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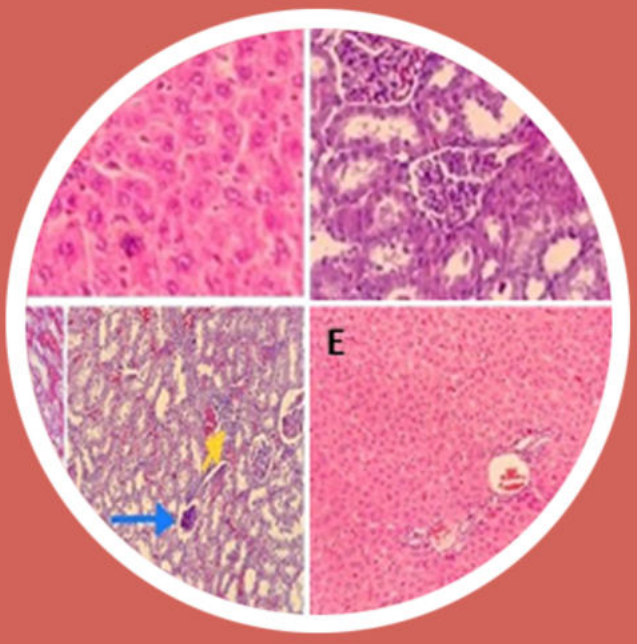

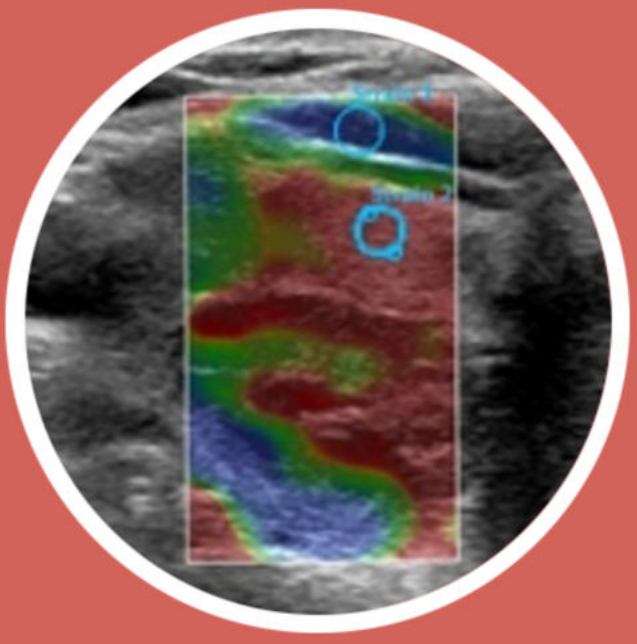

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Table of Contents

Original Articles

Reviews

Case Report

The European Research Journal 2022;8(3):320-325 Original Article

DOI: 10.18621/eurj.978538 *Microbiology*

Extraction and biological evaluation of *Mycobacterium bovis* extracellular vesicles as adjuvant and candidates for bovine tuberculosis vaccine

Mohammad Kazem Sharifi Yazdi[1], Seyed Davar Siadat[2,3],Alireza Monadi Sefidan[4], Ali Taheri Mirghaed[5], Mohammad Khalifeh-Gholi[6,7], Sarah Sharifi-Yazdi[8], Mohammad Saleh Safari[9], Sina Sharifi-yazdi[10]

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ABSTRACT

Objectives: BCG vaccine is the only virtual vaccine that has significantly helped control tuberculosis for 80 years. Bacteria naturally release extracellular vesicles (EVs) in different environments during the growth process. The use of extracellular vesicles is an alternative way to transfer ligands that are detected by host cells. Vesicles range in size from 50 nm to 250 nm in diameter and contain phospholipids, proteins, and lipopolysaccharides, and can carry additional factors such as toxins, adhesive, or immune system compounds that are important in pathogens. Therefore, this study aimed to evaluate these compounds as adjuvants or candidates for the bovine tuberculosis vaccine.

