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ORIGINAL ARTICLE

What is the ideal repair technique for triple hernia accompanied by umbilical and bilateral inguinal hernias?

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Abstract

Background: For patients with bilateral inguinal hernia (BIH) accompanied by an umbilical hernia (UH), there may be hesitations regarding the repair of all three hernias in the same session in terms of pain and possible problems in the healing process. Studies on these patients are rather rare in the literature. In the present study, the results of laparoscopic and open surgical treatment in patients with triple hernia accompanied by BIH and UH were compared.

Methods: The data of patients were analyzed retrospectively. Patients whose hernias were fully repaired with open surgery made up group 1, and patients who underwent laparoscopic transabdominal preperitoneal (TAPP) repair together with open UH repair made up group 2. There were 10 patients in group 1 and 11 patients in group 2. The patients' umbilical defect diameter, whether inguinal hernia (IH) was scrotal or not, applied surgical technique, length of hospital stay, duration of the operation, postoperative complications, and recurrence status were evaluated.

Results: While 19 (90.5%) of the patients were male, 2 (9.5%) were female. In group 2, recurrence of unilateral IH was seen in 1 (4.5%) patient. Complications developed in 3 (14.3%) patients: seroma in 1 patient in group 1; and seroma in 1 patient; and wound infection in another patient in group 2. There was no significant difference between the groups in terms of duration of the operation, length of hospital stay, or postoperative complications (p value, p=0.251, p=0.756, p=0.538, respectively)

Conclusion: In patients with triple hernia where umbilical and BIH occur together, open UH repair and IH repair can be performed safely by TAPP repair.

Keywords: Triple Hernia, Umbilical Hernia, Bilateral Inguinal Hernia, Transabdominal Preperitoneal Repair.

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INTRODUCTION

Inguinal and femoral hernias are referred to as inguinal hernias (IHs), and they are treated surgically. Although there is a lot of experience with IH and many studies have been performed, new scientific studies are needed due to continued recurrence, the excessive variety of hernia repairs, and the definition of modern surgical approaches. It is no longer a matter of discussion whether bilateral inguinal hernia (BIH) repairs can be performed either openly or laparoscopically in the same session (1). Although IHs can be repaired without any problems with the open technique, it is known that the laparoscopic approach provides advantages such as allowing intervention in both inguinal areas through the same incisions, less chronic pain, rapid recovery, and early return to work (2). In patients with BIH accompanied by an umbilical hernia (UH), there may be hesitations regarding the repair of all 3 hernias in the same session in terms of pain and possible problems in the healing process. Studies on these patients are rather rare in the literature. In the present study, the results obtained from laparoscopic and open surgical treatment in patients with triple hernia where BIH was accompanied by UH were compared.

MATERIALS AND METHODS

Patients with UH and BIH who were operated in Kahramanmaraş Elbistan State Hospital General Surgery Clinic between January 1, 2017 and December 31, 2021 were analyzed retrospectively. Patients whose hernias were fully repaired with open surgery made up group 1, and patients who underwent laparoscopic transabdominal preperitoneal (TAPP) repair together with open umbilical hernia repair made up group 2. Patients under the age of 18 were excluded from the study. There were 10 patients in group 1 and 11 patients in group 2. The patients' sex, age, body mass index (BMI), risk factors for hernia, radiological examinations, umbilical defect diameter, whether inguinal hernia was scrotal or not, existence of primary/recurrent hernia, American Society of Anesthesiologists (ASA) score, surgical technique applied, length of hospital stay, duration of the operation, postoperative complications, and recurrence status were evaluated. Approval for the study was obtained from the Clinical Research Ethics Committee of Kahramanmaraş Sütçü İmam University (date 06.04.2022, decision number 2022/07).

Surgical technique (Group 1): Spinal anesthesia was administered to all patients. A transverse incision was made just above the umbilicus. The fascia defect was then closed with primary sutures. Next, tension-free repair was performed with the darning technique using 2/0 prolene sutures in some patients and with prolene mesh in the others. An oblique incision was made in the inguinal region on one side. Polypropylene mesh approximately 6×12 cm in size was laid on the region and fixed (Lichtenstein technique). The same procedures were applied to the other inguinal region as well.

Surgical technique (Group 2): General anesthesia was administered in all of the patients. The surgery was performed in the supine and 10°-15° Trendelenburg position. Following the transverse incision over the umbilicus, a trocar was inserted using Hasson's open technique. The UH sac was opened and the contents of the sac were reduced. After the purse string suture and sliding-type knot were prepared, an 11-mm trocar was placed, the knot was tightened and the trocar was fixed (Figure 1). At the level of the umbilicus, a 5-mm trocar was placed on both the right and left midclavicular lines. On one side, the peritoneum was opened in the inguinal region, dissected, and the hernia sac was completely released. Polypropylene mesh approximately 12×15 cm in size was fixed to the pubis and anterior abdominal wall with 3-4 absorbable automatic mesh fixers. The same procedures were applied to the other inguinal region as well. In all cases, the peritoneum was closed with a mesh stabilizer. After the trocars were removed, the purse string suture in the umbilical defect was knotted. Afterwards, repair was carried out in some patients with 2/0 prolene sutures and in others with polypropylene mesh.

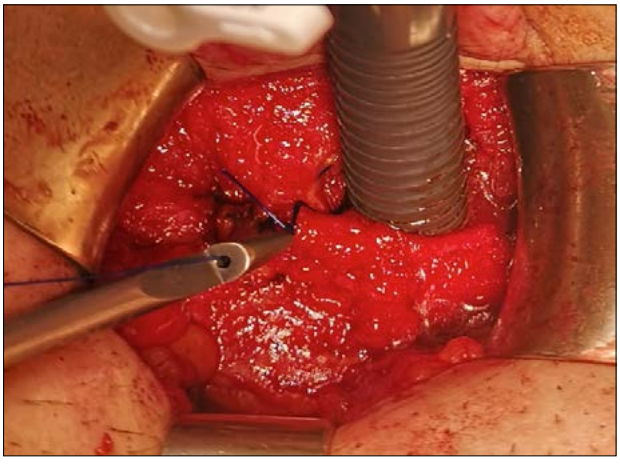

Figure 1. Fixation of the trocar inserted through the umbilical defect with a previously prepared purse string suture and sliding knot

Statistical Analysis: Categorical variables were presented as frequency (n) and percentage (%), normally distributed continuous variables as mean±standard deviation (SD), and non-normally distributed ones as median (min-max). The relationship between categorical variables was examined with Fisher's exact test. The assumption of normal distribution was checked with the Shapiro–Wilk test. The Mann–Whitney U test was used for non-parametric comparison of continuous variables between the study groups, and the independent t-test was used for parametric comparison. All analyses were performed with IBM SPSS 23.0 (IBM Corp., Armonk, NY, USA). P values less than 0.05 were considered statistically significant.

RESULTS

Among the 150 cases of BIH, 21 patients with accompanying UH were included in the present study. During the study period, 954 unilateral IH operations were performed. In this period, the rate of BIH repair was 13.6%. The rate of accompanying UH among the BIH was 14%. While 19 (90.5%) of the patients were male, 2 (9.5%) were female. The mean age was 54 (min 27 - max 76). In group 2, unilateral IH recurrence developed in 1 (4.5%) patient. Complications developed in 3 (14.3%) patients: seroma in 1 patient in group 1; seroma in 1 patient, and wound infection in another patient in group 2. There was no significant difference between the two groups in terms of age (p=0.061), sex (p=0.214), BMI (p=0.595), or chronic disease (p=0.183) distributions. Two (9.5%) patients in group 2 suffered recurrence. The median umbilical defect diameter was 2 (IQR: 2-2.5) cm in the group that underwent open surgery and 1 (IQR: 1-1.5) cm in the group that underwent surgery by laparoscopy, and the umbilical defect diameter was greater in the open surgery group (p= 0.002). Darning (100% and 40%, respectively) was used more frequently in the laparoscopically operated patients and grafting (60% and 0%, respectively) in the open surgery patients (p=0.004) (Table 1). There was no significant difference between the groups in terms of duration of surgery, length of hospital stay, or postoperative complications (Table 2).

Table 1. Comparison of demographic characteristics, ASA scores, and imaging methods of patients

	Group 1 (n=10)	Group 2 (n=11)	p
Age (years), *median (IQR)*	60,5(44-65)	45(31-55)	0.061
Sex, *n(%)*			
Female	2(20)	0(0)	0.214
Male	8(80)	11(100)	
BMI (kg/m^2), *mean±SD*	26.2±1.8	25.6±2.8	0.595
Chronic disease, *n(%)*	5(50)	2(18.2)	0.183
Emergency / Elective, *n(%)*			
Elective	9(90)	11(100)	0.476
Emergency	1(10)	0(0)	
Recurrent case, *n(%)*			
First repair	10(100)	9(81.8)	0.476
Recurrence	0(0)	2(18.2)	
Imaging, *n(%)*			
None	3(30)	6(54.5)	0.250
USG	4(40)	5(45.5)	
CT	3(30)	0(0)	
Umbilical defect diameter (cm), *median (IQR)*	2(2-2.5)	1(1-1.5)	**0.002**
Scrotum extension, *n(%)*	2(20)	2(18.2)	0.999
ASA, *n(%)*			
1	3(30)	8(72.7)	0.120
2	5(50)	3(27.3)	
3	2(20)	0(0)	
Umbilical technique, *n(%)*			
Graftless darning	4(40)	11(100)	**0.004**
Grafted	6(60)	0(0)	

ASA: American Society of Anesthesiologists, BMI: Body mass index, USG: Ultrasonography, CT: Computed tomography

Independent t-test, Mann–Whitney U test, Fisher's exact test. Data are given as median (minimum-maximum), mean±standard deviation, number, and percentage. If p<0.05, there is a statistically significant difference.

Table 2. Comparison of the duration of the operation, length of hospital stay, and postoperative complication status of the patients

Variables	Group 1 (n=10)	Group 2 (n=11)	p
Duration of the operation (min), *median (IQR)*	87.5(80-95)	85(80-90)	0.251
Length of hospital stay (days), *median (IQR)*	1(1-2)	1(1-2)	0.756
Complication, *n(%)*	1(10)	2(18.2)	0.538
Seroma	1(10)	1(9.1)	0.999
Wound infection	0(0)	1(9.1)	0.999
Recurrence, *n(%)*	0(0)	1(9.1)	0.999

Mann–Whitney U test, Fisher's exact test. Data are given as median (minimum-maximum), number, and percentage. If $p<0.05$, there is a statistically significant difference.

DISCUSSION

There is no single technique that can be considered standard for all IHs. The characteristics of the patient, anesthesia status, cost, and the surgeon's experience and preference affect the choice of technique. IH can be repaired with laparoscopic and open techniques. Although different methods have been developed in the open technique, the Lichtenstein technique is recommended more frequently due to its proven success and short learning curve. The laparoscopic approach, on the other hand, provides rapid recovery and a low risk of chronic pain despite the long learning curve. Therefore, a laparoscopic approach is recommended for BIH if the necessary surgical conditions are met (3-5). There is limited information in the literature about triple hernias when umbilical IH is accompanied by BIH (6).

In the present study, open and laparoscopic approaches in IH repairs were used at similar rates. Moreover, the hernia size and ASA score were greater in patients who underwent open repair, and spinal anesthesia was administered in all of them. It is thought that deciding to open repair in these patients might stem from concerns regarding both avoidance of general anesthesia complications, and possible surgical difficulty due to hernia size. Patients declining general anesthesia or choosing open surgery may also be the reasons behind this decision as well. Although triple hernias can be successfully repaired with the open technique, it is more cosmetically satisfactory to operate through three small incisions in the laparoscopic technique. In addition, the absence of contraindications for the laparoscopic approach in many open repair cases led to the opinion that the laparoscopic rates should be higher in future studies.

UH and other surgical pathologies that require intervention in the abdomen can be treated separately in the same session. However, the widespread application of modern endoscopic techniques in surgery enabled other surgical abdominal interventions to be performed simultaneously with UH repair with fewer incisions (7).

It was reported that the operation time is longer in laparoscopic inguinal hernia repairs (8). The instruments used in laparoscopic operations are more elaborate, and instrument preparation may take longer compared to open surgeries. However, the surgical repair times in the present study were similar between the approaches. It is thought that the smaller umbilical defect diameters in patients with laparoscopic IH and the greater monitoring of scrotal hernias in patients who underwent open inguinal repair may have reduced the time difference between the two techniques. In addition, the routine application of laparoscopic inguinal repairs and the completion of the learning curve by the surgeons that carried out the operations within the scope of the present study may have also caused the durations of the applied techniques to be similar (9).

Organ injury within the hernia sac is a potential risk during the insertion of a trocar or Veress needle through the hernia defect. In order to reduce this risk, it was recommended to insert the trocar via Hasson's technique (10). Therefore, in all of the patients in the present study, the trocar inserted in the umbilical region was placed using Hasson's open technique. In addition, after the content of the sac was reduced, a purse string suture was prepared in the fascia defect and a sliding knot was applied. It is thought that this placement of the trocar is highly beneficial in preventing insufflated gas from escaping from the trocar margin and narrowing the surgical field.

In a study conducted by Kamer et al., it was reported that 9.4% recurrence was observed with the primary suture

technique in umbilical defect repair and 5.6% with Mayo repair, while no recurrence was observed in patients who underwent tension-free repair with prolene mesh (10). In Harriott et al.'s study, hernias formed by the trocar site in the umbilical region in laparoscopic IH repairs were examined and it was stated that trocar site hernias occurred 3 times more frequently after triple hernia repairs in UH compared to BIH repairs (11). In the present study, although inguinal hernia recurrence was observed on one side of one patient in group 2, no UH recurrence was observed in either group. Results may vary in studies with more patients. Harriot et al. applied a prolene mesh plug for UH repair in 105 patients. They repaired the fascia by primary suturing the fascia one by one in 71 patients, and there was no difference in recurrence rates between the two groups. However, none of the patients in the present study underwent only primary repair. In group 1, the defect was primarily repaired. In group 2, the previously prepared purse string suture was knotted and the defect was closed. Then tension-free repair with polypropylene mesh was applied to some of the patients in both groups, while the others were repaired with 2/0 prolene suture darning. It is remarkable that there were no recurrences in patients who were repaired with darning.

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

In conclusion, TAPP and open UH repair are practical and safe approaches in patients with triple hernia when umbilical and bilateral inguinal hernias occur together. It is known that the application of prolene mesh in hernia repair significantly reduces recurrence rates. However, in cases with a small umbilical hernia defect diameter, non-absorbable suture darning is also a safe option. Nevertheless, studies involving more cases are needed.

Declarations

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

This study was approved by the Kahramanmaraş Sütçü İmam University Clinical Research Ethics Committee (Date 06.04.2022, Decision Number 2022/07).

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ORIGINAL ARTICLE

Percutaneous radiofrequency nerve ablation in patients with chronic heel pain

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Abstract

Background: Heel pain, known as fasciitis, runner's heel, tennis heel, or police heel, can sometimes become challenging to manage. Many conservative methods, including stretching or orthosis, steroid injection, and extracorporeal shock wave therapy, are used in treating heel pain. This study investigated the therapeutic effect of percutaneous radiofrequency nerve ablation (RFNA) in treating patients with chronic heel pain.

Methods: In this retrospective study, 78 (84 feet) patients with severe and chronic heel pain were included. Patients with heel pain related to calcaneal spur were resistant to conservative methods. Therefore, RFNA was performed on patients who met the criteria. Patients were assessed with a questionnaire and the visual analog scale (VAS) before and after the procedure.

Results: The mean VAS results were 9.3 ± 0.8 before and 3.4 ± 2.6 six months after the procedure, with statistically significant change observed. ($p<0.05$) Furthermore, 79.2% of participants found RFNA favorable.

Conclusions: According to the findings, we may say that RFNA successfully relieves chronic heel pain.

Keywords: Chronic Plantar Fasciitis, Radiofrequency Nerve Ablation, Calcaneal Spur.

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INTRODUCTION

Heel pain describes pain and discomfort around the plantar side of the rear foot. Plantar fasciitis, plantar fasciopathy, runner's heel, tennis heel, and police heel; are various eponyms of heel pain. The prevalence of plantar heel pain is around 9.6% among the population, and in athletes, it is about 5-18%(1).

Chronic heel pain, one of the most common foot conditions affecting the foot, accounts for 15% of all adult foot complaints requiring professional care. Heel pain is primarily common in active people over the age of forty. This increased prevalence may result from decreased plantar fascia elasticity and slowing the healing process with age. In addition, the physical condition affects heel pain; for example, obesity can cause heel pain because excess pounds stress the heels(2,3). Co-morbidities such as obesity or a sedentary lifestyle are related to a higher risk of the presence of heel pain(4).

Chronic heel pain's aetiology is multifactorial; other than plantar fasciitis, calcaneal nerve entrapments, calcaneal bursitis, broken bone spurs, and stress fractures of the calcaneus could cause heel pain. The biomechanical aetiology of plantar fasciitis is increased tension and fascia thickness of the plantar fascia(5). The formation of a calcaneal spur is a sign of prolonged plantar fasciopathy. The reason is plantar fasciitis if the pain starts at the first step and decreases with walking. The pain increases with walking after sitting and resting; passive stretching of the plantar fascia exacerbates the pain. Moreover, neurogenic heel pain is originated from irritation or entrapment of the nerves that innervate the heel region. The related nerves are the first branch of the lateral calcaneal nerve and medial calcaneal nerve. In addition, the medial calcaneal branch and the anterior and posterior portions of the inferior calcaneal branch of the posterior tibial nerve provide sensory feedback to the heel(6). Entrapment of the lateral branch of the calcaneal nerve (LBCN) occurs between the deep fascia of the abductor hallucis muscle and the quadratus plantar muscle's medial head. The entrapment rate of LBCN is about %15 in chronic heel pain(7) .

Heel pain is managed with conservative methods such as; rest, stretching, anti-inflammatory drugs, injections, and supportive orthosis(8).

According to a guideline by Thomas JL et al.; treatment modalities could be stratified into three steps(9). First-step consists of non-steroidal medication and stretching regimens, activity modifications, foot padding, and cortisone injections. Second-step; prefabricated and custom orthotic devices, repeated cortisone injections, and physical therapy. Third-step surgical treatment inventions are extracorporeal shock wave therapy (ESWT), and radiofrequency. By this guideline, we could treat most patients with conservative measures. 10-20% of patients with heel pain would need a third management step. This patient population has been suffering from heel pain for a long time, and this pain gets unbearable and restricts a person's daily life. Surgical treatments have successful outcomes, but complications of these interventions are not innoxious; persistent heel pain (5.6%), paraesthesia or numbness (4.3%), soft tissue healing problems (1.7%), and superficial infection (0.4%) could have been seen (10). Therefore, surgeons are very circumspect in their decision to operate the patients because of heel pain. Also, patients want to try all the methods before surgery. For these reasons, ESWT and nerve ablation are the preferred methods for patients and surgeons.

Radiofrequency lesioning or nerve ablation is not a new intervention for the medical community. Peripheral nerve ablation has been performed for patients stricken with chronic pain by algologists with RFNA devices for years. Nowadays, it has become a popular method because its fewer complication risks. This study hypothesizes that chronic heel pain can be treated with the RFNA method with a low risk of complications compared to the surgery. In this present study, we evaluate the effectiveness of RFNA in reducing chronic heel pain.

MATERIALS AND METHODS

In this retrospective study conducted with the local ethics committee's permission, 78 patients treated in an Orthopedics and Traumatology Department of Training and Research Hospital in 2018-2019 were reached. All patients had heel pain present for at least six months and were resistant to many conservative treatment methods. Inclusion criteria are; older than 18 years old, mid-term heel pain (at least six months), conservative treatment failure, including; stretching, physical therapy, steroid injection, oral anti-inflammatories, and arch-supporting orthosis. Exclusion criteria are; prior foot surgery

or calcaneal fracture of the affected side, peripheral neuropathy, tarsal tunnel entrapment, ischemic limb disease, allergy to local anesthetics, and open wounds on the foot. Patients meeting these criteria were asked to complete a questionnaire at the sixth-month follow-up after the procedure. The study was conducted following the principles of the Declaration of Helsinki and with the permission of the local ethic committee.

All patients' medical histories and visual analog scale (VAS) values for pain assessment before and after the procedure were noted from the medical records. We asked patients to fill out a questionnaire which we prepared to analyze the procedure's effectiveness. In the questionnaire; age, weight, height, occupation, localization of heel pain, duration of pain, treatments for heel pain, VAS scores before and after the procedure, how much percent of the patients' pain was resolved after the procedure, opinions about the success rate of the procedure and whether the patient would recommend this treatment to another relative with heel pain were asked. Pain levels and changes in pain levels were collected with a 10-cm VAS score sheet. Adverse events were asked about in the questionnaire. Patients filled the questionnaire alone, and a medical doctor helped if any patient did not understand the questions.

Under the supervision of a senior surgeon, various clinic surgeons with the RFNA device (COOLIEF ® Cooled Radiofrequency; Belgium) performed the procedures in the intervention room. Materials for nerve ablation are a grounding pad, needle (cannula) with stylet, electrode probe (with 5 mm long destructive tip), povidone-iodine, sterile gauze, and sterile cover. The first step is to get informed consent from the patient and check the pain area in the heel. The second is placing the RF grounding pad on the patient's ipsilateral calf. Skin antisepsis was performed with povidone iodine. After skin preparation, local anaesthesia was obtained with 2 ml 1% lidocaine hydrochloride injection to the heel's medial aspect where the pain is located. After checking the numbness, a sterile needle was inserted through the calcaneus' anteromedial aspect to target the calcaneus' anteromedial aspect, where sensory nerve branches of the heel were located. The medial calcaneal branch and the anterior and posterior portions of the inferior calcaneal branch of the posterior tibial nerve are targeted. These three nerves provide sensory feedback to the anterior medial tubercle of the calcaneus and medial and posterior portions of the calcaneus(6). Next, an RF electrode was inserted through the cannula. For checking the electrode placement, sensory stimulation was performed by increasing the voltage at 50 Hz from 0, until the patient began to feel a tingling sensation. After that, the procedure is continued according to the patient's feedback. The procedure is stopped if any adverse event (allergic reaction, severe pain) happens. The procedure starts when the patient feels the stimulus distinctly. For example, if the stimulus could not be felt distinctly by a patient at 1.0 volt, the surgeon's needle's position would be checked and replaced if necessary. Ideally, the sensation should occur with a stimulus of fewer than 0.5 volts at 50 Hz. This step aims to determine whether the probe is on the motor or sensory nerve. If the probe is on the motor nerve, involuntary muscle contraction and foot movements will be seen when up to 2 volts stimulus is given. Fasciculations such as contractions in the foot muscles indicate that the motor neuron is targeted. The probe is repositioned whenever there is doubt that the probe is on the motor neuron. Another distinctive feature is that the electrical signal required for motor neurons' stimulation occurs at a lower frequency, while sensory neurons can be stimulated at faster frequencies. For example, it is possible to stimulate the sensory neuron with a 50 Hz electrical signal, while the 2 Hz frequency of the electrical signal is sufficient to stimulate motor neurons. Therefore, after finding the calcaneal nerve (LBCN) sensory branch, 1 mm 0.5% bupivacaine was injected through the cannula to obtain painless nerve ablation. Subsequently, ablation begins with a maximum temperature of 90° C. Ablation lasts 90 seconds; this time and temperature settings are selected according to previous studies(6,11). It was the last step of the procedure; subsequently, the cannula was withdrawn, a sterile bandage was applied, and the patient was allowed to tolerate all normal activities, such as ambulation on the operated foot. In addition, anti-inflammatory drugs were prescribed for diminishing pain or ice compresses to the heel.

Statistical analysis

Data were analyzed using the SPSS ver. 20.0 (IBM Co., Armonk, NY). The variables were investigated using visuals (histograms and probability plots) and a Kolmogorov–Smirnov test to determine whether they were normally distributed. In reporting descriptive

statistics, the data were expressed as mean ± standard deviation and median (minimum-maximum) for continuous variables and as frequency and percentage (%) for nominal and categorical variables. In addition, a one-way analysis of variance tests was used to compare the patients' VAS scores.

RESULTS

In this retrospective study, we analyzed 78 patients (84 feet), 13 patients were male (17%), and 65 were female (83%). The mean age was age 52,3±10,7, and the mean BMI was 32±4,8. Twenty-one patients had bilateral heel pain, 21 had a right heel, and 36 had left heel pain. More than half of the patients, primarily women, were housewives, 61%. More than half of patients with hypertension (65%) and diabetes mellitus (44%) and coronary heart disease were seen in 20% of patients with heel pain. 97.4% of the patients felt pain under the heel, and 30% felt pain in the medial side. Forty-four patients (57%) were stricken to heel pain for more than one year, and the rest were more than six months. 81% of the patients used arc-supporting orthosis, and 62% did stretching exercises. (Table 1). Of the 84 feet, 10 (14.3%) received ESWT, 18 (25.7%) received steroid injections, and 57 (%81.4) patients used arch-support orthosis, 44 (62.9%) underwent a stretching regimen. All 84 feet were unresponsive to these previous treatments.

Ablation was performed on six patients, both heels, 30 patients' right heels, and 42 patients' left heels. The mean VAS score for all patients before the ablation procedure was 9.1±1.4, and six months after the procedure was 3.5 ±2.7 ($p<0.001$). According to patients' opinions, twenty-five of the patients think the procedure was successful, 37 think it was very successful, and 16 found it was unsuccessful (Figure-1).

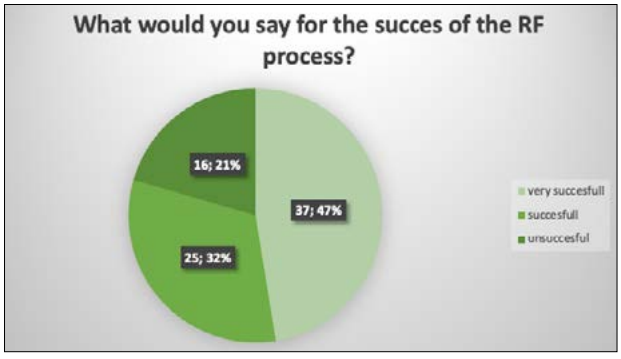

Figure 1. Success of RF therapy from the patients' eyes

According to the patient's report, 44.2% of patients said they almost had a painless heel after the procedure, and 27.3% said more than half of the pain was resolved. Also, 13% of the patients said less than 50% of the pain had a reduction, while 15% said there was less than a 10% reduction (Figure 2).80% of patients stated that they could recommend this procedure to relatives suffering from the same problem. After the procedure, one adverse event was stiffness in the medial heel region, resolved by ice compression and rest. Of the 78 patients, 6 (7.7%) had received RFNA to both heels. All 62 patients rated the treatment as either very successful or successful, of the 16 (20.5%) patients who had placed the treatment as either fair or poor.

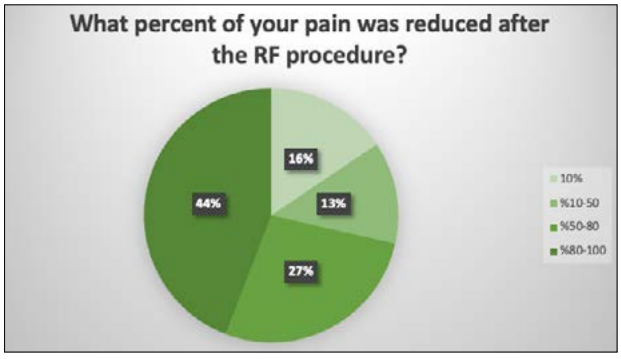

Figure 2. Pain reduction rate of RF procedure according to patients' report

DISCUSSION

We found that radiofrequency nerve ablation is a successful, non-surgical treatment option for chronic heel pain. Chronic heel pain is a disease that makes daily life unbearable and restricts the person from standing all day long. Although there are various conservative treatment methods for heel pain, surgical methods come into play when conservative methods are insufficient. The successful results of RFNA, an alternative to surgical procedures, are seen in this article.

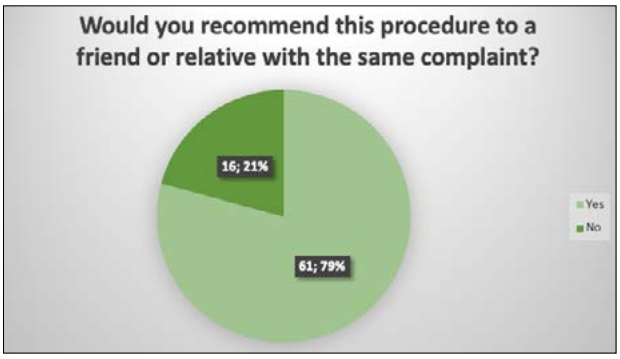

Figure 3. Patients were asked whether they would recommend the RFNA procedure to their relatives

Plantar heel pain is a common, disabling symptom among adults aged 40 years and over; in our study, the mean age was 52, and 84% of patients were female, as mentioned in the literature. Obesity and pronated foot posture are associated with heel pain and may be risk factors for developing chronic heel pain(3,4,12). Our study population's average body mass index was 32±4, supporting the current data. Diabetic patients are particularly at risk due to common risks and co-morbidities such as obesity or a sedentary lifestyle. Similarly, in the present study, 44% of patients with diabetes mellitus and 66% have hypertension, which may show us a sedentary lifestyle related to plantar heel pain. High physical activity was negatively associated with posterior heel pain in either heel; similarly to this information, more than half of the patients in this study were housewives(12).

Plantar fasciitis-related heel pain could be treated in three steps: first, non-steroidal medication, stretching regimens, and activity modifications. Also, calcaneal spur and nerve entrapment (FBLPN) are treated in the same conservative manner(13). If the first step of treatment fails, the second step of treatment consists of corticosteroid injection, orthotics, and physical therapy usually has 85% to 90% success rate in treatment within 2 to 3 months (14). All our patients failed these treatment modalities and were treated for at least six months. Consequently, pre-treatment VAS scores were high. A statistically significant difference between the VAS scores before RFNA and six months after treatment was observed in the present study, as Liden et al. Of the patients, 20-30 % with heel pain need the third step of treatment, consisting of extracorporeal shock wave therapy (ESWT) and nerve ablation or surgical release interventions, a solution for chronic heel pain(6). Plantar fasciopathies could be treated with the endoscopic or open release of the plantar fascia with successful outcomes. Still, in some cases, persistent heel pain (5.6%), paresthesias or numbness (4.3%), soft tissue healing problems (1.7%), and superficial infection (0.4%) could have been seen(10). Surgical decompression and neuroma excision should be considered if conservative treatments are ineffective; radiofrequency nerve ablating is a desirable method instead of surgical treatment or an option to the surgical procedure. These cases, which are resistant to the conservative manner, could benefit from RFNA.

There was one adverse event that happened and was cured with ice compression and elevation of the extremity. That shows us that RFNA is a safe, repeatable procedure. ESWT is another safe and effective method of reducing pain in chronic plantar fasciitis. It is unclear which mechanism in ESWT relieves heel pain, but one of the theories is that ESWT causes injury to the calcaneal nerves. ESWT's success rate improved overall VAS pain by 60% (15). Also, ESWT is an operator-dependent technique, and outcomes are still controversial. In the current study, we ablated the sensory nerve that innervates the heel area to ease the heel pain, as done before by Liden et al. and Arslan et al. (6,11). RFNA is also an operator-dependent method, but voltage tests to ensure the needle is on the sensory nerve make RFNA's specificity superior to ESWT. Patients' feedback guides proper cannula placement, which is the most crucial step of the procedure. The possible complications include periosteal burns, skin burns, fat pad atrophy, and entire plantar surface denervation. For practitioners, placement of the needle is becoming proper through repetitive procedures. Also, we can say it is not an easy method to learn. The possible complications include periosteal burns, skin burns, fat pad atrophy, and entire plantar surface denervation, hematoma, skin burns, and fat pad atrophy were not observed. In this study, only one patient reported stiffness in the medial heel region.

