min) | 250.0-590.0 | 350.0 | 351.7±86.0 |
| Bleeding (mL) | 1000.0-3000.0 | 1875.0 | 1861.1±601.6 |
| ICU (day) | 2.0-9.0 | 3.5 | 3.9±1.7 |
| Hospitalization (day) | 10.0-35.0 | 19.0 | 20.4±7.5 |
| | n | % | n |
| Ascites (+) | 11 | 61.1% | |
| ECOG PS | | | |
| 0 | 3 | 16.7% | |
| I | 10 | 55.6% | |
| II | 5 | 27.8% | |
| TAH-BSO | 10 | 55.5% | |
| PEL-PA LND | 17 | 94.4% | |
| Vaginal cuff resection | 1 | 5.6% | |
| Hemicolectomy | 1 | 5.6% | |
| Subtotal colectomy | 2 | 11.1% | |
| Total colectomy | 5 | 27.8% | |
| LAR | 8 | 44.4% | |
| Small bowel resection | 3 | 16.7% | |
| Appendectomy | 18 | 100.0% | |
| Omentectomy | 18 | 100.0% | |
| Peritonectomy | 18 | 100.0% | |
| DS/DR | | | |
| DS | 15 | 83.3% | |
| DR | 3 | 16.7% | |
| Liver metastasectomy | 3 | 16.7% | |
| Splenectomy | 7 | 38.9% | |
| Cholecystectomy | 3 | 16.7% | |
| Gastrectomy | 4 | 22.2% | |
| Hepatoduodenal LND | 2 | 11.1% | |
| Histologic type | | | |
| Serous | 13 | 72.2% | |
| Mucinous | 3 | 16.7% | |
| Mixed epithelial carcinoma | 1 | 5.6% | |
| Carcinosarcoma | 1 | 5.6% | |
| FIGO | | | |
| IIIC | 10 | 55.6% | |
| IV | 8 | 44.4% | |
| Cisplatin | 17 | 94.4% | |
| Paclitaxel | 1 | 5.6% | |
| CC0 | 9 | 50.0% | |
| CC1 | 7 | 38.9% | |
| CC2 | 2 | 11.1% | |
| PC | 7 | 38.8% | |
| IDC | 3 | 16.7% | |
| SC | 8 | 44.4% | |
| Complications* | | | |
| Anastomotic leak[2] | 1 | 14.3% | |
| Intestinal fistula[3] | 2 | 28.6% | |
| Wound infection[2] | 1 | 28.6% | |
| Rectovesical fistula[4] | 1 | 14.3% | |
| Ureteral injury[1] | 1 | 14.3% | |
| Re-operation | 1 | 5.6% | |
| Exitus | 5 | 27.8% | |

PCI = peritoneal carcinomatosis index, LN = lymph node, CRS+HIPEC = cytoreductive surgery+hyperthermic intraperitoneal chemotherapy, ICU = intensive care unit, ECOG PS = Eastern cooperative oncology group performance status, TAH+BSO = total abdominal hysterectomy bilateral salpingo-ooferectomy, PEL-PA LND = pelvic-paraaortic lymph node dissection, LAR = low anterior resection, DS = diaphragm stripping, DR = diaphragm resection, FIGO = International federation of gynecology and obstetrics, CC = completeness of cytoreduction PC = primary cytoreduction IDC = interval debulking cytoreduction, SC = secondary cytoreduction, *CTCAE = common terminology criteria for adverse events grade (1: mild, 2: moderate, 3: severe, G4: life-threatening), sd, standard deviation

## Discussion

EOC is the most frequently observed ovarian malignancy and is the leading cause of death among gynecological malignancies [2,3]. Recurrence develops within 3 years of treatment in approximately 60% of patients [13]. Although no consensus has been reached regarding the extent of CRS+HIPEC, the timing of administering this treatment, the chemotherapeutic agent to be administered, the doses, and the methods of administration of intraperitoneal chemotherapy in the treatment of advanced stage ovarian cancer, the results obtained in the last 20 years have been encouraging [14].

In a multicenter study during which CRS+HIPEC was performed at different times, the OS was 52.4 months and the DFS was 16.6 months. The patients were divided into eight subgroups, and the OS and DFS were longer in patients with PC than in those who received recurrent cytoreduction. The shortest survival among patients with PC was reported to be in those who did not respond after NACT. CC score and PCI were shown to be independent markers [15]. The results of another study comparing primary cytoreduction with and without HIPEC showed no difference between the 2 groups in OS (33.8 months vs. 33.6 months), and the DFS was significantly longer in the group receiving HIPEC (25.6 months vs. 20 months) [16]. In a prospective study by Spiliotis et al. [17] patients with recurrent grade IIIC and IV ovarian cancer were followed-up and those receiving CRS+HIPEC were compared with those receiving CRS alone. OS was significantly longer in patients sensitive to platin and receiving HIPEC (26.7 months vs. 13.4 months). Results of a previous study investigating the role of HIPEC in improving the survival in patients with recurrent

**Table 2.** Comparison of PCI ≤9 and PCI ≥10.

| | PCI ≤9 (n = 7) | | PCI ≥10 (n = 11) | | |
|---|---|---|---|---|---|
| | Mean ± SD | Median | Mean ± SD | Median | p¥ |
| Age (years) | 52.3±15.0 | 57.0 | 51.4±11.9 | 50.0 | 0.683 |
| CA-125 | 624.6±701.7 | 408.8 | 1214. 3±2062 | 139.2 | 0.892 |
| PCI | 6.1±2.4 | 7.0 | 14.0±4.1 | 14.0 | 0.004 |
| Number of LN metastases | 7.1±10.2 | 2.0 | 10.6±12.6 | 2.0 | 0.782 |
| Number of total LN | 34.1±20.2 | 29.0 | 37.3±18.8 | 36.0 | 0.650 |
| Duration of CRS+HIPEC (min) | 291.4±35.3 | 300.0 | 390.0±87.5 | 390.0 | 0.006 |
| Bleeding (mL) | 1535.7±548.3 | 1500.0 | 2068.2±560.0 | 2000.0 | 0.067 |
| ICU (day) | 3.9±1.2 | 3.0 | 3.9±2.0 | 4.0 | 0.780 |
| Hospitalization (day) | 19.3±7.1 | 16.0 | 21.2±8.1 | 21.5 | 0.591 |
| | n | % | n | % | |
| Ascites | 4 | 57.1% | 7 | 63.6% | 1.000 |
| ECOG PS | | | | | |
| 0 | 3 | 42.9% | 0 | 0.0% | |
| I | 1 | 14.3% | 9 | 81.8% | 0.043 |
| II | 3 | 42.9% | 2 | 18.2% | |
| TAH-BSO | 4 | 57.1% | 6 | 54.5% | 0.119 |
| PEL-PA LND | 7 | 100.0% | 10 | 90.9% | 1.000 |
| Vaginal cuff resection | 0 | 0.0% | 1 | 9.1% | 1.000 |
| Hemicolectomy | 1 | 14.3% | 0 | 0.0% | 0.389 |
| Subtotal colectomy | 0 | 0.0% | 2 | 18.2% | 0.497 |
| Total colectomy | 0 | 0.0% | 5 | 45.5% | 0.101 |
| LAR | 4 | 57.1% | 4 | 36.4% | 0.630 |
| Small bowel resection | 0 | 0.0% | 3 | 27.3% | 0.245 |
| Appendectomy | 7 | 100.0% | 11 | 100.0% | 1.000 |
| Omentectomy | 7 | 100.0% | 11 | 100.0% | 1.000 |
| Peritonectomy | 7 | 100.0% | 11 | 100.0% | 1.000 |
| DS/DR | | | | | |
| DS | 7 | 100.0% | 8 | 72.8% | 0.131 |
| DR | 0 | 0.0% | 3 | 27.2% | |
| Liver metastasectomy | 1 | 14.3% | 2 | 18.2% | 1.000 |
| Splenectomy | 2 | 28.6% | 5 | 45.5% | 1.000 |
| Cholecystectomy | 0 | 0.0% | 3 | 27.3% | 0.245 |
| Gastrectomy | 0 | 0.0% | 4 | 36.4% | 0.119 |
| Hepatoduodenal LND | 0 | 0.0% | 2 | 18.2% | 0.497 |
| Histologic type | | | | | |
| Serous | 7 | 100.0% | 6 | 54.5% | 0.101 |
| Mucinous | 0 | 0.0% | 3 | 27.3% | 0.245 |
| Mixed epithelial carcinoma | 0 | 0.0% | 1 | 9.1% | 1.000 |
| Carcinosarcoma | 0 | 0.0% | 1 | 9.1% | 1.000 |
| FIGO | | | | | |
| IIIC | 6 | 85.7% | 4 | 36.4% | 0.311 |
| IV | 1 | 14.3% | 7 | 63.6% | |
| Cisplatin | 7 | 100.0% | 10 | 90.9% | 1.000 |
| Paclitaxel | 0 | 0.0% | 1 | 9.1% | |
| PC | 3 | 42.9% | 4 | 36.4% | |
| IDC | 2 | 28.6% | 1 | 9.1% | 0.367 |
| SC | 2 | 28.6% | 6 | 54.5% | |
| Complications | | | | | |
| + | 5 | 71.4% | 6 | 54.5% | 0.637 |
| - | 2 | 28.6% | 5 | 45.5% | |
| Re-Operation | 0 | 0.0% | 1 | 9.1% | 1.000 |
| Exitus | 2 | 28.6% | 3 | 27.3% | 1.000 |
| CC0 | 5 | 33.3% | 4 | 44.4% | |
| CC1 | 1 | 22.2% | 6 | 11.1% | 0.146 |
| CC2 | 1 | 44.4% | 1 | 44.4% | |

¥Mann–Whitney U test and chi- square test (Fischer) were used for calculations. PCI = peritoneal carcinomatosis index, LN = lymph node, CRS+HIPEC = cytoreductive surgery+hyperthermic intraperitoneal chemotherapy, ICU = intensive care unit, ECOG PS = Eastern cooperative oncology group performance status, TAH+BSO = total abdominal hysterectomy bilateral Salpingo-ooferectomy, PEL-PA LND = pelvic-paraaortic lymph node dissection, LAR = low anterior resection, DS = diaphragm stripping, DR = diaphragm resection, FIGO = International federation of gynecology and obstetrics, CC = completeness of cytoreduction, PC = primary cytoreduction IDC = interval debulking cytoreduction, SC = secondary cytoreduction, sd, standard deviation

ovarian cancer showed an OS of 59.3 months and a DFS of 15.8 months. Compared with the control group, the group receiving CRS+HIPEC showed no improvement in survival [18]. Our results showed that the DFS was 18.8 months, which is consistent with findings reported previously. However, our study had a short follow-up period, we evaluated patients with primary and recurrent tumors together, and included only patients with grade IIIC and IV tumors, which may attribute to the shorter OS than that reported in previous studies.

**Table 3.** Comparison of CC0 and CC1+CC2.

| | CC0 (n = 9) | | CC1 +CC2 (n = 9) | | |
|---|---|---|---|---|---|
| | Mean ± s.d | Median | Mean ± s.d | Median | p¥ |
| Age (years) | 49.6±14.6 | 49.0 | 53.9±11.1 | 57.0 | 0.536 |
| CA-125 | 943.2±1011.4 | 826.9 | 1026.7±2200.6 | 136.2 | 0.354 |
| PCI | 9.3±5.6 | 9.0 | 12.6±4.6 | 11.0 | 0.132 |
| Number of LN metastases | 9.6±11.2 | 4.0 | 9.0±12.6 | 2.0 | 0.446 |
| Number of total LN | 36.9±21.9 | 31.0 | 35.2±16.5 | 34.0 | 0.965 |
| Duration of CRS+HIPEC (min) | 313.3±45.8 | 310.0 | 390.0±101.5 | 390.0 | 0.042 |
| Bleeding (mL) | 1583.3±484.1 | 1500.0 | 2138.9±600.9 | 2250.0 | 0.049 |
| ICU (day) | 4.0±2.1 | 3.0 | 3.8±1.4 | 4.0 | 0.892 |
| Hospitalization (day) | 20.2±8.5 | 16.0 | 20.6±6.9 | 20.5 | 0.772 |
| | n | % | n | % | |
| Ascites | 6 | 66.7% | 5 | 55.6% | 0.629 |
| ECOG PS | | | | | |
| 0 | 3 | 33.3% | 0 | 0.0% | |
| I | 5 | 55.6% | 5 | 55.6% | 0.206 |
| II | 1 | 11.1% | 4 | 44.4% | |
| TAH-BSO | 5 | 55.5% | 5 | 55.5% | 1.000 |
| PEL-PA LND | 9 | 100.0% | 8 | 88.9% | 1.000 |
| Vaginal cuff resection | 0 | 0.0% | 1 | 11.1% | 1.000 |
| Hemicolectomy | 1 | 11.1% | 0 | 0.0% | 1.000 |
| Subtotal colectomy | 0 | 0.0% | 2 | 22.2% | 1.000 |
| Total colectomy | 2 | 22.2% | 3 | 33.3% | 0.599 |
| LAR | 4 | 44.4% | 3 | 33.3% | 0.343 |
| Small bowel resection | 1 | 11.1% | 2 | 22.2% | 1.000 |
| Appendectomy | 9 | 100.0% | 9 | 100.0% | 1.000 |
| Omentectomy | 9 | 100.0% | 9 | 100.0% | 1.000 |
| Peritonectomy | 9 | 100.0% | 9 | 100.0% | 1.000 |
| DS/DR | | | | | |
| DS | 9 | 100.0% | 6 | 66.7% | 0.206 |
| DR | 0 | 0.0% | 3 | 33.3% | |
| Liver metastasectomy | 2 | 22.2% | 1 | 11.1% | 1.000 |
| Splenectomy | 1 | 11.1% | 6 | 66.7% | 0.016 |
| Cholecystectomy | 1 | 11.1% | 2 | 22.2% | 1.000 |
| Gastrectomy | 1 | 11.1% | 3 | 33.3% | 0.257 |
| Hepatoduodenal LND | 0 | 0.0% | 2 | 22.2% | 0.471 |
| Histologic type | | | | | |
| Serous | 8 | 88.9% | 5 | 55.6% | 0.114 |
| Mucinous | 0 | 0.0% | 3 | 33.3% | 0.206 |
| Mixed epithelial carcinoma | 1 | 11.1% | 0 | 0.0% | 1.000 |
| Carcinosarcoma | 0 | 0.0% | 1 | 11.1% | 1.000 |
| FIGO | | | | | |
| IIIC | 5 | 55.6% | 5 | 55.6% | 1.000 |
| IV | 4 | 44.4% | 4 | 44.4% | |
| Cisplatin | 8 | 88.9% | 9 | 100.0% | 1.000 |
| Paclitaxel | 1 | 11.1% | 0 | 0.0% | |
| PC | | | | | |
| IDC | 4 | 44.4% | 3 | 33.3% | |
| SC | 3 | 33.3% | 0 | 0.0% | 0.153 |
| | 2 | 22.2% | 6 | 66.7% | |
| Complications | | | | | |
| + | 6 | 66.7% | 5 | 55.6% | 0.629 |
| - | 3 | 33.3% | 4 | 44.4% | |
| Re-Operation | 0 | 0.0% | 1 | 11.1% | 1.000 |
| Exitus | 0 | 0.0% | 5 | 55.6% | 0.009 |