Methods: In the present study, *Mycobacterium bovis* standard CRBIP7.121 was used. Extraction of membrane vesicles after mass culture was performed by a method based on ultracentrifugation and deoxycholate. After preparation and staining, the vesicles were examined by electron microscopy. Sample analysis was also performed by SDS-PAGE. The presence of LPS in the sample was measured by the LAL test. In addition, the harmlessness of bacterial EVs and the absence of any toxic agents in the sample were confirmed by pyrogenic tests in rabbits.

Results: The protein content of membrane vesicles is equal to 1.25 and 1.32 mg/ml. In SDS-page evaluation, bands of 35, 40, 50, and 70 kDa were observed and then membrane vesicles were observed and confirmed by electron microscopy. The amount of vesicle toxin contained by the LAL test was reported in the permissible range.

Conclusions: Discussion of the data obtained from the above research shows that at different stages of the purification process, EVs fully retained their spatial and natural form and lacked impurities. Therefore, due to the importance of external vesicles in developing immune responses, EVs extracted from *M. bovis* CRBIP7.121 can be considered a useful and effective immunogen against Mycobacterium infections.

Keywords: Extracellular vesicles, *Mycobacterium bovis* CRBIP7.121, adjuvant and vaccine

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Bovine tuberculosis is important in terms of public health in human societies. The ease and frequency of the spread of the causative agent of tuberculosis from animals to humans, especially in environments where bovine tuberculosis is not controlled, can make this disease an important common disease (Zoonosis). Tuberculosis infection in humans is caused by drinking contaminated milk in children, although transmission of the infection can also be by respiratory [1-3]. Bovine tuberculosis is caused by *Mycobacterium bovis* and is one of the most common diseases between humans and animals around the world. This bacillus infects domestic animals and wild mammals and makes them a reservoir for the disease, which makes it difficult to control the disease [4]. Vaccination is the best way to prevent the consequences of infectious diseases in humans and animals [5]. Common vaccines mainly include attenuated live pathogens and subunit vaccines such as inactivated bacterial toxins. Adjutants are chemical or biological compounds that cause nonspecific stimulation of the immune system against the antigens with which it is injected [5, 6]. *M. bovis* Calmett-Guerin (BCG) is the only virtual vaccine that has been instrumental in controlling tuberculosis for more than 80 years. The vaccine can provide about 80% immunity against Mycobacterium and Miliary TB in infants and young children, however, the protective effects of BCG against lung disease vary at different ages [7]. Bacteria naturally release extracellular vesicles (EVs) in different environments at different stages of growth. Vesicles range in size from 50 nm to 250 nm in diameter and contain phospholipids, proteins, and lipopolysaccharides (periplasmic compounds) and can carry additional factors such as toxins, adhesive, or immune stimulants that are important in patency [8]. EVs production has been recorded in various pathogenic and non-pathogenic species of Mycobacterium, indicating that the release of EVs is a conserved feature among Mycobacterium species. Proteomic analysis has shown extensively that only EVs from pathogenic species have the TLR2 agonist lipoprotein and have played an important role in stimulating the immune system [9]. The aim of this study was to purify the external vesicles of *M. bovis* to perform immunological evaluations of extractive vesicles.

METHODS

Bacterial Strain Culture

The standard culture of *M. bovis* was cultured in Lunstein-Johnson (LJ) medium with McFarland 1 turbidity for 3-4 weeks at 37°C to confirm Zill Nelson staining and microscopic observation and biochemical tests such as nitrate reduction, niacin and Catalase was used.

OMV Eextraction

Extraction of outer membrane vesicles was performed based on ultracentrifugation using solutions containing deoxycholate and sucrose gradient. In summary, after preparing a thick mass from the inactive *Mycobacterium bovis* cell body and preparing the cell mass with solutions containing Tris, EDTA, and deoxycholate, successive centrifuges were performed at high speed and finally, the precipitate obtained was dissolved in 3% sucrose. The resulting solution was sterilized using 0.2 filters.

Nanodrop

The standard concentration of the proteins of the extracted vesicles was measured using a nanodrape. The basis of this device is spectrophotometry.