In the present study, we have seen that patients suffering from heel pain resistant to conservative treatment methods benefit from RFNA therapy. The RFNA method, which is a hope for these patients with chronic heel pain, has given us the capability to deal with this situation with another non-surgical procedure. In this study, more than one surgeon performed the RFNA procedure under the supervision of

a senior surgeon. Although each surgeon used the same method, we observed a few unsuccessful attempts during the learning phase of the method. Nevertheless, we found that 80% of the patients were content with RFNA, and the pain resolution rate was 70.5%, different from Sollitto et al. and Liden et al (6,16). In another study, Cione et al. retrospectively analyzed 75 patients with calcaneal neuritis treated with fluoroscopically guided RFNA and reported a clinical success rate of 93.3% (17). Erken et al. reported 39 feet that had received RFNA to the FBLPN sensory branches, with a success rate of 85.7% at the 2-year follow-up period(18) . We think that having more than one practitioner in the study increased the number of unsuccessful attempts at the learning stage, which caused us to obtain less successful results than other researchers.

The present study has several limitations: a small number of patients, a single-center study, the follow-up schedule was six months, and close follow-up could better understand the short-term VAS score results. Nevertheless, most of the data were taken from the patient's records from the outpatient clinics and confirmed by personal conversation.

CONCLUSION

This study demonstrates that RFNA may ease heel pain resistance to prior conservative measures but is not an absolute or definitive treatment method. We can say that RFNA can be recommended as a non-surgical treatment, such as ESWT. This study shows us that RFNA is a safe option for resistant heel pain patients.

Declarations

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

This study was approved by the clinical research Ethics Committee of the University of Health Sciences Diskapi Yildirim Beyazit Training and Research Hospital (Date: 19.10.2020, Number: 68/06).

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ORIGINAL ARTICLE

A different approach in primary spontaneous pneumothorax surgery: Lateral pleurectomy technique and results

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Abstract

Background: Primary Spontaneous Pneumothorax is a disease with a frequent recurrence rate. The uniportal videothoracoscopic surgery approach is the standard treatment for air leak and recurrence prevention. In common practice, apical pleurectomy is used after apical wedge resection. Our study aims to examine the results, especially the recurrence rate, of the lateral pleurectomy and apical pleural abrasion combination technique applied as an alternative to this technique.

Methods: A total of 50 patients who underwent uniportal videothoracoscopic surgery for primary spontaneous pneumothorax between November 2018 and May 2021 were included in the study. The follow-up period for recurrence of the patients was 12-42 months. Age, gender, pneumothorax side, operation indication, Body Mass Index, smoking history, incision length and location, air leakage and tube thoracostomy duration, Verbal Numeric Pain Scores, post-operative length of hospital stay, complications, and recurrence data were recorded.

Results: Of the 50 patients, 44 (88%) were male, and 6 (12%) were female, with a mean age of 22.3±5.9 years. Surgery was performed from the existing drain site in 32 (64%) cases and the former drain scar in 11 (22%) cases. The mean duration of tube thoracostomy was 2.7±0.97 days, and the mean length of hospital stay was 3.6±0.89 days. Complications developed in 2 (4%) cases, and recurrence was observed in 2 (4%) cases.

Conclusions: Lateral pleurectomy with the apical pleural abrasion technique is easy to apply and reduces the possibility of undesired hemorrhagic drainage. We believe that the lateral pleurectomy technique is safe, easily applicable, and less risky in terms of hemorrhage.

Keywords: Lateral Pleurectomy, Primary Spontaneous Pneumothorax, Uniportal Surgery, VATS.

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INTRODUCTION

Primary Spontaneous Pneumothorax (PSP) is a disease primarily seen in young adults who are thin, tall, and without a history of underlying disease or trauma. It usually occurs due to spontaneous rupture of subpleural blebs or bullae located at the apex of the lung (1). While the incidence of PSP is 7.4-18/100.000 per year in young men, it has been reported as 1.2-6/100.000 in young women (2). Secondary Spontaneous Pneumothorax (SSP) differs from PSP in that it is caused by Chronic Obstructive Pulmonary Disease (COPD) or trauma (iatrogenic, injury, etc.). Being a smoker is a significant risk factor for PSP (3).

In the literature, the lowest recurrence rate after PSP surgery is still in the PSP operation performed with a thoracotomy incision (4). However, in recent years, uniportal Videothoracoscopic Surgery (VATS) has become the standard treatment method in many clinics for PSP surgery, with less pain and surgical trauma, a shorter hospital stay, and a quicker return to routine life due to post-operative comfort (1, 5). Published uniportal VATS studies have described wedge resection and Apical Pleurectomy (AP) technique, primarily in the bullous area at the lung apex. It has become a widely accepted surgical procedure (3, 5, 6). Although similar recurrence rates were reported with total pleurectomy and apical pleural abrasion techniques, except for AP, in some studies, it was reported that apical pleurectomy is a more successful procedure in preventing recurrence (1, 5, 7). However, a substantial number of studies show that AP gives the same recurrence rates as apical pleural abrasion (7, 8). Moreover, advantages such as shorter operation time, less drainage, shorter hospital stay, and less pain were reported with the apical pleural abrasion technique than the AP technique in these studies (7-9).

Our study aims to analyze the results of Lateral Pleurectomy (LP) and apical pleural abrasion combination technique, which aims to investigate the advantages of AP and apical pleural abrasion techniques in uniportal VATS operation, on other parameters, especially on the recurrence rate.

MATERIALS AND METHODS

Study Design

This study was designed as a retrospective and observational study. The approval of the local ethics committee was obtained (Approval number: 2022/74). The authors confirmed compliance with the World Medical Association Declaration of Helsinki on the ethical conduct of research involving human subjects.

Fifty-five cases operated for PSP in a single center and by the same thoracic surgeon between November 2018 and May 2021 were included in the study retrospectively. Four of these cases were excluded from the study since relapse information for PSP could not be reached, and 1 case developed traumatic pneumothorax while doing sports on the operated side during the post-operative follow-up period. A total of 50 patients with a follow-up period of 12-42 months for pneumothorax recurrence were included in the study. Patients over 40 years of age and with SSP (COPD, emphysema, traumatic, etc.) were omitted.

Patient data were obtained from physical and digital archive files. Age, gender, pneumothorax side, operation indication, Body Mass Index (BMI), smoking history, incision length and location, air leakage and tube thoracostomy duration, pain scores, post-operative length of hospital stay (LOS), complications, and recurrence data were recorded. Recurrence information of the cases was obtained by physical and digital hospital files and telephone interviews.

Prolonged air leak (more than seven days), recurrence of PSP, and bilateral pneumothorax were the indication for PSP operation. The patients' pain was scored according to the Verbal Numeric Pain Scale (VNPS). VNPS is a verbal numerical scale in which patients are asked to verbally indicate a number between 0 and 10 corresponding to their current pain intensity. On this scale, zero corresponds to no pain, while ten corresponds to the greatest pain a person will ever experience. According to the VNPS score, 0 points were classified as no pain, 0-3 mild pain, 3-5 moderate pain, 5-7 severe, and 7-10 very severe.

Surgical Procedures

All patients were operated on with the same surgical procedure. After double-lumen intubation under general

anesthesia, operations were performed in the lateral decubitus position. Before performing the incision, a local anesthetic (2 mg/kg Bupivacaine) was used to block the area around the incision and lower and upper intercostal nerves. Although the incision sites differ in patients sent from external centers, a single incision line of approximately 2.5 cm in the anterolateral thorax, where the 7th-8th intercostal space intersects with the anterior axillary line, was preferred for tube thoracostomies and surgeries performed in our center. A 30-degree 10mm endoscopic camera, endoscopic grasper, endoscopic linear stapler, endoscopic nut, and energy device were used during the operation. When active air leakage was detected, wedge resection was performed with an endoscopic linear stapler (apex and the apical part of the lower lobe superior). Apical pleural abrasion and cauterization were performed with the help of endoscopic hazelnut and energy devices to the cupula and lateral pleural area in the upper part of the fourth intercostal space. The LP procedure was performed with blunt and sharp dissections with the help of endoscopic grasper, forceps, and dissectors in the 4th intercostal and below, extending to the 10th intercostal space, 3 cm from the descending aorta in the left hemithorax, and 3 cm from the esophageal bed in the right hemithorax. No pleurectomy was performed in the pleural area above the 4th intercostal space at the cupula or lower level (Figure 1). The removed lung wedge material and pleura were sent for pathological examination in separate containers. After a single 24-28 French (F) silicone drainage tube (underwater drainage system) was placed through the operation incision, the incision was closed by suturing the muscle layer with polyglactin suture material and by placing silk drain-retaining sutures on the skin. After all the cases were extubated in the operating room and followed up in the recovery room, the patients were taken to the inpatient service room in a stable and conscious-cooperative manner. The pain status of the patients was scored daily during hospitalization with VNPS, and an intercostal nerve block was performed with a local anesthetic drug and intravenous medical treatment for those with pain. Patients were mobilized, and balloon inflation exercises were started in the 4th-6th hours post-operatively. Daily follow-up was done with a posteroanterior chest X-ray. The amount of tube thoracostomy drainage was noted daily, and the drain of the patients who did not have air leakage and whose drainage amount was 100 cc serous was terminated. Control chest X-ray was seen 6 hours after the drain was removed, and patients with expanded lung appearance were discharged. On the 10th day after discharge, a control chest X-ray was seen in the outpatient clinic, and the sutures were removed by the thoracic surgeon who performed the surgery. Information on long-term recurrence of the cases was obtained from the phone numbers or e-mail addresses registered in the hospital data system.

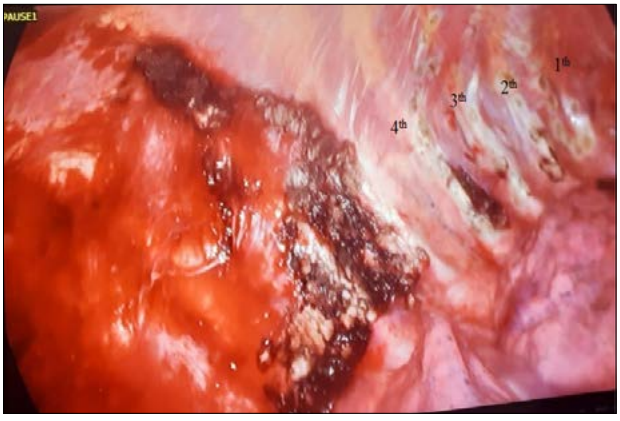

Figure 1. Picture of Lateral Pleurectomy (LP) and apical pleural abrasion combination technique obtained during uniportal VATS.

Statistical Method

Statistical analysis was performed using the "IBM SPSS Statistics for Windows. Version 22.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA)" program. Descriptive statistics of the research: frequency and percentage for categorical variables, mean and standard deviations for numerical variables.

RESULTS

The baseline demographic characteristics of the patients are given in Table 1. Table 2 shows the patients' per-operative information.

Table 1. Baseline Sociodemographic Characteristics of Patients

	n (%) mean ±SD
Gender	
Female	6 (12)
Male	44 (88)
Age	22.3+5.9
Pneumothorax side Right Left	 32 (64) 18 (36)
Operation indication	
Prolonged air leakage	18 (36)
First recurrence	12 (24)
Second recurrence	8 (16)
Recurrence in the opposite side Two-sided pneumothorax Recurrence after surgery	6 (12) 5 (10) 1 (2)
Smoking History Yes No	 15 (30) 35 (70)

Complications were seen in 2 (4%) cases. One case with major complications was hypotension due to hemorrhagic drainage that developed at the post-operative 4th hour. When a single incision site was explored, hemorrhage originating from the intercostal artery was detected and bleeding was controlled from the same incision site, and he was discharged three days later. The other case was wound infection and healing was achieved with wound debridement and suturing (Table 2).

Table 2. Per-operative variables of the patients

	n (%) mean ±SD
Incision length (cm)	2.8±3.9
Incision location Current drain incision Former drain scar New incision	 32 (64) 11 (22) 7 (14)
Air leakage duration (days)	2.96±4.1
Tube thoracostomy duration	2.7±0.97
Post-operative LOS	3.6±0.89
Complication No Yes	 48 (96) 2 (4)
Recurrence No Yes	 48 (96) 2 (4)
Pain Scores POD1 POD3 POD10	 5±1.19 3.3±1.16 0.32±0.65

Note: LOS: Length of hospital stay, POD1: post-operative day 1, POD2: post-operative day 2, POD 3: post-operative day 3.

The patients' pain scores on the 1st, 3rd, and 10th days post-operative according to VNPS are given in Table 2. On the first post-operative day, three patients (6%) described the pain as mild, 33 patients (66%) moderate pain and 14 patients (28%) very severe pain. On the third post-operative day, 32 patients (64%) described mild pain, 15 patients (30%) moderate, and two patients (4%) very severe pain. On the tenth post-operative day, 39 patients (78%) reported no pain, while 11 patients (22%) reported mild pain.

The cases were followed between 12 and 42 months after surgery for recurrence. Recurrence developed in only two patients (4%). One patient was addicted to inhaler drugs (cocaine-marijuana addict) and stated that he continued to use it extensively after discharge. He presented with recurrent pneumothorax only at the apex one month after the operation. He was discharged with an expanded lung after three days of follow-up with closed underwater drainage with a catheter thoracostomy (8 F). No recurrence was observed in the 3-year follow-up of the patient who stopped using drugs. The other case was admitted with recurrent pneumothorax in the 6th month post-operatively. The patient, who underwent a re-uniportal VATS procedure with apical wedge resection and total pleurectomy, was discharged without complications after four days of inpatient follow-up. During the recurrent pneumothorax operation, it was observed that the air leak was from the former stapler line, and wedge resection was performed by placing a new stapler under the stapler line. To prevent a recurrence, a pleurectomy was completed to total pleurectomy. The patient, diagnosed with granulomatous inflammation in favor of tuberculosis due to post-operative pleural pathology, Long-term (9 months) anti-tuberculosis treatment was applied to the patient, whose pathology result of his second operation showed granulomatous inflammation compatible with tuberculosis. No recurrence of pneumothorax was observed in the next two years. Furthermore, no evidence of secondary spontaneous pneumothorax was found in the patient's anamnesis or CT findings before the first operation.

DISCUSSION

The primary purpose of operations for PSP is to prevent air leakage and recurrence. There are many studies on the results of operations performed with standard posterolateral thoracotomy, axillary thoracotomy, tri-

portal VATS, and currently uniportal VATS procedure. Although the current uniportal VATS procedures successfully reduce the recurrence rate, it is still known that the thoracotomy procedure has the most successful rate in preventing recurrence (4, 9). However, the significant success of uniportal VATS in terms of minimal incision scar, post-operative pain advantage, drainage, LOS, and early return to routine life has made it the standard PSP surgical procedure today (10-12). This study was conducted to present the advantages of using uniportal VATS and lateral pleurectomy technique as an alternative to apical pleurectomy, which is used in many centers for PSP, in the light of case results.

Less pain and drainage, shorter tube residence time and LOS time, and better cosmetic results detected in studies showing the superiority of the uniportal approach compared to the tri-portal VATS approach in the literature were also confirmed in our study (11-13). The incision size reported in these large-series studies is similar to the mean length of 2.8 cm we found in our study (11-13). In addition, it was observed that the reported 2-5% post-operative complication rates were similar to those in our study (1, 11-15). Finding similar results in the LP procedure with Uniportal VATS as in the AP procedure indicates that it is a safe method that can be applied more easily and in a shorter time as a surgical technique. In addition, performing a pleurectomy over the internal mammarian artery and subclavian artery in AP carries the risk of severe intra-operative and post-operative hemorrhage. With LP, it is possible to avoid this risk.

Pain from parietal pleural origin due to pleurectomy is an inevitable outcome in the post-operative period. As there are many other ways to reduce pain, the intercostal nerve blocking method that the surgeon can perform, unfortunately, seems difficult or even impossible to apply in the AP technique in the high-level ribs and cupula region. However, in the combination technique of LP and apical pleural abrasion, the incision's lower and upper intercostal spaces and the 4-10th intercostal spaces can be easily reached, and intercostal nerve blockade can be applied at multiple levels. Thus, pain caused by post-operative pleurectomy in the LP technique is prevented locally and effectively. However, we would like to point out that this issue should be investigated with future studies involving large case series on this subject.

As Caecilia et al. (1) mentioned in their study, avoidance of pleurectomy in the internal mammarian artery-vein (IMA/IMV) and subclavian artery and vein areas in AP is an essential issue in preventing the possible complication of massive hemorrhage in AP. Although it is mentioned in some studies that AP with Uniportal VATS that AP completely covers the IMA/IMV and subclavian artery and vein areas, we think that it is a difficult pleurectomy procedure in practice (7, 14). The risk of undesired massive hemorrhage in the IMA/IMV and subclavian artery and vein areas with the LP and apical pleural abrasion technique is relatively low, and it is technically easier to apply. We believe that the recurrence rate in PSP surgery will be similar to that in the AP technique, with the surgeon's easier-to-apply LP technique. However, we would like to point out that comparative studies involving larger series are needed on this subject.

Uniportal VATS LP procedure was performed from the existing drain site in 32 (64%) cases and the old drain scar in 11 (22%) cases. This result shows us that the LP technique can also be applied from the existing or old drain site. The fact that no new incisions are made in patients indicates that the LP technique is compatible with achieving better cosmetic results, which is one of the goals of uniportal VATS surgery.

In meta-analysis studies published in the literature, it has been frequently mentioned that PSP patients are tall and thin people with typical physical features and smoking habits (4, 6, 14, 15). In our study, the mean BMI was 19.8 kg/m2, and 35 (70%) of the cases were smokers. These findings are compatible with classical literature knowledge.

Many studies have been published to reveal which method is better between AP and apical pleural abrasion for decreasing recurrence rates after PSP surgery. Although studies show that the effects of abrasion and pleurectomy on recurrence are close, meta-analysis studies have reported that AP has a statistically lower recurrence rate than abrasion (6, 13-15). Our study showed recurrence in 2 (4%) patients who underwent LP with uniportal VATS surgery. One of these patients relapsed during the post-operative continuation of heavy inhaler drug use. The patient did not give any information about inhaler drug use before the first operation. He shared information about

substance use after recurrence development. In addition, with the LP applied in the first surgery, the recurrence was limited only to the apical region. It was treated with a minimally invasive intervention such as a catheter thoracostomy. Similarly, in the literature, recurrence of PSP due to inhaler drug use has also been reported in cases who underwent the AP procedure (16, 17). In the second operation of the other relapsed patient, tuberculosis was found due to pleural pathological evaluation. It is pleasing that this patient did not experience complications secondary to advanced tuberculosis, such as empyema and non-expanded lung. The recurrence in this patient is thought to be related to parenchymal tuberculosis rather than the LP procedure. Before the first operation, there was no anamnesis, physical examination, or CT findings in this patient to suggest tuberculosis as a secondary cause of spontaneous pneumothorax. However, detection of leakage from the stapler line during the operation could be due to inadequacy of the surgical technique or tuberculosis. The follow-up time of our cases is 12-42 months, and 5- and 10-year follow-up results should have waited for precise results regarding recurrence. However, it was observed that the current recurrence rates were similar to the procedure performed with AP (1, 5, 7, 8, 18).

Limitations

The main limitations of our study are that the follow-up period has not yet reached at least five years, the number of patients is low, and the absence of a control group. We think that these limiting factors can be eliminated by presenting further studies with five and 10-year follow-up results in terms of PSP recurrence.

As a result, the AP or apical pleural abrasion technique applied with bulla or bleb excision with uniportal VATS, which is the current surgical approach of PSP, has become a standard procedure in many centers. AP is a relatively difficult and long-lasting surgical procedure with an increased risk of hemorrhage. The LP and apical pleural abrasion techniques we applied in our study both ease the application and reduce the risk of hemorrhagic drainage. We think the LP technique is safe, easily applicable, and less risky in terms of per-operative hemorrhage.

Declarations

The authors received no financial support for this article's research and/or authorship. There is no conflict of interest.

This study was approved by the clinical research ethics committee of the Ordu University (Date:25.03.2022, Approval Number: 2022/74)

Abbreviations

AP: Apical Pleurectomy

BMI: Body-Mass Index

COPD: Chronic Obstructive Pulmonary Disease

F: French

LOS: Length of Hospital Stay

LP: Lateral Pleurectomy

PSP: Primary Spontaneous Pneumothorax

SSP: Secondary Spontaneous Pneumothorax

VATS: Videothoracoscopic Thoracic Surgery

VNPS: Verbal Numeric Pain Scala

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Note: Our study, which consists of the majority of the patients in this study, was presented as an oral presentation at the 11th National Thoracic Surgery Congress held in Antalya on 24-27 October 2021.

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ORIGINAL ARTICLE

The effect of silymarin given before partial hepatectomy on liver regeneration in rats with ischemic preconditioning during liver resection

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Abstract

Background: The regeneration capacity of liver tissue after partial hepatectomy closely affects morbidity and mortality. We aimed to investigate the effect of silymarin and ischemic preconditioning (IPC) before partial hepatectomy on liver regeneration.

Methods: Thirty rats were randomly divided into 5 groups (n=6). Serum AST, bilirubin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) values were studied 48 hours after hepatectomies. Mitotic count, congestion, necrosis, cytoplasmic vacuolization, and presence of neutrophils were evaluated histopathologically. Proliferating cell nuclear antigen (PCNA) antibody was studied immunohistochemically.

Results: The AST value (2071.5±938.4) was the highest in the partial hepatectomy (PH) group. The Ischemic Preconditioning Partial Hepatectomy Group (IPC) (1535.5±204.8) and the Silymarin+Partial Hepatectomy Group (Silymarin group) (1192.3±526.3) had lower levels of AST values; however, the AST values were the lowest in the Silymarin+Ischemic Preconditioning+ Partial Hepatectomy Group (IPC+ Silymarin group) (1002.8±348.9). Likewise, the highest improvement in bilirubin levels was observed in the IPC+ Silymarin group (0.33±0.11). IL-6 (11.54±2.89) and TNF-α (39.2±22.73) values were the lowest in the IPC group, and interestingly, both were higher in the silymarin-treated groups. It was observed that these values increased in Silymarin group and IPC+ Silymarin group who received silymarin treatment compared to PH group and IPC group who did not receive silymarin. In histopathological analysis, it was found that the mitosis rate and PCNA percentage were higher in the Silymarin and IPC+ Silymarin groups.

Conclusions: The use of silymarin before hepatectomy and IPC during partial hepatectomy increase liver regeneration.

Keywords: Liver Resection, Silymarin, Ischemic Preconditioning, Liver Regeneration.

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INTRODUCTION

Partial resection of the liver is a procedure performed for various reasons, including trauma-related injury, donor hepatectomy, and the treatment of cysts, abscesses, and primary or secondary liver tumors (1). The regeneration capacity of the remaining liver tissue after partial resection of the liver closely affects morbidity and mortality. Ischemia/reperfusion (I/R) injury is a primary factor associated with the occurrence of liver failure in the postoperative period following liver resection (2). Ischemic preconditioning (IPC) is a method that is applied prior to procedures likely to cause I/R injury and has been suggested to prevent or ameliorate I/R injury in various organs, including the liver (3).

Hepatic I/R injury and IPC have been associated with the levels of reactive oxygen species, nitric oxide, adenosine, TNF-α and, chemokines (4, 5). The administration of IPC has been demonstrated to protect sinusoidal endothelial cells and hepatocytes through modulation of the endogenous oxidant/antioxidant system (5). The protective effects conferred by IPC after hepatic surgery have been associated with improved survival and smaller liver necrosis after I/R injury (6). Silymarin is a compound extracted from the seeds of Silybum marianum (colloquially known as Milk thistle) that may provide hepatoprotective effects through the inhibition of free radicals formed by the metabolism of critical toxic substances, such as acetaminophen (7, 8). Silymarin is established to have anti-fibrotic, anti-inflammatory, anti-apoptotic, and anti-oxidant effects, and thus, may alleviate possible dysfunctions of the liver. Consistently, there are numerous studies which have shown the protective and regenerative effects of silymarin in liver diseases. Put together, these suggest a role for silymarin in preventing or reducing I/R injury, either as a stand-alone treatment or in combination with IPC (8).

In addition to the surgical techniques applied to minimize the likelihood of hepatic dysfunction following partial liver resection, a number of chemical and herbal drugs have been used to increase regeneration and prevent potential adverse outcomes (9). In this experimental study, we aimed to investigate and compare the effects of silymarin and IPC in the prevention / alleviation of hepatic dysfunctions following partial hepatectomy in rats by measuring parameters associated with inflammation and liver functions.

MATERIALS AND METHODS

Animal care and study design

This study was carried out in accordance with the Declaration of Helsinki, after obtaining permission from Gazi University Animal Experiments Local Ethics Committee with code number G.U.E.T.-15.047.

Thirty Wistar-Albino male rats weighing between 200-300 grams were used in the study. Rats were randomly divided into 5 groups, each consisting of 6 rats. Rats were received by the Experimental Research Center and were acclimatized for 1 week before the study. All rats were kept in sufficiently large cages with standard environmental housing (21±2 °C, standard rodent chow, tap water, 45-65% humidity, 12-hour day/night cycles with automatized lighting).

Except for the sham group, all rats underwent partial hepatectomy. The treatments were performed based on the relevant groups and included silymarin administration and IPC. Silymarin (Carsil® 90 mg tablet, Sopharma PLC, Bulgaria) was administered at a dose of 200 mg/kg/day via orogastric tube. IPC was performed by subjecting rats to three cycles of 5-minute ischemia followed by 10-minute reperfusion (total duration 45 minutes).

Experimental groups

Sham group: The sham group underwent laparotomy and only hepatic manipulation was performed. Partial hepatectomy group (non-treatment): 70% liver resection was performed after laparotomy. IPC Group: The rats in this group underwent IPC before 70% liver resection. Silymarin group: These rats received silymarin for 6 weeks before the procedure. After 6 weeks, 70% liver resection was performed. IPC + silymarin group: These rats received silymarin for 6 weeks before the procedure. After 6 weeks, IPC was performed before 70% liver resection.

Surgical procedure

Surgeries were applied under intraperitoneally-administered general anesthesia using 50 mg/kg ketamine (Ketalar®, Parke Davis and Co. Inc., 40mg/kg) and 10 mg/kg xylazine (Rompun®, Bayer Ag, Leverkusen, Germany; 5mg/kg). Briefly, the abdomen was entered with a midline abdominal incision in the supine position, and for IPC, using a microvascular clip distal to the portal vein and hepatic artery, 5 minutes of ischemia followed by 15 minutes of reperfusion followed by 45 minutes of total hepatic ischemia was performed. In accordance with

the method described by Higgins et al., the left lateral and median lobes of the liver were tied with 4/0 silk sutures at the junction with the vena cava, and 70% liver resection was performed.(10). At the end of the experiment, after the samples were taken, the rat's heart was removed and euthanized.

Sample acquisition

Each rat was euthanized 48 hours after their respective procedure and tissue and blood samples were obtained. Total working time was kept equal in all groups. Tissue specimens were fixed with 10% neutral buffered formaldehyde. Blood samples were collected via cardiac puncture and were centrifuged at 1500×g for 10 minutes to obtain sera, which were stored at -20 ˚C until analysis.

Measurement of biochemical parameters

As inflammatory parameters, we measured serum TNF-α levels and IL-6 which were quantified with enzyme-linked immunosorbent assays (ELISA) (SunRed Rat TNF-α ELISA Kit and SunRed Rat IL-6 ELISA Kit). Serum AST and total bilirubin levels were studied in a Roche Cobas 8000 Autoanalyzer equipped with the necessary commercial kits at the Medical Biochemistry Laboratory of Gazi University Hospital.

Histopathological evaluation

After the macroscopic examination of tissues fixed with 10% neutral buffered formaldehyde, the tissue specimens were embedded in paraffin and cut at 5μm-thickness. Examination was carried out with H&E staining under a light microscope. Mitosis, congestion, necrosis, cytoplasmic vacuolization, and neutrophil presence were scored semi-quantitatively. The mitosis count was calculated from 30 consecutive high-magnification fields. Congestion was classified into three groups (mild, moderate, significant); presence of necrosis into four groups (absent, mild, moderate, severe); cytoplasmic vacuolization into four groups (absent, mild, moderate, severe); neutrophil inflammation into two groups (present, absent). Also, proliferating cell nuclear antigen (PCNA) was studied with immunohistochemical antibodies with the Ventana Benchmark XT immunohistochemistry automatic staining device.

Statistical analysis

Descriptive statistics are given as the median of the frequency percentage distribution. Nonparametric tests were used as it was an animal study. The Kruskal-Wallis test was used to compare three groups of quantitative data, and benforonia correction was made to find out which group the difference originated from. Mann-Whitney U test was used for comparison of 2 groups. $P<0.05$ was considered statistically significant.

RESULTS

Effects on serum TNF-α and IL-6 levels

When the groups were compared, it was found that PH increased IL-6 (20.2±14.42) and TNF-α (66.36±39.48) values; however, IL-6 (11.54±2.89) and TNF-α (39.2±22.73) values were the lowest in the IPC group; rivetingly, both values were higher in the groups that were given silymarin.

Effects on serum AST and Total Bilirubin levels

It was evaluated that (Table 1) the AST value (2071.5±938.4) was the highest in the PH group, but the AST value was lower in the IPC (1535.5±204.8) and Silymarin (1192.3±526.3) groups. It was observed that the AST value was the lowest in the IPC + silymarin group (1002.8±348.9) and significantly improved the AST level with a synergistic effect. Likewise, the highest improvement in bilirubin levels was observed in the IPC + silymarin group (0.33±0.11).

Table 1. IL-6, TNF-α, AST and Total Bilirubin values of the study groups

	IL-6 (Mean±SD)	TNF-α (Mean±SD)	AST (Mean±SD)	Total Bilirubin (Mean±SD)
Grup S	10.99±1.92	22.75±10.16	393.5±82.3	0.05±0.01
Grup PH	20.2±14.42	66.36±39.48	2071.5±938.4	0.38±0.20
Grup IPPH	11.54±2.89	39.2±22.73	1535.5±204.8	0.42±0.13
Grup SPH	38.78±26.27	97.40±59.11	1192.3±526.3	0.40±0.12
Grup SIPPH	26.74±10.59	77.39±20.20	1002.8±348.9	0.33±0.11

Histopathological Evaluation

When the histopathological scores were examined (Table 2), it was found that the mitosis rate and PCNA percentage were higher in the groups given silymarin (Silymarin group and IPC + silymarin group) compared to the PH group. Although the rates of congestion, necrosis, vacuolization, and neutrophils were worse in Silymarin and IPC + silymarin groups; it was thought that its hepatoprotective effect was due to preserving the mitosis rate and keeping the PCNA percentage high (Figure 1,2).