¥Mann–Whitney *U* test and chi- square test (Fischer) were used for calculations, PCI = peritoneal carcinomatosis index, LN = lymph node, CRS+HIPEC = cytoreductive surgery+hyperthermic intraperitoneal chemotherapy, ICU = intensive care unit, ECOG PS = Eastern cooperative oncology group performance status, TAH+BSO = total abdominal hysterectomy bilateral Salpingo-ooferectomy, PEL-PA LND = pelvic-paraaortic lymph node dissection, LAR = low anterior resection, DS = diaphragm stripping, DR = diaphragm resection, FIGO = International federation of gynecology and obstetrics, CC = completeness of cytoreduction, PC = primary cytoreduction, IDC = interval debulking cytoreduction, SC = secondary cytoreduction, sd, standard deviation

Multiple organ resection may be necessary for effective CRS, and this can increase the morbidity and mortality rates associated with the operation. Previous studies have reported morbidity rates of 14%–55%, 30-day mortality rate of 0%–4.9%, and a mortality rate of 0%–20% in the subsequent period [19,20]. Consistent with the results reported previously, results of our study showed that the rate of complications causing morbidity was 38.9%. While there was no perioperative mortality was observed during this study, the mortality rate in the subsequent period was 27%. The higher rate of mortality observed in this study than that in previous studies may be because CRS+HIPEC

was performed only in patients with grade IIIC and IV disease, and therefore, there was a higher rate of multiorgan resection

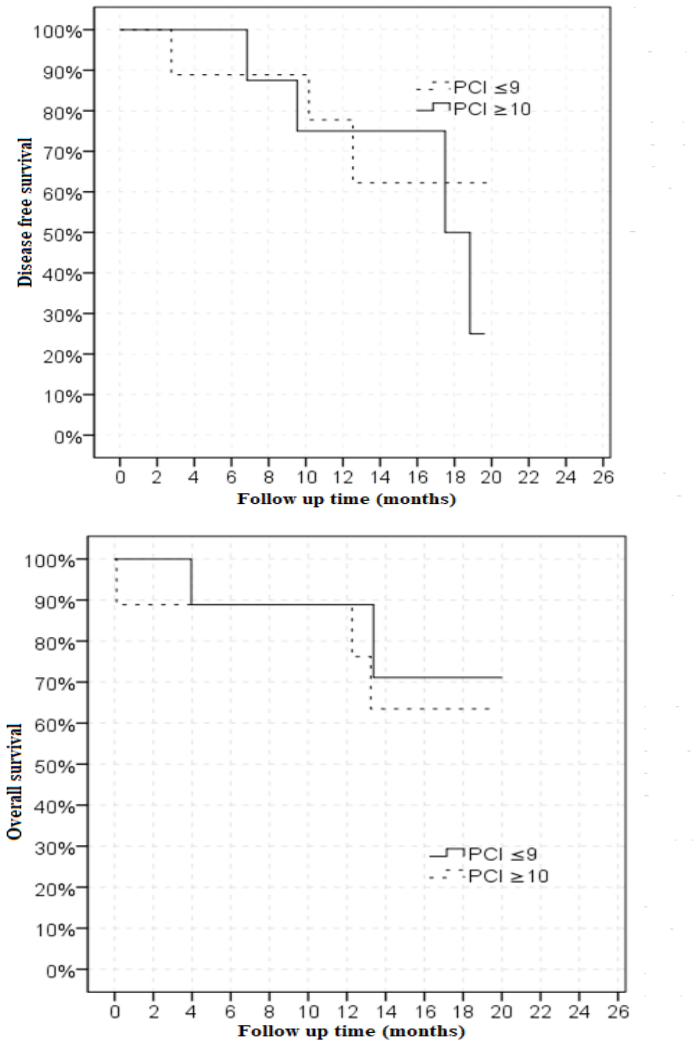


**Figure 1 a.** Kaplan–Meier curves of disease-free survival for patients with peritoneal carcinomatosis index (PCI) ≤9 and PCI ≥10, **b.** Kaplan–Meier curves of overall survival for patients with PCI ≤9 and PCI ≥10

Previous studies showed that a residual tumor <1 cm was sufficient for optimal cytoreduction. Recent studies indicate that optimal cytoreduction is evaluated using the CC score [12]. Optimal cytoreduction (CC0 and CC1) is directly related to positive results of the surgery and is a strong marker of survival [21]. A previous study showed that the survival in patients with CC0, CC1, and CC2 was 30.9, 23.9, 12.1 months, respectively [17]. Patients with CC0 had longer survival and a greater need for perioperative blood transfusion than those with CC >0 [15]. Unlike the results reported previously, our results showed that the amount of bleeding, operating time, and rates of splenectomy were significantly lower in patients with CC0, and the mortality rate was significantly higher in the CC1+CC2 group. The number of patients with CC1 were higher than those with CC2 in our study. Higher bleeding and longer operating time were attributed to the greater efforts made to ensure CC1 during the operation. Survival analysis showed that the OS and DFS were significantly longer in the CC0 than in the CC1+CC2 group, which was consistent with the results reported previously. No significant difference was determined between the groups in respect of morbidity, and all patients who died were in the CC1+CC2 group. Absence of mortality in the CC0 group during the follow-up period shows the importance of optimal cytoreduction. PCI, showing the tumor distribution within the

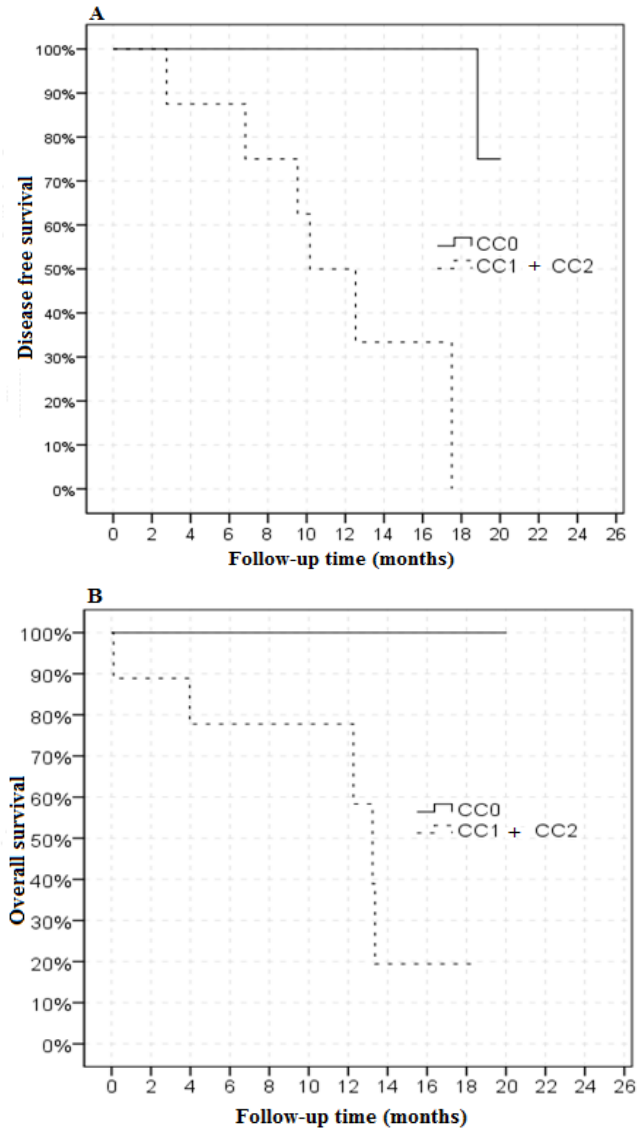


**Figure 2 a.** Kaplan–Meier curves of disease-free survival for patients with completeness of cytoreduction score 0 (CC0) and CC1+CC2 **b.** Kaplan–Meier curves of overall survival for patients with CC0 and CC1+CC2

abdomen, is a scoring system providing information of the applicability of optimal cytoreduction, and is calculated while making perioperative exploration [11]. The median PCI value in our study was 10. A previous study showed that the mean PCI was 12.7, and the mean survival was longer in those with PCI ≥12.7 [15]. A previous study in patients with secondary cytoreduction showed that patients with PCI >6 had a worse survival than those with PCI ≤6 [18]. The results of a retrospective study investigating the predictive value of PCI in patients with primary cytoreduction showed that high PCI values had a negative effect on OS and DFS. Additionally, operating time was significantly longer in patients with high PCI values [22]. Total colectomy, small intestine resection, liver metastasectomy, gastrectomy, and hepatoduodenal lymph node dissection were performed a greater number of patients with PCI ≥10, but the difference was not statistically significant. Similar to the results reported previously, our results showed an increase in operating time with an increase in PCI. However, unlike the results reported previously, PCI did not have an effect on OS and DFS. HIPEC can be performed using two different techniques, namely, open (coliseum) or closed. In the coliseum technique,

the heated chemotherapeutic agent is administered within the abdomen with catheters without closing the abdominal wall after the CRS. The most important advantage of this technique is that the heated chemotherapeutic agent can be homogenously distributed within the abdomen. The disadvantage of the open technique is that the chemotherapeutic agent may form an aerosol because of the high heat, and thus, may increase the exposure of the surgical personnel to the chemotherapeutic agent. The closed technique is performed by closing the abdominal wall, placing drains in the four quadrants and then administering the heated chemotherapy into the abdomen. Although homogenous distribution of the drug is more difficult using this technique than with the open technique, the most important advantage of the closed technique is less dissipation of heat [7, 23]. We used the closed technique in this study to maintain high intra-abdominal temperature and to reduce the exposure of the surgical personnel to chemotherapy.

This study had low number of patients, lacked a control group, and had a short follow-up period. Thus, additional, prospective, randomized, and comprehensive studies on this topic should be performed in the future. For many years, CRS+HIPEC has been performed in patients with primary and recurrent grade IIIC and IV ovarian cancer. Although a consensus has not been established thus far, HIPEC is believed to play a role in improving the survival, irrespective of the time when it is performed. In conclusion; we report the short-term results of CRS+HIPEC performed by the same team at a single center to a specific patient group. HIPEC alone is not sufficient and should be performed with optimal CRS.

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# The relationship between Koos classification and ADC-post-contrast signal intensity values of vestibular schwannoma - A cross sectional study

## Vestibüler schwanomlarda Koos sınıflaması ile ADC-post kontrast sinyal intensite değerlerinin ilişkisi – Bir kesitsel çalışma

**Umut Perçem Orhan Söylemez [1], Başak Atalay [2], Nesrin Gündüz [2]**

Abstract

Aim: We aimed to determine the inter-observer reliability of the Koos classification and to evaluate its correlation with Apparent Diffusion Coefficiency (ADC) and post contrast signal intensity values.

Methods: Vestibular schwannomas were retrospectively scanned from picture archiving and communication system over a 4-year period. Koos grade of tumor was assessed by two radiologists blinded to the clinical and pathological data. Cohen's Kappa was used to analyse inter-observer agreement of Koos grade. The correlation between ADC, signal intensity measurements and Koos grade of tumors was analysed with Kendall's tau b correlation coefficient.

Results: Thirty three patients (21 females, mean age 52.6±16.6 years) with 34 vestibular schwannomas were included the study. The reliability analyses revealed excellent inter-observer agreement for ADC (ICC: 0.93, 95% CI: 0.87-0.97, p<0.001) and signal intensity (ICC: 0.98, 95% CI: 0.96-0.99, p<0.001) measurements. The percentage agreement for Koos grading was 97% (k= 0.96, p<0.001). A correlation was not found between the Koos grade and ADC (_τ_b: 0.18). Also signal intensity remained similar between Koos grades (p=0.125). A significant difference was found for ADC values between Koos grades (p=0.039).

Conclusion: Koos classification is a practical and useful grading system with excellent inter-observer agreement for vestibular schwannomas. ADC values differs significantly between the tumor grades, but ADC and post contrast signal intensity values have no correlation with Koos grade.

Key Words: Vestibular Schwannoma, magnetic resonance imaging, Koos classification, apperent diffusion coefficient.

Öz

Amaç: Bu çalışmada Koos sınıflamasının gözlemciler arası güvenilirliğini ve Görünür Difüzyon Katsayısı (ADC) ve kontrast sonrası sinyal intensite değerleri ile korelasyonunu değerlendirmeyi amaçladık.

Yöntemler: Hastane veri tabanından 4 yıllık süre içerisinde radyolojik raporlamada vestibuler şıvannom tanısı yer almış olan MR görüntüleri retrospektif olarak tarandı. Lezyonların Koos sınıflamasına göre derecesi, klinik ve patolojik verilere kör olan iki bağımsız radyolog tarafından değerlendirildi. Koos sınıflamasının gözlemciler arası uyumunu analiz etmek için Cohen's Kappa; ADC, sinyal intensite ölçümleri ve Koos tümör derecesi arasındaki korelasyonu değerlendirmede Kendall's tau b korelasyon katsayısı kullanıldı.

Bulgular: 34 vestibüler şıvannom, 33 hasta (21 kadın, ortalama yaş 52.6±16.6 yıl) çalışmaya dahil edildi. Güvenilirlik analizleri, ADC (ICC: 0,93, %95 GA: 0.87-0.97, p<0,001) ve sinyal intensite (ICC: 0,98, %95 GA: 0,96-0,99, p<0,001) ölçümleri için gözlemciler arası mükemmel uyum saptandı. Koos evrelendirmesiiçin değerlendiriciler arasında %97 oranında uyum saptandı (k= 0,96, p<0,001). Koos evresi ile ADC (_τ_b: 0,18) arasında bir korelasyon saptanamadı. Ayrıca sinyal intensitelerinin farklı Koos evreleri arasında değişmediği saptandı (p=0,125). Ancak Koos evreleriarasında ADC değerleri açısından anlamlı fark olduğu saptandı. (p=0,039).

Sonuç: Koos sınıflaması, vestibüler şıvannomlar için mükemmel gözlemciler arası uyuma sahip, pratik ve kullanışlı bir evrelendirme sistemidir. ADC değerleri ile tümör evreleri arasında önemli ölçüde farklılık izlenirken, ADC ve kontrast sonrası sinyal intensite değerlerinin Koos derecesi ile korelasyonu yoktur.