SDS-PAGE

To determine the protein pattern in OMV and estimate their molecular weight at 30 µl of the purified sample was electrophoresed on a 12% gel and a protein marker was used to determine the protein weight (Fig. 1).

Electron Microscopy

Membrane vesicles were ultrasonically treated to disperse the vesicles followed by attaching to Formvar/carbon-coated nickel grids. Grids were washed with a 0.01 M PBS supplement, 0.1% gelatine (PBG), and 0.5% BSA. The vesicles on the grids were fixed with 1% glutaraldehyde in PBS at 4°C for 60 minutes and negatively stained with 1% potassium phosphotungstate pH 7.5. The grids were examined using a Zeiss EM10C transmission electron microscopy operated at 80 KV.

LAL Test

To measure the amount of LPS in the sample, Thermo Scientific Pierce LAL Chromogenic Endotoxin Quantitation Kit was used according to the manufacturer's protocol.

Pyrogen Test

The extracted external vesicles were tested on four healthy albino New Zealand rabbits. At the beginning of the study, the animal weight was limited between 1.8 and 3.8. Three rabbits were used for testing and one for control. The OMV was injected into rabbit peripheral ear veins at a dose proportional to the rabbit weights. The animals' rectal temperature was measured using a digital thermometer. If the rabbit did not showed a temperature rise of 0.6 C or greater or the sum of the three single temperature increases not exceeds of 1.4◦C, the test material has no toxicity.

Evaluation of the Level of total IgG Antibody against OMV in Mice Immunized by ELISA

After blood sampling and serum collection, the level of antibody against protein was evaluated by ELISA. Protein was prepared in PBS buffer at a concentration of 5 µg/100 µl. The mouse serum used was prepared in dilutions of 1: 250-1: 500-1: 1000 and 1: 2000. The mouse IgG concentration of 1.6000 was used and the samples were then read at 405nm wavelength.

RESULTS

Results of Electrophoresis of Membrane Vesicles

Electrophoretic motion analysis of proteins in the extracted vesicles of M. bovis showed protein bands in the 25, 40, and 50 kDa regions according to the markers.

Measurement of Protein using Laurie Test

Lowry method was used to estimate the concentration and quality of extracted membrane vesicles. The results of physicochemical analysis of vesicles extracted from M. bovis showed that the total protein