Table 2. Comparison of the histopathological features of the remaining liver tissue, 48 hours after partial hepatectomy according to the groups

	Grup S	Grup PH	Grup IPPH	Grup SPH	Grup SIPPH	P
Mitosis rate	0	42.0±21.24 37.0 (18-80)	23.0±25.76 14.0(1-60)	99.5±96.47 62.0 (13-236)	75.66±98.16 24.0(1-206)	0.27
Congestion (%)						0.001*
Mild	100	100	-	-	-	
Moderate	-	-	66.7	66.7	83.3	
Significant	-	-	33.3	33.3	16.7	
Necrosis (%)						0.006*
Absent	100	50	16.7	66.7	-	
Mild	-	50	50	16.7	-	
Moderate	-	-	33.3	16.7	100	
Severe	-	-	-	-	-	
Vacuolization (%)						0.39
Absent		-	-	-	-	
Mild	100	-	-	-	-	
Moderate	-	50	66.7	50	16.7	
Severe	-	50	33.3	50	83.3	
	-					
Neutrophil (%)						0.44
Absent	100	100	66.7	83.3	66.7	
Present	-	-	33.3	16.7	33.3	
PCNA (%)	0	13.3±8.57 11.5 (6-30)	30.83±5.84 32.5 (20-35)	48.33±17.1 47.5 (30-70)	74.16±3.76 75.0 (70-80)	0.001*

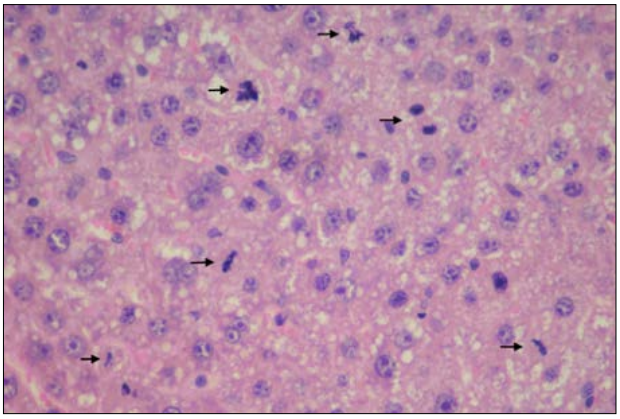

Figure I. Diffuse cytoplasmic vacuolization in hepatocytes and marked increase in mitosis as indicated by arrows in the group that underwent Silymarin + ischemic preconditioning followed by partial hepatectomy (HEX40)

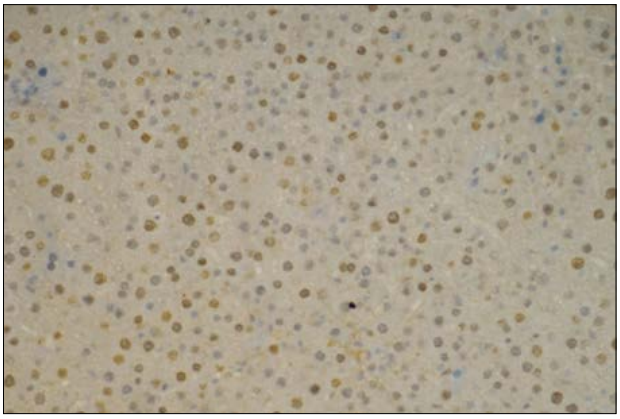

Figure 2. Widespread PCNA immunostaining in hepatocytes in the group that underwent Silymarin + ischemic preconditioning followed by partial hepatectomy (X20)

DISCUSSION

The ability of an organ to regain its mass as a result of tissue loss or loss of a part is defined as regeneration. Post-resection liver regeneration is a complicated process in which proinflammatory cytokines, hormones, and transcription factors play a role (11, 12).

Anaerobic metabolism in the ischemic process and post-reperfusion injury, which is mainly caused by free oxygen radicals, are closely related to dysfunction, morbidity, and mortality after hepatectomy.

Studies focused on IPC and intermittent clamping, and in a limited number of studies, it was shown that IPC was particularly effective in alleviating IRI (13).

Silymarin is a compound that has been used in the treatment of various diseases, especially liver diseases, for more than 2000 years (14). Zholobenko et al. showed that preconditioning of silymarin and its components affects signaling pathways (15). We thought that silymarin could increase hepatoprotection by increasing the effectiveness of IPC, via reducing the adverse effects of ischemia-reperfusion injury.

IRI induces cholestasis and temporarily reduces bile secretion. Changes in bile flow result in an increase in AST levels and plasma bilirubin levels and return to normal within 1-3 days (16). Therefore, we evaluated AST, bilirubin, and histopathological results to detect IRD.

AST levels were found to be statistically significantly lower in the Silymarin and IPC + silymarin groups compared to the other groups, but higher than the control group. Bilirubin values were found to be lower in the IPC + silymarin group. From these results, we thought that silymarin and ischemic preconditioning together reduced hepatocyte damage and increased their hepatoprotective activities.

In 2012, Wang et al. performed left lateral lobe and caudate lobe transplantation following IPC in rats in their study. They reported that histopathological damage was significantly lower in the preconditioning group, and TNF-α was higher in the preconditioning group in the early period and lower in the following periods. However, they reported that IL-6 expression increased significantly both in the early and late periods in the preconditioning group. Accordingly, they suggested that IL-6 regulates hepatocyte proliferation (17). In our study, TNF-α and IL-6 levels were statistically significantly higher in the other groups compared to the control group, and the values were lower in the IPC group compared to the other groups; on the contrary, it was found that both values increased significantly in the group that underwent silymarin+partial hepatectomy. Based on these results, it can be thought that silymarin increases IL-6 levels and ischemic preconditioning decreases TNF-α and hepatocyte damage. Cescon et al. showed that direct IPC did not change the histopathological findings (18). Esin H et al., in a study investigating liver regeneration with dipyridamole administration in rats that underwent partial hepatectomy, showed that mitotic index, PCNA, and relative liver weight (RA) were significantly higher in parallel with regeneration (19). In this study, we considered mitosis rate and PCNA percentages to evaluate liver regeneration in rats who underwent partial hepatectomy. When combined with other findings, we found that these values were significantly higher in the groups treated with silymarin and IPC and were an indicator of hepatoprotective effect.

Mitotic index and PCNA index were used frequently in studies on liver regeneration and played an important role in the interpretation of regeneration (20, 21). By Hou et al. mitotic index and PCNA index data were also used to determine the effect of an organic compound named FR167653 on liver regeneration in rats that underwent partial hepatectomy (22). In their study, it was found that the mitotic index and PCNA index, which were determined simultaneously from the liver sections of the control group rats who had undergone partial hepatectomy, were higher than the other groups. In our study, it was observed that the mitosis rate was higher in the silymarin group compared to the other groups, but there was no statistically significant difference compared to the other groups. It was found that the PCNA index was statistically significantly higher in the group that underwent Ischemic Preconditioning + Silymarin + Partial hepatectomy. With these results, it is thought that the application of IPC and Silymarin together is more effective on regeneration in groups that underwent partial hepatectomy.

In this experimental study, we compared the results of rats who underwent partial hepatectomy with those who underwent partial hepatectomy after using silymarin for

6 weeks before resection. We found that the regeneration ability of the remaining liver tissue was higher and the liver functions were less affected in the group given silymarin compared to the group that was not given. In addition, although it has been shown that IPC increases the regeneration of the remaining liver tissue and liver functions are less affected during partial hepatectomy, we found that the use of silymarin before hepatectomy increases regeneration more and affects liver functions less. With these results, it can be said that the use of silymarin and IPC before hepatectomy will increase liver regeneration. However, similar clinical studies are needed in order to apply these results in clinical practice.

Declarations

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

This study was approved by the Ethics Committee of the Gazi University (Date: 26.06.2015, Ref No: 2017/15.047).

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ORIGINAL ARTICLE

Treatment of talar osteochondral defect with peroneus longus tendon autograft

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Abstract

Background: Tendon autograft has been used in Freiberg's disease, capitellar osteochondritis dissecans, and osteochondral defect in the knee joint. The aim of this study was to evaluate the clinical and radiological results of patients treated with tendon autografts in the treatment of talus osteochondral defect (TOD), and to compare the results of this treatment with other treatment modalities in light of the literature.

Methods: The study was carried out with patients who were treated for TOD with peroneus longus tendon otograft between 2009-2017. 17 ankles of 15 patients were included in the study. The patients who were operated had osteochondral lesions that were Berndt and Harty stage III-IV on radiographs, and Hepple stage III-IV-V on magnetic resonance imaging (MRI). American Orthopedic Foot and Ankle Score (AOFAS) was used for clinical evaluation. Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) classification was used for postoperative radiological evaluation.

Results: The mean age of the patients was 31.9±14.1 (min 17-max 64) years. The mean follow-up period was 23.9±28.7 (min 6-max 120) months. The mean defect size was 1.7±0.7 (min 0.9-max 3.3) cm². The mean AOFAS score was 50.1±15.7 (min 24-max 77) preoperatively and 90.8±7.7 (min 70-max 100) postoperatively. The mean MOCART score was calculated as 87.1±3.1 (min 80-max 90). Postoperative osteoarthritis was not detected in any of the direct radiographs of the patients.

Conclusions: Tendon autograft was considered to be a reliable, easy, cheap and one-step method that can be used in TOD treatment.

Keywords: Ankle Joint, Osteochondral Defect, Scaffold, Tendon Autograft.

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INTRODUCTION

Talus osteochondral defect (TOD) is a pathological, acquired lesion in which the articular cartilage and subchondral bone are damaged (1). After the knee, the most common joint for cartilage lesion treatment is the ankle. TOD occurs in a significant proportion of ankle sprains or trauma (2). The avascular nature of the articular cartilage causes a limited ability to self-repair and regenerate (3). As a result, the probability of degeneration and subsequent osteoarthritis after the damage is quite high (4).

If the complaints cannot be resolved with conservative treatment applied for 6 months, it should be evaluated in terms of surgical treatment (5,6). The superiority of any treatment method over the other has not been conclusively proven so far. If the appropriate surgical method is not preferred in the treatment, an increase in the lesion stage, chronic ankle pain, and secondary osteoarthritis may develop (7,8).

Surgical treatment of TOD includes marrow stimulation techniques such as microfracture, anterograde-retrograde drilling and arthroscopic debridement, osteochondral autograft transplantation system (OATS), osteochondral allograft transplantation, autologous chondrocyte implantation (ACI), matrix induced autologous chondrocyte implantation (MACI), autologous matrix induced chondrogenesis (AMIC), and the use of auxiliary biological materials (9).

Tendon autograft has been used in restoration of the metatarsal head in Freiberg's disease, capitellar osteochondritis dissecans in the elbow, and osteochondral defect (OCD) in the knee joint (10-12). In an experimental study, tendon autograft has been used in OCD in the medial femoral condyle of the dog's knee (13).

The present study aimed to evaluate the clinical and radiological effectiveness of autologous tendon transplantation in selected TODs and to explore its potential as an alternative treatment for TODs.

MATERIALS AND METHODS

Study was carried out with 17 ankles of 15 patients who underwent TOD treatment with tendon autograft between 2009 and 2017. This study was approved by the Etics Commitee of the Karadeniz Technical University (Ref No: 2017/170). Berndt and Harty classification was used for preoperative radiographical evaluation of all patients and Hepple classification was used for magnetic resonance imaging (MRI) evaluation.

Patients who were followed-up for at least 6 months postoperatively in our clinic were included in this study. Adult patients not responding to conservative treatment, with active ankle pain or mechanical symptoms, no advanced osteoarthritis or other ankle diseases, no ankle instability, Berndt and Harty stage III-IV on radiographs, and Hepple stage III-IV-V lesions on MRI were operated.

Those who did not attend adequate follow-up, refused to participate in the study, did not sign the consent form, did not attend their appointments and did not come to the final controls, did not have regular follow-ups in our clinic, were treated in other clinics, did not have the first radiograph and had insufficient information in the file, and had another surgical procedure related to the operated ankle were not included in the study.

The American Orthopedic Foot and Ankle Score (AOFAS) was used in the preoperative and postoperative clinical evaluation of the patients. For the evaluation of osteochondral repair, patients underwent MRI before surgery and at the earliest 6 months after surgery. The status of cartilage repair in the MRI was evaluated using the Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) score. Mocart score includes parameters such as degree of defect repair and filling, integration with neighboring zones, repair tissue surface quality, repair tissue structure, repair tissue signal intensity, subchondral lamina, subchondral bone, adhesion, and effusion.

The subchondral bone parameter in the the original MOCART score could not be calculated on the postoperative MRI evaluation since the defect area was restored with an autologous tendon in the treatment procedure. In this regard, the patient's MOCART score was not 100 points. The highest score was 90 because all subchondral bone parameters were scored 0.

SPSS 23.0 statistical package program was used in the analysis of the data. Descriptive statistics of the evaluation results; numbers and percentages for categorical variables, mean, standard deviation, minimum and maximum for numerical variables. Kolmogorov-Smirnov test was used to evaluate the conformity of the data with normal

distribution. Comparison of two dependent groups was evaluated with the Significance of Difference Between Two Spouses Test (paired t-test). Statistical significance level was accepted as $p<0.01$.

SURGICAL TECHNIQUE AND POSTOPERATIVE CARE

In the preoperative period, medial malleolar osteotomy was planned for centralmedial and posteromedial lesions, which were located behind the anterior of the ankle joint in computed tomography (CT) scans taken while the ankle was in full plantar flexion, for which surgical treatment was considered. After the ankle anteromedial skin incision, the defect was reached by arthrotomy. The defect was debrided until intact cartilage was reached at the defect margins. The sclerotic subchondral tissue was curetted until the healthy subchondral tissue appeared. The subchondral bone was drilled at intervals of 2–4 mm using a 1 mm sized Kirschner wire to the mesenchymal stem cells to come to the defected area. Defect sizes were reevaluated and remeasured.

With the lateral incision of the same extremity, the peroneus longus tendon was removed as half of its thickness. The tendon graft was made into a ball by making a knot on itself and placed in the defect (Figure 1). The ankle was flexed and extended to allow the defect to take its full shape. It was checked whether the tendon graft overflows from the defect and whether it is stable (Figure 2). Insufficient or overflowing tendon grafts were reshaped to completely fill the defect.

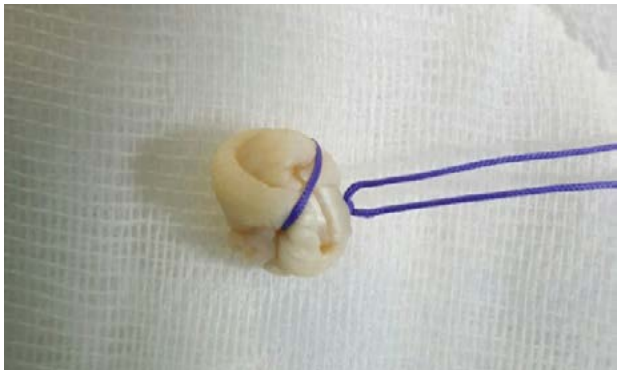

Figure 1: The tendon graft was made into a ball by making a knot on itself and ready to be transferred to the defect area.

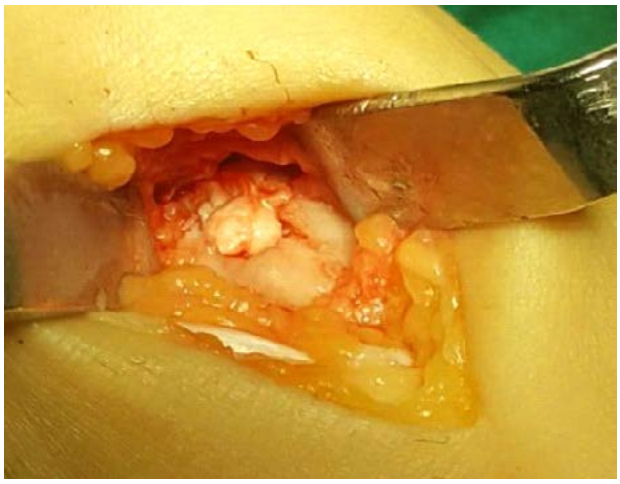

Figure 2. The left talus central medial defect was treated without the need for medial malleolus osteotomy. It can be seen that the tendon fits perfectly into the defect area.

In patients who underwent medial malleolus osteotomy (Figure 3), the osteotomy line was reduced and fixed with a 3.5 mm cannulated partially threaded cancellous screw. After the sugery, a short leg cast with a neutral ankle of 90 degrees was applied.

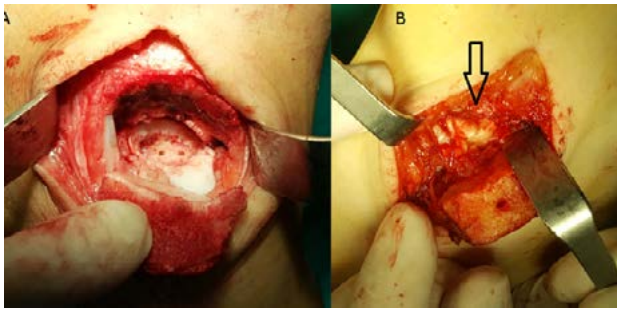

Figure 3. A: Since the right talus was located posteromedially behind the tibia anterior cortex, the defect was treated by performing a medial malleolus osteotomy. B: It can be seen that the tendon fits perfectly into the defect area.

The wound site was followed by opening the cover over the plaster. The sutures were removed on the 15th day after surgery. Walking plaster was applied to the patients who did not undergo osteotomy and were mobilized with a full load. Patients who underwent osteotomy were mobilized for 6 weeks without weight bearing. At the end of 6 weeks, the cast was removed and ankle exercises were started. After the cast was terminated, partial weight bearing was started in patients who had union on the osteotomy side on radiograph.

RESULTS

17 ankles of 15 patients who met our criteria and completed at least 6 months of follow-up were evaluated. 8 of the 15 patients were operated on the right, 5 on the left and 2 on bilateral ankles. 8 of the patients were male and 7 were female. The mean age of the patients was 31.9±14.1 (min 17-max 64) years. 1 patient was 17 years old and consent form was obtained from his parents. Others were over 18 years old and consent forms were obtained from them.

The mean follow-up period of the patients was 23.9±28.7 (min 6-max 120) months. 9 of the lesions were Berndt and Harty stage III and 8 were stage IV. 5 of the lesions were Hepple stage III, 3 were stage IV, and 9 were stage V. 8 of the lesions were in the posteromedial of the talus dome and 9 were in the mediocentral.

The mean size of lesions was 1.7±0.7 (min 0.9-max 3.3) cm^2. All 15 patients were adults. Preoperative and postoperative osteoarthritis was not detected in any of the direct radiographs of the patients (Figure 4).

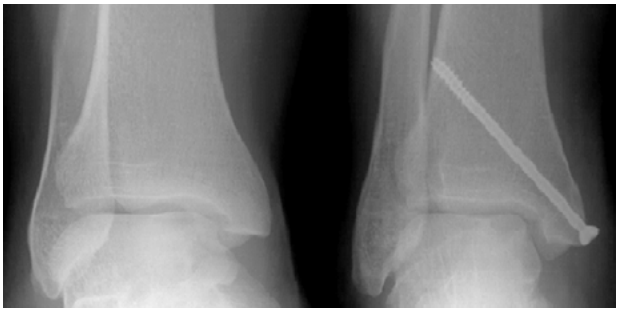

Figure 4. 12th month anterior posterior radiograph of a patient with right talus posteromedial defect who underwent medial malleolus osteotomy. No degeneration is seen in the joint.

Autologous peroneus longus tendon graft was used in all patients who underwent TOD treatment with a tendon graft. None of the patients developed complications related to the donor area.

In the clinical evaluation using AOFAS, the mean preoperative AOFAS score of the patients was 50.1±15.7 (min 24-max 77). In the postoperative period, the mean AOFAS score was 90.8±7.7 (min 70-max 100). The difference between preoperative and postoperative scores was statistically significant ($p<0.001$).

MRIs taken at the earliest 6 months after surgery were evaluated. The mean MOCART score was calculated as 87.1±3.1 (min 80-max 90). No complications developed in any of the patients after the operation. Any ankle disease requiring reoperation did not develop. The graft survival rate of the patients participating in the study was 100% (Figure 5 and 6).

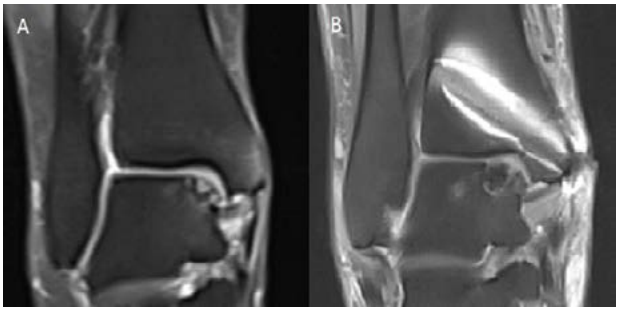

Figure 5. A: Coronal T2 section magnetic resonance images (MRI) of a grade 3 posteromedially located osteochondral defect of right talus (1 cm2 size) in 47-year old female; B: 6 months follow-up coronal T2 section MRI showing complete filling of the defect and establishment of smooth articular surface

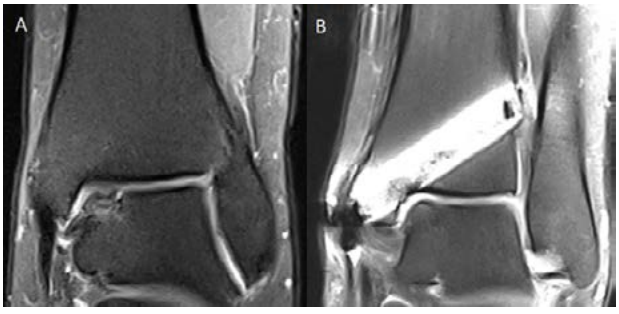

Figure 6. A: Coronal T2 section magnetic resonance images (MRI) of a grade 3 posteromedially located osteochondral defect of left talus (1.35 cm2 size) in 17-year old female; B: 1-year follow-up coronal T2 section MRI showing complete filling of the defect and establishment of smooth articular surface

All of the patients returned to their active working lives and daily activities without restriction, were satisfied with the surgery and stated that they could recommend it to other patients, stated that their ankle pain was relieved. Detailed patient demographics are shown in Table 1.

Table 1. Demographics Data of the Patients and Results

Variable	Value
Total number of patients	15 (17 osteochondral defects of the talus)
Age (year), mean ± SD (range)	31.9 ± 14.1 (range, 17–64)
Sex (female/male)	7/8
Follow-up time (months), mean ± SD (range)	23.9 ± 28.7 (range, 6–120)
Lesion area (cm^2), mean ± SD (range)	1.7 ± 0.7 (range, 0.9–3.3)
AOFAS, mean ± SD (range)	Preoperative 50.1 ± 15.7 (range, 24–77). Postoperative 90.8±7.7 (range, 70–100)
MOCART, mean ± SD (range)	Postoperative 87.1 ± 3.1 (range, 80–90)
Complication	No

Note. SD, Standard deviation. AOFAS, American Orthopedic Foot and Ankle Score. MOCART, Magnetic Resonance Observation of Cartilage Repair Tissue.

DISCUSSION

In this study, there was improvement in ankle scores. The mean AOFAS score was 50.1±15 preoperatively, and 90.8±7.7 postoperatively. The difference between preoperative and postoperative scores was statistically significant ($p<0.001$). We believe that this situation was caused by the viscoelastic and anisotrophic structure of the tendon autograft. The mean MOCART score was calculated as 87.1±3.1 (min 80-max 90). No complications developed in any of the patients after the operation and no patient required revision. The graft survival rate of the patients participating in the study was %100.

Peroneal tendon autografts have been used in the treatment of OCDs because the tendon has a solid structure, can resist mechanical loads, is composed of collagen fibers due to its structure, and can act as a scaffold. The tendon graft is a monophasic (monoblock) graft. It repairs the tissues in the defect by replacing both chondral and subchondral tissues together (10).

Tendons were traditionally known to contain only tenocytes (14-16). However, human and mouse tendons have also been shown to contain tendon stem cells (Tendon Stem Cells, TSC) (17). TSCs have the potential for multidifferentiation, including differentiation into adipocytes, chondrocytes, and osteocytes. In addition, Mos et al. showed that tendon-derived fibroblasts (Tendon Derived Fibroblasts, TDFs) from adolescent non-degenerative human hamstring tendons can differentiate into adipocytes, chondrocytes, and osteocytes (18). It has also been found that TSCs can differentiate into adipocytes, chondrocytes, and osteocytes in vitro, and form tendon-like, cartilage-like, and bone-like tissues in vivo (19). With these features, the tendon autograft we used supports that it can be used in the treatment of OCDs.

The main issue in the treatment of an OCD should be primarily to fill the defect with an autograft close to the mechanical properties of the tissues in the region. As a result, the geometry of the joint should be restored and this area should be included in load bearing, pain should be eliminated, joint functions should be preserved, and degeneration should be stopped (10).

Tendon autograft has been used and good results have been obtained in the restoration of the metatarsal head in Freiberg's disease and capitellar osteochondritis dissecans in the elbow (11,12). In addition, it has been used in an OCD in the medial femoral condyle of a dog's knee and the results have been found to be satisfactory in macroscopic and microscopic terms (13).

A previous study reported satisfactory results in a case where an autologous tendon has been used in the restoration of an OCD in the knee joint. As the results of the knee OCD treatment with tendon autograft were positive, we preferred its application in TOD treatment as well. As we treated TOD with peroneus longus tendon autograft, also Turhan et al. treated 20 patients (22 knees) with OCD of the femoral medial condyle with peroneus longus tendon autograft. The mean age of patients was 25.5± 6.8 years. The average defect size was 4.2 ± 2.1 cm^2. Total Knee Injury and Osteoarthritis Outcome Score (KOOS) was 29.4 ± 5.5 preoperatively, and 81.5 ± 5.9 postoperatively. The mean follow-up period was 68.7 ± 37.7 months. The mean MOCART score was 56.2 ± 10.7. 80% of the patients showed no radiological progress of osteoarthritis (10).

Radiological improvement was detected in all patients, and MOCART scores in MRI evaluation had satisfying results. In our study, the subchondral bone parameter in the original MOCART score could not be calculated in the postoperative MRI evaluation because monoblock repair is performed by replacing the tendon graft cartilage and subchondral bone together. In other words, it replaces

both cartilage and subchondral tissue function. Tendon grafts do the job of both tissues. In this regard, no patient's MOCART score was 100 points in our study. All subchondral bone parameters were scored 0. We attribute the absence of radiological degeneration to the repair of the defect, its participation in weight bearing and the improvement of joint functions. It is very important that tendon autografts are seen on MRI even after 1-8 years postoperatively and degeneration does not develop on radiographs.

The limited number of patients, short follow-up periods, the absence of a comparison group, and the lack of arthroscopic and histological evaluation are the limitations of this study.

The most important result of this study is that patients do not have pain, improvement of ankle functions are observed, and they return to work. Tendon autograft can be used in both primary and revision cases. Depending on the size of the defect, the diameter of the tendon node can be adjusted. Since the tendon tissue is flexible, it can adapt to any surface and joint geometry. While none of the patients in our study developed degeneration and arthrosis in the ankle, the tendon graft was in the place where it was placed in the control MRIs of all patients, any lysis, any resorption, and any insufficiency were not observed. No patient required a secondary operation. These results showed us that the defect was repaired with a tendon graft, the tendon adapted to the geometry of the joint, began to carry loads in the joint, and facilitated the movements.

CONCLUSIONS

It was concluded that our method is a single-session, easy, reliable, inexpensive method, which does not cause any loss for the patient, has a high level of patient satisfaction, and can be used for revision surgery in cases where other methods cannot be successful.

Declarations

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

This study was approved by the Ethics Committee of the Karadeniz Technical University (Date: 02.10.2017, Ref No: 2017/170).

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ORIGINAL ARTICLE

From a dermatologist point of view, enthesopathy and peripheral neuropathy in psoriasis patients

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Abstract

Background: Investigating psoriasis patients' enthesopathy and peripheral neuropathy, which dermatologists often neglect.

Methods: Seventy-four psoriasis patients' lower limb entheses were evaluated by ultrasonography using Glasgow Ultrasound Enthesitis Scoring Scale (GUESS). Sensory and motor nerve conduction studies in median and ulnar nerve, motor nerve conduction research of peroneal and tibial nerves and sensory conduction study on right sural nerve was performed in 25 patients.

Results: 172 of 730 entheses had ultrasonographic enthesopathy symptoms (23.56%). Enthesopathy was substantially more common in cases involving the nail ($p = 0.004$). The frequency of enthesopathy did not change significantly between symptomatic and asymptomatic patients ($p = 0.408$). Seven of 25 patients (28%) had a pathology in nerve conduction studies. With increasing GUESS scores, bilateral ulnar and right tibial nerve distal motor latencies were shown to become longer ($p = 0.001$, $p = 0.01$, $p = 0.019$), although left ulnar nerve sensory conduction velocity got slower ($p = 0.033$).

Conclusions: Enthesopathy and peripheral nerve dysfunction were frequently observed in psoriasis patients. Dermatologists should be mindful of neuromusculoskeletal disorders in psoriasis patients.

Keywords: Psoriasis, Enthesopathy, Neuropathy, Ultrasound.

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INTRODUCTION

Psoriasis is seen in 2% of the general population, and is accompanied by arthritis in 5-30% of these cases (1). Enthesopathy is inflammation in the area where tendons and ligaments attach to bone. Even though there are studies on the frequency and characteristics of enthesopathy in spondyloarthritis, studies on the entheseal areas of psoriasis patients irrespective of the presence of arthritis are still rare (2). In those studies, psoriasis cases that lacked clinical signs of enthesitis or joint damage were found to have some sort of joint involvement with ultrasonography (USG) (3). Even though larger studies with longer follow-up periods are required in this subject, primary studies indicated that the psoriasis cases with enthesopathy involvement have a greater risk of developing psoriatic arthritis. Regarding this fact, early term diagnosis of enthesopathy presence may be of great assistance in the early diagnosis and treatment of psoriatic arthritis (PsA), a progressive joint damage condition (4). Due to this, we decided to employ Glasgow Ultrasound Enthesitis Scoring Scale (GUESS) in addition to clinical diagnosis to examine entheseal regions using USG (5).

Peripheral neuropathy is an umbrella term used for almost all peripheral nervous system diseases (6). In the previous case studies of psoriasis and PsA patients found in the literature, peripheral neuropathy was reported (7). Even though there are previous studies on the occurrence of polyneuropathy due to pathogenesis or treatment of rheumatologic conditions, only one study has been conducted on this subject in psoriasis patients (7-9). This is the reason why we investigated the presence of polyneuropathy in our cases using electrophysiological methods.

This study aims to investigate the presence and frequency of enthesopathy using GUESS, the frequency of peripheral neuropathy in arm and leg by nerve conduction studies (NCS), the relationship between enthesopathy and clinical parameters, and to compare the frequency of symptomatic and asymptomatic enthesopathy in psoriasis patients.

MATERIALS AND METHODS

Prospectively, seventy-four individuals that are followed in our dermatology clinic, over 18 years of age with chronic plaque type psoriasis and no known musculoskeletal disease, recent trauma, surgery or injection were included in this study. Patients with previously diagnosed polyneuropathy, diabetes mellitus, thyroid disease, chronic kidney failure, autoimmune diseases, pernicious anemia, a history of alcohol abuse, chemotherapy, epilepsy or seizures were excluded from the NCS.

The dermatologist recorded the age, gender, height, weight, body mass index value (kg / m2), age at the diagnosis of psoriasis, duration of the disease, other known diseases, family history of psoriasis, presence of scalp, genital and intergluteal area and nail involvement, PASI ("Psoriasis Area Severity Index") score, and NAPSI ("Nail Psoriasis Severity Index") score. A physical therapy and rehabilitation physician evaluated the presence of pain and / or swelling in the knee and heel joints, as well as pain, tenderness, swelling and restriction of movement in the knee and heel joints. In laboratory exams, C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) values were recorded.

One week after their initial evaluation, all patients underwent USG for their knee and ankle joints at the Hacettepe University Physical Therapy and Rehabilitation clinic. A specialist conducted the study in a dark room using a 7-12 MHz linear probe (Logiq 5, GE Medical systems) without being informed of the clinical severity of the patients. With the patient supine and the knee flexed 30°, the superior pole of the patella (quadriceps tendon insertion), the inferior pole of the patella (patellar ligament origin) and the patellar ligament insertion at the tibial tuberosity was examined. The Achilles tendon and the plantar aponeurosis were evaluated with the patient supine and the feet at 90° of flexion over the edge of the examination table. In GUESS, the quadriceps tendon, patellar tendon (attachment areas to the lower pole of the patella and tibial tuberosity), Achilles tendon and plantar aponeurosis are evaluated in grey scale for tendon thickness, the presence of bursitis, erosion and enthesophyte. Bursitis was described as a well circumscribed, localized anechoic or hypoechoic region at the site of an anatomical bursa that was compressible by the transducer. Bony erosion was described as a cortical fracture with a step down contour defect, whereas an enthesophyte was defined as a step up bony prominence at the end of the normal bone contour. The maximum thickness of the ligament, aponeurosis, and tendon was measured proximal to the bony insertion.