Anahtar Kelimeler: Vestibular schwannom, manyetik rezonans görüntüleme, Koos sınıflaması, görünür difüzyon katsayısı

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## Introduction

Vestibular schwannomas (VS) are the most common cerebellopontine angle (CPA) tumors and the third most common intracranial benign tumors [1, 2]. Sensorineural hearing loss and tinnitus are the most common clinical symptoms, while vertigo, 5th and 7th cranial nerve neuropathies, brain stem compression findings and hydrocephalus are other symptoms [2, 3]. Histopathological confirmation is not always required for VSs because these lesions are detected with a great accuracy in MRI [2, 4]. Lesions have an intracanalicular component and enlarge the internal acoustic canal. Generally, they are iso/hypointense on T1-weighted (T1W) images and hyperintense on T2-weighted images (T2W), with homogeneous marked enhancement [4]. Cystic and hemorrhagic changes are seen in large lesions, while absence of calcification is typical [2]. T1W images are the best for post-operative evaluation of recurrence or residue, T2W images are important for differential diagnosis of the other conditions that affect the brain stem, fast imaging employing steady-state acquisition (FIESTA)/constructive interference in steady state (CISS) sequences are standart for 7th and 8th nerve assessment, post-contrast images are also indispensable for temporal imaging for differential diagnosis of masses [2]. CT is used to evaluate the petrous bone for the decision of surgical approach, but is a limited imaging method for internal acoustic canal [5].

Surgery and radiotherapy are the treatment options, also observation may be choosen for VSs [6]. Although decision of treatment or observation is determined by factors such as the patient's symptoms and comorbidities, the Koos classification is the most widely used grading system for these tumors [2]. Koos et al. had classified these tumors based on hearing protection after surgery and also defined subgroups according to the position of the cochlear nerve and evaluated the neurotopographic response [7]. Reliability [8], validity [8], response to stereotactic radiosurgery and radiotherapy according to this classification [9, 10] were previously evaluated. Reliability of apparent diffusion coefficient (ADC) and signal intensity (SI) according to tumor grade have not been well studied. This study evaluates ADC and SI measurements between VS grades according to Koos classification and also the inter-observer reliability of Koos classification.

## Material and methods

This cross-sectional study was approved by the university ethics committee (approval ID: 2021/0397). The records of the reports of patients who underwent contrast enhanced temporal MRI between 2017 and 2021 were retrospectively scanned. Research from the reports was conducted with the search of using keywords of ‘vestibular schwannoma’, ‘cerebellopontine angle tumor’ and ‘acoustic neuroma’. Patients having tumor which have compatible features with VS in CPA and internal acoustic canal were evaluated. MRI exams with diffusion weighted images, ADC maps and post contrast sequences with adequate image quality were included the study. Images with inadequate quality for assessment and tumors which have incompatible features with VS were excluded from the study.

### MR imaging parameters and evaluation

The side, maximum diameter of tumor, ADC (Fig.1), post contrast SI values (Fig.2), Koos grade of tumor were assessed by two radiologists blinded to the clinical and pathological data. According to Koos classification VSs are evaluated as; grade I (Fig.3); small intracanalicular tumor, grade 2 (Fig.4); small tumor with protrusion into the cerebellopontine angle but no contact with the brainstem, grade 3 (Fig.5); tumor occupying the cerebellopontine cistern with no brainstem displacement and grade IV (Fig.6); large tumor with brainstem and cranial nerve displacement [7].

All MRIs performed with General Electric Signa Excite 1.5 Tesla MRI device. T1 and T2-weighted sequences with a 3 mm slice thickness, 3D-FIESTA sequence with 0.5 mm slice thickness, also fluid-attenuated inversion recovery and diffusion-weighted sequence images were obtained. Post contrast images were obtained after 0.2 mL/kg gadolinium injection.

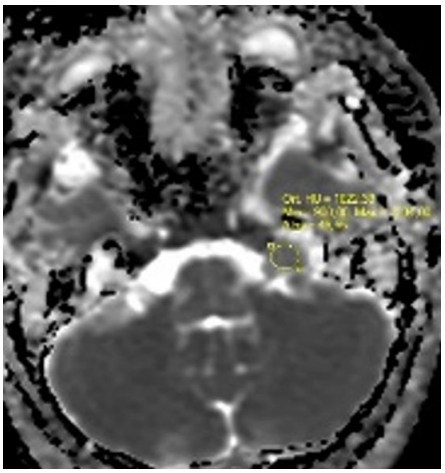

Figure 1: ADC measurement with region of interest in a left sided Koos grade 2 schwannoma.

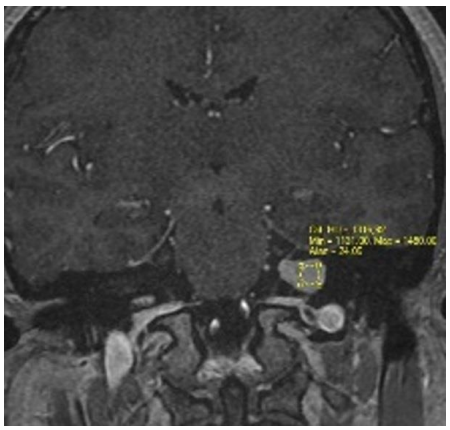

Figure 2: Post contrast signal intensity measurement in a left sided Koos grade 2 schwannoma.

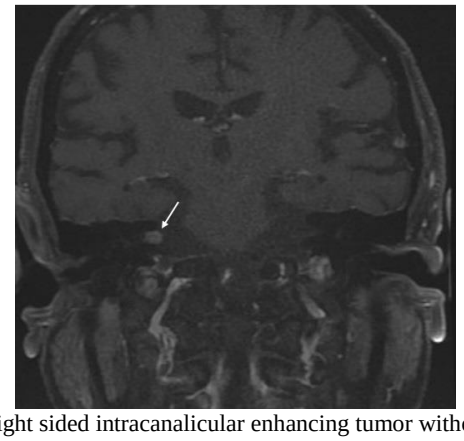

Figure 3: Right sided intracanalicular enhancing tumor without extension to cerebellar cistern (arrow); Koos grade 1.

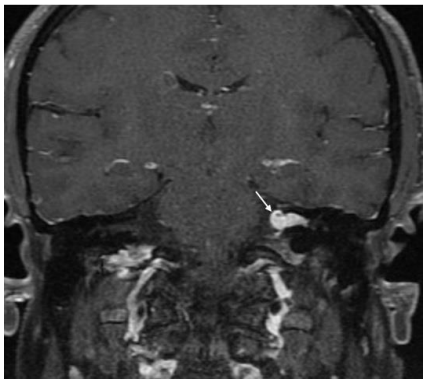

Figure 4: Left sided tumor with extension to cerebellar cistern, there is no contact with brain stem (arrow); Koos grade 2.

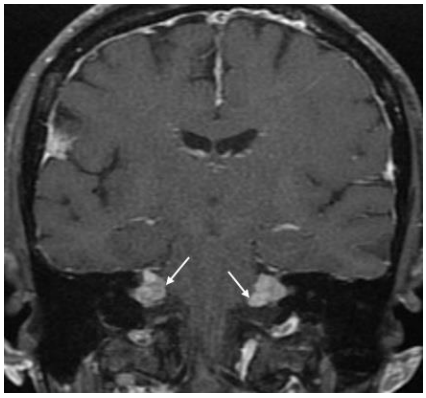

Figure 5: In a patient with NF type 2 bilateral tumors with marked enhancement, lesions are contacting brain stem but there is no compression (arrows); Koos grade 3.

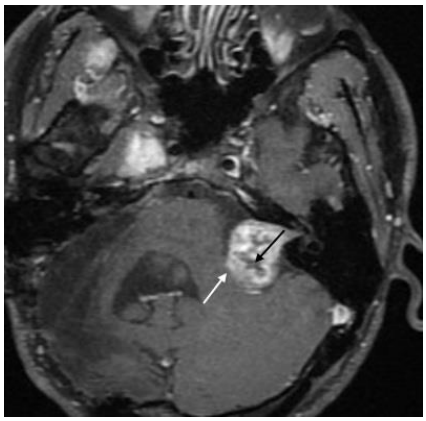

Figure 6: In another patient with NF type 2 left sided tumor with evidence of brain stem compression (white arrow), there is cystic changes in the center of the lesion (black arrow); Koos grade 4.

**Statistical analysis**

Data were analyzed using SPSS software (ver. 26.0; IBM Corp., Armonk, NY, USA). Cohen's Kappa was used to analyse inter-observer agreement of Koos grade. Values ≤ 0 as indicating no agreement and 0.01–0.20 as none to slight, 0.21–0.40 as fair, 0.41– 0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement. Kendall's tau b correlation coefficient was used to analyse correlation between ADC, SI measurements and Koos grade of tumors (p values <0.01 were considered to indicate statistical significance).

## Results

Thirty three patients [21 (61.8 %) females, mean age 52.6±16.6 years] with 34 VSs were included the study. Seventeen of the tumors were right sided and the remaining were left sided. There were bilateral schwannomas in a patient with a diagnosis of Neurofibromatosis type 2. The mean of the maximum tumor diameter was 15.03±7.13 mm. The means of whole tumor ADC and SI values were 1402±363 and 1400±512, respectively (Table 1). The frequencies for each tumor grade from 1 to 4 were, 3 (8.8%), 13 (38.2%), 9 (26.5%), and 9 (26.5), respectively (Table 2). The reliability analyses revealed excellent inter-observer agreement for ADC (ICC: 0.93, 95% CI: 0.87-0.97, p<0.001) and SI (ICC: 0.98, 95% CI: 0.96-0.99, p<0.001) measurements. The percentage agreement for tumor grading was 97%, with only one Grade 3 tumor being judged as Grade 4 by the second reader (Cohen's Kappa 0.96, p<0.001). The maximum size (p<0.001) and ADC values (p=0.039) differed significantly between the tumor grades, however, the SI values remained similar (p=0.125) (Table 2). A correlation was not found between the tumor grade and ADC values (Kendall's tau b correlation coefficient: 0.18).

Table 1: Summary of quantitative parameters.

| | Size (mm) | ADC ($mm^2/s$) observer 1 | ADC ($mm^2/s$ observer 2 | SI observer 1 | SI observer 2 |
|---|---|---|---|---|---|
| Minimum | 3 | $851.0\times 10^{-6}$ | 838.0 | 492.0 | 482.0 |
| Maximum | 32 | $2309.0\times 10^{-6}$ | 2193.0 | 2650.0 | 2619.0 |
| Median | 15 | $1349.0\times 10^{-6}$ | 1329.5 | 1498.0 | 1411.0 |

ADC: apperent diffusion coefficient, SI: signal intensity

## Discussion

This study reports a perfect inter-observer agreement for Koos classification. ADC values differed significantly between Koos grade of VSs, however ADC or SI values were not correlated with Koos grades. According to our knowledge this is the first study that investigates correlation of Koos classification and measurable MRI parameters such as post contrast signal intensity and ADC.

Guidelines offers observation for Koos grade I-II asymptomatic tumors, stereotactic radiosurgery for grade I-II symptomatic tumors and surgery or radiosurgery for grade III-IV tumors [2]. Particularly, Koos grade IV is important in terms of life-threatening brain stem compression and the only treatment option is surgery [2]. The size of the tumor is also important for the post-surgical persistence of facial paralysis which varies between 3 and 46% [11]. The part of the tumor is in the acoustic canal, size of tumor, and which nerve components it affects are the major in determining the surgical method and access. Therefore, MRI plays a big role for guidance the surgery. Koos classification has been widely adopted in terms of surgery and has been the subject of many clinical-surgical correlation studies [7, 9, 12, 13]. Although the classification is completely based on radiologic featured, there are not many radiological studies on this subject.

Table 2: Distribution of schwannomas according to Koos grades and size, ADC and signal characteristics

| | Koos grade | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | p |
| Number of patients, n (%) | 3 (8.8%) | 13(38.2) | 9(26.5) | 9(26.5) | |
| Size (mm) | 3 | 10.5 | 16 | 23.5 | <0.001 |
| ADC observer 1 | 1531±443 | 1330±359 | 1215±319 | 1631±317 | 0.039 |
| ADC observer 2 | 1506±407 | 1304±337 | 1228±255 | 1595±309 | 0.053 |
| SI observer 1 | 720±72 | 1360±537 | 1598±505 | 1542±384 | 0.125 |
| SI observer 2 | 734±15 | 1368±532 | 1529±513 | 1503±375 | 0.117 |

ADC: Apperent diffusion coefficiency, SI:Signal intensity

Idea in the evaluation of ADC and signal intensity values; it was thought that the water content and molecular structure of the growing tumor could be reflected in the measurable values. Size measurement can be misleading due to the different intracanalicular and cisternal components of the tumor and volumetric measurements are required. It is also known that after radiotherapy, there may be a temporary pseudo-size increase secondary to edema [14]. The hypothesis this study based on was; if ADC and/or signal intensity is associated with tumor grade, these parameters can be used for grading and follow-up of VSs. Knugelis KE. et al. reported that VSs, have between 1006-1563 × 10−6 mm2/s ADC values have worser facial nerve outcome after surgery [13]. Also ADC was reported to be useful in differential diagnosis of CPA schwannoma and meningiomas [15]. In current study ADC values showed a statistically significant difference between tumor grades. The reason for the lack of correlation between ADC and grade, may be the small number of patients and the different distribution of the tumor grades.

In previous studies some authors reported T2 signal intensity had association with soft tumor consistency and better facial nerve outcome although not statistically significance had been found [12] while some authors found no association [13]. Recent studies are generally focused on surgical planning, differential diagnosis of CPA tumors and post-operative facial nerve outcome [12, 13, 16]. In current study surgical results and facial nerve outcome were not assessed. Because, the main purpose was to correlate the grade with MRI in the patients in observation and candidate for surgery.

The main limitation of this study was the limited number of patients. And the second limitation was the diagnosis of tumors was not confirmed histo-pathologically.

In conclusion, Koos classification is a practical and useful grading system with excellent inter-observer aggreement for VSs. ADC values differs significantly between the tumor grades, but not correlated with grade. Therefore, it is not yet possible to say that ADC may be used in grading VSs. Nevertheless, it is a promising parameter in surgical planning, approach and predicting patient outcome. Future prospective studies with large patient groups are needed.

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# Does rectal application of Red-Ginseng have an effect on colorectal anastomosis healing? An experimental study

## Rektal yoldan uygulanan Red-Ginseng'in kolorektal anastomoz iyileşmesi üzerinde etkisi var mıdır? Bir deneysel çalışma

**Abdullah Şenlikçi [1], Koray Koşmaz [1], Abdullah Durhan [1], Marlen Süleyman [1], Yusuf Murat Bağ [2], Mevlüt Recep Pekcici [1], Mehmet Şenes [3], İknur Alkan Kusabbi [3], Özlem Tanas Işıkçı [4], Yılmaz Ünal [1], Sema Hücümenoğlu [4]**

Abstract

Aim: To evaluate the possible effects of red-ginseng on colon anastomosis healing in an experimental rat model.