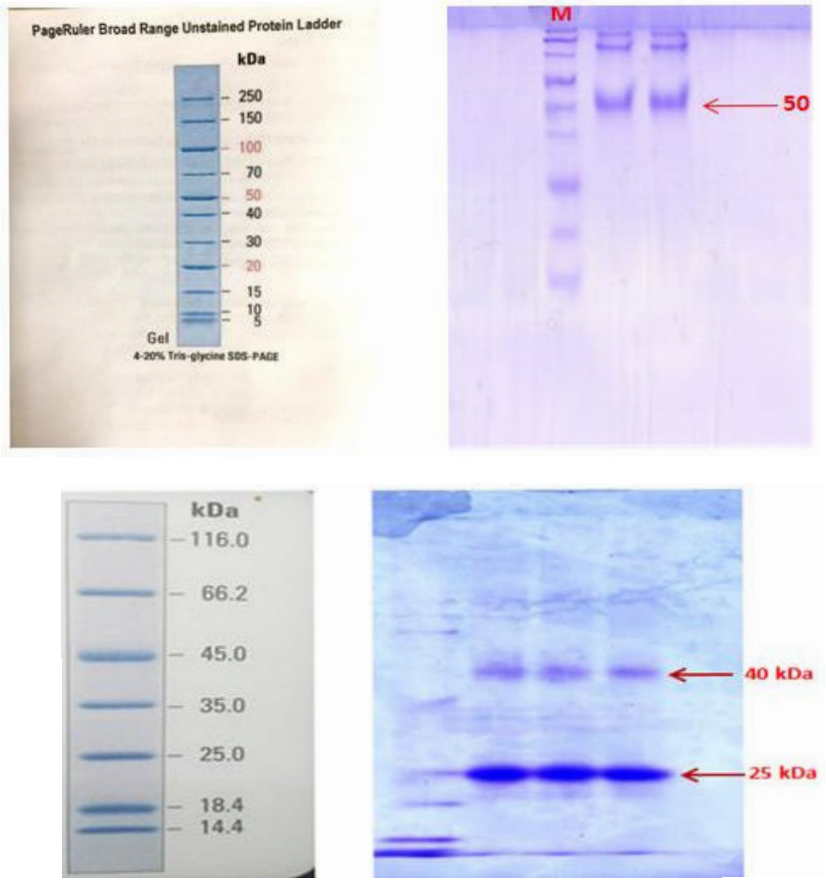

Fig. 1. Protein pattern of outer membrane vesicles by SDS_PAGE method

content of membrane vesicles was 1.32 mg/ml, which is an acceptable amount.

Electron Microscopy

The stability of the natural form of membrane vesicles at different stages of the purification process was investigated by electron microscopy. The spatial properties of the extracted membrane vesicles in negative contrast staining with the EM 900 electron microscope are shown below. As can be seen in the Fig. 2, the extracted vesicle is about 50-50 nm and retains its spatial properties at various stages of extraction and purification.

Determination of Endotoxin Levels in Membrane Vesicles by Chromogenic Limulus Amebocyte Lysate (LAL)

Results in this semi-quantitative test showed that the extracted vesicles were allowed to be used in the animal model within the safe limit (less than 300 IU).

Pyrogenic Test

After the test, no significant increase in temperature was observed in rabbits, indicating a lack of pyrogenicity.

DISCUSSION

The BCG vaccine was first introduced in 1921. This vaccine is by far the most widely used vaccine in the world. The current BCG vaccine, based on live attenuated bacteria from the *Mycobacterium bovis* strain that has been passed sequentially, is available and is used to protect infants and children against tuberculoses. However, this vaccine provides unreliable protection against pulmonary tuberculosis in the adult population. This vaccine is known to be the only effective live vaccine so that the killed BCG vaccine cannot provide protection in the animal sample. Also, *Mycobacterium tuberculosis* killed in humans provides poor protection and instability, and the level of immunity produced by the BCG vaccine varies from 0 to 80% [8, 9]. The BCG vaccine is largely safe, but in some cases has side effects such as abscesses at the injection site and local lesions such as osteoporosis [10, 11]. Another challenge in the development of bovine tuberculosis vaccines is the lack of knowledge of dominant immunogenic antigens. It has been suggested that the BCG vaccine used does not adequately express immunogenic antigens, and therefore mutant strains containing antigenic proteins are suggested for the development of improved BCG vaccines [12]. Recent studies have shown that infants with HIV are routinely vaccinated with the BCG vaccine at birth. Such infants and asymptomatic individuals, as well as those who have developed symptoms of AIDS, are at risk for developing BS [13]. The existence of such problems indicates the need to change the BCG vaccination strategy and produce a new generation of vaccines with lower risk and greater safety against subsequent consequences. Therefore, it seems that designing and introducing a new vaccine candidate can be considered a necessity. Various species to improve the BCG vaccine or to produce new vaccines are currently under investigation in many clinical trials. Vaccines currently on the production line are mainly based on protein subunits with adjuvants or viral vectors and some inactive bacteria from Mycobacterium strains [14]. A study of *M. tuberculosis* vesicles has shown