The following criteria were used to determine abnormal structure thickness: quadriceps tendon thickness >6.1 mm, proximal and distal patellar ligament thickness >4 mm, Achilles tendon thickness >5.29 mm, plantar aponeurosis thickness >4.4 mm. Total GUESS score ranges from 0-36 (5).

Electromyography (EMG) and NCS tests were performed without the use of needles on patients without an underlying cause for polyneuropathy such as a pre-existing illness or history. According to Oh, the latency and conduction velocities (NCV) of patients were measured (10). The median and ulnar nerves of the upper extremities were measured for sensory and motor NCV, the peroneal and tibial nerves of the lower right extremities were measured for motor NCV, and the sural nerve was measured for sensory NCV. Another physician performed the procedure at the Hacettepe University Physical Therapy and Rehabilitation Clinic using a 5-channel EMG instrument (Medelec Synergy TECA machine; Oxford Instruments Medical, Surrey, United Kingdom) at room temperature, while the skin temperature was over 30° C.

All statistical reviews were performed using SPSS 15.0 for Windows. The Kolmogorov-Smirnov test was used to determine if the numerical data were normally distributed or not. The "significance test of the difference between the two means" was used to compare normally distributed numerical variables between two groups, and the "Mann-Whitney U test" was used to compare non-normally distributed numerical variables. In the comparison of more than two independent groups, the Kruskal-Wallis test was used for data that did not meet the parametric test assumptions, but the "One-way Analysis of Variance (ANOVA)" or "Welch ANOVA" tests were used for data that satisfied the parametric test assumptions. To establish the link between categorical variables, a Chi-square test (Pearson Chi-square, Yates Corrected Chi-square, or Fisher-Exact Chi-square) was employed. As descriptive statistics, parametric tests provided mean±standard deviation, while non-parametric tests provided median (minimum-maximum). Spearman's rho coefficient was used to analyze correlations between variables. P values below 0.05 were considered as statistically significant.

The study was conducted with the approval of the Ethics Committee of Hacettepe University Faculty of Medicine Clinical and Pharmaceutical Research Local Ethics Committee with decision number HEK 12/70.

RESULTS

Using USG, 740 entheses from 74 patients (39 female (52.7%), 35 male (47.3%)) were evaluated. Due to extremely thick psoriasis plaques on the skin, ten enthesis regions from 3 patients (bilateral patellar ligament proximal and distal entheses in 2, left patellar ligament proximal and distal entheses in 1 patient) could not be seen. In 55 of 74 patients (74.32%), at least one enthesopathy finding was present in 172 of the 730 entheses (23.56%). Achilles' enthesis was the most common area where enthesopathy was diagnosed (59 / 730) (8.08%). The most prevalent symptom was enthesophytes (95 / 730) (13.01%) and the most common site of enthesophyte was the calcaneus posterior pole in Achilles tendon enthesis (26 / 74 patients, 50/730 entheses). Table 1 shows the findings of USG.

Table 1. USG findings of entheses

	Quadriceps enthesis (n = 148)	Proximal Patellar enthesis (n = 143)	Distal Patellar enthesis (n = 143)	Achilles enthesis (n = 148)
Tendon thickness*	2	2	1	1
Bursitis	19	0	3	6
Bone erosion	2	7	29	5
Enthesophyte	34	5	3	50
Inflammation	21	2	4	7
Chronic Symptoms	36	12	32	55

*Quadriceps tendon ≥ 6.1 mm, proximal patellar ligament ≥ 4 mm, distal patellar ligament ≥ 4mm, Achilles tendon ≥ 5.29 mm, plantar aponeurosis tendon ≥ 4.4 mm
n = Enthesis number

The mean GUESS score was determined to be 2.51 ± 2.49 (0 - 13). Nineteen patients had a GUESS score of 0, while the remaining 55 scored at least 1. There was no statistically significant correlation between the GUESS score and gender, disease onset age, disease duration, family history, scalp involvement, intergluteal / perianal area involvement, genital area involvement, PASI, NAPSI, body mass index, ESR and CRP values, knee and / or heel symptoms ($p > 0.05$). This link was statistically significant ($p = 0.022$, $r = 0.266$): as age increased, so did the GUESS score. Patients with nail involvement had substantially higher GUESS scores when compared to patients without nail involvement ($p = 0.004$, $r = 0.338$), although there was

no statistically significant difference in age between the two groups (p = 0.309). Table 2 depicts the relationship between the GUESS score and demographic and clinical data, and Figure compares the GUESS scores of patients with and without nail involvement.

Table 2. The relationship between the GUESS score and demographic and clinical data

	GUESS score Median (minimum-maximum)	p
Gender		
Female (N = 39) Male (N = 35)	2 (0 - 7) 2 (0 - 13)	0.721
Family history		
No (N = 55) Yes (N = 19)	2 (0 - 13) 1 (0 - 7)	0.187
Scalp involvement		
No (N = 15) Yes (N = 59)	2 (0 - 7) 2 (0 - 13)	0.589
Intergluteal/perianal involvement		
No (N = 48) Yes (N = 26)	2 (0 - 13) 2 (0 - 7)	0.633
Genital area involvement		
No (N = 44) Yes (N = 30)	2 (0 - 7) 2 (0 - 13)	0.45
Nail involvement		
No (N = 30) Yes (N = 44)	1 (0 - 5) 3 (0 - 13)	**0.004**
Knee and/or heel symptom		
No (N = 45) Yes (N = 29)	2 (0 - 13) 2 (0 - 7)	0.408

When 29 patients with symptoms on the knee and/or heel were compared with 45 patients without symptoms, no significant difference was found between these two groups in terms of GUESS score (p = 0.408). 25 of 74 patients went under NCS. 7 of the 25 patients (28%) were diagnosed with a pathology. As a result of NCS, 1 patient (4%) had decreased motor NCV in the peroneal nerve, 1 patient (4%) had increased motor latency in right tibial distal nerve, 1 patient (4%) had decreased left median sensory NCV, 2 patients (8%) had decreased sensory NCV in bilateral median nerve, and 2 patients (8%) were diagnosed with right carpal tunnel syndrome. Right ulnar (p = 0.001), left ulnar (p = 0.01) and right tibial nerve distal motor latency (p = 0.019) increased as GUESS scores went up, while left ulnar NCV decreased (p = 0.033). Other clinical and laboratory parameters were not found to correlate with latency and conduction velocities.

DISCUSSION

Psoriasis is a chronic, recurrent, inflammatory dermatosis accompanied by 5-30% PsA. In the seronegative spondyloarthritis (SpA) group, PsA is clinically characterized by joint, tendon and entheses involvement. It is hypothesized that the inflammation in spondyloarthropathies starts at the first entheses and subsequently spreads to the adjacent joint (3). The early detection of enthesopathy using USG can be a very useful method in the early diagnosis and treatment of PsA, a progressive disease characterized by joint destruction (4).

In one study that employed GUESS scores to investigate subclinical enthesis involvement in psoriasis patients, 62.5% of the patients were reported to have enthesopathy (11), and in another study, at least one symptom of enthesopathy was observed in 32.9% of 450 entheses evaluated (12). 62% of psoriasis patients in a recent study using the MASEI (Madrid Sonography Enthesitis Index) score (an enthesis score which contains power doppler and upper limb examinations in addition to GUESS score) for enthesis evaluation had at least one entheseal abnormality (13). These results are similar to our study.

In our investigation, there was no statistically significant correlation between GUESS score and disease period or PASI scores. This outcome is consistent with previous studies (2,12,13,14). Nail involvement is a risk factor for developing PsA. Future PsA development is 2.24 times more likely in patients with nail involvement (15). In a study comparing psoriasis patients with and without nail involvement, Ash et al. found that patients with nail involvement had a significantly higher USG mean score than those without nail involvement. In addition, this study demonstrated that USG scores increase with the severity of nail involvement (14). Similarly, in our study, patients with nail involvement had higher GUESS scores (p = 0.004, r = 0.338), however there was no significant relationship between GUESS scores and the severity of nail involvement (p = 0.224). Regardless of the level of nail involvement we discovered a substantial correlation

between nail involvement and enthesitis formation in psoriasis patients. Therefore, all psoriasis patients with nail involvement should be reviewed for enthesopathy presence, regardless of the severity of nail involvement.

Only 3-8% of early-stage PsA patients exhibit articular symptoms (4). Using GUESS scores, Gisondi et al.'s study on 30 patients without articular symptoms revealed at least one enthesopathy finding in every patient (2). De Simone et al.'s study about the review of Achilles tendon alterations using USG reported that 53.6% of 41 asymptomatic psoriasis patients had some kind of pathology in USG (16). De Filippis et al. found that 33% of 24 asymptomatic patients had developed enthesopathy (17). Comparing 29 symptomatic patients to 45 asymptomatic patients in our study revealed no significant difference in GUESS scores ($p = 0.408$). However, during USG review, 75.5% of 45 patients displayed at least one symptom of enthesopathy. These results support the idea that psoriasis patients with no musculoskeletal symptoms or signs frequently have enthesopathy.

Due to the fact that autoimmune collagen tissue diseases can directly affect the nerves, cause nerve entrapment secondary to arthritis and synovitis, or be caused by the medications used to treat these conditions, peripheral neuropathy can be observed in these conditions. In case reports from previous studies in the literature, neuropathy coexisted with psoriasis and psoriatic arthritis patients (18,19). 7 out of 25 NCS patients (28%) were diagnosed with pathological disorders, with 2 (8%) of them being diagnosed with right carpal tunnel syndrome. In a study of peripheral nerve dysfunction in 32 psoriasis patients, Chroni et al. did not identify any patients with a pathological condition (7). In another study comparing Ankylosing spondylitis (AS) patients and healthy control groups, AS patients had a 46% rate of peripheral neuropathy whereas in the healthy control group this rate was 3.3% (9). Furthermore, epidemiological studies revealed that peripheral neuropathy was observed in 7% of the elderly population and in 10% of patients with connective tissue disease (20,21). In this regard, our research is groundbreaking since it demonstrates that psoriasis and peripheral nerve involvement are connected. To more precisely identify this relationship more clearly, further controlled studies are necessary.

According to the findings of this study, bilateral ulnar and right tibial nerve distal motor latencies appear to increase when GUESS scores are higher. There is no evidence in the published literature of a correlation between enthesopathy and peripheral neuropathy. Entrapment of nerves between joint structures swollen with edema, rheumatoid synovitis, nodules and calcified ligaments is one of the peripheral neuropathy mechanisms in rheumatologic illnesses (9). In enthesopathy, increased tendon thickness, edema, and newly developed bony structures known as enthesophytes can also cause nerve compression. In our study, we only examined lower extremity entheses, however, we found a correlation between GUESS scores and ulnar nerve measurements (direct proportion with right ulnar latency ($p = 0.001$) and left ulnar latency ($p = 0.01$), opposite proportion with left ulnar nerve sensory NCV ($p = 0.033$)). Similar changes may occur in the upper extremity entheses as they do in the lower extremities. In conclusion, our study is the first to suggest a possible relationship between enthesopathy and peripheral nerve dysfunction. To clarify this relationship's etiology, more controlled studies with more patients are necessary.

Our study was limited by the absence of a control group, the small number of patients who underwent NCS, and the analysis of only the lower extremity entheses with USG.

CONCLUSION

Our findings indicate that nail involvement and enthesopathy are closely related, that psoriasis is associated with a significant prevalence of asymptomatic enthesopathy, and that enthesopathy may be associated with peripheral nerve dysfunction. Dermatologists should keep in mind the neuromusculoskeletal disorders in psoriasis patients.

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Declarations

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This study was approved by the clinical research Ethics Committee of the Hacettepe University Faculty of Medicine Clinical and Pharmaceutical Research (Date: 11.05.2012 Number: 12/70-32).

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ORIGINAL ARTICLE

Evaluation of the opinions of patients applying to the emergency department in the north of Syria about COVID-19 vaccine

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Abstract

Background: In north of Syria, health care is provided with humanitarian aid. Mass vaccinations, seen as a way out of the pandemic in Syria, are to be carried out with the support of WHO and under the supervision of Turkey. In this study, we assessed the knowledge and opinions of people who applied to hospital emergency departments in north of Syria Region about COVID-19 and the vaccine.

Methods: A face-to-face survey was conducted by trained interviewers among patients and their relatives who applied to the emergency department of Azez Vatan and Çobanbey Hospitals in North of Syria Region.

Results: A total of 331 subjects, 40.2% males and 59.8% females, participated in the study. The mean age was 36.06±12.69 years. While 42.9% of the participants wanted to be vaccinated, 42% said they did not want to be vaccinated, and 15.1% were undecided. When those who answered "No and I am undecided" to the question "Would you like to be vaccinated?" were asked about their hesitations about the vaccine, 129 people indicated that they did not think the vaccines were safe, and 107 people were not sure whether the vaccines provided protection. Belief was found to have no significant effect on the desire to be vaccinated.

Conclusions: It is believed that vaccine awareness in the global sense, misconceptions and concerns about vaccines are effective and should be taken into account in vaccination activities and pandemic response in Syria as well as around the world.

Keywords: COVID-19, Emergency Department, Syria, Vaccine.

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INTRODUCTION

COVID-19 (Coronavirus Disease 2019) was first detected in Wuhan in the last quarter of 2019 and declared a pandemic by the World Health Organization (WHO) (1). The COVID-19 pandemic, which has spread globally and caused international concern since its first appearance (2,3), has caused more than 4 million deaths and nearly 200 million diseases by July 2021 (4); the global economy affected by the epidemic contracted by 3.4-7.6% and caused a 7.9% loss of working hours worldwide (5). The pandemic disrupted many aspects of human activity and led to global socioeconomic challenges, including rising unemployment, income, food and housing insecurity, and intimate partner violence (6-9).

Even if the spread of the virus can be limited by medical services and mask-distance-hygiene measures in the community, problems in economic and social life will continue until effective mass immunity is achieved through vaccination (10). Indeed, many governments, as well as global health authorities, have indicated that mass vaccination against the virus is the easiest and possibly only path to normalcy and stability (11,12). In response to this evolving global crisis, in order to normalize human activity, institutions have had to take protective and preventive measures in addition to developing vaccines at a pace unprecedented in the history of vaccine production (13,14). While a typical vaccine development trial takes as long as up to 73 months, accelerated vaccine trials during the COVID-19 pandemic shortened that time to 14 months (15). Unfortunately, the speed of vaccine production has led to reservations about vaccines in society (16).

The Strategic Advisory Group of Experts on Immunization (SAGE) defines the term "vaccine hesitation" as a delay or refusal to vaccinate despite the availability of vaccination services. There are three major key factors responsible for vaccination hesitancy: First, the thought that the vaccine is unnecessary because the severity of the disease is not understood; second, low belief in the efficacy and safety of the vaccine and the thought that the health care system and its staff are inadequate; third, concerns that the vaccine is readily available, affordable, and easy to transport (17,18).

In the Middle East Syria has been on the rise for about 10 years. The country has been affected by internal unrest and half of its population has been displaced by internal and external migration (19). In northern Syria, health care is not under the control of a central health system, but is provided with humanitarian aid (20). This makes the region even more vulnerable to the pandemic. Mass vaccinations, seen as a way out of the pandemic in Syria, are to be carried out with the support of WHO and under the supervision of Turkey (21,22).

In this study, we assessed the knowledge and opinions of people who applied to hospital emergency departments in the Northern Syria Region, where internal unrest is ongoing, about COVID-19 and the vaccine. We aimed to shed light on the community-based barriers to vaccination trials in the region and contribute to regional and global vaccination trials.

MATERIALS AND METHODS

Study Design

Before the start of the study, The Ethics Committee of Hatay Mustafa Kemal University for non-interventional research (resolution number: 16) approved the study in 06.05.2021. The type of study is cross-sectional research. Between 15.05.2021-30.05.2021 a face-to-face survey was conducted among patients and their relatives who applied to the emergency department of two hospitals (Azez Vatan and Çobanbey Hospitals with 200 beds each) in Northern Syria Region. It is a survey study that included 331 participants. Addition, the study was conducted in accordance with the ethical principles of the Declaration of the World Medical Association of Helsinki.

Selecting participants

During the planning phase of the study, sample size calculation was made with A Priori power analysis. The minimum sample size needed for the study was calculated as 323 (Effect size w=0.30, α err probe=0.05, Power (1-β err probe) = 0.95, Df=17.). The sample size of the study was calculated with the program G*Power 3.1.9.7 (Franz Foul, Unversitat Kiel, Germany).

All participants are Arabs residing in Northern Syria. Informed consent was obtained from the participants in the survey and personal information was kept confidential.

People with mental disabilities and psychiatric illnesses and children under 18 years of age were excluded from the study. At the time of the study, only healthcare workers in the region had received the COVID-19 vaccine. None of the participants included in our study had yet received the COVID-19 vaccine.

Obtaining the data

The original questionnaire was created by us by scanning the literature. Firstly, demographic, medical and social questions such as age, education level, comorbidity, district of residence, type of housing, family type, and average income were asked. In addition, questions were organized to evaluate the perspectives of the participants on COVID-19 and its vaccine. The questionnaires were prepared in Arabic by local Syrian physicians and administered by trained interviewers.

Statistical Analysis

Statistical analyses of the study were performed using Statistical Package software for Social Sciences version 25.0 for Windows (IBM SPSS Statistics for Windows, version 21.0. Armonk, NY: IBM Corp., USA). The normality assumption was tested with the tests Kolmogorov-Smirnov and Shapiro-Wilk. Explanatory statistics of variables are reported as mean ± standard deviation, median (Min-Max) and n (%). For univariate analyses, the Chi-Square test and the Fisher-Freeman-Halton test were used, depending on the nature of the variables and the availability of assumptions.

RESULTS

A total of 331 subjects, 133 (40.2%) males and 198 (59.8%) females, participated in the study. The mean age was 36.06±12.69 years. 31 (15.7%) of the female participants were pregnant and 38 (19.2%) were nursing mothers. Of the participants, 171 (51.7%) lived in urban areas, 160 (48.3%) lived in rural areas and camps and 24.2% (n=80) had no house as accommodation. The average number of people living in the same house was 6. There were 260 participants (78.5%) living in nuclear families and 144 (43.5%) were unemployed. 73 of the respondents (22.1%) had a history of chronic illness. 84 of the participants (25.4%) had a university degree. The income level of 230 participants (69.5%) ranged between 501TL-3000TL (Figure 1).

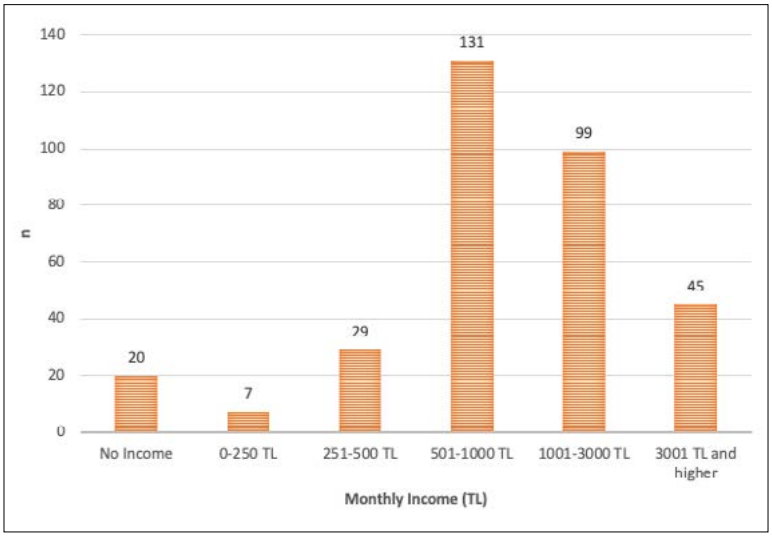

Figure 1. Income levels (n=331) of participants.

When asked who should receive the COVID-19 vaccine first, 253 participants (76.4%) responded as health care workers, 33 (10.0%) as education workers, 45 (13.6%) as religious officials, security personnel, and officers. When participants' views on COVID-19 in Syria were evaluated, 158 (47.7%) participants thought that COVID-19 was not common in Syria. The views of the participants about COVID-19 disease and what it is are shown in Figure 2. While 142 (42.9%) of the participants wanted to be vaccinated, 139 (42.0%) said they did not want to be vaccinated and 50 (15.1%) were undecided.

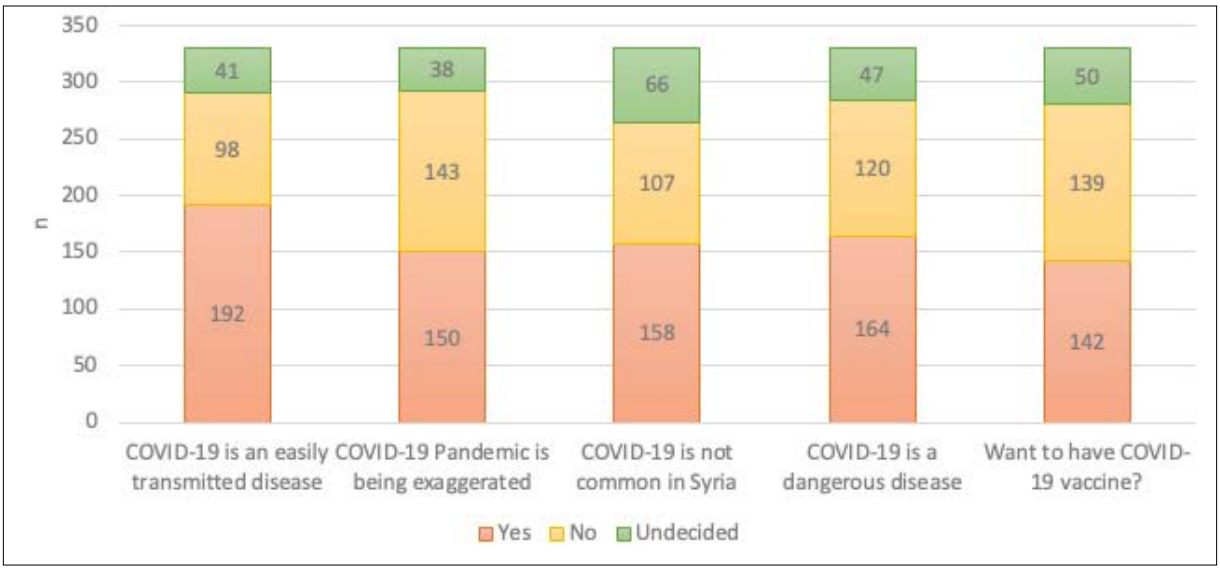

Figure 2. Participants' views of COVID -19 (n=331).

When those who answered "No and I am undecided" to the question "Would you like to be vaccinated?" were asked about their hesitations about the vaccine, 129 people (68.3%) indicated that they did not think the vaccines were safe, 114 people (60.6%) feared that the vaccine would cause harm, and 107 people (56.6%) were not sure whether the vaccines provided protection (Figure 3).

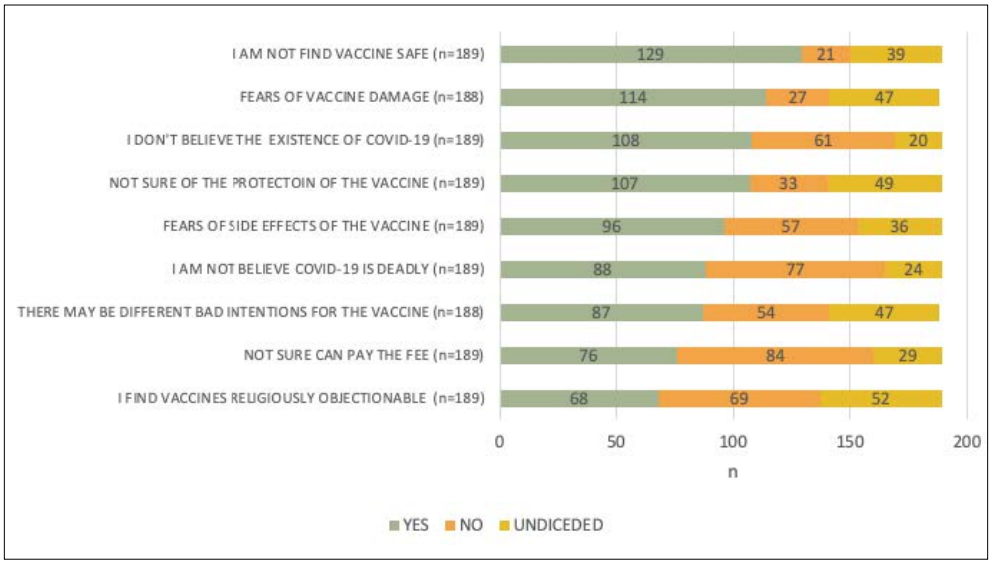

Figure 3. Reason for hesitation of those who answered "No and I am undecided" to the question "Do you want to get vaccinated?".

There was no significant association between gender, pregnancy, and breastfeeding status and the desire to be vaccinated (p=0.259, p=0.085, p=0.985 respectively). Although there was no significant difference between place of residence and desire to be vaccinated (p=0.082), the affirmative response (56.3%) of participants who lived in urban areas was higher than those who lived in rural areas and camps (43.7%). 33.8% (n=47) of those who wanted to be COVID-19 vaccinated and 35.4% (n=17) of those who were undecided had a university degree or higher level of education. The level of education was found to have a statistically significant relationship with participants' willingness to be vaccinated against COVID-19 (p=0.007) (Table 1).

Table 1. Comparison of desiring to be vaccinated against COVID-19 with demographic and chronic disease conditions

DESIRE TO BE VACCINATED AGAINST COVID-19						
Demographic and Chronic illness status		Yes n(%)	No n(%)	Undecided n (%)	Test Value	p
					Chi-Square test	
Gender (n=331)	Male (n=133)	51(38.3)	63(47.4)	19(14.3)	2.703	0.259[a]
	Female (n=198)	91(46.0)	76(38.4)	31(15.6)		
Working status (n=331)	Employed (n=187)	77(41.2)	79(42.2)	31(16.6)	0.921	0.631[a]
	Unemployed (n=144)	65(45.1)	60(41.7)	19(13.2)		
Chronic illness (n=331)	Yes (n=73)	31(42.5)	29(39.7)	13(17.8)	0.572	0.751[a]
	No (n=258)	111(43.0)	110(42.6)	37(14.3)		
Pregnancy (n=198)	Yes (n=31)	9(29.0)	17(54.8)	5(16.1)	4.928	0.085[a]
	No (n=167)	82(49.1)	59(35.3)	26(15.6)		
Breastfeeding (n=198)	Yes (n=38)	17(44.7)	15(39.5)	6(15.8)	0.030	0.985[a]
	No (n=160)	74(46.3)	61(38.1)	25(15.6)		
					Fisher-Freeman-Halton Test	
Place of residence (n=331)	City (n=171)	80(46.8)	69(40.4)	22(12.8)	10.210	0.082[b]
	Village (n=103)	43(41.7)	38(36.9)	22(21.4)		
	Camp (n=54)	19(35.2)	29(53.7)	6(11.1)		
	Others (n=3)	0(0.0)	3(100.0)	0(0.0)		
Education (n=324)	Illiterate (n=47)	15(31.9)	27(57.4)	5(10.6)	**23.664**	**0.007[b]**
	Literate (n=37)	16(43.2)	15(40.5)	6(16.2)		
	Primary school (n=47)	21(44.7)	19(40.4)	7(14.9)		
	Middle school (n=52)	23(44.2)	22(42.3)	7(13.5)		
	High school (n=57)	17(29.8)	34(59.6)	6(10.5)		
	University and Higher (n=84)	47(56.0)	20(23.8)	17(20.2)		

a :Chi-Square test, b :Fisher-Freeman-Halton Test

DISCUSSION

Although there is general consensus that mass vaccination is the fastest and probably the only way to end the pandemic of COVID-19 that has spread worldwide (23) and caused international concern since its first appearance in December 2019, vaccination opponents and hesitation is also a major issue on the agenda (24, 25).

In our study, we sought to learn the opinions and concerns of people in the region about COVID-19 vaccines by a questionnaire made to patients and their relatives who applied to the emergency services of the hospitals as part of the humanitarian response in northwestern Syria. The majority of participants were women and the average age of participants was 36.06 years. Although there is no complete information about the current population, this could explain the decrease of male population in the society after the war and the high percentage of women among the participants who accepted the questionnaire. Looking at the number of participants, 51.7% of them live in the city. This could be because people who live in the city have easier access to the hospital. Considering the social lives of the participants, the average number of people living in the same house was 6. In poor regions where underdevelopment and civil crowded are prevalent, there is crowded family life for various reasons. Knowing the views of an crowded family on infectious diseases and COVID-19 will be important for these and similar regions.

The mass vaccinations that began in November 2020 are not yet sufficient to prevent the COVID-19 pandemic. However, effective vaccination is important to minimize the risk of health system collapse. For this reason, health workers have been prioritized in vaccination trials in many countries, including Turkey and England, and in South Korea vaccination trials that began in February 2021 were initiated by health workers and caregivers. Because in community-based matrix studies, it has been shown that the risk of COVID-19 transmission to healthcare workers is higher than the general population (26-28). In a prospective population-based cohort study, it is estimated that 10-20% of COVID-19 cases are healthcare workers (29). In the study by Seyhan et al, it is shown that the number of healthcare workers who have COVID-19 is relatively high among the already small number of healthcare workers in the study conducted in Northwest Syria according to WHO (30). When participants in our study were asked to whom the COVID-19 vaccine should be administered first, the majority of them answered in line with the literature: healthcare workers. In this case, the preference for health workers in immunization activities launched in Northwest Syria Region in parallel with global policies may indicate that this strategy could be acceptable to the region's society.

Studies of awareness of COVID-19 in Arab societies generally consist of surveys conducted via social media or websites. In an online survey conducted in Jordan, Saudi Arabia and Kuwait, the general COVID-19 level of knowledge was 66.1%, while the level of knowledge about COVID-19 transmission was 43.3% (31). In an online survey conducted throughout Syria, the general awareness level of COVID-19 was found to be 75.6% and the knowledge level was 67% (32). In our study, the awareness level was found to be lower among the participants of Northeast Syria Region compared to the general Syrian population. In our study, which included Northwest Syria and evaluated participants' perspectives on COVID-19, most participants agreed that COVID-19 is an easily transmitted disease. However, about half of the participants thought that this epidemic is exaggerated, that COVID-19 is not prevalent in Syria, and that COVID-19 is not a dangerous disease. Since our study is a face-to-face survey, it can be expected to be more beneficial and more objective than similar studies in terms of incorporating the views of other segments of society that digital platforms cannot reach. When our study is evaluated together with other studies on this topic, it can be assumed that about half of the Syrian population does not have the desired level of knowledge and awareness about COVID-19.

In relation to mass vaccination, global and international studies in the literature have shown that 52.1% of participants worldwide refuse an approved vaccine. From a regional perspective, the countries with the lowest vaccine acceptance rate (<50%) were Egypt and Libya in Africa, and Afghanistan and Bangladesh in Asia. On the other hand, the vaccine acceptance rate was found to be 87% or more in Australia and other companion countries (33). In a study conducted in 19 different countries with an online panel, 71.5% of participants stated that they would agree to a possible COVID-19 vaccination. The highest rate of positive responses in accepting a vaccine with proven safety and efficacy was in China (88.6%), while the

highest rate of negative responses was in Poland (27.3%) and the lowest positive response rate was found in Russia (54.9%) (34). In a similar study, 66-88% of participants from 12 countries agreed to get vaccinated themselves and 67-91% of participants stated that they would recommend the vaccine to their relatives (35). In developed countries, acceptance of the vaccination was 62.1% in a European study (36). In a survey conducted by telephone and in person in the United States, 57.6% of participants said they would agree to be vaccinated (37). In a web-based survey study in the Arab world, only 12.6% of Arab respondents in 23 Arab countries and 122 other countries with Arab residents said they would accept an appropriate and safe vaccine, while 21.7% were undecided and 40.4% rejected it (38). In country-based studies, the rate of those who accepted the vaccine was 36.8% in Jordan, 53.1% in Kuwait, and 64.7% in Saudi Arabia (3,39,40). In a social media study among Syrians living in Syria or outside Syria, with a survey in Arabic, 35.92% of 1222 respondents accepted the COVID-19 vaccine (41). In a study conducted among Syrian immigrants in Lebanon, it was found that 66% of the participants tended to get vaccinated and 28.8% refused (42). It was found that 42.9% of the participants in our study wanted the vaccine for COVID-19. Compared to the literature, the acceptance of vaccination in our study is higher than in most Arabic countries and developing countries. Considering that almost all the world literature is written in English and with Latin alphabet, it can be assumed that access to accurate information in Arab societies may be limited due to the lack of Arabic sources in the literature and the absence of standard literature in Arabic language in Arab countries. In our study and similar studies, the low awareness of COVID-19 in Syria and other Arab societies can be explained by similar reasons.