Methods: In Group 1 (sham), laparotomy and colonic mobilization were performed. Colonic transection and anastomosis were performed to Groups 2 and 3. In Group 2 (control), 2 ml of saline was administered rectally to all rats for 10 days. Group 3 (drug group) rats were given 2.5 gr/kg ml red ginseng extract rectally for 10 days.

Results: The rats in the sham group had the highest bursting pressure and the rats in the red ginseng group had higher bursting pressure than in the control group ($p<0.05$). A significant difference was observed only for the total SH level between the control group and the red ginseng group ($p= 0.008$). The red ginseng group had the highest scores for all parameters in the histopathological evaluation and the differences were significant compared to both the sham group and the control group.

Conclusion: Rectally administered red-ginseng significantly increased wound healing parameters and increased anastomotic burst pressure. This is the first study in the literature on the beneficial effects of red-ginseng on colorectal anastomoses.

Keywords: Red-ginseng, anastomosis, antioxidant, rat

Öz

Amaç: Red-ginsengin kolon anastomozu iyileşmesindeki olası etkilerini değerlendirmek.

Metot: Grup 1'de (sham) laparotomi ve kolonik mobilizasyon yapıldı. Diğer 2 grupta (grup 2+3) kolonik transeksiyon ve anastomoz yapıldı. Grup 2 (kontrol)'de tüm ratlara 10 gün boyunca rektal yoldan 2 ml saline verildi. Grup 3 (ilaç grubu)'de tüm ratlara 10 gün boyunca 2,5 gr/kg ml red ginseng ekstresi verildi.

Sonuçlar: Sham grubundaki ratlar en yüksek patlama basıncına sahipti ve red ginseng grubundaki ratlar kontrol grubuna göre daha yüksek patlama basıncına sahipti ($p<0,05$). Kontrol grubu ile red ginseng grubu arasında sadece toplam SH düzeyi için anlamlı bir fark gözlendi ($p= 0,008$). Red ginseng grubu, histopatolojik değerlendirmede tüm parametreler için en yüksek puanlara sahipti ve farklılıklar hem sham grubu hem de kontrol grubu ile karşılaştırıldığında anlamlıydı.

Sonuç: Rektal yoldan uygulanan red-ginseng belirgin olarak yara iyileşme parametrelerini arttırmış, anastomoz patlama basıncını arttırmıştır. Bu red-ginsengin kolorektal anastomozlar üzerindeki yararlı etkileri hakkında literatürdeki ilk çalışmadır.

Anahtar Kelimeler: Red-ginseng, anastomoz, antioksidan, rat

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## Introduction

Anastomotic leakage after colon resection and anastomosis is a serious problem for surgeons. Colorectal anastomotic leakage can cause devastating acute and long-term consequences, such as the need for additional intervention, prolonged hospital stay, and higher morbidity and mortality rates [1]. For many years, studies have been conducted not only for the development of new surgical techniques but also for the evaluation of locally or systemically applied products to prevent anastomotic leakage, but no method that is still widely used has been found as yet [2].

Red ginseng is the boiled root of Panax ginseng CA Meyer and has a long history of use in East Asia as a medicinal herb. Recent studies have reported various effects of red ginseng, including antioxidant, antitumor, antimutagenic, antidiabetic, and immunomodulatory activities [3]. Studies have shown that red ginseng has positive effects on wound healing [4].

Knowing the positive effect of red ginseng on wound healing, the aim of this study was to evaluate the effect of rectal application of red ginseng on rats with colorectal anastomosis.

## Material and methods

All procedures in this experimental study were applied in accordance with the National Laboratory Animals Use and Care Guidelines and approved by the Animal Ethics Committee of Ankara Training and Research Hospital (date of approval: 27.01.2021 , protocol number:0064).

### Animals and Experimental Study Procedure

The study included a total of 30 female Wistar-Albino rats with a mean weight of 230 ± 18 grams. All rats were fed with commercial rat chow and kept in wire cages under standard laboratory conditions. Food was withdrawn from the rats 12 hours before anesthesia and water 2 hours before. The anesthesia and surgical procedure were performed by the same team under sterile conditions. Before the procedure was started, all rats were anesthetized intramuscularly with 50 mg / kg ketamine hydrochloride and 5 mg / kg Xylazine. Enteral and parenteral antibiotics were not given during the study.

The 30 rats were randomly separated into 3 groups of 10. In Group 1 (sham) laparotomy and colonic mobilization were performed (Figure 1). Colonic transection and anastomosis were performed in Group 2 and Group 3 (Figure 2,3). The colonic transection was performed in the descending colon, in a 2 cm area kept 3 cm above the peritoneal reflection. Following the transection, end-to-end anastomosis was applied with 5-0 prolene to all rats by the same physician. The abdominal layers were closed with 3-0 silk continuous sutures. In Group 2 (control), 2 ml of saline was administered rectally by feeding tube (6 F) for 10 days. In Group 3 (drug group), 2.5 g / kg ml of red ginseng extract was administered rectally via a feeding tube (6 F) for 10 days. Prior to the daily administration of saline or red ginseng, anaesthesia of 8 mg / kg ketamine hydrochloride was administered intramuscularly. The rats were placed in the Trendelenburg position and the feeding tube was advanced 5 cm into the anal canal and saline or red ginseng extract was administered.

On the postoperative 11th day, all the rats were sacrificed using high-dose anesthetic. Then, a U-shaped incision was made and the anastomosed segments were resected. In Group 1, the colon segment of the same region was resected. The anastomotic bursting pressure of all the specimens in the 3 groups was measured. After division into 2 parts longitudinally and histopathologically, the tissue malondialdehyde (MDA), total sulfhydryl (t-SH) and catalase (CAT) levels were measured.

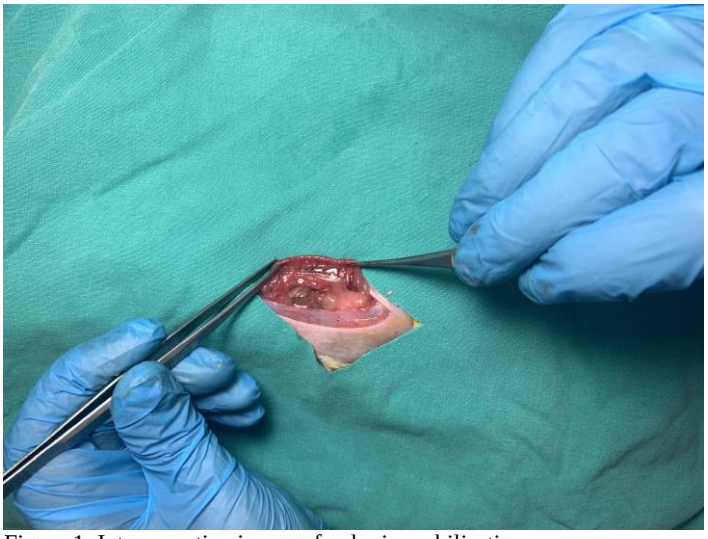

Figure 1. Intraoperative image of colonic mobilisation.

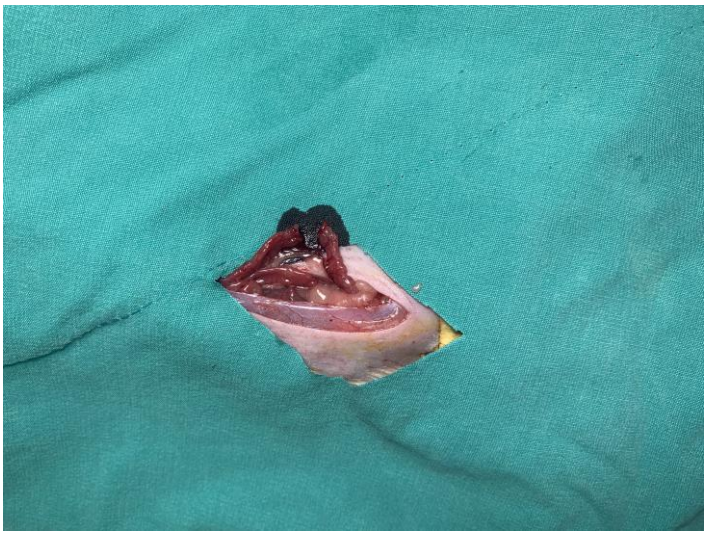

Figure 2. Intraoperative image of colonic transection.

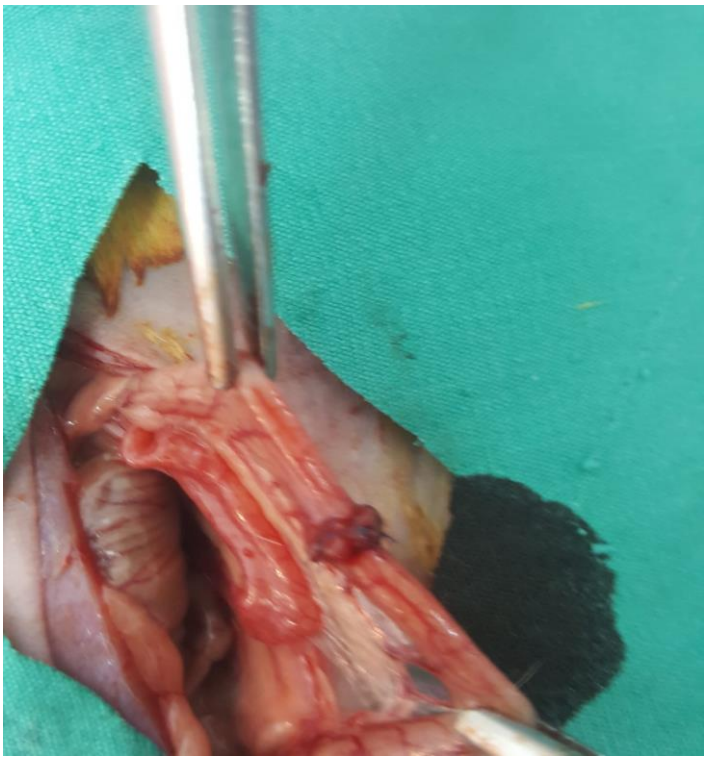

Figure 3. Intraoperative image of colonic anastomosis.

**Anastomotic bursting pressure measurement**

Excision was made to include the surrounding tissue 2.5 cm proximal and distal to the anastomosis. The distal of the anastomosis was tied with a 3-0 silk suture. A plastic catheter was placed on the unattached side and tied with 3-0 silk suture to prevent air leakage. The plastic catheter was connected to a pump connected to a barometer. The bowel segment was placed in a container full of water. The pump pressure was increased, and when air bubbles were observed, this pressure was considered to be the anastomotic bursting pressure, and was recorded in mmHg.

**Measurement of malondialdehyde (MDA), total sulfhydryl (t-SH) and catalase (CAT) levels**

The parameters of oxidative stress were evaluated in the Biochemistry Department of Ankara Training and Research Hospital. Liver tissues were kept at -80°C until the day of the analysis. The malondialdehyde (MDA), total sulfhydryl (t-SH) and catalase (CAT) levels were determined.

Tissues were homogenized in phosphate buffer for MDA, t-SH and CAT measurements.

The levels of MDA were measured using the fluorometric method, as described by Wasowicz et al. [5] Following the reaction that occurs between thiobarbituric acid (TBA) and MDA, the reaction product was isolated in butanol. Then spectrofluorometric measurement was made at a wave length of 547 nm for emission and 525 nm for excitation. The standard was 0 to 5 µmol/L 1,1',3,3' tetraethoxypropane solution. 50 µL homogenate was introduced into 10 ml glass tubes, each containing 1 ml of distilled water in order to measure MDA levels in tissue. A 1 mL solution containing 29 mmol/L TBA was added to acetic acid and mixed. The samples were then exposed to heat of between 95° and 100°C for a period of 1 hour using a water bath. After cooling the heated samples, they were mixed with 25 µL of 5 mol/L hydrochloric acid (HCL) and agitation was used for a period of 5 min to extract the mixture of the reaction using 3.5 mL n-butanol. The butanol phase was separated through centrifugation for 10 min at 1500 g, then a fluorometer (HITACHI F-2500) was used to measure the fluorescence in the butanol extract at 547 nm and 525 nm wavelengths for emission and excitation, respectively. The levels of MDA were shown as nmol/g protein.

Determination of CAT levels was made with spectrophotometric measurement, as defined by Hadwan [6]. The test is for biological samples and depends on the rapid formation of a stable and colored carbonato-cobaltate (III) complex. The method is based on the conversion of cobalt (II) to cobalt (III) by hydrogen peroxide in biological tissues in the presence of bicarbonate. First, the samples were incubated with hydrogen peroxide solution for 2 minutes. The working solution containing the cobalt-bicarbonate reagent was then added. This reagent evaluates unreacted hydrogen peroxide. CAT activity is always directly proportional to the rate of decomposition of hydrogen peroxide. Hydrogen peroxide acts as oxidizing cobalt (II) to cobalt (III) in the presence of bicarbonate ions; this process ends with the production of a carbonato-cobaltate (III) complex ([Co (CO3) 3] Co). The final product formed has two maximum absorbance wavelengths: 440 nm and 640 nm. The 440 nm wavelength was used to evaluate CAT activity. The value of CAT activity was determined using the first order reaction rate constant as kU. CAT activity level was reported as kU/g protein.

The t-SH measurement was performed according to the principle described by Taylan et al, in which this method was applied by adapting the Ellman reaction to the microplate method [7]. Methanolic 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) solution, which reacts with sulfhydryl groups, was used in this study. The reaction products were measured using a multi-measurement mode microplate reader containing the monochromator. The measurement was carried out at 412 nm. 200 µL of Tris buffer (0.25 M Tris-HCl, pH 8.2 containing 20 mM EDTA) was pipetted and 25 µL of homogenate was added. 10 µL of DTNB reagent (4 mg / mL in methanol) was added to each well and incubated for 15 minutes at room temperature, then the absorbance of yellow 5-thio-2-nitrobenzoic acid (TNB) at 412 nm (A2) was measured against blank (A1). Glutathione standards were also tested under the same conditions, and the curve was generated by linear regression analysis. The content of the t-SH group in the samples corresponding to the net absorbance (A2-A1) was calculated from the standard curve generated using reduced glutathione (GSH) (between 0 and 450 µM). t-SH levels are given as µmol/gr protein.

**Histological Evaluation**

The anastomosis line was incised longitudionally and fixed in 4% formaldehyde solution and embedded in paraffin with a routine procedure. Sections 3 mm in thickness were cut from tissue blocks and stained with hemotoxylin and eosin. The anastomosis line was graded in a blinded fashion using the Ehrlich and Hunt numerical scale (8) from 0 to 4, as modified by Philips et al (9). Inflamatory cell infiltration, fibroblast activity, neo-angiogenesis and collagen deposition were graded from 0 to 4 as follows: 0:no evidence, 1: occasional evidence, 2: light scattering, 3: abundant evidence and 4: confluent cells or fibers (Figure 4-6).