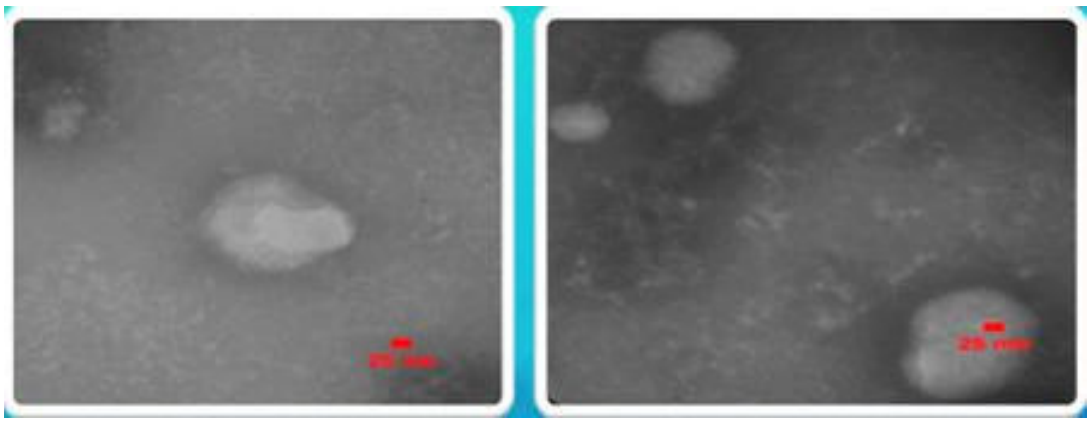

Fig. 2. Electron micrograph of outer membrane vesicles

that these vesicles contain TLR2 agonist lipoproteins so that macrophages infected with these vesicles are able to produce T2-dependent chemokines and T-dependent cytokines. It has also been shown that injecting extracellular vesicles into the lungs can stimulate an inflammatory response against them. The present study investigates the production of extracellular vesicles by the standard *Mycobacterium bovis* strain. In this study, the pattern of proteins extracted from this strain was protein bands in the 25, 40, and 50 kDa regions. Also, the extracted vesicle was 200-50 nm in size, and in terms of vesicle consistency, it maintained its spatial properties in different stages of extraction and purification. To study the function and structure of *Mycobacterium bovis* extracellular vesicles, no study has been done so far and all hypotheses are based on *M. tuberculosis* extracellular vesicles. The vesicles of this bacterium contain various compounds from the bacterial cell wall such as lipoarabinomannan and TLR2-stimulating lipoproteins, etc. In this bacterium, the VirR gene is responsible for the production of these vesicles, which play a very important role in controlling the stimulation of the immune system. Bacillus vesicles are also produced in large quantities under iron deficiency, which indicates the important role of these vesicles in iron deficiency conditions [15]. Due to its clinical use, meningococcal OMV vaccines, which have been used since 1980 and are an important tool in combating the spread of *Neisseria meningitidis* serogroup B, are one of the targets of vesicle extraction to produce vaccines. In fact, as of 2010, OMV was the only commercially approved vaccine against *N. meningitidis* reported [16]. According to these studies, it may be possible to evaluate the compounds in *M. bovis* vesicles and use them as alternative vaccine candidates. In addition to the vaccine role, the adjuvant role of these compounds is also considered. The goal of vaccination is to create a strong immune response to provide long-term protection against infection, and to achieve this goal, unlike attenuated live vaccines, the whole organism is usually killed or subunit vaccines need to add an adjuvant to be effective. [17]. Mycobacterium extracts have been widely used in vaccines as adjuvants. Among these cases is the complete adjuvant of Freund, which contains components of Mycobacterium. Mycobacterium cell wall contains a variety of antigens, including peptidoglycans, arabinoglycans, mycolic acids, proteins, phosphatidyl inositol, lipomannas, and arabinomans. These components stimulate dendritic cells via mannose and NOD2 receptors [18, 19]. In a study by Lee *et al*. [20], they showed that Mycobacterium vesicles contained cell wall compounds such as lipoproteins X, A, and G, and other compounds that appear to stimulate immune cells. Therefore, these compounds may be used as adjuvant [20]. Therefore, the vesicle extracted from *M. bovis* due to its preservation nature can be considered as a candidate for possible use for vaccines and adjuvant, which requires further research in this field.

Limitations

Further research of the Molecular pathobiology and immunological properties would lead to the development of better and safer vaccines.

CONCLUSION

The results indicate that these structures may be suitable candidates for vaccination and be considered as a new generation of vaccines against M. bovis infections, although further studies in this field are needed to evaluate antibody subclasses and responses.