Among the studies that looked at vaccine refusal and hesitation, a study conducted in Kuwait indicated that 83.7% of participants who did not consent to vaccination had concerns about the side effects of the vaccines, 71.8% did not think the vaccines were safe, and 69.9% had doubts about the vaccine's effectiveness (39). In a study conducted with hospital patients in Nigeria, 31.7% of patients did not think vaccines were safe, 6.9% found them religiously objectionable, 43.4% did not trust the companies that produce vaccines, 9.4% believed that people are branded with vaccines, 20.2% believed that vaccines have harmful side effects, 9.7% believed that the vaccine does not work, and 20% believed that vaccines would make them sick (43). In another study on the Middle East, while 53.1% of participants were concerned about the effectiveness of the vaccine, 59.4% were concerned about the safety and side effects of the vaccine, 24.5% said that the virus was made in a lab because of the vaccine and for financial reasons, 14.5% said that the vaccine would put a chip in people (3). In a survey conducted with Arab participants from 23 Arab countries and 122 other countries with Arab residents, 61.4% of the participants fear an unknown side effect of the vaccine, 33% do not trust the vaccine manufacturers, 21.8% believe that the vaccine will change our genetic structure, and 13.5% believe that the vaccine is lethal (38). In an international survey study involving Syrians, the percentage of those who fear the side effects of the vaccine is 66.2% among those who are hesitant about the vaccine and 52.1% among those who reject the vaccine, the percentage of those who said they doubt the vaccine's effectiveness was 33.4% among those who are hesitant about the vaccine and 41.82% among those who rejected it (41). In another survey conducted among Syrian immigrants in Lebanon, participants who did not want to be vaccinated expected to receive slightly more information about the vaccine (35%); 21% thought more public health interventions were needed and did not think the vaccine was necessary; 23% were afraid of the side effects of the vaccine, thought it might interact with other medications, and did not consider vaccination because they did not have confidence in the system (42). In our study, the majority of participants (68.3%) who do not want to be vaccinated and who are undecided did not think the vaccine was safe, feared that the vaccine would cause harm (60.6%), and felt that it would not effectively prevent the disease (56.6%). In addition, almost half of those who do not want to be vaccinated and who are undecided stated that they were afraid of the side effects of the vaccine, thought the vaccine was inconvenient because of their religious beliefs, that there might be hidden and evil intentions behind the vaccine, and that they thought they could not afford the vaccination fee. It is believed that these and similar misconceptions and concerns, which are widespread around the world, are effective and should be taken into account in vaccination activities and pandemic response among Syrians.

Among the studies evaluating vaccine hesitancy, vaccine rejection, or factors affecting vaccination, studies among societies in the US and the Middle East, it was shown that as the level of education increases, so does COVID-19 knowledge and vaccine acceptance rates (16,20,21). In our study, it was observed that the desire to be vaccinated against COVID-19 was highest in those with a university degree or higher education.

In this study, which investigates the opinion of the society in the Northern Syria Region on the COVID-19 epidemic and vaccines, it was found that although the majority of participants in the region accept that COVID-19 is an easily communicable disease, about half of them believe that COVID-19 is not prevalent in Syria, that this epidemic is exaggerated, and that COVID-19 is not a dangerous disease. The acceptance of vaccination in our study is higher than in most Arabic countries and developing countries. It has been found that the majority of participants who do not want to be vaccinated and who are undecided do not think the vaccine is safe; they fear that the vaccine will harm them and believe that it will not protect them from the disease; almost half fear the side effects of the vaccine; they find the vaccine reprehensible because of their religious beliefs; there may be hidden and evil intentions associated with the vaccine, and they believe that they cannot afford the vaccination fee. In line with the literature, our study found that most of those who agreed to be vaccinated had a university degree or higher level of education. It is believed that vaccine awareness in the global sense, misconceptions and concerns about vaccines are effective and should be taken into account in vaccination activities and pandemic response in Syria as well as around the world.

The COVID-19 pandemic is one of the largest pandemics that humanity has ever experienced. Although developed countries seem to be containing the COVID-19 epidemic at the national level thanks to diagnosis, treatment, and vaccination attempts at the national level, the failure to control the epidemic in neighbouring and underdeveloped countries suggests that national efforts may be in vain. In combating the COVID-19 pandemic, the only way seems to be for all countries to act with global common battle plans and to ensure social immunity in a global sense.

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Declarations

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This study was approved by the Ethics Committee of the Hatay Mustafa Kemal University for non-interventional research (Date: 06.05.2021, Ref No: 2021/16).

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ORIGINAL ARTICLE

Risk of morbidity and mortality in preterm infants born to advanced maternal age pregnancies

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Abstract

Background: Little is known about the effect of advanced maternal age on preterm morbidity and mortality. This study aimed to evaluate the possible relationship between maternal age and morbidity and mortality in premature infants born at a gestational age ≤32 weeks.

Methods: Premature infants born at ≤32 weeks of gestation and admitted to the neonatal intensive care unit were divided into three groups by maternal age: <35, 35-39, and ≥40 years. Infant and maternal demographic and clinical characteristics, and preterm morbidity and mortality were compared between the groups.

Results: A total of 827 preterm infants were included. Their distribution by maternal age was as follows: 659 infants in the <35 years group, 120 in the 35-39 years group, and 48 in the ≥40 years age group. Older maternal age was associated with higher gravidity, frequency of assisted reproductive technology use, preeclampsia, gestational diabetes mellitus, and caesarean delivery (P=0.004, P<0.001, P=0.007, P=0.004, and P<0.001, respectively). Respiratory distress syndrome, patent ductus arteriosus, and necrotising enterocolitis were significantly more frequent in preterm infants aged ≥35 years (P=0.014, P=0.029, and P<0.001, respectively).

Conclusions: In addition to the maternal risks associated with pregnancy at older ages, some prematurity morbidities may also increase in frequency. Although this novel study presents important results, further studies are needed to evaluate the relationship between advanced maternal age and preterm morbidity.

Keywords: Maternal Age, Preterm Infants, Morbidity, Mortality, Aged.

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INTRODUCTION

Worldwide, the childbearing age for women extends into 30s and sometimes 40s. The Centre for Disease Control and Prevention have reported that pregnancy in women over 40 years of age is becoming more common in developed countries. The prevalence of first pregnancy at the age of >35 years was 9% in developed countries in the 2000s, but has increased to 23% in 2014 (1). The most common reason for this is that women with higher socioeconomic status and education levels tend to postpone motherhood until after their 30s. As a result, the use of assisted reproductive technology (ART) has increased (2).

Although the impact of childbirth in older mothers on maternal and perinatal outcomes has been extensively studied, there is no universal consensus on the definition of advanced maternal age (AMA). The term AMA refers to the prolongation of a woman's reproductive life in later years and is usually used for women aged ≥35 years (3,4). Additionally, the term extremely advanced maternal age (EAMA) has been used for pregnancies in women over 40 years of age (5).

Women with AMA (>35 years) are at risk of obstetric complications and serious interventions (6). The AMA increases the risk of pregnancy complications, such as ectopic pregnancy, miscarriage, fetal chromosomal abnormalities, congenital anomalies, placenta previa, placental abruption, gestational diabetes mellitus (GDM), preeclampsia, and caesarean delivery. These maternal complications may lead to preterm delivery and an increased risk of perinatal death (1). Despite this information, data regarding the clinical outcomes of preterm infants born to mothers under 35 years of age compared to those born to mothers ≥35 years of age are lacking. It is not known whether premature infants born at ≤32 weeks gestation, which is already a high-risk group in terms of preterm morbidity, have a higher risk of morbidity and mortality when born to mothers with AMA (7). We hypothesised that preterm infants born to mothers with AMA are at a greater risk of morbidity and mortality than those born to younger mothers. The present study was conducted to test this hypothesis by comparing morbidity and mortality of premature infants born at ≤32 weeks gestation to mothers with and without AMA.

MATERIALS AND METHODS

Study design and patients

This retrospective study included infants admitted to the neonatal intensive care unit (NICU) of our hospital between September 2019 and December 2021. Patient data were obtained by reviewing the medical records. Infants born at ≤32 weeks of gestation and admitted to the NICU were included in this study. Infants born after 32 weeks of gestation were excluded from this study. The study was carried out after obtaining approval from the local clinical ethics committee (Date:02.02.2022, Approval No: 2022/E2-22-1362). All the authors conducted the study in accordance with the principles of the Declaration of Helsinki. During the study period, care and feeding conditions were similar for all newborns.

Demographic and clinical characteristics

For all infants included in the study, the following data were obtained from the medical records: maternal age, gravidity, use of ART, antenatal steroid therapy, congenital anomaly, presence of preeclampsia, maternal thyroid disease, maternal chorioamnionitis, and GDM, mode of delivery, intubation in the delivery room, GA, birth weight (BW), sex, Apgar scores at 1 and 5 min, presence of severe congenital anomaly, small for gestational age (SGA), duration of mechanical ventilation (MV), non-invasive ventilation (NIV), supplemental oxygen, presence of early onset neonatal sepsis (EOS), late-onset neonatal sepsis (LOS), respiratory distress syndrome (RDS), patent ductus arteriosus (PDA), bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP) requiring treatment, intraventricular haemorrhage (IVH; grade≥3), necrotic enterocolitis (NEC; ≥2), day of full enteral feeding, length of NICU stay, and mortality.

Definitions of morbidities of prematurity

Small for gestational age was defined as BW below the 10th percentile for GA on the Lubchenco curve (8). All significant clinical and echocardiographic PDAs were regarded as haemodynamically significant PDA (9). All preterm infants requiring >30% oxygen at a postmenstrual age of 36 weeks were classified as having moderate/severe BPD (10). The ROP was defined according to the third edition of the International Classification of Diseases (11). Intraventricular haemorrhage on cranial ultrasound was defined according to the Volpe criteria (12). Necrotic enterocolitis was defined using the Bell criteria (13). Sepsis was defined as EOS if it occurred within the first 72 h of life, and as LOS if it occurred after the first 72 h (14). The need for surfactants was determined according to the national guidelines, and infants that received surfactants were classified as having RDS (15).

The infants were divided into three groups by maternal age: mothers aged <35, 35–39, and ≥40 years. The three groups were compared in terms of infants' demographic and clinical characteristics, morbidity, and mortality.

Statistical Analysis

Data were analysed using Statistical Package for Social Sciences (SPSS) version 18 for Windows (SPSS Inc., St. Louis, MO, USA). Non-normally distributed continuous variables were compared between the groups using the t-tests. Categorical variables were analysed using the chi-squared or Fisher's exact tests. Continuous variables are presented as mean ± standard deviation (SD) and/or median (minimum-maximum). Categorical variables were expressed as frequency and percentage distributions. Analysis of variance (ANOVA) with Bonferroni correction was used for comparisons among three groups. Results with P values <0.05 were considered statistically significant in all comparisons.

Sample size

Sample size calculation was based on the morbidity variable. The power calculation was performed according to the data from a previous study which was conducted in the relationship of neonatal morbidity in advanced maternal age pregnancy (16). The total sample size of 138 (46 patients per group) will be sufficient to detect power of 80% and a significance level of 5%.

RESULTS

This study included 827 premature infants born at ≤32 weeks of gestation. When grouped by maternal age, 659 infants were in the <35 years age group, 120 were in the 35-39 years group, and 48 were in the ≥40 years age group. The mean (± SD) maternal age was 26.1 ± 4.5 years in the <35 years age group, 36.2 ± 1.2 years in the 35-39 years age group, and 41.6 ± 2.2 years in the ≥40 years age group. ANOVA method was used to compare the groups. Older maternal age was associated with higher gravidity and frequency of ART, preeclampsia, GDM, and caesarean delivery (P=0.004, P<0.001, P=0.007, P=0.004, and P<0.001, respectively. Other demographic characteristics showed no significant differences between the groups (P>0.05) (Table 1). The RDS, PDA, and NEC were significantly more frequent in preterm infants born to mothers aged ≥35 years (P=0.014, P=0.029, and P<0.001, respectively). The other infant clinical outcomes and morbidity rates were similar between the groups (P>0.05) (Table 2).

Table 1. Demographic characteristics of patients

Characteristics	Maternal Age Groups			P value			
	< 35 years (I) (n = 659)	35–39 years (II) (n = 120)	≥ 40 years (III) (n = 48)	ANOVA	I vs. II	I vs. III	II vs. III
Gravidity [a]	1 (1–4)	3 (1–6)	3 (1–6)	0.004*	0.018*	0.001*	0.107
Assisted reproductive technology [b]	34 (5.1)	19 (15.8)	10 (20.8)	<0.001	<0.001*	<0.001*	0.373
Antenatal steroid [b]	453 (68.7)	84 (70)	33 (98.7)	0.946	0.753	0.930	0.809
Preeclampsia [b]	123 (18.6)	26 (21.6)	18 (37.5)	0.007*	0.443	0.002*	0.035*
Maternal chorioamnionitis [b]	52 (7.8)	11 (9.1)	6 (12.5)	0.512	0.330	0.307	0.474
Maternal thyroid disease [b]	16 (2.4)	3 (2.5)	2 (4.1)	0.466	0.551	0.187	0.306
GDM [b]	21 (3.1)	10 (8.3)	6 (12.5)	0.004*	0.002*	0.001*	0.036*
Gestational age (weeks) [c]	28.1±1.2	28.0±1.1	27.7±1.2	0.385	0.487	0.199	0.464
Birth weight (g) [c]	1058±230	1065±219	1064±208	0.944	0.758	0.853	0.975
Cesarean delivery [b]	342 (51.8)	79 (65.8)	39 (81.2)	<0.001*	0.002*	<0.001*	0.021*
Male sex [b]	345 (52.3)	62 (51.6)	23 (47.9)	0.259	0.890	0.110	0.171
Apgar score at 1 min [a]	5 (1-8)	5 (1-7)	5 (1-7)	0.711	0.798	0.303	0.355
Apgar score at 5 min [a]	8 (2-10)	8 (3-9)	8 (2-9)	0.481	0.893	0.158	0.270
Intubation in the delivery room [b]	51 (7.7)	10 (8.3)	4 (8.3)	0.711	0.497	0.510	0.833
SGA [b]	47 (7.1)	11 (9.1)	6 (12.5)	0.219	0.332	0.115	0.187
Congenital anomaly [b]	5 (0.7)	2 (1.6)	1 (2)	0.212	0.105	0.067	0.153

GDM, gestational diabetes mellitus; SGA, small for gestational age.
*Statistically significant p values are highlighted.
[a] Data are given as median (minimum-maximum)
[b] Data are presented as n (%)
[c] Data are given as mean ± standard deviation.

Table 2. Clinical outcomes and morbidities

Variables	Maternal Age Groups			P value			
	< 35 years (I) (n = 659)	35–39 years (II) (n = 120)	≥ 40 years (III) (n = 48)	ANOVA	I vs. II	I vs. III	II vs. III
Duration of MV (days) [a]	3.8±3.0	4.4±2.6	3.9±2.6	0.442	0.488	0.273	0.173
Duration of NIV (days) [a]	7.6±5.6	8.3±5.4	8.2±6.1	0.762	0.515	0.679	0.941
Duration of supplemental oxygen (days) [a]	24.3±13.5	26.2±15.5	25.7±14.8	0.683	0.445	0.668	0.899
ENS [b]	14 (2)	4 (3.3)	2 (4.1)	0.830	0.586	0.872	0.807
LOS [b]	141 (21.3)	27 (22.8)	10 (20.8)	0.092	0.083	0.182	0.335
RDS [b]	398 (60.4)	86 (71.6)	35 (72.9)	0.014*	0.019*	0.005*	0.248
PDA [b]	238 (36.1)	58 (48.3)	24 (50)	0.029*	0.011*	0.008*	0.594
BPD (moderate/severe) [b]	119 (18.1)	19 (15.8)	6 (12.5)	0.561	0.614	0.319	0.525
ROP [b]	59 (8.9)	10 (8.3)	5 (10.4)	0.363	0.804	0.174	0.189
IVH (grade≥3) [b]	58 (8.8)	9 (7.5)	4 (8.3)	0.678	0.413	0.643	0.860
NEC (grade≥2) [b]	9 (1.3)	9 (7.5)	3 (6.2)	<0.001*	<0.001*	0.003*	0.067
Full enteral feeding (days) [a]	16±7.3	17.4±7.9	16.4±4.2	0.199	0.100	0.708	0.460
NICU stay (days) [a]	55.1±30.1	57.6±32.7	57.4±31.5	0.649	0.708	0.621	0.640
Mortality [b]	106 (16.1)	21 (17.5)	7 (14.5)	0.884	0.431	0.609	0.969

BPD, bronchopulmonary dysplasia; EOS, early-onset neonatal sepsis; IVH, intraventricular hemorrhage; LOS, late-onset neonatal sepsis; MV, mechanical ventilation; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; NIV, non invasive ventilation; PDA, patent ductus arteriosus; RDS, respiratory distress syndrome; ROP, retinopathy of prematurity; SGA, small for gestational age

*Statistically significant p values are highlighted.

[a]Data are given as mean ± SD

[b]Data are presented as n (%)

DISCUSSION

The risk of maternal morbidity and preterm birth increases at later maternal age. A higher risk of preterm birth is associated with a higher risk of perinatal morbidity and mortality. However, no previous study has investigated whether preterm babies born to mothers with AMA have an increased risk of morbidity and mortality compared to preterm infants born to younger mothers. In our study, the frequencies of ART, preeclampsia, GDM, and caesarean delivery were higher in AMA mothers than in mothers <35 years of age. Additionally, preterm infants born to mothers with AMA had significantly higher rates of RDS, PDA, and NEC than preterm infants born to mothers <35 years.

As women age, the number of pregnancies increases, but the rate of live births may decrease. Therefore, the use of ART is on the rise (1,17). Clinical studies have shown that adverse obstetric and perinatal outcomes may be more common in mothers over 35 years of age (1,18-23). Unfavorable neonatal outcomes have been demonstrated in studies on term, early term, and late preterm infants. There are insufficient data on maternal age-related outcomes in preterm infants. In this study, we observed that negative maternal outcomes and some prematurity morbidities increased in frequency in association with maternal age.

The adverse obstetric consequences of AMA may also have unfavourable effects on the foetus. Advanced maternal age mothers are known to have a four-fold higher risk of preterm delivery than younger mothers (18). A higher risk of preterm birth is associated with lower BW and GA, lower Apgar scores, greater need for resuscitation, and

higher rates of respiratory distress and NICU admission (18-20,24). More importantly, greater prematurity in infants born to mothers with AMAs increases mortality risk (18,20,23). In fact, an unfavourable maternal obstetric history associated with AMA is followed by an increase in the risk of preterm birth, and increased preterm morbidity is the last link in the chain. Numerous studies conducted in different centres have revealed maternal outcomes similar to those in our study (18-20,24). However, whether preterm infants born to older mothers have a greater morbidity and mortality risk than infants born at ≤32 weeks of gestation to mothers <35 years of age has not been investigated.

Previous studies have evaluated infants born to mothers with AMA in terms of short-term clinical outcomes, such as in the delivery room and indications for NICU admission (1,18-20,24). Our study is the first to evaluate the possible relationship between morbidity in preterm infants and their mothers by maternal age. The results of our study indicated that RDS, PDA, and NEC were more common among infants born at ≤32 weeks of gestation when the maternal age was ≥35 years. Low BW and GA are the most important risk factors for RDS, PDA, and NEC (7). The BW and GA were similar among the groups in our study. This demonstrates the main finding of our study that maternal age ≥35 years was associated with a higher frequency of RDS, PDA, and NEC. Adverse intrauterine conditions in mothers with AMAs may also increase the frequency of neonatal respiratory distress. Placental factors are not only responsible for unfavourable intrauterine conditions but have also been implicated in neonatal respiratory distress (7,25). As histopathological examination of the placenta could not be performed in our study, the influence of placental factors on RDS could not be evaluated.

Our results indicate that preterm infants born to mothers with AMA have a higher rate of RDS; however, the underlying pathophysiology is not clear. The aetiology may involve impaired foetal surfactant production, resulting from unfavourable intrauterine conditions with increasing maternal age. This in turn results in greater neonatal surfactant use. A surfactant deficiency causes hypoxaemia, which improves with surfactant administration. As hypoxaemia resolves, the neonatal pulmonary arterial pressure decreases. This results in faster systemic arterial blood flow to the pulmonary artery via the PDA. All of these pathophysiological changes may cause PDA to remain open. Additionally, PDA can contribute to a reduction in gastrointestinal blood flow and NEC development (9). These pathophysiological mechanisms might explain the increased prevalence of RDS and subsequent increases in PDA and NEC observed in preterm infants born to AMA mothers in our study (7,9). The results of our study suggest that maternal age had no effect on other prematurity morbidities. A possible reason for this may be that the surfactant therapy shortened the duration of respiratory support in infants with RDS, which may explain why the groups had similar respiratory support times. The lack of significant differences between the groups in terms of morbidities, such as sepsis, BPD, ROP, IVH, and NICU stay and mortality may be related to their comparable respiratory support duration, BW, and GA (7,9-11).

Study limitations

Our study had certain limitations, owing to its single-centre and retrospective design. An investigation of placental histopathology, cord blood laboratory values, prenatal NST or umbilical cord Doppler, maternal drug use, and maternal BMI data was not possible. Therefore, our results must be evaluated based on available data.

This study showed that the prevalence of RDS, PDA, and NEC was significantly higher among preterm infants (GA≤32 weeks) born to mothers aged ≥ 35 years. Further studies, including placental histopathology and hormonal and biochemical markers, should be conducted to explain the increase in morbidity associated with AMA.

Declarations

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

The study was carried out after obtaining approval from the Ethics Committee (E2) of Ankara City Hospital (Date: 02.02.2022, Approval No: 2022/E2-22-1362).

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ORIGINAL ARTICLE

Ameliorative effects of varenicline and bupropion on morphine-induced conditioned place preference in rats

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Abstract

Background: Rewarding properties of morphine constitue the principal reasons for drug-craving behaviors which appear during morphine addiction. Varenicline and bupropion were reported to have some positive effects on addictive substances by different studies. In this study, the effects of varenicline and bupropion on morphine rewarding properties were investigated via conditioned place preference (CPP) in rats.

Methods: Conditioning was performed by intraperitoneal (i.p.) administration of morphine (10 mg/kg, i.p., 1, 3, 5, and 7 days) and saline (2,4, 6, and 8 days). To evaluate the development of dependence, subcutaneous administration of varenicline (0.5, 1 and 2 mg/kg, s.c.) or bupropion (5, 10 and 20 mg/kg, i.p.) was carried out 15 minutes before the administration of morphine. To evaluate the expression of dependence, varenicline or bupropion was administered 15 minutes before the test on 9th day. To investigate the extinction of the reward effect, drugs were tested daily on days 14, 18, and 22 and evaluated for reinstatement on 23rd day.

Results: Systemic morphine administration statistically significant produced CPP. Varenicline and bupropion did not reduce the development of morphine-induced CPP. In addition, varenicline and bupropion decreased expression, reinstatement and accelerated the extinction of morphine-induced CPP. Unlike varenicline, bupropion statistically significant produced CPP and altered locomotor activity.

Conclusions: These data suggest that varenicline and bupropion may be useful therapeutic pharmacological agents to reduce morphine dependence. The results of our research provide preliminary evidence to highlight the importance of the effects of varenicline and bupropion on morphine dependence. In the future, it would be appropriate to conduct mechanistic studies to explain the underlying mechanisms by using different methods on the subject.

Keywords: Morphine, Dependence, Varenicline, Bupropion, CPP.

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INTRODUCTION

Drug and substance dependence or addiction are a chronically relapsing disease, described by a compulsion to search and use the drug and substance, failure of control in limiting drug consumption, and the emergence of a negative emotional state the same anxiety, irritability, and dysphoria when access to the drug is blocked (1). The opioids are a broad class of medicines related in structure to the natural plant alkaloids, which exist in opium, Papaver somniferum. The opiates are classified as natural alkaloids that include morphine and codeine (2). The development of approaches for the rational use of morphine and other opioids have become an emergent call globally, as a response to the escalating emergency of prescription opioid abuse and misuse (2). Prescription opioids are the second several prevalent types of abused medication subsequent to marijuana (3, 4). In fact, it has been approximated that 16 million people worldwide have an opioid use disorder associated with the prescription of opioids, constituting a drug abuse epidemic (2). Morphine, a broadly used opioid analgesic drug, uses diverse behavioral and molecular effects (5). Addiction to morphine is an important public health issue (2).

Varenicline is an $\alpha 4\beta 2$ nicotinic acetylcholine receptors (nAChR) partial agonist and an$\alpha 7$ nAChR full agonist approved by the US Food and Drug Administration (FDA) for the treatment of nicotine addiction, significantly decreases nicotine craving and inhibits relapse (6). The literature review shows a direct and indirect role of nAChRs in drug addiction and dependence (7, 8). nAChR activation affects neurotransmitter systems, such as the choline, dopamine, serotonin, glutamate, gamma-aminobutyric acid, adrenaline, and endocannabinoid systems. These changes, in turn, influence cognitive functioning with a role in drugs and substance addiction (7, 8). nAChR plays a principal role in nicotine addiction and dependence, morphine dependence, alcohol consumption, methamphetamine dependence, and cue-induced cocaine craving (7). The activation of nicotinic receptors is closely linked to the rewarding effect of morphine (9). In similar studies, activation of nicotinic receptors has been reported to decrease the withdrawal symptoms related to morphine and increased the analgesic effect related to morphine (10, 11). In a different study, activation of nicotinic receptors has been shown to reduce opioid withdrawal (12, 13). Pre-treatment opioid receptor agonists reduced withdrawal symptoms due to accelerated nicotine with the nicotinic receptor blocker mecamylamine (13, 14). Furthermore, altering the effect of chronic nicotine exposure on endogenous enkephalin synthesis and relieving this by naloxone via reducing nicotine abstinence symptoms, as reportedin a study (15). The administration of nicotine in male and female mice μ formed up-regulation of opioid receptors (16). It is known that dopamine levels are decreased with morphine abstinence. A decrease in dopamine levels in morphine withdrawal reveals the it's association with drug-seeking behavior. Based on this hypothesis, it can be concluded that varenicline can reduce withdrawal symptoms by increasing the dopamine level which is decreasedin morphine dependence (8, 17, 18). The results of all these studies show that there is a close relationship between the opioidergic system and the nicotinic system.

Bupropion is a norepinephrine-dopamine disinhibitor approved by FDA for the treatment of depression and smoking cessation. Multiple investigations have highlighted the effectiveness of bupropion for the attenuate of nicotine, amphetamine, methamphetamine addiction (19, 20). Another research has shown that bupropion reduces both morphine tolerance and physical dependence (20). Dopamine is known to play an important role in the development and maintenance of morphine addiction (21). It has been reported that when dopamine receptor agonist is given before testing, it reduces the expression of morphine addiction (10, 21). In a similar study, the dopamine receptor agonist morphine addiction prevented its development and expession (22). Sympathetic hyperactivation is observed with morphine withdrawal. It is known that this sympathetic hyperactivation is due to the increased firing of noradrenergic neurons in the locus coeruleus (23, 24). Bupropion dose is depended on decreasing the firing of noradrenergic neurons in the locus coeruleus (25). The results of these studies suggest that bupropion may be an alternative in morphine dependence.

The conditioned place preference (CPP) method is a conventional preclinical and clinical behavioral paradigm applied to investigate the rewarding/drug-craving and aversion/avoidance effects of drugs and substances (26-29). CPP has also been confirmed with copulatory activity, food, and other rewarding motives. Opiates, such as

morphine, buprenorphine, heroin, besides drugs from other classes, including the CNS depressants ethanol and diazepam, psychostimulants, cocaine, and nicotine have been found to produce CPP (26, 27, 30). CPP method is regarded as an entrenched and reliable animal model to examine the rewarding effect of various substances and drugs of addiction, including morphine (27, 31). Based on the scientific data, purpose of the current investigation was to examine the effect of bupropion and varenicline morphine-induced CPP in rats.

MATERIALS and METHODS

Animals

Male Wistar albino rats (260-320 g) housed (4-5 per cage) under the controlled environmental conditions at 21-23◦C and 12:12 h light/dark cycle. The animals were allowed to food and water ad libitum. This work was supported by the Istanbul University Local Ethics Committee on Animal Experiments Date: 29.12.2011 No: 2011/164 and were in accordance with the EU Directive 2010/63/EU on the safety of animals utilized for scientific purposes.

Drugs

Varenicline tartrate and bupropion hydrochloride (Sigma, St. Louis) were dissolved in saline. Morphine hydrochloride was purchased from (Macfarlan Smith LTD., Edinburgh, UK). The doses of varenicline tartrate were administered to the animals by subcutaneously (s.c.) in volumes of 1 ml/kg. Bupropion hydrochloride and morphine hydrochloride solutions were given intraperitoneally in a volume of 1 ml/kg. Drug stocks were prepared freshly each morning of the experiment day. The control animals were administered saline (i.p.).

Apparatus

CPP paradigm consisted of a two-chambered apparatus (61 × 31 × 13 cm) with an optional sliding door. The walls of both the chambers were of black color. One chamber was paired with a grid rod floor and another chamber with a mesh sheet floor. To provide different contact stimuli, the floor of one of the chambers is striped (3 mm in diameter, 7 mm apart), the surface of one is perforated (29 cm x 29 cm), with a removable part (2 cm). After each application, the assembly was cleaned with a wet (70% alcohol) and dry cloth. Eight independent, identical CPP setups, in which the experiments were carried out, were placed in a room with conditions suitable for behavioral studies. The testing room was saved in a soundproof place with neutral masking wait noise.

Handling and habituation

It was done for the animals to get used to the experimental conditions (such as handling, injection) and the paradigm. The middle chamber of the conditioning box was removed, and the animals were allowed to roam freely in both compartments for 5 minutes**.**

Pre-conditioning test

All rats were in the CPP compartment without an injection and were entitled to voluntarily explore two-compartment for 15 min. The initial baseline preference was noted by observing the time spent by rats in each compartment to determine the conditioned place preference before drug administration. Animals that spent more than 600 (>66%) seconds or less than 300 (<33%) seconds in the pre-test were considered indicative of place preference or avoidance and were excluded from the experiment.

Conditioning

On days 1, 3, 5, and 7, the rats were administered an injection of morphine (10 mg/kg, i.p.), varenicline (0.5, 1 and 2 mg/kg, s.c.), bupropion (5, 10 and 20 mg/kg) or saline, and then they were quickly put into the drug-paired compartment of the CPP apparatus for 15 min. On days 2,4, 6, and 8, all rats were given saline and then they were quickly placed into the contrary compartment (the saline-paired compartment) for 45 min.

Post-conditioning test

The 15 min place preference tests were conducted on the 9thday in a drug-free state following the conditions like to pre-conditioning. The sliding door was removed, and all rats were located in the central line and allotted voluntary entrance to both the compartments. Then spent time in the co-drugs paired compartment was reported and the results were compared with the saline group and morphine-paired compartment.

Effects of varenicline and bupropion on the development of morphine-induced CPP

To study the influence of varenicline and bupropion on the development of morphine-induced CPP, the rats, which were treaeted with varenicline (0.5, 1 and 2 mg/kg, s.c.) and bupropion (5, 10 and 20 mg/kg, i.p.), or its vehicle 45 minutes before every morphine administration injection during the conditioning test, as defined above.