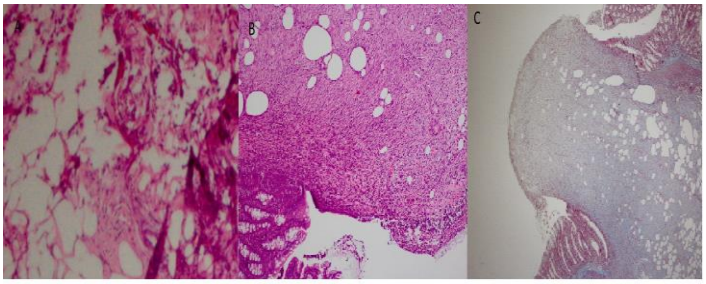


Figure 4. Histopathological appearance of the control group (H&E x4 magnification, H&E x10 magnification, trikrom x4 magnification).

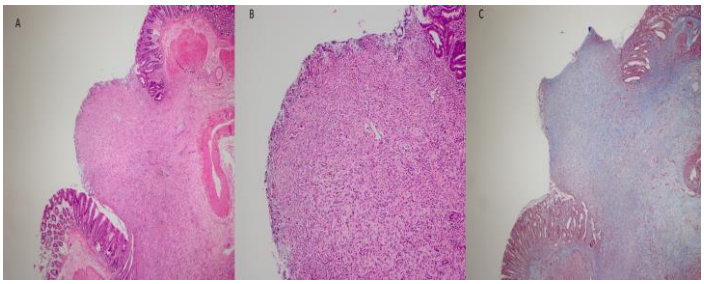


Figure 5. Histopathological appearance of the red-ginseng group (H&E x4 magnification, H&E x10 magnification, trikrom x4 magnification)

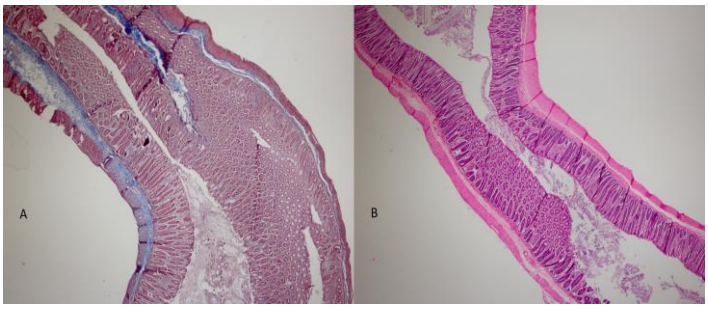


Figure 6. Histopathological appearance of sham group (H&E x4 magnification, trikrom x4 magnification).

**Statistical analysis**

The conformity of numerical variables to normal distribution was analyzed with the Shapiro-Wilk test. Numerical variables were given as mean ± standard deviation or median (minimum-maximum) values and were compared with the one-way ANOVA test followed by Tukey's HSD post hoc test or the Kruskal-Wallis test followed by Tamhane's T2 post hoc test, as appropriate. A value of $p< 0.05$ was considered statistically significant. Data analysis was performed using IBM SPSS Statistics for Windows, version 25.0 software (IBM Corp., Armonk, N.Y., USA).

## Results

No mortality or complications occurred during the postoperative period.

The anastomotic bursting pressure values are given in Table 1. The rats in the sham group had the highest bursting pressure and the rats in the red ginseng group had higher bursting pressure than in the control group. The differences between the control group and the sham group and the red ginseng group were statistically significant ($p= 0.002$ and $p=0.013$, respectively).

Table 2 shows the MDA, catalase, and total SH levels in the anastomosis. No significant difference was determined between the groups in respect of MDA levels. Catalase and total SH levels were significantly higher in the sham group compared to the control group ($p= 0.017$ and $p= 0.002$, respectively), with a significant difference observed only for the total SH level between the control group and the red ginseng group ($p= 0.008$).

The histopathological scores of the anastomosis are summarized in Table 3. The red ginseng group had the highest scores for all parameters and the differences were statistically significant compared to both the sham group and the control group. In addition, with the exception of inflammation, the control group had significantly higher scores for all the parameters compared to the sham group.

Table 1. Results and comparisons of anastomotic bursting pressure.

| | Sham group (Group 1) (n=10) | Control group (Group 2) (n=10) | Red ginseng group (Group 3) (n=10) | P Value |
|---|---|---|---|---|
| Bursting pressure (mm Hg) | 193.00 ± 25.84 | 157.50 ± 22.72 | 185.50 ± 12.79 | 0.001* |
| | p (1-2) | p (1-3) | p (2-3) | |
| | 0.002* | 0.693* | 0.013* | |

* One-way ANOVA test followed by Tukey's HSD post hoc test.

Table 2. Malondialdehyde, catalase, and total sulfhydryl levels in the anastomosis.

| | Sham group (Group 1) (n=10) | Control group (Group 2) (n=10) | Red ginseng group (Group 3) (n=10) | P value |
|---|---|---|---|---|
| MDA | 77.84 ± 13.84 | 101.66 ± 22.72 | 87.66 ± 39.90 | 0.174* |
| Catalase | 811.94 ± 134.29 | 519.35 ± 161.88 | 733.62 ± 319.53 | 0.018* |
| Total SH | 201.06 (116.53 -255.36) | 118.32 (83.25-130.67) | 184.08 (146.72-360.22) | <0.001** |
| | p (1-2) | p (1-3) | p (2-3) | |
| MDA | 0.152* | 0.711* | 0.504* | |
| Catalase | 0.017* | 0.710* | 0.095* | |
| Total SH | <0.001** | 0.002** | 0.829** | |

MDA: Malondialdehyde, SH: Sulfhydryl, * One-way ANOVA test followed by Tukey's HSD post hoc test, ** Kruskal-Wallis test followed by Tamhane's T2 post hoc test.

Table 3. The histopathological scores of the anastomosis.

| | Sham group (Group 1) (n=10) | Control group (Group 2) (n=10) | Red ginseng group (Group 3) (n=10) | P value |
|---|---|---|---|---|
| Inflammation | 2 (2-2) | 3 (1-4) | 4 (3-4) | <0.001* |
| Fibroblastic activity | 1(1-1) | 3 (3-4) | 4 (3-4) | <0.001* |
| Neoangiogenesis | 0 (0-0) | 2 (2-3) | 3 (2-3) | <0.001* |
| Collagen deposits | 0 (0-0) | 3 (2-4) | 4 (3-4) | <0.001* |
| | p (1-2) | p (1-3) | p (2-3) | |
| Inflammation | 0.360* | <0.001* | 0.012* | |
| Fibroblastic activity | <0.001* | <0.001* | 0.015* | |
| Neoangiogenesis | <0.001* | <0.001* | 0.015* | |
| Collagen deposits | <0.001* | <0.001* | 0.034* | |

* Kruskal-Wallis test followed by Tamhane's T2 post hoc test.

## Discussion

The findings of this study show that red ginseng has positive effects on anastomosis healing. In the group applied with red ginseng, anastomotic burst pressures were higher, and this was observed to have contributed to the healing of anastomosis histopathologically.

Anastomotic leakage is still one of the main causes of morbidity and mortality in surgical patients after colorectal surgery. The incidence of anastomotic leakage varies between 1-26%. Good oxygenation at all stages is important for wound healing. During the inflammatory phase, polymorphonuclear cells require oxygen to produce superoxide free radicals to kill microorganisms and avoid infection [10]. Anastomotic leakage is difficult to treat and may require relaparotomy and stoma opening. Therefore, it is most important to prevent anastomotic leakage. Numerous experimental and clinical studies have also been conducted to achieve better wound healing after colon anastomosis [11].

Burst pressure is an accurate method for the evaluation of anastomotic healing in the early period. The best known mechanical measurement methods are burst pressure, burst wall pressure and tensile strength measurement. As the burst method only assesses transmural pressure, it is closely related to the clinical situation [12]. In the current study, anastomotic burst pressure was used for anastomosis evaluation and a statistically significantly higher burst pressure was observed in the red ginseng group than in the control group.

Inflammation plays a central role in the regulation of anastomotic healing. In the inflammatory phase, white blood cells are involved in the mobilization and activation of fibroblasts and epithelial cells [13]. Chemotactic agents, cytokines and inflammatory cells are concentrated in the anastomotic area from the second postoperative day [14]. In the current study, inflammation was more common in the red ginseng group than in the sham group and control group. Collagen is an essential protein at all stages of the wound healing process. Anastomotic integrity strength is essential for complete anastomotic healing. Matrix metalloproteinase 8 (neutrophils, macrophages, and wound-side bacteria) and de novo collagen synthesis form the amount of wound collagen in the first three days of wound healing [15]. The current study result showed that the amount of collagen in the red ginseng group was higher than in the other groups.

Ginseng (Panax ginseng C.A. Meyer, root of Araliaceae) is often used as a raw material and taken orally as a traditional medicine in Asian countries. When ginseng is poured at 98-100°C, it is called red ginseng, the main component of

which is ginsenoside Rg3 and ginsenoside Rb1 [16]. Considering the mechanism of action of ginsenosides, anti-inflammatory effects have been proven by purifying ginsenosides. Expressions of pro-inflammatory cytokines (TNF-alpha, IL-1 and IL-6) have been shown to induce M1 and M2 polarization of macrophages or microglia while inhibiting iNOS and COX-2 enzyme expressions, which was found to be the anti-inflammatory mechanism of ginsenosides [17]. In a study by Kosmaz et al., red ginseng was given to rats that underwent splenectomy, and its antioxidant and anti-inflammatory activity was demonstrated. In that study, red ginseng was shown to have significantly reduced MDA values, which indicate intracellular oxidative stress, and significantly increased the level of catalase, an important antioxidant [18]. In another study by Durhan et al., in which oral red ginseng was evaluated in experimental occlusive jaundice, it was observed that MDA value decreased and catalase value increased [17]. However, in the current study, MDA value decreased and catalase value increased, but no statistically significant difference was observed between the control and red ginseng groups.

In conclusion, the results of this study demonstrated that rectal administration of red-ginseng increased the anastomotic burst pressure and provided histopathological improvement. Although it decreased the MDA level, which indicates oxidative stress, and increased the antioxidant catalase level, no statistically significant difference was observed. Although the positive effects are thought to be due to its antioxidant and anti-inflammatory properties, it would be appropriate to conduct more extensive studies on the subject.

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# Experimental comparison of autograft and DBM Flex (Grafton) for spinal lumbar fusion in rabbits

## Tavşanlarda spinal lomber füzyon için otogreft ve DBM Flex'in (Grafton) deneysel karşılaştırması

**Cem Demirel [1], Dursun Türköz [2], Tuncal Yılmaz [2]**

Abstract

Aim: The choice of graft materials used for spinal fusion possesses a great importance due to their crucial roles in bone remodelling. Although autogenous bone grafts are the "gold standard" for spinal fusion surgeries, they can cause various complications. Aim of this study was to compare the efficacy of demineralized bone matrix (DBM) and autograft in lumbar spinal fusion in a rabbit model of spinal lumbar fusion (SLF).

Methods: Twenty New Zealand rabbits were randomly divided into two groups and underwent SLF by using either iliac crest autologous bone graft (Autograft, n=10) or DBM Flex (Grafton, n=10). Eight-weeks after surgery, animals were sacrificed and spinal fusion was evaluated by computerized tomography (CT), manual palpation, macroscopic analyses, and histological assessments.

Results: CT results revealed that autograft led to significantly higher fusion scores than DBM Flex (p=0.0004). Mobility was significantly lower in autograft group (p=0.0007). Significantly lower bone formation scores were observed in DBM Flex group compared to autograft group (p<0.0001). Histology of spine in the autograft group was significantly better than DBM Flex group (p=0.0002).

Conclusion: Autograft was superior than DBM flex in SLF and these results indicate that autograft will continue to be the "gold standard" in SLF in the future.

Keywords: Autograft, DBM Flex, Demineralized bone matrix, Grafton, Spinal fusion surgery

Öz

Amaç: Spinal füzyon için kullanılan greft materyallerinin seçimi, kemiğin yeniden şekillenmesindeki önemli rolleri nedeniyle büyük önem taşımaktadır. Otojen kemik greftleri spinal füzyon ameliyatlarında "altın standart" olmasına rağmen çeşitli komplikasyonlara neden olabilir. Bu çalışmanın amacı, tavşan spinal lomber füzyon (SLF) modelinde demineralize kemik matrisi ve otogreftin etkinliğini karşılaştırmaktı.

Yöntem: Yirmi Yeni Zelanda tavşanı rastgele iki gruba ayrıldı ve iliak krest otolog kemik grefti (Autogreft, n=10) veya DBM Flex (Grafton, n=10) kullanılarak SLF uygulandı. Ameliyattan sekiz hafta sonra hayvanlar sakrifiye edildi ve spinal füzyon bilgisayarlı tomografi (BT), manuel palpasyon, makroskopik analizler ve histolojik değerlendirmelerle değerlendirildi.

Bulgular: BT sonuçları, otogreftin DBM Flex'ten anlamlı derecede daha yüksek füzyon skorları sağladığını ortaya koydu (p=0,0004). Hareketlilik otogreft grubunda anlamlı olarak daha düşüktü (p=0,0007). DBM Flex grubunda, otogreft grubuna kıyasla anlamlı derecede daha düşük kemik oluşum skorları gözlendi (p<0,0001). Otogreft grubundaki omurga histolojisi, DBM Flex grubuna göre anlamlı olarak daha iyiydi (p=0,0002).

Sonuç: Otogreft, SLF'de DBM flex'ten daha üstün bulunmuştur ve bu sonuçlar da otogreftin gelecekte de SLF'de "altın standart" olarak kabul edileceğini göstermektedir.

Anahtar kelimeler: Otogreft, DBM Flex, Demineralize kemik matrisi, Grafton, Spinal füzyon cerrahisi

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## Introduction

Functional spinal unit (FSU) is defined as the smallest segment exhibiting biomechanical characteristics of entire spine and consists of two adjacent vertebrae, intravertebral disc, facet (zygapophyseal) joints, joint capsules and supporting ligamentous structures [1]. Spinal instability is the development of pain and neurological deficits that prevent daily activities due to the inability of the spine to maintain its integrity, to prevent abnormal displacements, to prevent damage to the spinal cord and nerve roots under physiological loads on the spine as a result of acute or chronic insufficiency in FSU [2].

Spinal fusion surgeries with or without instrumentation have been widely performed to treat the patients with spinal pathologies. There are numerous factors such as surgical methods, graft materials, postoperative rehabilitation [3] and comorbidities [4] may affect the success of the fusion surgery. Since the fusion does not occur in a short time after fusion surgery is performed and systemic complications due to long-term immobilization may develop, use of spinal instrumentation with fusion surgery has been brought to the agenda [5, 6]. On the other hand, instrumentation without fusion is not a long-term solution for fixation as in a long-term, loosening will occur, therefore, long-term stabilization will only be possible with osseous fusion [7, 8]. The choice of the graft materials used for the spinal fusion possesses a great importance as they play important roles in bone remodelling and therefore, an optimal graft should have the properties as osteoconductive, osteoinductive and osteogenic [9].