Authors' Contribution

Study Conception: SS-y; Study Design: SS-Y; Supervision: ATM; Funding: AMS; Materials: AMS; Data Collection and/or Processing: ATM;, MS-S Statistical Analysis and/or Data Interpretation: MKSY, SDS; Literature Review: ATM; Manuscript Preparation: ATM and Critical Review: ATM, MK-G.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The authors disclosed that they did not receive any grant during conduction or writing of this study.

Ethics Committee Approval

This article is not a study with human participants. There are no experiments on animals. This article does not contain any studies on human participants or animals performed by the author. There is no identifying

information of participants.

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REFERENCES

1. Saad J, Baron S, Lagier JC, Drancourt M, Gautret P. Mycobacterium bovis pulmonary tuberculosis after ritual sheep sacrifice in Tunisia. Emerg Infect Dis 2020;26:1605-7.
2. Pozo P, VanderWaal K, Grau A, de la Cruz ML, Nacar J, Bezos J, et al. Analysis of the cattle movement network and its association with the risk of bovine tuberculosis at the farm level in Castilla y Leon, Spain. Transbound Emerg Dis 2019;66:327-40.
3. Acevedo MA, Dillemuth FP, Flick AJ, Faldyn MJ, Elderd BD. Virulence-driven trade-offs in disease transmission: a meta-analysis. Evolution 2019;73:636-47.
4. Allen AR, Ford T, Skuce RA. Does Mycobacterium tuberculosis var. bovis survival in the environment confound bovine tuberculosis control and eradication? A literature review. Vet Med Int 2021;2021:8812898.
5. Sharifi Yazdi MK, Siadat SD, Khalifeh-Gholi M, Sharifi-Yazdi S, Fayaz-Bakhsh A, Saleh Safari M. Extraction and biological evaluation of external membrane vesicles of Brucella abortus as a candidate for brucellosis vaccine. J Surg Med 2020;4:540-4.
6. Lorente-Leal V, Liandris E, Castellanos E, Bezos J, Domínguez L, de Juan L, et al. Validation of a real-time PCR for the detection of Mycobacterium tuberculosis complex members in bovine tissue samples. Front Vet Sci 2019;6:61.
7. Pozo P, VanderWaal K, Grau A, de la Cruz ML, Nacar J, Bezos J, et al. Analysis of the cattle movement network and its association with the risk of bovine tuberculosis at the farm level in Castilla y Leon, Spain. Transbound Emerg Dis 2019;66:327-40.
8. Trewby H, Wright D, Breadon EL, Lycett SJ, Mallon TR, McCormick C, et al. Use of bacterial whole-genome sequencing to investigate local persistence and spread in bovine tuberculosis. Epidemics 2016;14:26-35.
9. Cowie CE, Hutchings MR, Barasona JA, Gortázar C, Vicente J, White PCL. Interactions between four species in a complex wildlife: livestock disease community: implications for Mycobacterium bovis maintenance and transmission. Eur J Wildl Res 2016;62:51-64.
10. Rodriguez-Campos S, Navarro Y, Romero B, de Juan L, Bezos J, Mateos A, et al. Splitting of a prevalent Mycobacterium bovis spoligotype by variable-number tandem-repeat typing reveals high heterogeneity in an evolving clonal group. J Clin Microbiol 2013;51:3658-65.
11. Hlokwe TM, de Klerk-Lorist L-M, Michel AL. Wildlife on the move: a hidden tuberculosis threat to conservation areas and game farms through introduction of untested animals. J Wildl Dis 2016;52:837-43.
12. Grange JM, Yates MD, de Kantor IN & World Health Organization. Emerging and other Communicable Diseases, Surveillance and Control. Guidelines for speciation within the Mycobacterium tuberculosis complex, 2nd ed. World Health Organization. 1996.
13. Kronvall G, Stanford JL, Walsh GP. Studies of mycobacterial antigens, with special reference to Mycobacterium. Infect Immun 1976;13:1132-8.
14. Matsuo K, Yasutomi Y. Mycobacterium bovis Bacille Calmette-Gu´erin as a Vaccine Vector for Global Infectious Disease Control. Tuberc Res Treat 2011;2011:574591.
15. Petrovsky N, Aguilar JC. Vaccine adjuvants: current state and future trends. Im Ce Bio 2004;82:488-96.
16. Prados-Rosales R, Baena A, Martinez L, Luque-Garcia J, Kalscheuer R, Veeraraghavan U, et al. Mycobacteria release active membrane vesicles that modulate immune responses In a TLR2 dependent manner in mice. J Clin Invest 2011;121.:1471-83.
17. Kuehn MJ, Kesty NC. Bacterial outer membrane vesicles and the host-pathogen interaction. Genes Dev 2005;19:2645-55.
18. Nicolson M. Biomedicine in the twentieth century: practices, policies and politics. Soc Hist Med 2009;22:193-4.
19. Rath P, Huang C, Wang T, Wang T, Li H, Prados-Rosales R, et al. Genetic regulation of vesiculogenesis and immunomodulation in Mycobacterium tuberculosis. Proc Natl Acad Sci 2013;110:E4790-7.
20. Lee J, Kim SH, Choi DS, Lee JS, Kim DK, Go G, et al. Proteomic analysis of extracellular vesicles derived from Mycobacterium tuberculosis. Proteomics 2015;15:3331-7.
21. Delbos V, Lemée L, Bénichou J, Berthelot G, Deghmane AE, Leroy JP, et al. Impact of MenBvac, an outer membrane vesicle (OMV) vaccine, on the meningococcal carriage. Vaccine 2013;31:4416-20.