Effects of varenicline and bupropion on the expression of morphine-induced CPP

To determine the effects of varenicline and bupropion on the expression of morphine, various groups of rats were given with varenicline (0.5, 1 and 2 mg/kg, s.c.) and bupropion (5, 10, and 20 mg/kg, i.p.) on the test day, 15 minutes previous to the post-conditioning test.

Effects of varenicline and bupropion extinction of morphine-induced CPP

The conditioning box was used as half its floor with rods and half with holes. To investigate the extinction (extinction) of morphine induced CPP, varenicline (0.5, 1 and 2 mg/kg, s.c.) and bupropion (5, 10 and 20 mg/kg, i.p.) were given on days 14, 18, and 22 (4).

Effects of varenicline and bupropion on reinstatement of morphine-induced CPP

On the 23rd day, saline was applied to the saline group. Bupropion (5, 10, and 20 mg/kg) and varenicline (0.5, 1 and 2 mg/kg, s.c.) injections were administered to the other groups 15 minutes before a single dose of morphine (10 mg/kg) injection. Immediately after the last injection, the animals were placed in the apparatus with the partition removed, and the time they spent in the chamber coupled with drugs was determined for 15 minutes.

Measurement of effects of varenicline and bupropion treatment on locomotor activity

Locomotor activity was evaluated in a conditioned place preference setup. The floors of the setup were divided into 8 equal squares, and the number of square crossings of the animals were evaluated during 15 minutes during the test period (32, 33).

Statistical analysis

All data were analyzed using Prism software, and expressed as the mean ± S.E.M. (GraphPad). The change in preference was measured as the comparison of difference between time spent in the treatment drug-paired chamber post-conditioning. The results of CPP and locomotor activity analyses were presented as mean preference. Data were analyzed by one-way analysis of variance (ANOVA) followed by Post hoc Newman-Keuls's multiple comparison tests. A value of $p<0.05$ was considered as significant.

This work was approved by the Istanbul University Local Ethics Committee on Animal Experiments (2011/164) and were in accordance with the EU Directive 2010/63/EU on the safety of animals utilized for scientific purposes.

RESULTS

Effect of varenicline on morphine-induced CPP

The treatment of morphine significantly increased the place preference for the drug-paired chamber ($p<0.01$). The rats were given varenicline 30 minutes before the morphine injection, failed to change the effect of morphine on CPP ($p>0.05$; Figure 1). The rats administered with only varenicline did not demonstrate any CPP, compared with the saline control group ($p>0.05$; Figure 1). The results from ANOVA explained that varenicline pre-treatment attenuates the establishment of morphine-induced CPP [$F(4, 28) = 20.28$; $p<0.001$]. Post hoc Newman-Keuls's multiple comparison test demonstrated that varenicline (2 mg/kg, s.c.) significantly decreased expression the effect of morphine on CPP as compared to the morphine groups ($p<0.05$, Figure 2). In addition, two doses of varenicline (0.5 and 1 mg/kg, s.c.) wasn't significantly effective ($p>0.05$).

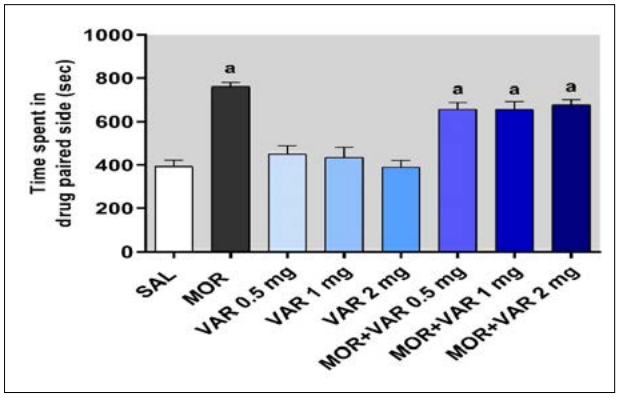

Figure 1. Effect of varenicline development on morphine-induced CPP. Data are presented as Mean ± S.E.M. (n=7-8/ group). One-way ANOVA with Newman-Keuls post hoc test. Significantly different from its control/saline[a]: ([a]p<0.001). Saline-SAL, Morphine-MOR. Varenicline-VAR.

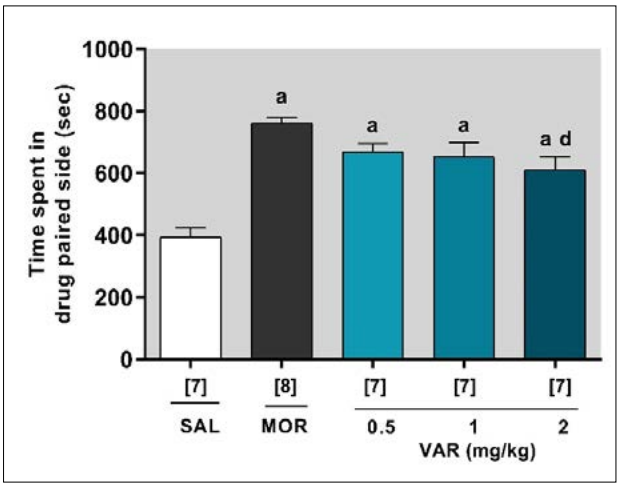

Figure 2. Effect of varenicline expression on morphine-induced CPP. Data are presented as Mean ± S.E.M. (n=7-8/ group). One-way ANOVA with Newman-Keuls post hoc test. Significantly different from its control/saline[a]. Significant difference between morphine[d]: ([a]p<0.001, [d]p<0.05). Saline-SAL, Morphine-MOR. Varenicline-VAR.

The effect of varenicline on extinction and reinstatement of morphine-induced CPP

The time-dependent effects of varenicline for the extinction and reinstatement of morphine-induced CPP in rats were evaluated. One-way ANOVA showed a significant group difference in extinction day 14, ext 1; [F (4, 29) = 9.436, p<0.05] (Figure 3 A), day 18, ext 2; [F (4,29) = 6.843, p<0.05] (Figure 3 B). Nonetheless, one-way ANOVA revealed that there was previously no significant group difference on extinction 3, [F (4, 29) = 1.450, p>0.05] (Figure 3 C), day 22, ext 3. The post hoc analysis expressed that varenicline significantly extenuated the time spent in drug-paired chamber at a dose of varenicline (2 mg/kg, s.c.) through extinction 1, when compared to the morphine group (p<0.05; respectively). In addition, Newman-Keuls multiple comparison test indicated varenicline (2 mg/kg) was able to extinguish morphine-CPP on ext3 (p < 0.05) compared to the morphine-paired chambers. No significance was detected among all groups on the extinction 3 (p>0.05, ext 3) (Figure 3 A, B, C).

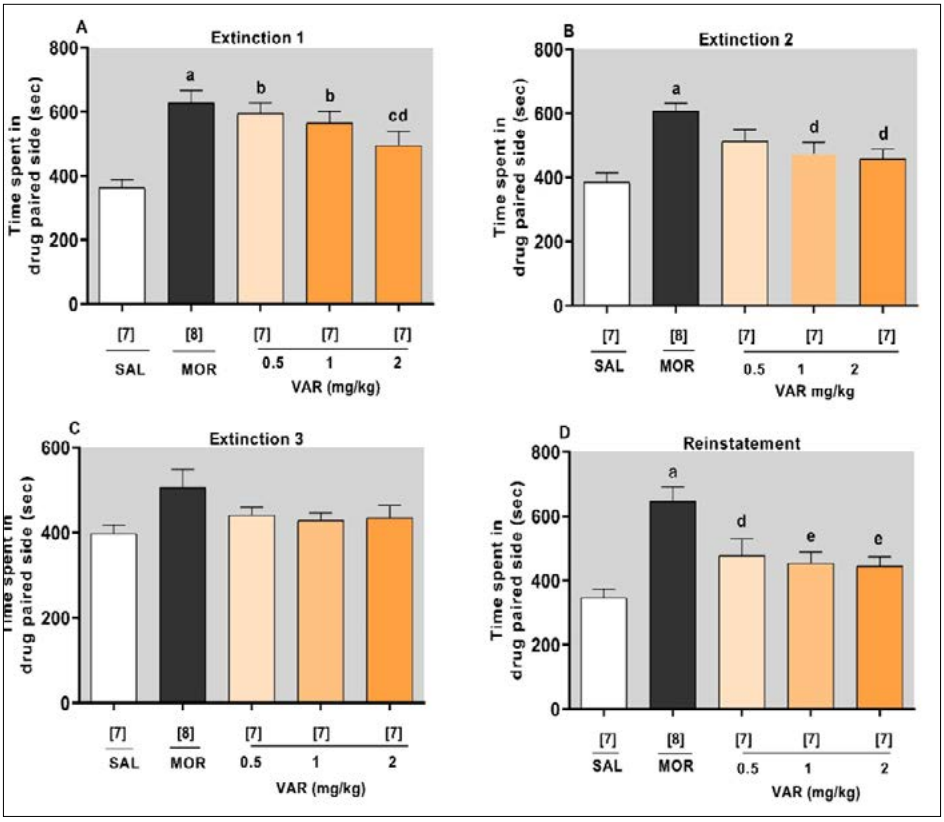

Figure 3. Effect of varenicline extinction and reinstatement on morphine-induced CPP. Data are presented as Mean ± S.E.M. (n=7-8/ group). One-way ANOVA with Newman-Keuls post hoc test. Significantly different from its control/saline[a,b,c]. Significant difference between morphine[d,e]: ([a]p<0.001, [b]p<0.01, [c]p<0.05; [d]p<0.05, [e]p<0.01: A-extinction 1, B-extinction 2, C-extinction 3, and D- reinstatement). Saline-SAL, Morphine-MOR. Varenicline-VAR.

The influence of varenicline on morphine (10 mg/kg, i.p.) priming produced CPP is present in Figure 3. One-way ANOVA showed that morphine produced place preference to the drug paired chamber [F (4, 29) = 8,710, p<0.001]. Newman-Keuls test displayed that the time spent in the drug-paired side on the reinstatement day after a priming morphine (10 mg/kg, i.p.) was significantly increased when compared to the time spent in the saline-paired side (p<0.001). Post hoc Newman-Keuls's multiple comparison test demonstrated that varenicline (0.5, 1, and 2 mg/kg, s.c.) significantly attenuated the reinstatement on CPP as compared to morphine (p<0.05, p<0.05 and p<0.01, respectively) (Figure 3 D).

Bupropion effect of development and expression of morphine-induced CPP

The treatment of morphine significantly increased the place preference for the drug-paired chamber (Figure 4, p<0.001). Bupropion treatment 30 minutes before (development) the morphine is given not changed of the effect of morphine on CPP (p>0.05, Figure 4). ANOVA explained that bupropion pre-treatment (expression) attenuates the establishment of morphine-induced CPP [F (4, 28) = 20.23; p<0.05]. Post hoc Newman-Keuls's multiple comparison test demonstrated that bupropion (10 and 20 mg/kg, i.p.) significantly decreased the effect of morphine on CPP as compared to the morphine group (Figure 5; p<0.05 and p<0.01; respectively). Additionally, Post hoc Newman-Keuls's multiple comparison test demonstrated that high-dose bupropion (20 mg/kg, i.p.) itself produced CPP (p<0.05). In addition, a lower dose of bupropion (5 mg/kg, i.p.) wasn't significantly effective (p>0.05; Figure 4).

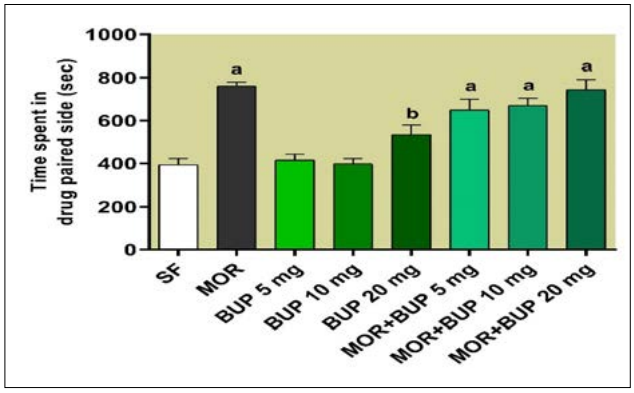

Figure 4. Effect of bupropion development on morphine-induced CPP. Data are presented as Mean ± S.E.M. (n=7-8/ group). One-way ANOVA with Newman-Keuls post hoc test. Significantly different from its control/ saline[a,b]: ([a]p<0.001, [b]p<0.05). Saline-SAL, Morphine-MOR. Bupropion-BUP.

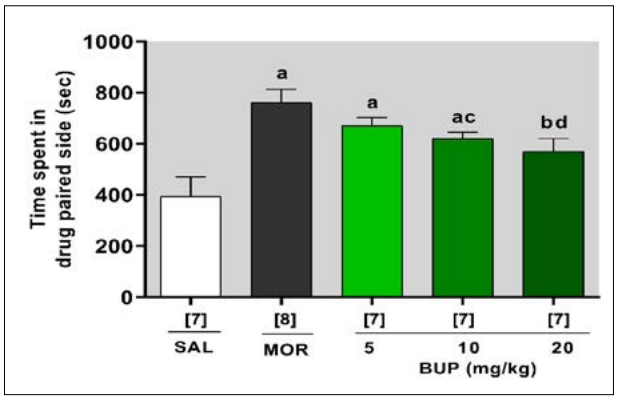

Figure 5. Effect of bupropion expression on morphine-induced CPP. Data are presented as Mean ± S.E.M. (n=7-8/ group). One-way ANOVA with Newman-Keuls post hoc test. Significantly different from its control/saline[a,b,c]. Significant difference between morphine[d]: ([a]p<0.001, [b]p<0.01, [c]p<0.05; [d]p<0.05). Saline-SAL, Morphine-MOR. Bupropion-BUP.

Effects of bupropion on extinction and reinstatement of morphine-induced CPP

The time-dependent effects of bupropion for the extinction and reinstatement of morphine-induced CPP in rats were assessed. One-way ANOVA showed that there was a significant group difference in extinction day 14, extinction 1; [F (4, 28) = 8.342, p<0.05] (Figure 6 A), day 18, extinction 2; [F (4,29) = 7.019, p<0.05] (Figure 6 B). Nonetheless, one-way ANOVA revealed that there was previously no significant group difference on extinction 3, [F (4, 28) = 2.275, p>0.05] (Figure 6 C), day 22. Post hoc Newman-Keuls's analysis expressed that bupropion significantly extenuated the time spent in drug-paired chamber at a dose of bupropion (10 and 20 mg/kg, i.p.) through extinction 2, when compared to the morphine group (p<0.05 and p<0.01; respectively) (Figure 6 A, B, C).

The effects of bupropion on morphine (10 mg/kg, i.p.) priming produced CPP is present in Figure 6 D. One-way ANOVA showed that morphine produced place preference to the drug paired chamber [F (4, 29) = 8,710, p<0.01]. Newman-Keuls test displayed that the time spent in the drug-paired side on the reinstatement day after a priming morphine (10 m/kg, i.p.) was significantly increased when compared to the time spent in the sline-paired side (Figure 6 D, p<0.01). Post hoc Newman-Keuls's multiple comparison test demonstrated that bupropion (5, 10, and 20 mg/kg, i.p.) significantly attenuates the reinstatement on CPP as compared to morphine (p<0.05, p<0.05 and p<0.01; respectively) (Figure 6 D).

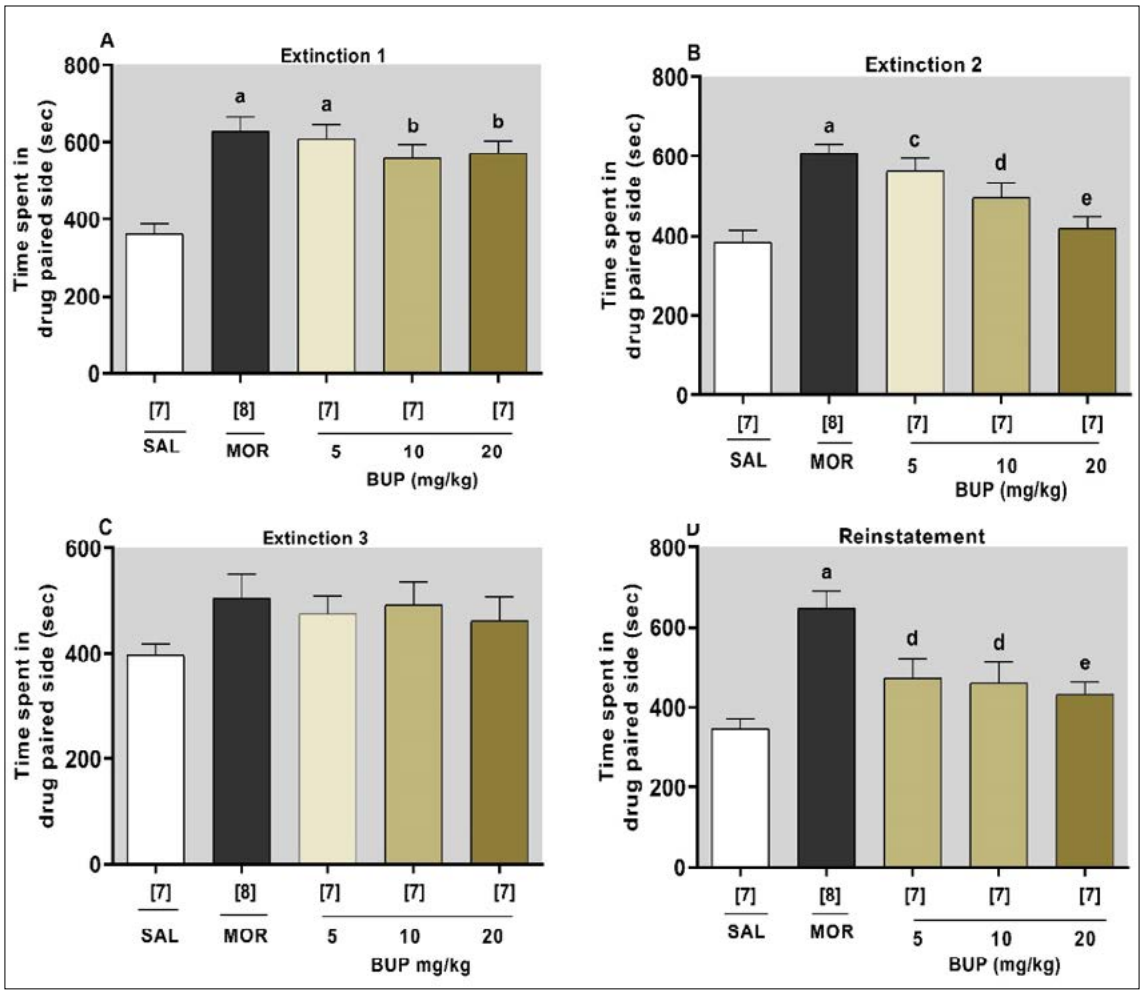

Figure 6. Effect of bupropion extinction and reinstatement on morphine-induced CPP. Data are presented as Mean ± S.E.M. (n=7-8/ group). One-way ANOVA with Newman-Keuls post hoc test. Significantly different from its control/ saline[a,b,c]. Significant difference between morphine[d,e]: ([a]p<0.001, [b]p<0.01, [c]p<0.05; [d]p<0.05, [e]p<0.01: A-extinction 1, B-extinction 2, C-extinction 3, and D- reinstatement). Saline-SAL, Morphine-MOR. Bupropion-BUP.

Effect of the varenicline and bupropion on locomotor activity of morphine-induced CPP

One-way ANOVA demonstrated that the morphine itself did not induce any influence on locomotion flowing the test period ($p>0.05$). Post hoc analysis presents the effect of the various doses of varenicline (0.5, 1 and 2 mg/kg, s.c.) itself, and co-administration morphine any effect on locomotion while they were treated flowing the morphien-induced CPP. However, in addition, Post hoc Newman-Keuls multiple comparison demonstrated that the administration of bupropion (20 mg/kg, i.p.) and co-administration with morphine statistically changed locomotor activity in the expression phase of CPP ($p<0.05$). The effects of varenicline and bupropion on locomotor activity are shown in Table 2 in the developmental phase of addiction and in Table 2 in the expression phase.

Table 1. Summary of development phases of varenicline and bupropion on locomotor activity in rats.

Groups	SAL	MOR 10 mg/kg	BUP 5 mg/kg	BUP 10 mg/kg	BUP 20 mg/kg	MOR+BUP 5 mg/kg	MOR+BUP 10 mg/kg	MOR+BUP 20 mg/kg
Development; Means/errors	32.57±2.918	36.25±4.043	42.14±4.872	47.50±5.915	61.00±4.946*	48.14±2.963	49.33±6.19	53.50±5.91^
Groups	SAL	MOR 10 mg/kg	VAR 0.5 mg/kg	VAR 1 mg/kg	VAR 2 mg/kg	MOR+VAR 0.5 mg/kg	MOR+VAR 1 mg/kg	MOR+VAR 2 mg/kg
Development; Means/errors	32.57±2.918	36.25±4.043	35.67±4.014	41.33±4.410	48.00±4.320	36.57±4.082	38.00±3.777	42.50±3.871

Locomotion was calculated for 15 minutes for each C.P.P. test (development and reinstatement tests) simultaneously. Locomotor activity evaluated the number of crossings from one square (8 equal-sized) to another within 15 minutes. Values are means ± S.E.M. (n=7-8/ group). Significantly different from its control/saline*. The significant difference between morphine^. One-way ANOVA with Newman-Keuls post hoc test. One-way ANOVA with Tukey post hoc test (*p<0.05, ^p<0.01). Saline-SAL, Morphine-MOR, Varenicline-VAR, Bupropion-BUP.

Table 2. Summary of expression and reinstatement phases of varenicline and bupropion on locomotor activity in rats.

Groups	SAL	MOR 10 mg/kg	MOR+VAR 0.5 mg/kg	MOR+VAR 1 mg/kg	MOR+VAR 2 mg/kg
Expression; Means/errors	32.57±2.918	36.25±4.043	36.00±3.077	39.17±4.230	43.17±4.956
Reinstetmen; Means/errors	34.43±2.644	35.00±2.928	37.33±2.261	39.17±4.028	41.83±3.646
Groups	SAL	MOR 10 mg/kg	MOR+BUP 5 mg/kg	MOR+BUP 10 mg/kg	MOR+BUP 20 mg/kg
Expression; Means/errors	32.57±2.918	36.25±4.043	43.67±5.619	48.83±6.156	60.17±7.002^*
Reinstetmen; Means/errors	34.43±2.644	35.00±2.928	45.29±6.209	43.50±4.938	40.33±4.991

Locomotion was calculated for 15 minutes for each CPP test (development and reinstatement tests) simultaneously. Locomotor activity evaluated the number of crossings from one square (8 equal-sized) to another within 15 minutes. Values are means ± S.E.M. (n=7-8/ group). Significantly different from its control/saline^. The significant difference between morphine*. One-way ANOVA with Newman-Keuls post hoc test. One-way ANOVA with Tukey post hoc test (*p<0.05, ^p<0.01). Saline-SAL, Morphine-MOR, Varenicline-VAR, Bupropion-BUP.

DISCUSSION

Drug abuse and dependence have been significantly influencing government health, economic development, and social harmony, both historically and contemporarily. Approximately 16 million people worldwide have morphine and another opioid use disorder associated with the prescription of opioids, which causes a drug abuse epidemic (2). Morphine, a broadly used opioid analgesic, carries diverse behavioral and molecular effects. Morphine addiction is a significant public health issue (2). The present investigation consistently examined the effects of varenicline and bupropion on the rewarding characteristics of morphine as measured following various phases of CPP (i.e., acquisition/development, extinction, and reinstatement). In this study, the treatment with varenicline and bupropion doses were chosen from the effective doses determined in a previous study based on nicotine and alcohol-induced CPP (34-36). Thus the results of the present study suggest that morphine at a dose of 10 mg/kg induces CPP in rats, which is consistent with the results of the previous study (4). There was no statistically significant difference between the varenicline and bupropion groups in the development phase. In addition, varenicline and bupropion decreased expression and reinstatement and expedited the extinction of morphine-induced CPP.

Methadone and buprenorphine are FDA approved drugs indicated for the treatment of opioid use disorder (37). Buprenorphine attenuates the expression of cocaine-induced CPP (38, 39). Moreover, in the study of O'Neal

et al., buprenorphine reduced heroin-induced CPP (40). However, buprenorphine itself produces CPP. Narasingam et al. have demonstrated that methadone reduces heroin-induced CPP (37). Methadone produces conditioned place preference in the rat (41). Caffeine produces a significant place preference (42). In addition, caffeine reduces alcohol and methamphetamine-induced CPP (43, 44). Pandy et al. have shown that bupropion reduces methamphetamine-induced CPP (45). In our study, the chronic administration of bupropion produced CPP. However, the chronic administration of bupropion with morphine did not change morphine-induced CPP. These results can be interpreted as follows: the substance with addictive potential reduces the addiction of the substance with higher addictive potential.

Similar to our results in a biased design model previously performed on mice, bupropion was found to be ineffective on the development of morphine dependence (46). In addition, the expression of addiction in this study is different from our results. In a study by McKendrick et al., which is different from our method, a biased design was preferred (46). Results may differ depending on the biased and unbiased design. In addition, the chosen animal species and experimental protocols can effect the results. In our study, high-dose bupropion statistically altered the locomotor activity. In addition, a high-dose administration of bupropion produced CPP. Alteration of locomotor activity may alter the CPP results. Varenicline did not change locomotor activity at any investigated doses. These results are similar to those reported in the literature (36, 47, 48). Varenicline from multiple pharmacological mechanisms may display an attenuation effect on morphine-induced CPP. It has been shown in a previous research that stimulation of the nAChR to change and modulate cell firing in the brain is critical for the maintenance of drugs/substance addiction and dependence (7, 8). Furthermore, a current strategy for the therapy of side effects of drugs of abuse potential utilizes the use of varenicline, as they can show efficacy and moderate toxicity (6-8, 49-51). In previous research, varenicline prevented apnea caused by fentanyl (52). Varenicline, a nicotine receptor agonist, has been shown to be effective in opioid-dependent in adults with chronic pain undergoing opioid detoxification (49). In a different study, morphine-induced CPP was inhibited by naloxone-induced avoidance by administering nicotine (53).

Morphine and nicotine, two common abuse substances, share multiple behavioral and rewarding characteristics, such as hypothermia, catalepsy, antinociception, and place aversion. The activation of nicotinic receptors is closely linked to the rewarding effect of morphine (9). Morphine acts as a partial weak agonist at $\alpha 4\beta 2$ and a weak antagonist at $\alpha 3$ nicotinic acetylcholine (54). In similar studies, nicotine decreased the withdrawal symptoms related to morphine and increased the analgesic effect related to morphine (10, 11). In a different research, nicotine reduced opioid withdrawal (12, 13). Pre-treatment with opioid receptor agonists reduced withdrawal symptoms due to accelerated nicotine with the nicotinic receptor blocker mecamylamine (13, 14).

In addition, in another study in which nicotine was chronically given, altering the endogenous enkephalin synthesis, which was revealed by giving naloxone, reduced nicotine withdrawal symptoms (15). Nicotine administration in male and female mice μ formed up-regulation of opioid receptors (16). It is known that dopamine levels are decreased with morphine withdrawal. A decrease in dopamine levels in morphine withdrawal reveals drug-seeking behavior. Based on this hypothesis, it can be thought that varenicline can reduce drug-seeking behavior by increasing the dopamine levels which is decreased in morphine dependence (8, 17, 18). Varenicline can contribute to the reduction of morphine-induced CPP by using these mechanisms.

It has been reported in a previous study that nicotine-induced CPP is reversed (reinstatement) with a single dose of morphine (36). In this study, it was interpreted that morphine addiction and nicotine addiction are closely related. Also, reversion of CPP (reinstatement) by morphine was statistically significantly inhibited by varenicline (36). Varenicline significantly increases D2/D3 level in brain reward centers in rats (55, 56). Moreover, varenicline decreases alcohol consumption in animals and humans (7, 8). Various investigations have highlighted the effectiveness of varenicline for attenuating nicotine ethanol and opioid addiction (6, 7, 49, 50, 57, 58). In addition, morphine addiction has an important role in the GABAergic system. GABA transmission decreases in brain areas during morphine withdrawal (59). Varenicline increases GABA transmission in the similar regions of brain (60). The literature review shows that GABA agonists

reduce morphine-induced-CPP (61, 62). Varenicline can reduce morphine-induced CPP by affecting the above mechanisms.

Bupropion, by multiple pharmacological mechanisms, may display an attenuation effect on morphine-induced CPP. It is a norepinephrine-dopamine disinhibitor approved for the treatment of depression and smoking cessation (20). Bupropion is a second-generation trimethylated monocyclic anti-depressant, which differs structurally from most anti-depressants, and resides in a new mechanistic class that has no direct effect on the serotonin system (20). Multiple investigations have highlighted the effectiveness of bupropion for the attenuate of nicotine, amphetamine, methamphetamine addiction (19, 20). In another research, it has been shown that bupropion reduces morphine tolerance and physical dependence (20). Dopamine is essential in developing and maintaining morphine-induced CPP (21). A dopamine receptor agonist was given before testing to reduce the expression of morphine-induced CPP (10, 21).

In a similar study, the dopamine receptor agonist morphine addiction prevented its development and expression (22). Sympathetic hyperactivation is seen with morphine withdrawal. It is known that this sympathetic hyperactivation is due to the increased firing of noradrenergic neurons in the locus coeruleus (23, 24). Bupropion dose depending on decreases the firing of noradrenergic neurons in the locus coeruleus (25). Although the exact mechanism for bupropion effectiveness in morphine-induced CPP is unexplored, its ability to reduce the reuptake of dopamine and noradrenaline may contribute to the attenuation of morphine-induced CPP. Bupropion may modulate morphine-induced CPP by affecting this mechanism.

In various studies, morphine-induced CPP.was reduced by different antidepressants. In the study by Kang et al., mirtazapine reduced morphine-induced CPP and morphine withdrawal (63). In another study by Charkhpour et al., the antidepressant drug duloxetine reduced all morphine-related withdrawal syndrome (64).

Therapeutic approaches for the therapy of morphine and other opioids dependence and addiction are proposed at decreasing the three most critical viewpoints: craving, withdrawal/abstinence symptoms, and relapse (3, 4, 65). Detoxification is frequently the primary step in treating individuals with morphine and other opioids addiction. The medicines utilized to support this detoxification include opioid receptor agonists, which allow fractional elimination of the narcotic from the brain by decreasing the severity of the abstinence symptoms even if they are considered highly addictive (i.e., methadone) (3, 4). In another way, opioid receptors antagonists (naltrexone, naloxone,) can be utilized, which can occur in the unexpected displacement of the drug that is matched by different withdrawal symptoms and prevalent relapses. Nevertheless, among the pharmacological approaches that have frequently been used to decrease withdrawal/abstinence symptoms, few can diminish the drug and substance craving, and they are also seldom efficient in blocking relapse (3, 4, 66). Consequently, various researches are devoted to the investigation of new strategies for pharmacological agents that can prevent or decrease both the discomfort caused by the withdrawal/abstinence symptoms, and the compulsive desire (craving) that drives uncontrolled usage of drugs and substances which are among the principal causes of relapse (3, 4).

Lastly, relapse is an important aspect of drug or substances addiction and dependence and the principal problem in the treatment of drug addiction (67, 68). Varying motives can enhance craving, and the following vulnerability to relapse subsequent detoxification. Despite this, various preclinical and clinical investigations have explained that re-exposure to the drug (priming) is the primary factor linked to drug-seeking and drug-craving behavior in animals and human addicts (6, 33, 69). Accordingly, since the blocking of relapse is the principal purpose of dependence treatment and it is still the major barrier in drug treatment, we also applied the method of CPP to evaluate the role of varenicline and bupropion in the reinstatement of drug-seeking behavior induced by priming.