Autogenous bone grafts, the grafts that have been harvested from another anatomical location of the patient and transplanted to another location, have been considered as the "gold standard" for spinal fusion surgeries [10]. Although the success rate of them with strong fusion capacity without the risk of rejection, they have some disadvantages such as the variations in graft quality due to the metabolism and age of the patients [11] and risk of blood loss and pain at the site of harvest [12], and autograft harvest-associated morbidity was estimated to be observed in 10-39% of the patients [13].

The alternatives materials for autografts including allograft, demineralized bone matrix (DBM), various synthetic materials such as calcium sulphates and calcium phosphates growth factors and cell- or platelet-based therapies have become alternatives for the autografts [9, 14]. DBM is the bone that has been treated with acid to demineralize the bone while keeping the organic matrix and growth factors intact [15]. Its osteoconductive and osteoinductive capacities are limited [15, 16], however, it has no osteogenic capacity [15]. On the other hand, DBM does not lead to any immunological reaction in the host because of acid treatment during the preparation of the material [17]. Moreover, DBM has been shown to induce spine fusion successfully in the experimental animals [18, 19].

In this study, we aimed to compare the efficacies of DMB flex (Grafton) and autograft on a model of posterior spinal incision of New Zealand white rabbits.

## Material and methods

### Experimental Groups and Animal Husbandry

This study was approved by the local Animal Experiments Local Ethics Committee. Skeletally mature 20 New Zealand White Rabbits (3 - 4 kg body weight) with both sexes were randomly divided into two groups as DMB flex (n = 10) and iliac crest autograft (n = 10) groups. All animals were housed under controlled temperatures (21 ± 1°C) and controlled lighting conditions (12-h light/ dark cycle) in individual cages with ad libitum access to food and water.

### Surgical Procedures

One hour previous to the experiments, each animal was coded with a number and received 20 mg/kg cefazolin as antibiotic prophylaxis. For surgery, animals were anesthetized by intraperitoneal administration of 3 mg/kg xylazine (Rompun®- Bayer) and 40 mg/kg ketamine hydrochloride (Ketalar®- Parke Davis). After anaesthesia was achieved, animals were positioned in prone position on operating table, surgical site was shaved, and skin was cleaned with 10% povidone iodine solution.

In DBM flex group of animals, a five cm midline incision was carefully performed, and subcutaneous layers were dissected. Fascia was opened from sinister to the midline in line with the L4-L6 vertebrae. Lamina of L5 and facet joints at L4/5 and L5/6 were decorticated by using a high-speed burr with 3 mm ball-end. DBM flex (Grafton) with an approximate size of 20 x 15 mm was grafted between the facet joints, and closure of fascia and subcutaneous tissues was achieved by using 3/0 vicryl sutures and skin closure was achieved by using 3/0 silk suture following homeostasis. The incisions were cleaned with 10% povidone iodine solution and animals were placed in their individual cages.

In iliac crest autograft group of animals, a five cm midline incision was carefully performed. After lumbar fascia was dissected bilaterally, the soft tissues on the iliac crest was dissected carefully. The bone graft with a size of 20 x 15 x 4 mm was harvested by bone rongeur. Homeostasis of the iliac crests was achieved by bone wax application. The autograft bone material was decorticated by using a high-speed burr with 3 mm ball-end. Lamina of L5 and facet joints at L4/5 and L5/6 were bilaterally decorticated by using a high-speed burr with 3 mm ball-end. Surgical region was cleaned, homeostasis was achieved, and iliac crest graft was placed. The closure of fascia and subcutaneous tissues was achieved by using 3/0 vicryl sutures and skin closure was achieved by using 3/0 silk suture following homeostasis. The incisions were cleaned with 10% povidone iodine solution and animals were placed in their individual cages.

### Postoperative Care

After the surgery, 2 mg/kg meperidine for analgesia and 20 mg/kg cefazolin sodium for antibacterial activity were administered intraperitoneally to each animal. General condition, neurological findings, mobility and infection findings in the animals were closely monitored and recorded for a week. Neurological deficits and alteration vital signs were not observed in any of the subjects in the early postoperative period. Animals were fed with standard diet and followed for eight weeks. Eight weeks later the animals were sacrificed by high dose lysthenon administration.

### Computerized Tomography

After sacrification, animals were taken to the radiology department and surgical region was scanned by thin slice computerized tomography (CT) and reconstructed into 3D images. Images were evaluated in terms of fusion and bone

formation. The fusion was scored as follows: net fusion = 2, likely fusion = 1, no fusion = 0.

**Manual Palpation and Macroscopic Evaluation of Bone Formation**

After CT scans, the spinal region between L7 and L2 including the objective lumbar spine (L4-L6) was dissected and surrounding soft tissue was removed. Fusion was assessed by manual palpation as previously described [20, 21]. The motion was assessed by a blinded examiner, and in case of that no motion was detected in the segments (L4/5 and L5/L6), the implanted graft was assessed as fused and scored as 2. If there was a motion in the segments assessed, the implanted graft was assessed as not fused and scored as 1.

Bone formation was also evaluated macroscopically and scored as follows: no new bone formation = 0, minimal new bone formation = 1, moderate new bone formation = 2, obvious new bone formation = 3.

**Histological Assessments**

The spinal segment that was dissected en bloc and fixed in 10% formalin solution for 24 h and dehydrated in ethanol solutions. After dehydration, harvested samples were decalcified in 10% nitric acid for seven days and embedded in paraffin. Longitudinal slices with five µm thickness were taken from the paraffin embedded samples and were stained with Hematoxylin and Eosin (H&E) by using the standard protocol. The success of the fusion was evaluated by an experienced pathologist who were blinded to groups as previously described by Emery et al. [22].

**Statistical Analysis**

Statistical analyses were conducted by using the Statistical Package for the Social Sciences (SPSS) version 15.0 (IBM). Data are expressed as mean ± standard deviation (SD). Normal distribution of the data was evaluated by Shapiro-Wilk normality test. Data with normal distribution was evaluated by independent Student's t-test while the data without normal distribution was evaluated by Mann-Whitney U test. The associations between the variables were evaluated by Chi Square test. A p value lower than 0.05 was accepted as statistically significant.).

## Results

**Computerized Tomography**

CT results revealed that one animal had likely fusion and the rest of the animal did not have any fusion in DBM Flex group (Figure 1A). On the other hand, six animals received autograft had net fusion, three animals had likely fusion and one animal had no fusion (Figure 1B). Mean fusion scores was significantly higher in the autograft group compared to DBM Flex group (Figure 1C; p = 0.0004).

**Manual Palpation and Macroscopic Evaluation of Bone Formation**

Examination by manual palpation showed that in iliac crest autograft group, no motion was observed in any of the animals while in DBM Flex group, motion was detected in eight of the animals (p = 0.0007).

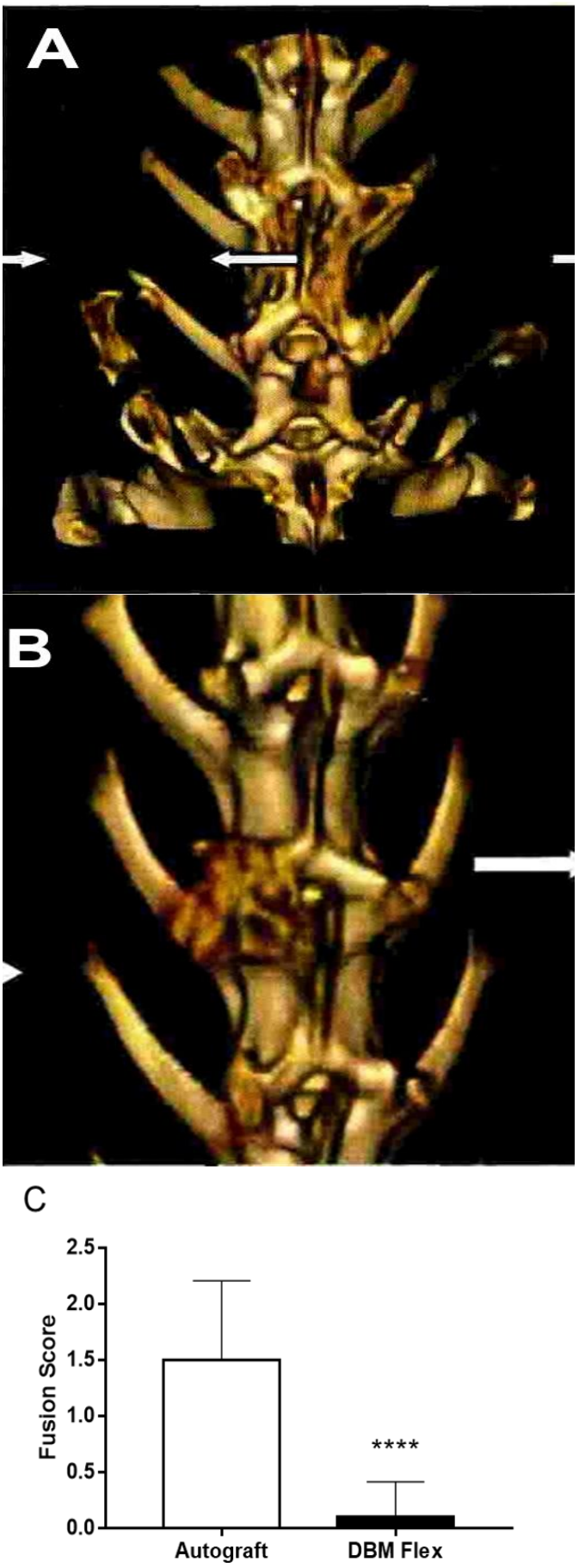


Figure 1. Representative 3D reconstruction of CT images of (A) Autograft, (B) DBM Flex groups. Arrows indicate the grafting region and bone formation four weeks after the grafting. (C) Fusion score of both groups. (Statistical analyses: Mann-Whitney U test. ***p = 0.0004).

DBM Flex grafting led to no new bone formation in nine of the animals and minimal new bone formation in one animal in the macroscopic evaluation of the bone formation. However, in the autograft group, an obvious new bone formation was observed in nine animals and moderate new bone formation in one animal ($p < 0.0001$).

**Histological Assessments**

DBM Flex group of animals exhibited significantly lower histopathological score (1.20 ± 1.40) compared to autograft group of animals (3.50 ± 2.07; Figure 2A - C; $p = 0.0002$).

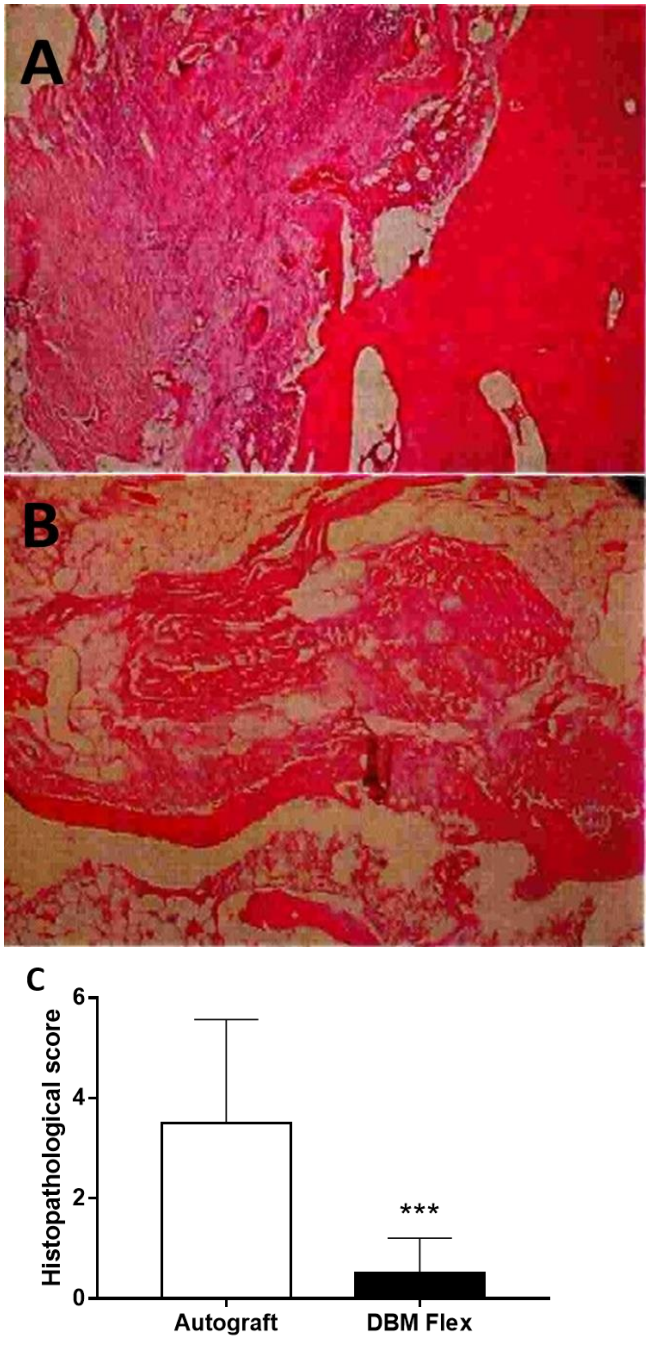


Figure 2. Representative histopathological images of (A) autograft group and (B) DBM Flex group. (C) Histopathological scores of both groups. (Statistical analyses: Mann-Whitney U test. ***p = 0.0002).

## Discussion

Achieving satisfactory spinal fusion has been the most important issue in the surgery of spinal pathologies [14, 23, 24]. Today, although we can provide spinal stabilization in the early period by using complicated spinal instruments, the success in long term is achieved with the development of fusion. In this study, we compared the efficacy of autograft and DBM Flex (Grafton) in spinal fusion and found that autograft is superior than DBM Flex in spinal fusion.

The most commonly used substitute materials in spinal fusion are autograft and allograft bones [25] and autogenous bone graft has been implicated as the "gold standard" for spinal fusion grafting [10]. Iliac crest bone grafts are the most commonly used substitute in spinal fusion [26]. Although autografts have various advantages including promotion of extensive fusion with perfect histocompatibility, autograft harvest-associated morbidities can be observed in a significant part of the patients [13]. Therefore, use of different graft materials were required in spinal fusion surgeries.

DBM is a synthetic substitute that is particularly osteoinductive and is partially osteoconductive [23]. On the other hand, bone formation was indicated to be supported by DBM through osteoconductive mechanisms [19]. The results regarding the success achieved in spinal fusion by use of DBM are variable. A study conducted by Cook et al. on dogs revealed that either alone or together with allograft, DBM is not effective in formation of stable spinal fusion [27]. Helm et al., in their study indicated that DBM has an inhibitory role on spinal fusion in dogs [28]. In rabbits, although autograft was found to yield higher spinal fusion rates compared to allograft and allograft together with DBM, the difference between the groups were not significant [29]. However, several animal studies indicated that different forms of DBM act as a graft extender/enhancer and provide superior fusion rates than autograft only [19, 30]. Similar controversial results regarding the success after DBM grafting in spinal fusion surgery were also obtained by various clinical studies [31-37]. On the other hand, autograft alone was shown to induce bone formation to a higher extent than DBM alone in dogs [30].