The European Research Journal 2022;8(3):326-332 Original Article

DOI: 10.18621/eurj.904343 *Orthopedics and Traumatology*

The effect of additional staple in the tibial fixation of anterior cruciate ligament reconstruction: a biomechanical study in sheep tibia

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ABSTRACT

Objectives: The aim of this study was to evaluate the necessity of staple use and the importance of screw diameter in the anterior cruciate ligament (ACL) reconstruction.

Methods: Twenty-eight sheep hind legs were divided into four groups in terms of tibial side fixation type as 7 mm bioabsorbable screw (Group 1), 7 mm bioabsorbable screw and staple (Group 2), 8 mm screw (Group 3), and 8 mm screw and staple (Group 4). The maximum force causing insufficiency in the graft, and the fixation strength between the groups were recorded. The results were compared among the groups.

Results: As a result of the statistical analysis regarding the necessity of using staple, group 2, with 7 mm screws and staple, was found to be significantly more resistant than group 1 ($p = 0.018$). In consequence of the biostatistics study, staple use was found not to create a statistically significant difference between group 3 in which an 8 mm bioabsorbable screw was used and group 4 ($p = 0.805$). In the statistical study to understand the significance of screw diameter, the samples of group 1 and group 3, in which no staple was used, were compared. It was found that group 3 samples with 8 mm screws had higher strength than those with 7 mm screws ($p = 0.011$). Between group 2 and group 4 in which a staple was used, despite the increase in screw diameter, the value of maximum strength had no remarkable difference statistically ($p = 1.00$).

Conclusions: Fixation strength is higher when fixation was done with a screw 1 mm larger than tibial tunnel diameter. However, using either a screw with 1 mm wider than tibial tunnel diameter and the screw in the same diameter with the tibial tunnel and additional staple have similar fixation strength on the tibial side. Therefore, we conclude that screw width is more important in maintaining stability of the graft fixation.

Keywords: ACL reconstruction, biomechanics, tibial fixation, bioabsorbable screw, staple

Anterior cruciate ligament (ACL) is the most frequently injured ligament in the knee, especially during sportive activities. Arthroscopic ACL is usually performed in patients who are at young ages. During ACL reconstruction, the tibial side is the weakest point of the reconstruction [1-4]. There are two main reasons for that. Firstly, bone density of the proximal tibia is lower than the distal femur and secondly, force direc-

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tion on the graft is parallel to the tibial tunnel [5]. It has been shown by many studies that graft fixation, especially on the tibial side, is the key point for the graft to remain stable in the early physical therapy program [4, 6]. Therefore, tibial fixation must be strong enough to withstand an early effective rehabilitation program. A significant number of studies have shown that long screws with large diameter provide stronger fixation [6-9]. However, the larger screws may cause damage in the autograft [10].