In summary, varenicline and bupropion treatment did not prevent the development of morphine-induced CPP in rats. However, varenicline and bupropion decreased expression and reinstatement and accelerated the extinction of morphine-induced CPP. The data suggest that varenicline and bupropion may be helpful as therapeutic pharmacologic agents to reduce morphine dependence. This study provides preliminary evidence to highlight

the importance of varenicline and bupropion effects on morphine addiction. It would be more appropriate to carry out future comprehensive research on this subject.

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Declaration

All authors declare that they have no conflict of interest to disclose.

This work was approved by the Istanbul University Local Ethics Committee on Animal Experiments Date: 29.12.2011 No: 2011/164 and were in accordance with the EU Directive 2010/63/EU on the safety of animals utilized for scientific purposes.

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ORIGINAL ARTICLE

Elderly burns; Our clinical experiences

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Abstract

Background: Though mortality rates have decreased with better understanding of burns' pathophysiology and advanced monitoring, elderly burn patients are still a challenging health problem. The elderly population is increasing worldwide. The aim of this study is to investigate the outcomes of burns for elderly patients and increase medical, public and governmental awareness of the issue. Elderly people should be trained in preventive measures to diminish the number of burn accidents.

Methods: Hospitalized patients between 2011 and 2019 were retrospectively studied. Age, gender, burned total body surface area (TBSA), burn agent, comorbidities, location of the burn injury, whether a house fire accompanied, and mortality rates were compared between adult patients (18 to 64 years old) and those who were elderly (65 years or older).

Results: Of the 2258 patients, 285 (12.6%) were aged 65 or older. The burned TBSAs of the adult patients were larger than those of the elderly patients ($p=0.019$). Scalding and contact burns were more frequent among the elderly than the adults ($p=0.001$). The elderly had greater co-morbidities, were mostly burned at home and more house fires accompanied their injuries than those of the adults ($p<0.001$). Despite having lesser burned TBSAs, mortality rates were significantly higher in the elderly ($p<0.001$).

Conclusions: As the elderly population increases worldwide, burn treatment facilities should be prepared for their increase in numbers and co-morbidities. To enforce preventive measures, awareness of the growing issue should be raised and public authorities should be alerted to their need to act.

Keywords: Burns, Elderly, Mortality, Co-morbidity.

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INTRODUCTION

'Elderly' is defined as 65 years old or older and 'advanced elderly' as 80 years old or older. The elderly age group is the most rapidly growing segment of Turkey's population (1). As the elderly population increases, so does the number of elderly patients, and many branches of medicine purport to deal with the health problems of this sensitive group. In particular, it is inevitable that more elderly patients will require treatment for burns in the future. Limited mobility, sight and hearing disorders and slowed reflexes make elderly people more prone to burns and burn-related injuries (2). Elderly burn patients often live alone and may have inadequate social and family support, which is the frequent reason for late admission to health care services after injury. Burn treatment outcomes are worse for the elderly than for adults aged under 65 years (3).

Almost 8% of physical traumas experienced by the elderly occur via burning (4). Failures in burn treatment for the elderly are attributable to factors such as skin atrophy, changes in the wound healing cascade, concomitant diseases, failure to adapt to stressful conditions, immobilization, digestive disorders and protein energy malnutrition and mental regression (5). Skin atrophy is the thinning of the skin. It causes difficulty in depth estimation, diminished graft take-rate, delayed donor site healing, longer length of hospital stays and higher hospital-related infection risk (6). All of these factors contribute to elderly mortality. At the site of the wound, inflammation slows, collagen synthesis decreases and epithelialization is delayed. Concomitant systemic diseases, such as diabetes-related neuropathy and atherosclerotic plaque formation resulting in insufficient perioperative perfusion and oxygenation, complicate burn management (7).

The fragile physical and systemic conditions of elderly people also complicate the treatment of burn shock and the provision of maintenance treatment. All known formulas and protocols should be revised individually, considering the patient's systemic health status; close, meticulous monitoring is a must.

Ankara City Hospital Burn Treatment Center is located at the capital city of Turkey and is a third referral center with eighteen ward beds, six intensive care beds and two operating theaters. Our study aimed to see the etiologic and demographic differences between the elderly and adults, aged under 65 years and treatment outcomes among them at our hospitalized patient population and prepare the Turkish healthcare system for dealing with an increasing number of elderly burn victims.

The study was carried out after obtaining approval from the Ethics Committee (E1) of Ankara City Hospital (Date: 17.03.2021, Approval No: 2021/E1-21-1607).

MATERIALS AND METHODS

All hospitalized patients aged 18 years and older between 2011 and 2019 were included in our retrospective study. Data were retrieved from the patients' files. The following data were recorded: age; gender; burned total body surface area (TBSA); burn agent; comorbidities; location of the burn injury; whether a house fire accompanied; and mortality rates. The patients were divided into two groups: adult and elderly. The adult group patients were between 18 and 64 years old. The elderly group patients were aged 65 years or older. Statistical analysis was presented with percentages (%), mean ± standard deviations, Chi-square testing and Mann-Whitney U testing. A p-value less than 0.05 is determined statistically significant.

RESULTS

Of the 2258 inpatients, 1973 (87.4%) were aged between 18 and 64 years (mean 36±12) and composed the adult group. The remaining 285 (12.6%) were 65 years old or over (mean 74±7) and composed the elderly group. The burned TBSAs of the adult patients were significantly larger than those of the elderly patients (18.65±21.02 and 15.52±20.82, respectively; p=0.019). Although there was no significant difference between groups third-degree burns were more prevalent in the elderly group (43.9% compared to 39.4% in the adult group [p=0,087]); however, fourth-degree burns were more frequent in the adult group (9.4% compared to 7.0% in the elderly group [p=0.116]).

While males were more frequently burned in the adult group (73.4% compared to 26.6% females), the distribution of the burns by gender was almost equal in the elderly (50.5% males and 49.5% females) (p<0.001, Table 1).

Scalding was the most frequent cause of burns among the elderly, followed by flame burns (43.5% and 36.8%, respectively); however, flames were the leading cause of burns among adults, followed by scalding (44.2% and 28.5%, respectively). While electrical burns were the third most common cause of burns among adults, contact burns were the third etiology among the elderly, (12.6%). There was a significant difference between adults and elderly (Table 1).

Table 1. Patients Characteristics and Demographics

	Age		Total	p
	18-64	>65		
Gender				
Female	525 (**26.6**%)	144 (**50.5**%)	669 (29.6%)	<0.001
Male	1448 (**73.4**%)	141 (**49.5**%)	1589 (70.4%)	
Etiology				
Scald	563 (28.5%)	124 (**43.5%**)	687 (30.4%)	
Contact	102 (5.2%)	36 (12.6%)	138 (6.1%)	
Flame	873 (**44.2%**)	105 (36.8%)	978 (43.3%)	<0.001
Electrical	319 (16.2%)	9 (3.2%)	328 (14.5%)	
Chemical	83 (4.2%)	7 (2.5%)	90 (4.0%)	
Frost bite	6 (0.3%)	0	6 (0.3%)	
Others	27(1.3 %)	4(1.4%)	21 (1.4 %)	
Burn place				
Home	989 (50.5%)	241 (**85.8**%)	1230 (54.9%)	
Workplace	474 (24.2%)	1 (0.4%)	475 (21.2%)	<0.001
Open place	447 (22.8%)	34 (2.1%)	481 (21.5%)	
House Fire				
No	1425(95.9%)	203 (90.6%)	1628 (95.2%)	<0.001
Yes	61(**4.1%**)	21 (**9.4%**)	82 (4.8%)	
Mortality8				
No	1774(89.9%)	231(81.1%)	2005(88.8%)	<0.001
Yes	199(**10.1%**)	54(**18.9%**)	253(11.2%)	
Total	1973	285	2258	

Table 2: Co-morbidities in 2258 patients

		Age (years)		
		18-64 (n, %)	>65 (n, %)	p
Epilepsy	No	1885 (95, 5%)	279 (97, 9%)	**0.036**
	Yes	88 (**4, 5**%)	6 (2, 1%)	
Diabetes Mellitus	No	1897 (96, 2%)	224 (78, 6%)	**<0.001**
	Yes	75 (3, 8%)	61 (**21, 4**%)	
COPD	No	1967 (99, 7%)	280 (98, 2%)	**0.007**
	Yes	6 (0, 3%)	5 (**1, 8**%)	
CVD	No	1950 (98, 8%)	262 (91, 9%)	**<0.001**
	Yes	23 (1, 2%)	23 (**8, 1**%)	

COPD= Chronic Obstructive Pulmonary Disease; CVD= Cardiovascular Diseases

The prevalence of co-morbidities was higher in the elderly group (109/285, 38.2%) than in the adult group (206/1973, 10.4%) (p<0.001). Diabetes mellitus, chronic obstructive pulmonary disease (COPD) and cardiovascular disease (CVD) rates were significantly higher among the elderly; however, epilepsy was significantly more common in adult patients (Table 2). When the effects of comorbidities on the burn depth were analyzed, patients with epilepsy were found to have significantly higher rates of third- and fourth-degree burns; no significant effect of other comorbidities was found (Table 3).

Table 3: Fourth and third degree burns and epilepsy correlation regarding to age groups.

Epilepsy						
Age	Burn Depth		No	Yes	Total	p
			N (%)	N (%)	N (%)	
18-64	3th degree	No	1158 (61.9%)	37 (36.3%)	1195 (60.6%)	
		Yes	713 (38.1%)	65 (**63.7%**)	778 (39.4%)	**0.000**
	Total	n	1871	102	1973	
>65	3th degree	No	152 (57.4%)	8 (40.0%)	160 (56.1%)	
		Yes	113 (42.6%)	12 (60.0%)	125 (43.9%)	0.102
	Total	n	265	20	285	
Total	3th degree	No	1310 (61.3%)	45 (36.9%)	1355 (60.0%)	
		Yes	826 (38.7%)	77 (**63.1%**)	903 (40.0%)	**0.000**
	Total	n	2136	122	2258	
18-64	4th degree	No	1703 (91.0%)	85 (83.3%)	1787 (90.6%)	
		Yes	168 (9.0%)	17 (**16.7%**)	185 (9.4%)	**0.012**
	Total	n	1871	102	1973	
>65	4th degree	No	249 (94.0%)	16 (80.0%)	265 (93.0%)	
		Yes	16 (6.0%)	4 (**20.0%**)	20 (7.0%)	**0.041**
	Total	n	265	20	285	
Total	4th degree	No	1951 (91.4%)	101 (82.8%)	2052 (90.9%)	
		Yes	184 (8.6%)	21 (**17.2%**)	205 (9.1%)	**0.002**
	Total	n	2136	122	2258	

In the elderly group, burn injuries occurred at a significantly higher rate at home (85.8%), whereas in the adult group 50.5% of the injuries occurred at home, 24.2% in the workplace and 22.8% in open spaces (Table 1). The charts of 1710 patients were noted for whether or not there had been a concomitant house fire. Burn injuries were associated with a significantly higher rate of house fires in the elderly group (9.4%) than in the adult group (4.1%) (Table 6). When the house fires were analyzed in relation to comorbidities, it was found that having a comorbidity increased the rate of house fires for both groups; as such, the rate of house fires was 66.7% for the elderly group (Table 4). Epilepsy, as a comorbidity, was encountered in 6.9% (5/81, p=0.253) of the house fires. However, diabetes mellitus was a significantly effecting comorbidity in 11.9% (13/82, p=0.002) of the house fires, especially among the elderly (Table 4).

Table 4 : House fires regarding the comorbidities and age.

Age	Comorbidity	House Fire		
		None	Yes	
		N (%)	N (%)	p
18-64	None	1275 (89.5%)	47 (77.0%)	
	Yes	150 (10.5%)	14 (**23.0%**)	**0.005**
	n	1425	61	
>65	None	146 (71.9%)	7 (33.3%)	
	Yes	57 (28.1%)	14 (**66.7%**)	**0.001**
	n	203	21	
Whole	None	1421 (87.3%)	54 (65.9%)	
	Yes	207 (12.7%)	28 (**34.1%**)	**<0.001**
	n	1628	82	
	Diabetes Mellitus			
18-64	None	1368 (95.9%)	56 (96.6%)	
	Yes	59 (4.1%)	2 (3.4%)	0.570
	n	1427	58	
>65	None	163 (94.2%)	40 (78.4%)	
	Yes	10 (5.8%)	11 (21.6%)	**0.002**
	n	173	51	
Whole	None	1531 (95.7%)	96 (88.1%)	
	Yes	69 (4.3%)	13 (11.9%)	**0.002**
	n	1600	109	

Although mean burned TBSA was lesser in the elderly, this group had a significantly higher mortality rate (Table 1). Having a comorbidity significantly increased mortality in the adult and elderly groups, as well as in both groups, combined ($p<0.001$, $p<0.001$, and $p<0.001$, respectively).

DISCUSSION

Given that age is an increased risk factor for burn injury and that Turkey's elderly population is increasing rapidly, a greater number of elderly burn patients can be expected in the nation's hospitals. 12.6% of our hospitalized burn patients were elderly; this number will be higher in the future.

According to demographic studies, young male patients are significantly more prone to burn injury than female patients. However, our results, consistent with results from the literature, showed that female and male burn victims are similar in proportion among elderly (8).

Although adult patients most commonly have burns caused by fire, followed by scalding; the elderly had significantly more burns from scalding, followed by fire. Electrical burns and contact burns were the third most frequent burn agents among adults and the elderly, respectively.

An analysis of our elderly patients' contact burns and the existence of epilepsy among the patients revealed a significant relationship between epilepsy and third and fourth degree burns, which were the most common type of contact burn.

Mabrouk et al. have found that nearly 80% of elderly patients burn themselves at home, and about 4% in the workplace (9). Concordant with the literature, our study shows the proportions of the accident sites to be more distributed among the adult population, with 50.5% of burns occurring at home, 24.2% in the workplace and 22.8% in open spaces, and the majority of the elderly

patients' burns (85%) occurring at home. Although the majority of our elderly patients burned themselves at home, this may vary among countries, as the retirement age of each country is different. Physical or mental illness, diminished alertness, slower reaction time, reduced mobility and poor eyesight and hearing can result in home accidents, especially in the kitchen and the bathroom (10).

House fires are important events that increase the severity of burn injuries and result in accommodation problems. House fires were accountable for the burn injuries of 9.4% of our elderly patients and 4.1% of our adult patients. Elderly patients who suffer from burn injuries generally live alone and have inadequate social support. Smoking, low income and poor building amenities and conditions (such as cooking over an open flame or with other heating equipment; lack of extinguisher systems) further increase the risk of fire. Fire injury and death commonly occur midmorning and early afternoon (11,12). The use of alcohol and medications such as sleeping pills or tranquilizers is an additional factor in the increased risk of a house fire. According to our results, having a comorbidity also significantly increases the incidence of house fires for all age groups. While the prevalence of epilepsy had no effect on the incidence of house fires, diabetes mellitus was found to significantly increase the incidence of house fires among elderly burn patients.

Elderly patients frequently have coexisting or premorbid conditions such as diabetes and cardiovascular disease. Mabrouk et al. showed an incidence of premorbid conditions of 54.6% in patients aged 60 years older (9). These pre-existing medical conditions render elderly people more susceptible to various complications after burn injury (13). In our study, diabetes and cardiovascular diseases were the most common co-morbid diseases in the elderly (21.4% and 8.1%, respectively), and epilepsy was the most common co-morbid disease in adult burn patients (4.5%). According to these results, the types of co-morbid diseases in elderly burn patients in Turkey (a developing country) and developed countries are similar, and living conditions, awareness and preventive measurements should be improved at this group.

Elderly diabetic patients are more likely to sustain burns during home accidents, especially due to neuropathy and diminished motor activity. Furthermore, the incidence of complications among diabetic burn patients is significantly higher due to the patients' triads of vasculopathy, neuropathy and immunopathy (14). According to population-based studies of diabetes and risk characteristics of diabetes in Turkey (TURDEP I and II), diabetes is moderately common in Turkey by international standards. The incidence of Diabetes in Turkey had increased by 90% over the last 12 years (from 2002 to 2013). The prevalence of diagnosed diabetes was 16.5% (15,16). In our study, diabetes was common among the elderly (21.4%), and house fires were frequently accountable for the burns of diabetic patients. To our finding, increased diabetic burn victim numbers with concomitant house fires will be more frequent health and social problem for Turkey and may be for all countries.

Epileptic patients most commonly have accidents and sustain injuries at home. These accidents are correlated with the patient's neurologic impairment and number of seizures. Burns affecting epileptic patients are severe and deeper due to the patient's loss of consciousness during seizures (17). Epileptic seizures were found to lead to a higher proportion of third- and fourth-degree burns in our adult and elderly patient groups.

Many scoring systems have been used in order to quantify the severity of burns and predict the mortality of burn patients. Baux Score, defined as the sum of the age in years and the burned percentage of the body, has been used by generations of clinicians to predict the probability of mortality following burn trauma (18). Over the past three decades, via the advancements in burn treatment, the Lethal Dose 50 (LD50) burn size has been increased. The mortality rate for adult burn patients has decreased to 5.5%. On the other hand, mortality among elderly burn patients still ranges from 7.4 to 66%, with an average of 30% (19).

Farinas et al. suggest a high conversion rate from partial to full thickness burns in the elderly; clinically, this is understood to be a deepening of the burn wound, as it makes the burn wound dynamic (20). Similar to previous studies, our clinical experiences showed that, although their TBSA was significantly smaller, the elderly group had a significantly higher mortality rate (18.9%). Elderly skin tissue undergoes significant physiological changes, such as decreased epidermal turnover, decreased skin appendages, thinning of the dermis, decreased dermal vasculature, decreased collagen and matrix and impaired

neurosensory perception (6). Therefore, elderly burn patients face deeper wounds and a longer healing time than adults with similar TBSA (7). Prolonged healing time results in longer hospital stays and a higher risk of hospital-acquired infections.

The physiological changes brought about by aging; age associated immune dysfunction; immunosenescence and pre-existing medical problems; malnutrition; and hypermetabolism all contribute to poorer outcomes for elderly burn patients (5).

Improvements in burn management and a reduction in mortality lead to more elderly patients being discharged from acute care hospitals alive. Rehabilitation, social reintegration and long-term survival are important goals for burn treatment. According to previous research, 90% of adults with a major burn return to a home environment within a year, compared to approximately 50% of elderly patients (19). As long-term disability is much greater among the elderly, aggressive rehabilitation to avoid early loss of function and muscle strength is very important (21).

In order to provide more reliable data on burn trauma and its consequences faced by the elderly population in our country, multicenter studies with large series including outpatients and inpatients are needed throughout the country.

Elderly burn patients face a significantly higher risk of mortality. Natural aging processes lead to delayed burn wound healing and increased morbidity and mortality. Pre-existing co-morbid conditions and impaired inflammatory responses are indicators of poor outcomes for the elderly. The elderly's impaired reflexes impede actions such as extinguishing the fire and cooling the burned site by applying tap water, and cause delays in seeking medical advice (which results in delayed treatment and deeper wounds). Elderly people should be trained in preventive measures to diminish the number of burn accidents. As most elderly burn injuries are preventable, passive and active prevention strategies should be encouraged.

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The results of study will be discussed with the public authorities.

Declarations

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The study was carried out after obtaining approval from the Ethics Committee (E1) of Ankara City Hospital (Date: 17.03.2021, Approval No: 2021/E1-21-1607).

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ORIGINAL ARTICLE

Clinical and microbiological characteristics of *Candida* meningitis/ventriculitis in children

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Abstract

Background: *Candida* meningitis/ventriculitis is rather rare during childhood. In this study, we investigated the clinical characteristics, risk factors, treatment, and prognosis of patients with *Candida* meningitis/ventriculitis.

Methods: Patients under the age of 18 years who were diagnosed with *Candida* meningitis/ventriculitis were evaluated retrospectively.

Results: A total of 10 cases with *Candida* meningitis/ventriculitis were analyzed. Three patients (30%) were below the age of one, and two (20%) were neonates. The two most common underlying conditions were hydrocephalus shunt and prematurity. Predisposing factors were a history of broad-spectrum antibiotic use, external ventricular drainage, total parenteral nutrition, central venous catheter, and staying in intensive care. The cerebrospinal fluid culture was positive in all patients, and 10% (1/1) had bacteremia. Of the isolates, 50% were *C. albicans*, 30% were *C. tropicalis*, 10% were *C. lusitaniae*, and 10% were *C. dubliniensis*. Fluconazole treatment was initiated in four (40%) and voriconazole in three (30%) patients. Two patients received combined treatment (amphotericin B + fluconazole/voriconazole). The median treatment duration was 38.6 days (range: 16–70 days). Three patients received intraventricular Amphotericin *B.Central* nervous system devices which were assumed to be infected were removed. A complication of endophthalmitis developed in one patient. The mortality rate was 10%.

Conclusions: Among agents causing meningitis/ventriculitis, *Candida* should also be kept in mind in premature neonates and patients with ventricular-peritoneal shunts. The history of antibiotic use and external ventricular drainage are important predisposing factors. It can be successfully treated with fluconazole, voriconazole, amphotericin B, and removal of the central nervous system device.

Keywords: *Candida*, Ventriculitis, Meningitis, Treatment, Children.

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INTRODUCTION

Central nervous system (CNS) involvement is very rare among invasive *Candida* infections in children. *Candida* species occupy the most common meninges within the CNS (1). *Candida* species usually cause acute meningitis during dissemination (2). In addition to hematogenous spread, they can reach the brain with craniotomy and ventriculoperitoneal shunt or other CNS devices (1). And they cause serious mortality and morbidity (3). *Candida* meningitis (CM) is most commonly seen in newborns with candidemia (2). 5%-9% of newborns with candidemia are accompanied by meningitis (4). Very low birth weight premature (PM) infants are a significant risk group for CM (5). Although CM is seen in healthy people, people with weak immune systems are also at risk (5). Previous antibiotic and steroid use, a central venous catheter (CVC), recent neurosurgery/ Cerebrospinal fluid (CSF) drainage system, abdominal surgery, and intravenous drug use have been reported as risk factors for CM (3, 6). Generally, the clinical symptoms are like acute bacterial meningitis, however, it can rarely cause chronic meningitis symptoms (1). Furthermore, hematogenous spread and symptoms related to eye, skin, and endocardial involvement can also be seen. There are limited information in the literature on CM in children.

In this study, we investigated the demographic, clinical characteristics, risk factors, treatment, and prognosis of patients diagnosed with *Candida* meningitis.

MATERIALS AND METHODS

Patients under the age of 18 and diagnosed with *Candida* meningitis followed between January 2015 and December 2021 were included in the study. Patients with *Candida* species grown in the CSF culture were obtained from microbiology laboratory records. The diagnosis of *Candida* ventriculitis/meningitis (CVM) was determined as patients with bacterial meningitis symptoms and signs according to age group, and as patients who had pleocytosis, low glucose, high protein in CSF analysis, and *Candida* growth in CSF culture. Patients without risk factors for CNS infection, without signs and symptoms of CNS infection, and without CSF glucose, protein, and normal pleocytosis were excluded from the study.

Demographic characteristics of the patients, predisposing factors, clinical management, CSF examination results, details of antifungal treatment, antifungal sensitivity results, culture results, echocardiography, and eye examination results were analyzed and recorded in the leaflets. CSF was obtained by lumbar puncture or CNS devices (lumbar drainage, external ventricular drainage [EVD], or shunt pump). For *Candida* identification and antifungal susceptibility tests, MIC values against amphotericin B, caspofungin, micafungin, fluconazole, flucytosine, and voriconazole were determined using the VITEK 2 Compact® (bioMeriéux, France) system and identification cards (YST) and antifungal susceptibility cards (AST-YST01).

With improvement of clinical symptoms and signs, normalization of CSF glucose, protein, pleocytosis, and absence of culture growth, patients who resolved completely on MRI in the presence of cerebral abscess were considered cured. The duration of treatment was decided according to the improvements. In addition to intravenous therapy, the infected intraventricular device was removed. Intrathecal (IT) treatment was not routinely applied. Intrathecal therapy was applied to the patients who did not improve despite appropriate antifungal treatment, whose CSF findings did not regress, and whose CSF culture growth continued. We administered amphotericin B deoxycholate through the device into the ventricle at a dose of 0.01 to 0.5 mg in 2 mL 5% dextrose in water (7, 8). Because of the lack of clear information about the IT duration, the decision was made on a patient basis. It was discontinued in patients with improved pleocytosis and sterile CSF.

In patients diagnosed with CM, the death that occurred within 30 days after *Candida* growth in CSF/Blood culture was determined as death ascribed to *Candida*.

Our study was conducted in accordance with the Declaration of Helsinki. Approval was obtained from Çukurova University Non-Interventional Clinical Research Ethics Committee (Date: 03/06/2022, Decision No:123).

Statistical Analysis

For statistical analyzes, SPSS version 23.0 was employed. Continuous measurements were summarized as means, deviation, and minimum-maximum, whereas categorical measurements were presented as numbers and percentages.

RESULTS

A total of 10 cases with CVM were analyzed. The median age was 41.3 months, (range: 2–180 months). Three patients were under the age of one, and two (20%) were premature infants. Seven (70%) patients were female. The most prevalent symptoms were vomiting, fever, proneness to sleep, and headache (50%, 30%, 20%, and 20%, respectively).

All patients had an underlying condition, and the two most common underlying conditions were hydrocephalus shunt and prematurity. Of the CVM, 60% (6/10) were associated with hydrocephalus shunt, where the postoperative, and spontaneous meningitis/ventriculitis manifestations accounted for 20% (2/10), and 20%(2/10) cases, respectively. Patient demographics, clinical characteristics, treatment, and prognoses are summarized in Table 1.

Table 1. Patient demographics, clinical characteristics, treatment and prognosis

Case	Age (Months) /Sex	Application Complaint	Underlying Disease	Risk Factors	Species / Repeat Number	CSF Analysis	PCT/ CRP	Tx and Duration	Additional Tx	Prognosis
1	20 / M	Vomiting , Proneness to sleep	H, V-P shunt	-	*C. tropicalis*/3	Glu:0 P:1316 Diffuse WBC	0.4/297	AmB(20) + FLU (13), after VCZ (20)	Shunt removal	Cure
2 **	2/F	Deterioration in general condition	PM (27 weeks)	Antibiotic use, Admission to intensive care unit CVC, TPN	*C. albicans*/5	Glu:1 P:234 60/mm^3 WBC	0.08/-	AmB (30) + FLU (39), after AmB(20) + VCZ (20)	IT AmB (14 day)	Cure
3	40/F	Fever	H, V-P shunt	EVD, Antibiotic use,	*C. tropicalis*/2	Glu:42 (SG:80) P:196 20/mm^3 WBC	0.08/3	VCZ(21)	EVD replacement	Cure
4	26/M	Fever, Vomiting , Clouding of consciousness	OBT, V-P shunt	EVD, Antibiotic use, CVC, TPN Admission to intensive care unit, History of surgical operation	*C. albicans*/9	Glu:11 (SG:127) P:193 70/mm^3 WBC	0.06/8	FLU(33)	EVD replacement + IT AmB (10day)	Cure
5*	5/F	Vomiting	H, V-P shunt	EVD, Antibiotic use, CVC	*C. lusitaniae*/3	Glu:82 (SG:90) P:420 330/mm^3 WBC	0.06/7	VCZ(49)	EVD replacement	Cure
6	14/F	Fever	H, V-P shunt	EVD Antibiotic use	*C. albicans*/2	Glu:4 (SG:81) P:417 120/mm^3 WBC	0.04/14	FLU(38) FLU(32)	EVD replacement	Cure
7	180/F	Headache , Proneness to sleep	H, V-P shunt	EVD, Antibiotic use,	*C. albicans*/3	Glu:76 (SG:108) P:420 Diffuse WBC	0.2/228	VCZ (24)	EVD replacement	Cure
8	72/E	Headach, Vomiting	OBT, V-P shunt	Antibiotic use, Admission to intensive care unit, History of surgical operation, Steroid use	*C. albicans*/1	Glu:51 (SG:192) P:1100 350/mm^3 WBC	0.01/12	AmB(32) after VCZ (16)	Shunt removal + IT AmB (12 day)	Died
9	2/F	Vomiting , Apnea	PM (30 weeks)	Antibiotic use, Admission to intensive care unit, TPN	*C .tropicalis*/1	Glu:46 (SG:192) P:95 0/mm^3 WBC	0.8/6	FLU (16)	-	Cure
10	52/M	Convulsion	H, V-P shunt	Antibiotic use	C. *dubliniensis*/1	Glu:9 (SG:89) P:3100 30/mm^3 WBC	0.06/4	FLU (42)	Shunt removal, Abscess drainage	Cure

*Concurrent blood culture has growth

**Case with endophthalmitis

F: Female, M: Male, H: Hydrocephalus, PM: Premature, V-P: Ventriculoperitoneal, OBT: Operated Brain Tumor, TPN: Total Parenteral Nutrition, CVC: Central Venous Catheter, EVD: External Ventricular Drainage, Tx: Treatment. IV: Intraventricular, Glu: Glucose (mg/dl), SG: Serum Glucose (mg/dl), P: Protein (mg/dl), WBC: White Blood Cell, PCT: Procalcitonin (Normal range: 0-0.5 ng/ml), CRP: C-Reactive Protein (Normal range: 0-8 mg/L), AmB: Amphotericin B, FLU: Fluconazole, VCZ: Voriconazole, IT: Intrathecal , CSF: Cerebrospinal Fluid.

The most common infections associated with CNS devices, which were a risk factor, were due to EVD, followed by ventriculoperitoneal shunt. Two patients with spontaneous meningitis were premature infants. Predisposing factors, nine patients had a history of broad-spectrum antibiotic use. Subsequently, EVD, total parenteral nutrition (TPN), a long stay in intensive care, and CVC, were the most prevalent predisposing factors (Table 1).

In hospital-acquired cases, culture growth was observed after a median of 30.1 days (range: 5–82 days) of hospitalization. The most common CSF abnormalities were pleocytosis in eight (80%) cases, elevated protein concentrations in 100%, and hypoglycorrhachia in seven (70%). CSF culture was positive in all patients, and 10% (1/1) had bacteremia (Case 5). Of the isolates, 50% were *C. albicans*, 30% were *C. tropicalis*, 10% were *C. lusitaniae*, and 10% were C. *dubliniensis*. Complications other than endophthalmitis were detected in only one patient

Case 2 was 27 weeks premature and on the 58th day of neonatal intensive care unit admission, CSF taken due to deterioration in his general condition was compatible with meningitis and C. albicans grew in his culture. Endophthalmitis was detected in the general scan. Amphotericin B and fluconazole-combined treatment was started (Since there is no flucytosine in our country). As growth continued in CSF cultures, external ventricular drainage was inserted and IT amphotericin B was added to the treatment. Treatment because CSF findings did not regress and C. albicans growth was 5 times. Amphotericin B and voriconazole were switched. *Candida* meningitis was healed after 59 days of treatment.

Case 6 was hospitalized with a ventriculoperitoneal shunt infection. The shunt was removed. Fever occurred again on the 19th day of EVD and systemic antibiotic therapy. *C. albican*s grew in CSF culture. Fluconazole was started. It improved after 38 days of treatment. 13 days after the antifungal was discontinued, growth occurred again in the CSF culture. Fluconazole was started again and completely recovered after 32 days of treatment.

No resistance was detected against amphotericin B (AmB), caspofungin, mycofungin, fluconazole (FLU), flucytosine (5- FC) and voriconazole (VCZ) in any patient.80% of the patients received single antifungal therapy. Of the patients who received single antifungal therapy, four of them received FLU, and three they received VCZ. One patient was initially started on AmB therapy. VCZ treatment was started due to resistant hypopotassemia (Case 8). One patient was treated with AmB and FLU combined, however, due to continued growth, a single VCZ treatment was started (Case 1).

The median treatment duration was 38.6 days (range: 16–70 days). In addition to systemic therapy, three (30%) patients received IT AmB treatment (Cases 2, 4, 8). IT AmB was added due to recurrent growth in CSF culture in cases 2,4, and IT AmB was added in case 8 due to continued pleocytosis. IT treatment was discontinued when CSF became sterile, and pleocytosis regressed. The duration of treatment applied was between 10 and 14 days.