Scoring was used in all three examinations, since spinal fusion has stages of inflammation, vascularization, osteoinduction, osteoconduction and remodeling. We observed that autograft yielded significantly higher lumbar spinal fusion than DBM Flex as revealed by the manual palpation and fusion score. Moreover, use of autograft for spinal fusion surgery led to a significantly better histology than DBM Flex grafting. This might be attributed to the extensive osteoinductive, osteoconductive and osteogenic properties of autografts [38-40].

In conclusion, our results suggest that autograft is superior than DBM Flex in spinal fusion. In our opinion, use of autograft in spinal fusion surgery will continue to be the "gold standard" for spinal fusion in the future. Further studies to find a suitable and efficient candidate substitute for autografts are required to avoid the complications that may arise due to autologous bone harvesting.

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# Timing of percutaneous endoscopic gastrostomy in COVID-19 infection: Endoscopic surgery unit experience

## COVID-19 enfeksiyonunda perkütan endoskopik gastrostomi zamanlaması: Endoskopik cerrahi ünite deneyimi

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Abstract

Aim: The COVID-19 pandemic has negatively affected the whole world and health systems. Although the literature includes recommendations regarding the timing of Percutaneous endoscopic gastrostomy (PEG) in COVID-19 patients, there are no significant clinical studies yet. Therefore, we aim to contribute to the literature by sharing our data on this subject.

Materials and Methods: Patients who underwent PEG between March 2020 and March 2021 were retrospectively evaluated in our clinic. The patients were compared statically in terms of age, gender, medical indications, comorbid diseases, hospitalization in the intensive care unit (ICU), blood tests, and post-intervention complications. PEG was inserted routinely in PCR-negative patients. Patients who underwent PEG were compared as outpatients and inpatients in the ICU. Moreover, patients who underwent PEG while hospitalized in the ICU were divided into two groups according to the presence of COVID-19 infection; patients noninfected with COVID-19 (group 1) and COVID-19 infected patients (group 2).

Results: PEG was performed in 66 patients during the COVID-19 pandemic. These patients predominantly consisted of those with SVH, Alzheimer's, or traumatic brain injury. In the present study, thirty-two (%48.5) patients were female with a mean age of 69.4±17.6, and forty-seven patients underwent PEG in the ICU. Furthermore, eleven of these patients were COVID-19, infected patient group (group-2). There was no statistical difference in blood albumin levels, CRP, hemogram results, and 30-day mortality results between group 1 and group 2 (P>0.05).

Conclusion: PEG is a minimally invasive intervention that is commonly used for enteral feeding. The timing of the procedure is crucial for inpatients with COVID-19. Although the most appropriate timing is the 30th day after the COVID-19 infection process, we think that PEG may insert on the 10th day in eligible patients.

Keywords: Percutaneous Endoscopic Gastrostomy, COVID-19, infection, Cerebrovascular disease, minimally invasive

Öz

Amaç: COVID-19 pandemisi tüm dünyayı ve sağlık sistemlerini olumsuz şekilde etkisi altına almıştır. Literatürde COVID-19 hastalarında Perkütan endoskopik gastrostomi (PEG) zamanlaması ile ilgili öneriler yer alsa da henüz önemli bir klinik çalışma bulunmamaktadır. Bu konudaki verilerimizi paylaşarak literatüre katkı sağlamayı amaçlıyoruz.

Gereç ve Yöntem: Mart 2020-Mart 2021 tarihleri arasında PEG takılan hastalar geriye dönük olarak değerlendirildi. Hastalar yaş, cinsiyet, tıbbi endikasyonlar, eşlik eden hastalıkları, kan testleri ve müdahale sonrası komplikasyonlar ile işlem sırasında hastanın yoğun bakım ünitesinde olup olmadığı kaydedildi. PCR negatif hastalarda rutin olarak PEG yapıldı. PEG uygulanan hastalar ayaktan ve yoğun bakım ünitesinde yatan hastalar olarak karşılaştırıldı. Daha sonra yoğun bakım ünitesinde yatarken PEG uygulanan hastalar, COVID-19 enfeksiyonu varlığına göre iki gruba ayrıldı. ( grup-1 ve grup-2)

Bulgular: Pandemi sırasında uygun endikasyonları olan 66 hastaya PEG yerleştirildi. Bu hastalar ağırlıklı olarak SVH, Alzheimer veya travmatik beyin hasarı olanlardan oluşuyordu. Çalışmada otuziki (%48.5) hasta kadındı ve yaş ortalaması 69.4±17.6 idi. Ayrıca, kırkyedi hastaya yoğun bakımdayken PEG uygulandı; bu hastalardan 11'i COVID-19 enfekte hasta grubuydu (grup-2). Grup-1 ve grup-2 arasında kan albümin düzeyleri, CRP, hemogram sonuçları ve 30 günlük mortalite sonuçları açısından istatistiksel fark yoktu (P>0.05)

Sonuç: Minimal invaziv bir işlem olan peg enteral beslenme amacıyla yaygın olarak kullanılmaktadır. COVID-19 enfekte hastalara işlemin zamanlaması önemlidir. En uygun zamanlama enfeksiyon sürecinden sonraki 30. gün olmakla birlikte uygun hastalarda 10. Gün peg uygulanabilceğini düşünmekteyiz.

Anahtar Kelimeler: Perkütan Endoskopik Gastrostomi, COVID-19, enfeksiyon, Serebrovasküler hastalık, minimal invaziv

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## Introduction

Coronavirus, which has spread rapidly worldwide, is a respiratory RNA virus that can lead to clinical issues with severe respiratory failure [1]. Symptomatic patients often transmit the infection through sizeable droplets produced during coughing and sneezing [2]. As a result of this global enigma, new guidelines to protect patients and healthcare professionals from confirmed and suspected cases are frequently issued. At the same time, health authorities strive to establish the optimal approach [3]. Recommendations for invasive surgical procedures in patients infected with COVID-19 have also been reviewed, and a delay to surgery of 3-4 weeks is recommended in the case of patients who cannot be fed orally and so require feeding tube insertion [4].

In 1980, Gauderer et al. described the percutaneous endoscopic gastrostomy (PEG) primarily for elderly patients with swallowing difficulties linked to neurological disorders [5,6]. This minimally invasive method is more advantageous than other procedures, providing a secure long-term feeding route with low morbidity and complication rates [7,8]. Therefore, its efficiency and practicality make PEG a preferred intervention for long-term enteral nutrition [9].

Currently, the literature includes minimal data on the topic of placing PEG in COVID-19 patients. Therefore, this study aims to evaluate PEG applications in our center over one year during the pandemic and present our data on PEG timing in patients infected with COVID-19.

## Material and methods

### Patients and Ethics

This study includes patients who underwent PEG in the General Surgery Endoscopy unit between March 2020 and March 2021, incorporating the first and second pandemic waves. The PEG procedure was performed on patients with gastrointestinal system continuity but could not take oral nutrition for about 30 days. Data concerning patient age, gender, medical indications, comorbid diseases, hospitalization in intensive care unit (ICU) at the time of the procedure, laboratory parameters, PCR tests, and complications were accessed from patient records and documented. First, the patients were compared as inpatients and outpatients in the ICU. Second, the patients were divided into two groups: patients in the intensive care unit not infected with COVID-19 (group 1) and patients infected with COVID-19 (Group 2). The groups were compared in terms of clinical features. Approval for this study was granted by the ethics committee of Sakarya University (05/03/2021; 71522473-050.01.04-21455-212).

### Patient selection process, diagnosis of COVID-19, and prevention of infection

PEG was administered to patients who were PCR negative. The procedure was not performed on patients with impaired bleeding profiles or symptoms of sepsis. COVID-19 diagnosis was made in symptomatic patients through PCR positivity and thorax computed tomography (CT) findings. All patients were given a COVID-19 PCR test before the procedure. In both PCR-positive patients and cases diagnosed with COVID-19 by tomography, PEG was delayed until after a negative PCR could be obtained, and symptoms of the disease had passed. While standard precautions were taken for non-virus-infected patients (wearing of masks by all team members and 30-minute room ventilation), special precautions were taken for infected patients (N95 mask, visor, disposable gowns, 1-hour room ventilation, and disinfection of the room after the procedure).

### PEG procedure

Before the procedure, patients were monitored, and nasal oxygen was initiated at 4-6 L/minute. The patients received 1-2 mg midazolam, and one mcg/kg fentanyl, with 0.5 mg/kg propofol added after the procedure had started for sedation. The cases got a lidocaine hydrochloride spray and 2cc prilocaine to the skin at the incision site for local oropharyngeal anesthesia. Antibiotic prophylaxis with cefazolin sodium was administered to the patients, except for cases already under antibiotic treatment. The patients did not receive enteral feeding for at least 8 hours before the procedure. An experienced team performed the process in the general surgery endoscopy unit.

The PEG procedure was performed by applying the "pull-through" technique by observing the indentation made by the finger and the transillumination created by the endoscope through the stomach. As a standard, a 20Fr PEG tube was placed in all patients. All interventions were made using a Fujinon VP-4450HD (Fujifilm company, Minato-ku, Tokyo, Japan) fiber endoscope. Enteral feeding was started 16 hours after the feeding tube was inserted.

In the present study, one patient whose PCR test was positive during the peg procedure and three patients who could not have the PEG procedure were excluded. In addition, while endoscopic gastric tube revisions were included in the study, gastric tube replacements performed without endoscopy were excluded.

### Statistical analysis

The frequency of sociodemographic, clinical data, and descriptive statistics were calculated as numbers, distribution, and percentages. The control groups, continuous variables with normal distribution were compared using the independent sample t-test; variables not showing a normal distribution with the Mann-Whitney U test, and independent group rates were compared using Fisher's exact test. A p-value $<0.05$ was considered significant. Analyses were performed using SPSS statistical software (IBM SPSS Statistics, Version 26.0. Armonk, NY: IBM Corp.).

## Results

During the pandemic, 66 patients had appropriate indications for PEG, and these procedures were planned. However, PEG was not performed because of co-morbidities, high-risk American Society of Anesthesiologists (ASA) scores, unsuitable anatomy or infection status. Patients undergoing PEG frequently had cerebrovascular disorder (CVD), Alzheimer's disease, or traumatic brain injury. The primary and comorbid conditions of these patients were shown in Table 1.

Most patients were in the ICU at the time of the procedure. A comparison was made between the intensive care patients and those who were prepared for PEG in the outpatients' clinic. (Table 2). The results of this comparison showed the 30-day mortality rate to be significantly higher in intensive care patients ($p = 0.01$). In addition, the C reactive protein (CRP) and white blood count (WBC) values of the patients hospitalized in the intensive care unit were significantly higher ($p = 0.001$ and $p = 0.01$, respectively), while albumin and hematocrit levels were significantly lower ($p <0.05$). All of the virus-infected patients were inpatients in the intensive care unit (Table 2).

In the present study, ten patients had complications with their feeding tubes due to the deterioration of the tube in five patients and wound infection in the remaining five patients. In comparison, the antibiotic treatment was sufficient in the five patients who had wound infection; in the remaining cases, the tube had to be removed and replaced with a new feeding tube.

The diagnosis of virus-infected patients (group 2), who were all in intensive care, was made by PCR in 4 patients and tomography in 7 patients. Patients with ICU have been divided into two groups: non-virus-infected (group 1) and virus-infected (group1) (Table 3). There was no significant difference between the two groups regarding age, gender, blood albumin levels, WBC and hematocrit values, blood gas, or lactate values (Table 3).

**Table1.** Primary diseases and comorbid diseases of patients with PEG

| | All Patients (n%) | Patients with COVID-19 (n%) |
|---|---|---|
| Diagnosis | | |
| Cerebrovascular disease | 25 (37.9) | 5 (45.4) |
| Alzheimer disease | 13 (19.7) | 3 (27.3) |
| Trauma | 7 (10.6) | |
| General condition disorder | 4 (6.1) | 1.1 (9.1) |
| Hypoxic or anoxic brain injury | 5 (7.6) | 1.1 (9.1) |
| Cranial tumor | 3 (4.6) | 1.1 (9.1) |
| Dementia | 2 (3.0) | |
| Parkinson disease | 2 3.0) | |
| Amyotrophic lateral sclerosis | 1 (1.5) | |
| Cerebral palsy | 1 (1.5) | |
| Polyneuropathy | 1 (1.5) | |
| Lip or tongue cancer | 1 (1.5) | |
| Esophageal cancer | 1 (1.5) | |
| Total | 66 (100) | 11(100) |
| Co-Morbidities | | |
| Hypertension | 20 (29.9) | 4 (33.3) |
| Senility | 17 (25.4) | 6 (50) |
| Lung failure | 9 (13.4) | 5 (41.7) |
| Coronary artery disease | 8 (11.9) | 1 (8.3) |
| Diabetes Mellitus | 8 (11.9) | 2 (16.7) |
| Chronic obstructive lung disease | 7 (10.4) | 1 (8.3) |
| Malignity | 6 (9.0) | |
| Congestive heart failure | 5 (7.5) | 2 (16.7) |
| Thrombocytopenia | 2 (3.0) | |
| Schizophrenia | 1 (1.5) | |
| Substance abuse | 1 (1.5) | |
| None | 23 (34.3) | |
| Total | 106 | 21 |

## Discussion

The COVID-19 infection is associated with a high mortality rate in patients with multiple co-morbidities. Patients undergoing PEG procedures have had generally long-term and severe co-morbidities. Therefore, the timing of PEG insertion is essential in the presence of the co-existence of the COVID and PEG process.

The ICU-specific nutrition guidelines have suggested early nutrition (EN) within 24-48 hours following the intervention [10]. Furthermore, the influence of hypoalbuminemia, serum C-reactive protein levels, metastatic cancer, and other comorbid diseases on mortality after PEG is well-documented [11]. Both albumin levels and hemogram values of the patients undergoing PEG in the ICU were significantly lower than in the other patients; their CRP levels were significantly higher; and the 30-day mortality was also higher in these ICU patients, as expected. Thus, the importance of early enteral nutrition in this patient group is evident.

Wound infection is the most common complication associated with the PEG procedure. The surgical site infection can frequently respond to antibiotic treatment. [12]. In half of our patients, the feeding tube had to be removed due to wound infection, but after medical treatment, the PEG was reinserted in a second session.

A positive PCR test is used to make a COVID-19 diagnosis in clinically suspected patients. In case of a false negative, thorax CT may assist the diagnosis [13]. In this study, tomography was used to confirm the diagnosis of COVID-19 in 7 patients.

**Table 2.** Descriptive statistics of Outpatient and ICU patients

| | Insertion of PEG in outpatient (n=19) | Insertion of PEG in ICU (n= 47) | *P* |
|---|---|---|---|
| Gender (M/F) | 12(63.2%)/7(36.8) | 21(44.7)/26(55.3) | 0.155 |
| Age (year) | 68.5±22.8 | 69.7±15.4 | 0.616 |
| CRP (mg/L) | 45.8±45.7 | 98.7±61.0 | <0.01 |
| Albumin (g/L) | 32.5±6.9 | 26.4±4.7 | <0.01 |
| WBC (K/uL) | 7.2±2.6 | 9.2±2.9 | 0.01 |
| Hematocrit (%) | 35.1±4.2 | 29.4±5.4 | <0.01 |
| Hemoglobin (g/dl) | 11.4±1.4 | 9.6±1.7 | <0.01 |
| Complication and mortality | | | |
| Diagnosis of COVID-19 | 0 | 11(23.4%) | 0,014* |
| Leakage around the PEG | 2 (10.5%) | 7(14.9%) | 0,901* |
| Alteration of the PEG | 6 (31.6%) | 4(8.5%) | 0.017 |
| First 30-day mortality (%) | 0 | 12(25.5%) | 0.01 |

CRP: C-Reactive protein: Intensive care unit, PEG: Percutaneous endoscopic gastrostomy, WBC: White blood cell, Mann Whitney U test, *,Fisher Exact

**Table 3**. Descriptive statistics of Group 1 and Group 2

| | Patients without COVID-19 (Group 1) (n=36) | Patients with COVID-19 (Group 2) (n=11) | P |
|---|---|---|---|
| Patients (female/male) | 14/22 (38.9/61.1) | 6/5 (54.5/45.5) | 0.369 |
| Age (year) | 68.3±14.9 | 72.6±16.9 | 0.421 |
| CRP (mg/L) | 99.8±61.8 | 102.8±58.8 | 0.885 |
| Albumin (g/L) | 26.5±4.6 | 25.0±4.1 | 0.511 |
| WBC (K/uL) | 9.2±3.1 | 9.5±2.5 | 0.757 |
| Hematocrit (%) | 29.6±5.3 | 27.8±4.9 | 0.199 |
| Hemoglobin (g/dl) | 9.7±1.7 | 9.0±1.5 | 0.150 |
| Arterial blood gases ph | 7.4±0.1 | 7.4±0.06 | 0.680 |
| Complication and the hospital mortality | | | |
| Leakage around the PEG | 7 (21.2%) | 0 | |
| The insertion time of PEG after COVID-19 diagnosis (days) | - | 30 (10/50) * | - |
| Hospital mortality (First-30 days) (%) | 9 (25.0%) | 3 (27.3%) | 0.295 |

CRP: C-Reactive protein, PEG: Percutaneous endoscopic gastrostomy, WBC: White blood cell, Mann Whitney U test, * median(min/max)

In our study, there was a strong association between virus-infected patients who underwent PEG and COVID-19. The literature suggests that COVID-19 may be more severe in individuals with a history of COVID-19 and describes the effects of accompanying cardiovascular diseases in these patients [14]. Morbidity and mortality rates are higher in COVID-19 infected patients with comorbidities such as diabetes, cardiovascular disease, chronic respiratory diseases, and in patients over the age of 60 [15]. Patients who undergo PEG have multiple risk factors. After the early insertion of PEG, an increase in 30-day mortality can be expected. In the cases in this study, the 30-day mortality rate was similar to other patients hospitalized in the ICU.

Patients can be infected by the environment retching, coughing, and drooling during upper GI endoscopy. Therefore, measures should be taken to protect both patients and healthcare professionals in the pandemic period [16, 17]. It should be emphasized that at the peak of the pandemic, only urgent endoscopic procedures should be performed and that the decision to place endoscopic feeding tubes should be made on a patient-by-patient basis [18]. Moreover, PEG intervention should not be achieved unless complications related to the nasoenteral tube develop in active infection [19]. As perioperative COVID-19 conditions are known to carry an increased risk of death and pulmonary complications, international collaborative studies recommend delaying non-urgent procedures, where possible [20]. Meanwhile, current studies also suggest the optimal time for surgical intervention to be after an asymptomatic period of 7 weeks following virus infection [21]. Also, for PEG, a delay of 3-4 weeks with the pull technique is recommended [4].

This study has some limitations. First, there was a small sample size and retrospective planned research. On the other

hand, the present study can be critical for emphasizing the timing of the PEG procedure in patients with PEG.

PEG, which is a minimally invasive procedure, is widely utilized to supply enteral nutrition. Since surgical procedures increase the severity of the disease in COVID-19 patients, the timing of the intervention is essential in infected patients. Although the most appropriate timing is on the 30th day after the infection has cleared, it is possible to use PEG on the 10th day in proper patients who have a negative PCR and no disease symptoms..

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# An atypical co-existence of sialadenitis and thyroiditis in a COVID-19 patient: A case report

## Bir COVID-19 hastasında sialadenit ve tiroidit atipik birlikteliği: Bir olgu sunumu

**Deniz İncaman [1], Gamze Gül Güleç [2]**

Abstract

COVID-19 (SARS-CoV-2) is an infectious disease that causes respiratory tract infection in humans. This disease, which first appeared in Wuhan, China, has spread worldwide, causing a pandemic. COVID-19, which is constantly came into question with new complications, remains the leading problem all over the world. In this case report, it was aimed to present a COVID-19 case with both sialadenitis and thyroiditis. Although separate cases of thyroiditis and sialadenitis have been reported, we think that it may contribute to the literature since there is no similar case with together.

Keywords: COVID-19 disease, sialadenitis, splenomegaly

Öz

COVID-19 (SARS-CoV-2) insanlarda solunum yolu enfeksiyonuna neden olan bulaşıcı bir hastalıktır. İlk olarak Çin'in Vuhan kentinde ortaya çıkan bu hastalık tüm dünyaya yayılarak bir pandemiye neden oldu. Sürekli yeni komplikasyonlarla gündeme gelen COVID-19, tüm dünyada hala en önde gelen sorun olmaya devam ediyor. Bu olgu sunumunda hem sialadenit hem de tiroidit ile seyreden bir COVID-19 vakasının sunulması amaçlandı. Tiroidit ve sialadenit için ayrı vakalar bildirilmiş olsa da birlikte benzer bir vaka olmaması nedeniyle literatüre katkı sağlayabileceğini düşünüyoruz.

Anahtar kelimeler: COVID-19 hastalığı, sialadenit, tiroidit

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Informed Consent: The written consent was received from the patient who was presented in this study.
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## Introduction

COVID-19 disease caused by SARSCoV-2 was accepted as a pandemic by the World Health Organization on March 11, 2019 [1]. Initially, COVID-19 was thought to involve only the pulmonary system, but with the progression of pandemics extra pulmonary symptoms have been reported [2]. Subacute thyroiditis (De Quervain's thyroiditis, subacute granulomatous thyroiditis) and sialadenitis are the inflammation of the thyroid and salivary glands respectively. These inflammatory diseases could be observed following viral infections. Data on COVID-19-related thyroiditis and sialadenitis have begun to emerge [3, 4].

Here in, COVID-19 patient with both sialadenitis and thyroiditis is presented.

## Case report

A 52-year-old female patient was admitted to the internal medicine outpatient clinic of our hospital with complaints of pain and swelling that radiated to her chin under both ears. Her complaint started about 7 days ago and was accompanied by fatigue. The patient did not use any medication during this period. She has been suffering from hypertension for 10 years and has been using oral ramipril. In the first physical examination of the patient in the outpatient clinic, her temperature was 37.5°C, her pulse was 88 beats/min, her oxygen saturation was 98%, and her blood pressure was 135/80 mmHg. Edema, hyperemia, and increased body temperature were observed in the anterior neck and from both tragus to the chin. On respiratory examination, bilateral diffuse end-inspiratory crepitant rales were heard. At the first examination of the patient, we obtained the following results: White blood cell count 2500 $10^9$/L, lymphocyte 720 $10^9$/L, platelet count 113.000 $10^9$/L, hemoglobin 11.2 g/dL, glucose 98 mg/dL, urea 32 mg/dL, creatinine 0.76 mg/dL, aspartate aminotransferase 30 U/L, alanine aminotransferase 10 U/L, ferritin 241 ng/mL, CRP 31 mg/dL, sedimentation 63, lactate dehydrogenase 296 u/L, TSH 0.01 mIU/L (range 0.38-5.33), fT4 2.2 ng/dL (range 0.61-1.12), fT3 5.4 pg /mL (range 2.6-4.4), d-dimer 4.4 mg/L.

In the superficial tissue ultrasound taken, both parotid gland parenchyma was distinctly heterogeneous, and they had a patchy pseudocyst appearance. The thyroid gland was diffusely heterogeneous. Both submandibular gland parenchyma was heterogeneous (sialadenitis in both glands) secondary to their appearance with patchy hypoechoic pseudocysts. There were reactive lymph nodes in the neck, with a fatty hilus measuring 9 mm on the short axis of the common larger one at all levels. In the non-contrast lung tomography, in both lungs, there were common nodular consolidated areas, the largest measuring 34x18 mm, prominent in the upper lobes and the center, and the spleen was reported to be 16 cm larger than normal in the cut areas (Figure). Splenomegaly was confirmed by abdominal ultrasound.

COVID-19 PCR nasal swab test and other viral-autoimmune markers were sent from the patient. After the COVID-19 PCR test was positive, subacute thyroiditis, viral pneumonia, acute sialadenitis were considered in the patient, and the treatment of 5-day favipiravir tablet, levofloxacin tablet, deltacortil tablet, low molecular weight heparin was started, and the filiation teams were informed about the home quarantine rules. Other viral markers sent were negative, anti-TPO, anti-TG, and thyroid receptor antibodies were negative. The patient was followed up during the quarantine period, and he was interviewed by phone every 48 hours. The patient had no fever, cough, and shortness of breath did not develop. On the 15th day after the quarantine, she applied to the internal medicine outpatient clinic again. Her vital signs were stable, her breathing sounds were normal, and the swelling and redness on her face had improved. In control examinations, we found these laboratory investigations: White blood cell count 4900 $10^9$/L, lymphocyte count 1300 $10^9$/L, platelet count 149.000 $10^9$/L, hemoglobin 11.6 g/dL, glucose 90 mg/dL, urea 36 mg/dL, creatinine 0.70 mg/dL, aspartate aminotransferase 29 U/L, alanine aminotransferase 102 U/L, amylase 41 U/L, lipase 31 U/L, TSH 1.2 mIU/L, fT4 0.79 ng/dL, fT3 2.7 pg/mL ferritin 30 ng/mL, CRP 6 mg/dL, sedimentation 12, lactate dehydrogenase 210 u/L, d-dimer 0.4 mg/L. It was reported that the inflammation in the parotid glands regressed in the control superficial tissue ultrasound, the thyroid gland was heterogeneous, and the lymph nodes in the neck were smaller compared to the previous ultrasound. In the abdominal ultrasound, the spleen dimensions were measured as 15 cm, a reduction was observed compared to the previous one.

The patient gave consent to have personal health information published without divulging personal identifier.

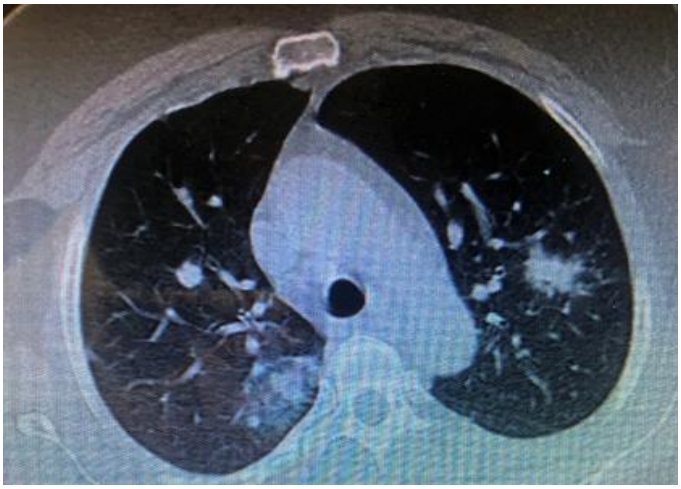

Figure 1: Non-contrast chest computerized tomography image of the patient.

## Discussion

Subacute or de Quervain's thyroiditis is a granulomatous thyroiditis that usually occurs during or after viral infection in patients with a genetic predisposition [5]. Mumps virus, Adenovirus, Measles virus, Coxsackie virus, Epstein-Barr virus, Measles viruses can cause inflammation in the thyroid and salivary glands. Subacute thyroiditis has been reported most frequently in women (3-5 times more), between the ages of 30-50. Thyroiditis presents with pain in the neck region, difficulty in swallowing, and fever 15-21 days after viral infections [6, 7]. Our case was a 52-year-old female patient with COVID 19 pneumonia who had complaints for 7 days. Generally, improvement is seen within 4-6 weeks in thyroiditis, but sometimes it may cause prolonged symptoms. In our case, the process continued for 2 weeks. The mechanism for the physiopathology of thyroiditis in COVID-19 patients is claimed to be related to angiotensin-converting enzyme 2 (ACE-2), which is expressed in the thyroid gland and essential for SARS-CoV-2 to be able to invade human cells [8]. In a review including 22 cases reported until May 2021, it was concluded that de Quervain's thyroiditis is an extra-pulmonary symptom of SARS-CoV-2 infection and SARS-Cov-2 should be kept in mind during the etiological workup of de Quervain's thyroiditis, especially in women [8]. However, in a more recent study in

Italy, it was showed that there is no increase in the incidence of subacute thyroiditis during the pandemic [9].

Acute sialadenitis is usually caused by bacterial infections, followed by viral infections and some inflammatory diseases (sarcoidosis, Sjogren's). Most viral transmission to the salivary glands usually occurs via the hematogenous spread, but it may occur more rarely with retrograde ductal migration [10]. The salivary gland pathogens can be detected with the nasopharyngeal swab sample, which is the diagnostic method of respiratory tract viruses. However, since the mucus produced from the salivary glands is also in the nasopharynx and lungs, it supports the idea that the virus infects the salivary glands [11]. Like the thyroid gland, acinar epithelial cells of salivary glands express ACE 2. It was hypothesized that the development of acute sialadenitis during SARS-CoV-2 infection is related to ACE2 [12]. Until now, there are a few case articles on salivary and parotid gland inflammations associated with COVID-19 [13]. In our case, involvement was observed in both submandibular glands and parotid glands.

In conclusion, the cases of COVID-19-related thyroiditis and sialadenitis continue to be reported in increasing COVID-19 studies [3, 4]. To our knowledge, this is the first report of sialadenitis and subacute thyroiditis co-existence in a COVID-19 patient. Further studies with more samples are needed to establish the relationship between COVID-19 and thyroiditis and sialadenitis.

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