The infected CNS devices were removed from the patients with CVM (8/8) (V-P shunt was removed in 2 patients, EVD was changed in 6 patients). Abscess drainage was performed in one patient (Case 10). The mortality rate was 10% (Case 8).

DISCUSSION

Fungal infections of the central nervous system are rare and life-threatening serious infections. *Candida* is the most common cause of CNS fungal meningitis. In a study conducted on children, they reported that 94.5% fungi isolated in CSF were *Candida* isolates (9). However, it may cause an isolated intracranial abscess or may be associated with meningitis (1, 10). In our study, we detected ventriculitis/abscess in only one patient. Premature neonates are at risk of CM and systemic candidemia. *Candida* often causes CM in the presence of other CNS devices, hematogenous or craniotomy, and V-P shunt, during disseminated candidiasis in premature neonates. As a complication of neurosurgical procedures, CM is an important cause of mortality and morbidity in neonates. Among the invasive *Candida* infections of neonates, CM was detected at a rate of 22% and their mean age was reported to be 26.2 weeks (11). Only two of our patients were premature neonates. Although no accompanying candidemia was detected, one patient had hydrocephalus due to intracranial bleeding and 27-week-old invasive *Candida* risk factors (Case 2).

Most cases of CM occur in patients with neurosurgery and CNS devices (lumbar drainage, external ventricular drainage, VP shunt). In a study conducted in the literature, it was reported that 71.4% (30/42) of those with *Candida* growth in their CSF were neurosurgery patients (12). 80% of our patients had a CNS device. While most of them were V-P shunt patients, only two patients had EVD inserted after brain tumor surgery. Fungal infection should be kept in mind when meningitis/ventriculitis develops in pediatric patients who have a CNS device inserted for any reason. In cases where there is no response to antimicrobial treatment, even if there is no culture growth, a treatment plan in this direction can be life-saving. Apart from PM and CNS devices, important risk factors are known as prolonged antibiotic use, CVC, TPN, immunosuppressant corticosteroid use, chemotherapy, recent neurosurgery or intra-abdominal surgery, and intravenous drug abuse (3, 6). Most of our patients (9/10) were also using antibiotics before. Only one patient had used steroids due to cerebral edema (Case 8). We did not any patients receiving immunosuppressive or KT. Although the possibility of invasive fungal infection is high in immunosuppressive patients, the two most common and most important risk factors in CM are the use of antibiotics, addition to brain surgery or CNS device.

It has been reported that *C. albicans* is mostly isolated in patients diagnosed with CM (6, 11). Although *C. albicans* is a very common species, there has been an increase in non-albicans *Candida* species recently. In our study, *C. albicans* was isolated in half of the patients and *C. tropicalis* was isolated in 30% of our patients. Lijun Xu et al. reported that they isolated 28.6% *C. albicans* and 35.7% *C. parapsilosis* in their study and reported results supporting that non-albicans *Candida* species were more common (12). In a study consisting only of pediatric patients with cancer, it was reported that they isolated *C. tropicalis* in all patients (13). Chen et al. reported that they detected *C. albicans* in half of their patients (14). In the literature, *C. famata, C. tropicalis, C. glabrata, C. dubliniensis, C. lusitaniae* and *C. util* can cause non-albicans *Candida* (11, 12, 14–16). Meningitis caused by *C. duplinienis* has been reported in adult cases, but no pediatric cases have been reported before. We had one case of *C. lusitaniae* and one case of *C. dubliniensis that* we isolated. These results suggest that there will be an important problem in rare fungi together with non-albicans *Candida* in the future.

For treating CM, liposomal AmB alone or along with oral 5-FC is recommended (7). If the patient is sensitive, FLU is also recommended. AmBdeoxycholate is recommended for initial treatment in the neonatal period. Baratgar et al. reported that they successfully treated six patients under the age of six months, with V-P shunt infection due to *Candida* with AmB (17). Fernandez et al. started AmB in all the 23 neonates with CM, and they combined five patients' treatments with 5-FC (11). They reported that at the beginning of treatment in adult patients with CM, they used FLU and VCZ, and they also switched to VCZ while using FLU (12). In our study, we initiated most of the patients with FLU or VCZ because of good CSF transmission. We treated two patients with a combination of AmB and FLU/VCZ antibiotics. Since there is no 5-FC in our country, we could not use it in treatment. The treatment period of CNS *Candida* infections can last from weeks to months and this period varies from patient to patient. In the case series presented by Chen et al., they reported a case in which they gave treatment for as long as 23 weeks (18). In our study, we had a case that we treated for up to 70 days. Although intraventricular therapy is not routinely recommended, intravenous therapy may be inadequate from time to time in patients with CNS drainage such as V-P shunt. Cases in which successful results were obtained with the application of intraventricular AmB have been reported in the literature (19, 20). We successfully treated three of our patients by administering intrathecal AmB. CNS devices that are assumed to be a source of and colonized with *Candida* should be removed, this has great importance in the success of treatment. In our study, we either changed all the CNS devices or removed them from all patients.

The prognosis of *Candida* meningitis may vary according to many factors such as age, underlying disease, antifungal drug resistance, and time of diagnosis. In a study on patients under six months of age in the literature, a mortality rate of 0% was stated (17). It has been reported that six of the 23 patients with CM in the neonate period were expired (11). Lijun Xu et al. reported 50% mortality in their CM cases (12). In a study conducted on patients other than neonates, 42% of CM-related deaths were reported (21). Chen et al. detected a mortality rate of 11.1% in neurosurgery patients (14). In our study, the mortality rate was 10%. Mortality rates differ in studies reported in the

literature. We believe that keeping CM in mind in patients who are in the risk group, and who do not respond to antibiotic treatment and initiating antifungal therapy early on will reduce prognosis and mortality.

In conclusion, *Candida species* are rare agents in the etiology of meningitis/ventricülitis in childhood. The clinic is nonspecific. It is a more common species in premature neonates and patients who have undergone neurosurgery and have a CNS device. Long-term use of antibiotics is the most important predisposing factor. Most patients can recover with systemic FLU, VCZ, AmB, and intraventricular AmB and the removal of CNS devices.

Decleration

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

Our study was conducted in accordance with the Declaration of Helsinki. Approval was obtained from Çukurova University Non-Interventional Clinical Research Ethics Committee (Date: 03/06/2022, Number of Meeting/Decision No:123).

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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ORIGINAL ARTICLE

Effect of pioglitazone on oxidative stress of skeletal muscle in the insulin resistance rat model induced by high sucrose diet

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Abstract

Background: Metabolic syndrome is associated with some medical disorders such as central obesity, being overweight, insulin resistance and hypertension. This study was designed to determine the effect of pioglitazone on oxidative stress in the insulin resistance rat model.

Methods: In this study, the model was induced by high sucrose (935 mm) diet for 20 weeks. Three groups were used in the experiment. Control group received standard laboratory diet and drinking water. Metabolic syndrome induced group received 32% sucrose containing drinking water for 20 weeks. Pioglitazone-treated metabolic syndrome group has received pioglitazone treatment (30 mg/kg/day, via oral gavage) for two weeks at the end of the 18th week of metabolic syndrome group. After experimental period, skeletal muscle tissues were homogenized to measure important enzymes such as aspartate aminotransferase, lactate dehydrogenase and as the marker of oxidative stress; total-antioxidant-status, total-oxidant-status and malondialdehyde. Western blot technique was used to determine protein level of thioredoxin1.

Results: Aspartate aminotransferase and lactate dehydrogenase levels increased in metabolic syndrome group but pioglitazone treatment decreased these levels. In metabolic syndrome group the oxidative stress status increased but the treatment of pioglitazone decreased the level of oxidative stress in the skeletal muscle. In addition, thioredoxin1 decreased in metabolic syndrome group but administration of pioglitazone increased this level.

Conclusions: There was an elevated effect of oxidative stress in high sucrose fed rats but the treatment of pioglitazone improved glucose tolerance and insulin sensitivity.

Keywords: Metabolic Syndrome X, Oxidative Stress, Pioglitazone, Wistar Rat, TRX1 Protein.

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INTRODUCTION

Insulin resistance (IR) comprises a common prevalent metabolic disorder, which seems to govern the pathophysiology of diabetes mellitus, metabolic syndrome (MS), and obesity (1). It is a central feature of MS, showing a strong association with most components of the syndrome (2). The metabolic consequences, hyperinsulinemia, hyperglycemia, lipid, and lipoprotein dysregulation act in synergy to sustain the pathologic state of insulin resistance (3). With the evolution of insulin resistance, endothelial dysfunction, inflammation, and atherosclerosis worsen progressively (4).

Skeletal muscle tissue is a primary tissue responsible for 85% of total body insulin-stimulated glucose uptake and oxidative metabolism (5). It is well established that skeletal muscle insulin resistance and impaired glucose metabolism are both due in part to reduced insulin action and glucose uptake (6). It plays a central role in the whole-body insulin resistance, as well as in the subsequent development of metabolic syndrome, type 2 diabetes (7).

Thiazolidinediones (TZDs) are a class of oral antidiabetic agents that improve insulin sensitivity and glucose homeostasis in type 2 diabetic patients (8,9). TZDs activate the transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ), which is predominantly expressed in adipose tissue (10). Intramyocellular lipid (IMCL) is predominantly stored as intramuscular triglyceride in lipid droplets and is utilized as metabolic fuel during physical exercise. Pioglitazone is one of the members of TZDs; reduce IMCL content in insulin-resistant states (11), which is the most likely mechanism for TZDs to improve peripheral insulin sensitivity (12).

Impaired thioredoxin1 (TRX1) responses enforce a negative impact on antioxidant defense and tissue protection in experimental diabetes (13). An early study showed that serum TRX levels were higher in type 2 diabetics compared to controls (14).

The aim of the present study was to see whether oxidative stress occurs in skeletal muscles and to examine the possible ameliorative effect of pioglitazone on oxidative stress if oxidative stress has occurred in a skeletal muscle.

MATERIALS AND METHODS

Animals and Experimental Model

Three-months-old male Wistar Albino rats (200-250g) were used. Animals were kept under standard conditions (12-hour (h) light/ dark cycle, 24±2°C, 35-60% humidity). Rats were fed both with standard laboratory chow which includes (as percentage) torula yeast 30.0, corn oil 2.0, sucrose 59.0, DL-methionine 0.3 and AIN-76 TM mineral mixture 5.0 and AIN-76 TM vitamin mixture 1.0 with digestible energy 12.59 MJ/kg from Horland Tekland, Madison, WI, USA and had free access to water. The animals were randomly divided into the three groups consisting of 8 rats each. Control group (Con) received standard laboratory diet and drinking water. MS group received 32% sucrose (935 mm) containing drinking water for 20 weeks (15). Pioglitazone-treated MS group (MS-PGZ) was given pioglitazone (30 mg/kg/day, via oral gavage) for two weeks. At the end of the experimental period, animals were anesthetized after being deprived of food for 12 h. Skeletal muscles were removed and used for measurements. In each experimental group, body weight (g), blood glucose level (mg/dl), insulin (ng/ml), triglyceride (mg/dl), homeostatic model assessment-insulin resistance (HOMA-IR), and HOMA-β scores were measured as previously described in our study (16). All animal procedures and experiments described in the present study were approved by the Animal Ethics Committee of Ankara University Faculty of Medicine (Date: 02/02/2015, Number: 2015-2-37).

Tissue Homogenization

Skeletal muscles were homogenized with a motor-driven Teflon to glass homogenizer in cold buffer: (mm) TrisHCl 20 (pH 7.4), NaCl 150, KCl 2, EDTA 2, DTT 0.5, protease inhibitor cocktail 100, PMSF 0.4 and 2% NP-40. And then centrifugation process was performed to separate the cell membrane and cytosol. Protein content of cytosol was used in biochemical assays and western blot measurement.

Biochemical Assays

After homogenization of skeletal muscle tissues, protein content was analyzed using the Bradford method (Bio-

Rad), and bovine serum albumin was used as the standard. Then, the important enzymes such as AST and LDH (Biovision, Cusabio; respectively) were measured in tissues using commercial kits. As the marker of oxidative stress, TAS and TOS (Rel assay diagnostics) were determined in tissues using commercial kits. MDA levels were determined by the thiobarbituric acid (TBA) method and the pink color produced by these reactions was measured spectrophotometrically at 532 nm.

Western Blot

Protein level of TRX1 was determined by Western blot. Shortly, equal quantities of proteins (20 μg) from samples were loaded and separated spread on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions. After electrophoresis (150 V, 1.5 h), the samples were electro-blotted onto a polyvinylidene difluoride (PVDF) membrane (20 V, 2 h). TRX1 contents in the samples were identified using anti-TRX1 (12 kd, 1/1000, rabbit, Abcam) antibody. Using the ECL plus detection system, immunoreactive protein bands were visualized. TRX1 protein levels in skeletal muscle tissues normalized according to the β-actin levels in skeletal muscle tissues from experimental group.

Chemicals

Unless specified, the reagents used were obtained from Sigma-Aldrich Chemie (Steinheim, Germany). Molecular weight markers and PVDF membranes were supplied from Bio-Rad. To detect proteins with Western blotting, enhanced chemiluminescence (ECL) plus reagents were used and supplied from GE Healthcare.

Statistical Analysis

All parameters were expressed as mean ± standard error of mean (S.E.M.). Statistical analyses were performed using one-way analysis of variance followed by Bonferroni post-hoc analysis. The p values less than 0.05 were considered to be statistically significant.

RESULTS

General parameters of experimental groups

The sucrose-fed rats exhibited several characteristics of MS, including central obesity, adiposity, and insulin resistance. MS animals had significantly high blood glucose, insulin, triglyceride, and HOMA-IR scores compared with control animals (data not shown).

Aspartate aminotransferase and lactate dehydrogenase levels

Table 1 represents the effects of metabolic syndrome and pioglitazone treatment on AST enzymes in tissues of skeletal muscle. The activities of AST were significantly increased ($p<0.05$) in MS as compared to the Con group. In MS group, the LDH level significantly increased as compared to the Con group. Pioglitazone treatment reduced significantly the LDH level in MS group as compared with both MS group and Con group.

Table 1. Biochemical parameters in skeletal muscle of experimental groups.

	Con (n=8)	MS (n=8)	MS-PGZ (n=8)
AST (mU/mL)	0.3 ± 0.05	0.6 ± 0.10*	0.4 ± 0.08
LDH (mU/mL)	0.4 ± 0.03	0.9 ± 0.05*	0.6 ± 0.03*≠

All parameters were expressed as mean ± standard error of mean (S.E.M.). Con: control, MS: metabolic syndrome, MS-PGZ: pioglitazone-treated metabolic syndrome group, n number of rats. *$p<0.05$ versus Con, ≠$p<0.05$ versus MS.

Oxidative damage markers

The level of TAS in skeletal muscle tissue from experimental group was shown in Figure 1A. TAS level was significantly decreased ($p<0.05$) in MS as compared to the Con group. But the treatment of pioglitazone significantly restored ($p<0.05$) these changed TAS levels to that of Con group. TOS level of MS group was significantly increased ($p<0.05$) as compared to the Con group. Furthermore, administration of pioglitazone decreased the level of TOS in MS group as compared to the MS group. MDA level of MS group was significantly increased ($p<0.05$) as compared to the Con group. The level of MDA was significantly decreased ($p<0.05$) in pioglitazone-treated MS group as compared to the MS group.

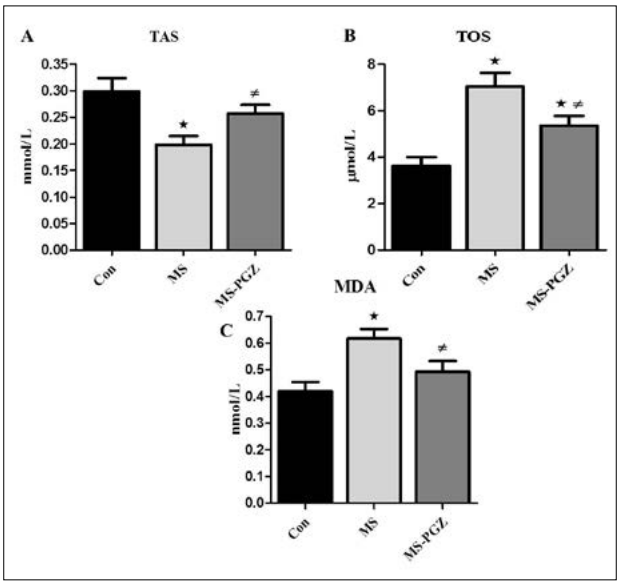

Figure 1. Changes of total antioxidant status (TAS) (A), changes of total oxidant status (TOS) (B) and malondialdehyde (MDA) (C) in skeletal muscles of experimental groups. Bar graph was expressed as mean ± standard error of mean (S.E.M.) from control group (Con, n=8), metabolic syndrome group (MS, n=8), pioglitazone-treated metabolic syndrome group (MS-PGZ, n=8). * p<0.05 versus Con, ≠p<0.05 versus MS.

Thioredoxin1 protein

Figure 2 showed TRX1 protein level in experimental groups. TRX1 protein level in MS group significantly decreased (p<0.05) as compared to the Con group. Pioglitazone treatment increased TRX1 protein level in MS group, but not significantly.

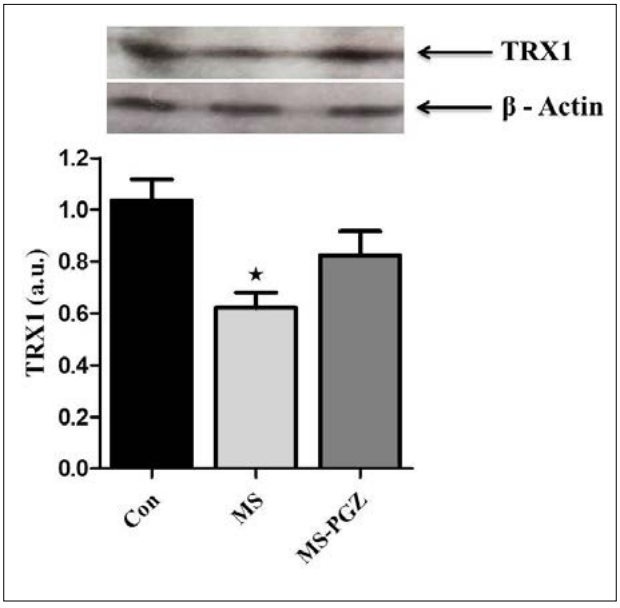

Figure 2: Representative western blots of thioredoxin1 (TRX1) for skeletal muscle in experimental groups is given at the top of the bar graphs. Densitometric results are expressed as a percentage of the band obtained with control in each of the experiments. Bar graphs were expressed as mean ± standard error of mean (S.E.M.) from control group (Con, n=8), metabolic syndrome group (MS, n=8), pioglitazone-treated metabolic syndrome group (MS-PGZ, n=8) * p<0.05 versus Con.

DISCUSSION

The present study demonstrated that pioglitazone has a favorable effect on oxidative stress of skeletal muscles in the insulin resistance rat model induced by high sucrose diet. This investigation provided new information about the oxidative stress markers and TRX-1 of skeletal muscles in a rat model of high sucrose diet. Additionally, the treatment of pioglitazone reduced the high sucrose induced elevated levels of AST and LDH in skeletal muscles of the MS group.

The defect is situated in peripheral tissues such as skeletal muscles and adipose tissue resulting in impaired insulin stimulated glucose utilization. Non-insulin dependent diabetes mellitus (NIDDM) is associated with insulin resistance and often accompanies hypertension, coronary heart disease or obesity (17). Association of these disorders with an atherogenic lipid profile, procoagulant state and other defects is usually referred to as metabolic syndrome (18). In the present study, the rats receiving 32% sucrose in the drinking water had higher daily water intake, higher glycemia, triglyceridemia, higher insulin resistance, and higher insulin values than those observed in control animals. As sucrose feeding altered total body weight, the observation of heavier fat depots suggests that the treatment increased the body adipose tissue mass, probably due to hypertrophy of the adipocytes. Treatment of pioglitazone significantly decreased the body weight compared to the MS group due to the decrement of both insulin resistance and daily uptake of calorie of rats as previously shown in our study (16).

AST and LDH, which are used as biomarkers for myotoxicity in both humans and animals (19), were elevated in patients with skeletal muscle injury due to various etiologies in the documented absence of liver disease in the study of Nathwani et al. (20). In accordance with this information, in the present study AST and LDH levels of MS groups were increased in skeletal muscles. This elevation may be caused by a defect of the skeletal muscle. The decrease in these elevated levels of AST and LDH via application of pioglitazone supports this information.

TAS shows the antioxidant levels in serum or related tissues (21). In a study, it was observed that the antioxidant levels of the alpha-lipoic acid group were significantly higher in the hind limb than that of the control group (22). In the present study, TAS level significantly decreased in the skeletal muscles in MS group. Treatment of pioglitazone significantly increased the TAS levels of the skeletal muscles when compared to the MS group. TOS shows the oxidant levels in serum or related tissues. Kartal et al. observed that the alpha-lipoic acid reduces the TOS levels of the hind limb in ischemia reperfusion group. In our study, the TOS level of the MS group was significantly higher than Con group. Also, the TOS level of the MS-PGZ group was significantly lower than that of the MS and Con groups. These results show that pioglitazone reduces the oxidative stress status in skeletal muscles.

MDA increases in proportion to the severity of lipid peroxidation, but it is not specific (23). In a previous study, fibromyalgia rats showed significantly elevated muscle levels of MDA. PGZ administration resulted in a decrease of MDA levels, which were similar to levels of normal healthy rats (24). Our findings come in agreement with this study; PGZ treatment decreased the elevated MDA levels of MS compared to the Con group levels. This result may explain that PGZ treatment confers protection against metabolic syndrome-induced alterations.

In previous studies, serum, plasma, and lymphocyte levels of TRX1 were significantly elevated in type 2 diabetes when compared to the healthy controls (14, 25). Miyamoto et al. (26) showed that the levels of plasma TRX were higher in diabetes mellitus and impaired glucose tolerance groups than in normal glucose tolerance group. In contrast to these reports, it is found that the TRX1 level significantly decreased in skeletal muscle of MS group. Treatment of PGZ increased this level to the control level but not significantly. The decrease of TRX1 may be responsible for the enhanced oxidative damage in skeletal muscle.

These observations in the present study may be interpreted as that both insulin resistance and hyperglycemia increase oxidative stress and may be one reason behind the changing's levels of oxidative stress markers and TRX1 expression in MS group. Our findings of beneficial effects of PGZ on biomarkers, oxidative markers and TRX1 levels are important, since changes in levels of biomarkers and oxidative stress are considered to be among the most important molecular mechanisms leading to complications in MS group.

Declarations

This study was supported by TUBITAK-SBAG-115S827 and Ankara Yıldırım Beyazıt University Projects Office-2864.

This study was approved by the clinical research Ethics Committee of the Animal Ethics Committee of Ankara University. (Date: 02/02/2015, Number: 2015-2-37)

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CASE REPORT

Heavy hemolysis after organophosphate poisoning by chlorpyrifos ethyl

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Abstract

Organophosphates (OPs) inactivate the acetylcholinesterase enzyme, thus increasing the cholinergic effect. In a clinical setting, patients with OP poisoning may experience nausea and vomiting, diarrhea, and abdominal pain, or in severe cases respiratory failure, seizures, and death. Rarely, acute hemolysis has also been observed. The existing literature contains few descriptions of several delayed diseases associated with OP poisoning. This article presents the case of a patient who experienced severe hemolysis approximately one week after OP exposure. Only a small number of similar cases have been previously examined.

Keywords: Hemolysis, Organophosphate Poisoning, Chlorpyrifos Ethyl.

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INTRODUCTION

Organophosphates (OPs) are often used as insecticides in agricultural areas. Mortality and morbidity rates are high in cases of OP poisoning caused by suicidal or accidental exposure. The primary target of OPs is the acetylcholinesterase (AChE) enzyme. The inhibition of AChE by OPs causes the accumulation of acetylcholine in cholinergic synapses and the overstimulation of muscarinic and nicotinic cholinergic receptors. It has three clinical manifestations: cholinergic syndrome, intermediate syndrome, and delayed polyneuropathy (1). Rarely, acute hemolysis has also been observed (2). The existing literature contains few descriptions of several delayed diseases associated with OP poisoning. In rare cases, such as ours, OPs may cause life-threatening hemolysis in the subacute poisoning period.

CASE REPORT

A 45-year-old male patient was admitted after presenting with weakness, fatigue, and an icteric appearance. He was hospitalized with a provisional diagnosis of autoimmune hemolytic anemia upon detection of the following values: WBC:4.4 K/uL, Hgb = 10.4 gr/dl; Plt = 148 K/uL; reticulocyte = 1.56%; total bilirubin = 10.8 mg/dL; indirect bilirubin = 9.71 mg/dL; and LDH = 459 U/L, macro & micro spherocytes were found in the peripheral smear. He declared no disease except immune thrombocytopenia (ITP), which was in remission with no continuing treatment. The patient was administered a 250-mg pulse methylprednisolone treatment. However, a direct coombs test was negative. The tests for paroxysmal nocturnal hemoglobinuria (PNH) and ADAMTS13 were normal. G6PD was normal. Haptoglobin could not be studied.

Despite the treatment, the patient's Hgb value decreased to 3.9 g/dl on the second day of hospitalization. On the third day, rapid increases were observed in total bilirubin (to 82 mg/dl) and indirect bilirubin (to 45 mg/dl). Therefore, a total plasma exchange process was started. Since hemolytic anemia had not been proven, a bone marrow (BM) biopsy was performed. The aspiration of BM showed incremental and significant dysplasia of the erythroid lineage (M/E ratio = 1/3; **Figure 1**). When a more thorough anamnesis was conducted, the relatives of the patient revealed that the patient had sowed seeds with the OP pesticide chlorpyrifos ethyl (CPF) about one week before the onset of the disease, and he had subsequently washed the device that he used by spraying it with water.

The patient's cholinesterase level was normal. However, since time had passed since the event, subacute OP poisoning was considered a diagnosis. The total bilirubin of the patient, who underwent a total of four plasmapheresis treatments, declined to 17 mg/dl and then continued to regress spontaneously without further plasmapheresis. Nine units of erythrocyte suspension replacement were provided. The reticulocyte level increased to 8.29% on the 10th day of hospitalization, and the Hgb level began to stabilize around 9.5 g/dl without transfusion. The LDH level decreased from 1,034 to 593 U/L. In the first month following discharge, the patient's Hgb level was 13 g/dl, and bilirubin levels were normal. In the second month, a control bone marrow aspiration showed a significant decrease in erythroid dysplasia (**Figure 2**) and the Hgb level increased to 15 g/dl.

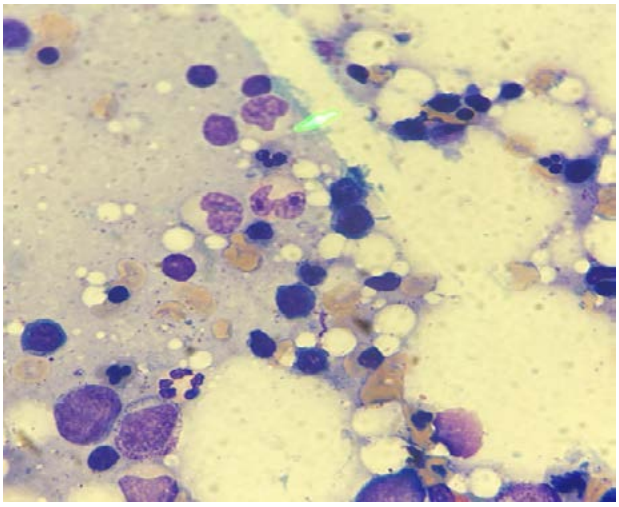

Figure 1: Dysplasia of erythroid lineage

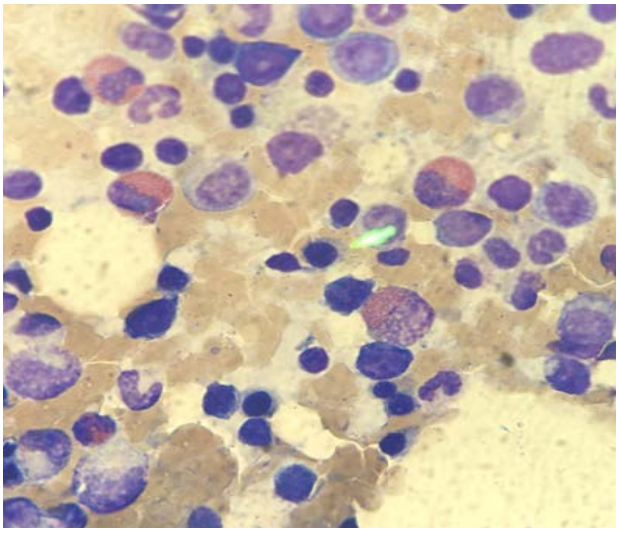

Figure 2: Control bone marrow aspiration

DISCUSSION

Organophosphates inhibit the function of carboxylic ester hydrolases, such as chymotrypsin, AChE, plasma, or butyrylcholinesterase (BChE), plasma and hepatic carboxylesterase (aliesterase), paraoxonase (esterases), and other non-specific esterases within the body. However, the most prominent clinical effects of poisoning with OPs result from their inhibition of AChE.

It is suggested that oxidative tissue damage plays a role in the pathogenesis of toxic effects (neurotoxicity, hepatotoxicity, immunotoxicity, embryotoxicity and genetic toxicity) resulting from acute and chronic OP applications (3). It has also been noted that free radicals, especially DNA, proteins, and cell phospholipids, form as a result of oxidative stress and have the ability to react with many organic and inorganic compounds, especially polyunsaturated fatty acids (4). These free radicals cause the exhaustion of the enzymatic and nonenzymatic antioxidant systems that protect the cell. It has been suggested that lipid peroxidation of cells, DNA damage, and changes in proteins occur due to oxidative damage resulting from these effects.

As a lipophilic molecule, CPF enters the cytoplasm of cells and begins to damage the cellular molecules inside the cells. It demonstrates its main toxic effect through its metabolite, chlorpyrifos-oxon (CPO), to which it is converted by cytochrome p-450. This metabolite carboxylesterase binds to AChE with high affinity, as in butyrylcholinesterase, and produces toxic effects by inhibiting AChE (5). Acute CPF toxicity includes other mechanisms that are unrelated to AChE inhibition, including oxidative stress. Oxidative damage begins primarily with the production of reactive oxygen species and causes damage to macromolecules such as lipids, DNA, and proteins.

The clinical picture depends on the specific agents, the amount that was absorbed, and the mode of exposure. If the amount absorbed in OP poisoning is high enough, the effects may be apparent within minutes. Most patients begin to show symptoms within 8 to 24 hours after intake. Parasympathomimetic effects are dominant and may include salivation, lacrimation, sweating, urinary incontinence, diarrhea, vomiting, bradycardia, bronchospasm, bronchorrhea, hypoxia, and miotic pupil. OP poisoning has also been associated with certain delayed manifestations, such as OP-induced delayed neuropathy, myonecrosis, personality changes, schizophrenia, and pancreatitis (6,7). However, the development of hemolysis occurs less frequently. In their case report, Kadeli and Hanjagi observe that hemolysis developed after one week (2). Similarly, in our case, hemolysis developed one week after exposure. Our case is similar to the other case in the literature in terms of the age and gender of the patient, and a significant decrease in hemoglobin levels and an increase in LDH levels were observed in both individuals. It was thought that the reticulocyte count was normal due to dysplasia in the bone marrow. However, in our case, the cholinesterase level was normal since it was measured after plasmapheresis.

Thus, it should be noted that OP poisoning may have unusual clinical manifestations. It should also be considered in the differential diagnosis of antiglobulin negative hemolysis. Ultimately, a physical examination and a detailed anamnesis are always the best guides.

Declarations

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Ethical Committee approval was not required. Informed consent was obtained from all participants.

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