On the other hand, fixation of graft on the tibial side with a staple in addition to bioabsorbable screw is a frequently used method to prevent failure as much as possible. In addition to the staple, post screw or washer screw can be used for fixation. Nevertheless, the use of additional fixation material has complications such as the subcutaneous sensation of the implant and pain. Therefore, a second surgical intervention may be required [11]. The advantage of fixation with staple among them is that it remains almost at the same level as the bone after insertion. Therefore, the staple is usually preferred due to the possibility of the patient to feel the implant is greatly reduced.

There are many publications advocating that bioabsorbable screws and staple should be used, and many others are defending and opposing the idea that fixation should be done with screw 1 mm wider than the tunnel width. However, currently there is no consensus regarding optimal tibial fixation in ACL reconstruction. In the present study, by testing two variables (staple usage and screw diameter) together, we intended to examine the relationship between them and the importance of each in the tibial fixation of ACL autograft. We aimed to evaluate the necessity of staple as an additional fixation method and the importance of screw diameter in the anterior cruciate ligament (ACL) reconstruction. We hypothesized that additional staple fixation increases the stability of graft when a screw of the same diameter was used.

METHODS

In the study, two years old on average, showing no additional pathology and slaughtered not more than 24 hours before the test, 28 sheep hind legs were used.
In this biomechanical study, the tests were carried out in the Strength and Biomechanics Laboratory of the Mechanical Engineering Faculty of Istanbul Technical University. MTS 858 Mini Bionix 2 test machine (MTS System Corporation 14000 Technology Drive Eden Prairie, MN USA, 55344) was used for study. The study was conducted according to the Decleration of Helsinki. Ethical approval was obtained from the institutional review board (771/08/2018). The ideal of the study is to do it in a young human cadaver. However, sheep bone was preferred because of its easy accessibility, its weight close to human weight and its long bones that can be implanted. Taken on the morning of the experiment from an abattoir where animals are slaughtered daily, sheep hind legs cut in the last 24 hours were dissected (Fig. 1). Soft tissues were removed. The tibia and femur were separated. The tibia was cut 12 cm distal to the knee joint level (Fig. 2). In

Fig. 1. Sheep tibia before fleksor digitorum tendon is removed.

Fig. 2. Sheep tibia after flexor tendon graft is removed and cut below the knee joint level.

a previous study, the digitorum tendon was found to be an appropriate graft for ACL recontruction [12]. The flexor digitorum tendon was taken from the same leg of the same sheep as a graft (Fig. 3).

The grafts folded in half and were prepared to be 7 mm wide. With an average of 4 cm, the tunnel was drilled with a 7 mm drill in the tibial joint in such a way that it would come out of ACL stump. The graft was passed through the tibial tunnel with 20mm 'Continuous-Loop' (CL) (Arthtrex®) and was inserted through the device to be tested via CL to prevent weak region formation in the graft (Fig. 4).

The samples were divided into four separate groups. In Group 1, only 7 mm wide bioabsorbable screws (Tulpar biointerference screws®) were used for tibial fixation. In Group 2, the graft was fixed to the tibial side with a 7 mm wide bioabsorbable screw and staple (Tulpar ligament staple®). While 8 mm wide bioabsorbable screws were used in Group 3, 8 mm wide bioabsorbable screws and staple were used in Group 4.

To increase the involvement of the apparatus to which the tibia is fixed distally, two cross-K wires were sent and fixed to the apparatus to be tested with synthetic paste. CL was placed on the device with the help of a proximal steel hanger (MTS 858 Mini Bionix 2). The force was applied to compare fixation methods in the same direction as the tibial tunnel, the most challenging situation possible [13]. Thus, it was aimed to prevent the tension due to the angulation between the graft and the articular surface. Firstly, with a frequency of 2 Hz, and a maximum of 50 N, 100 cycle force was applied to the samples with force control, and then the

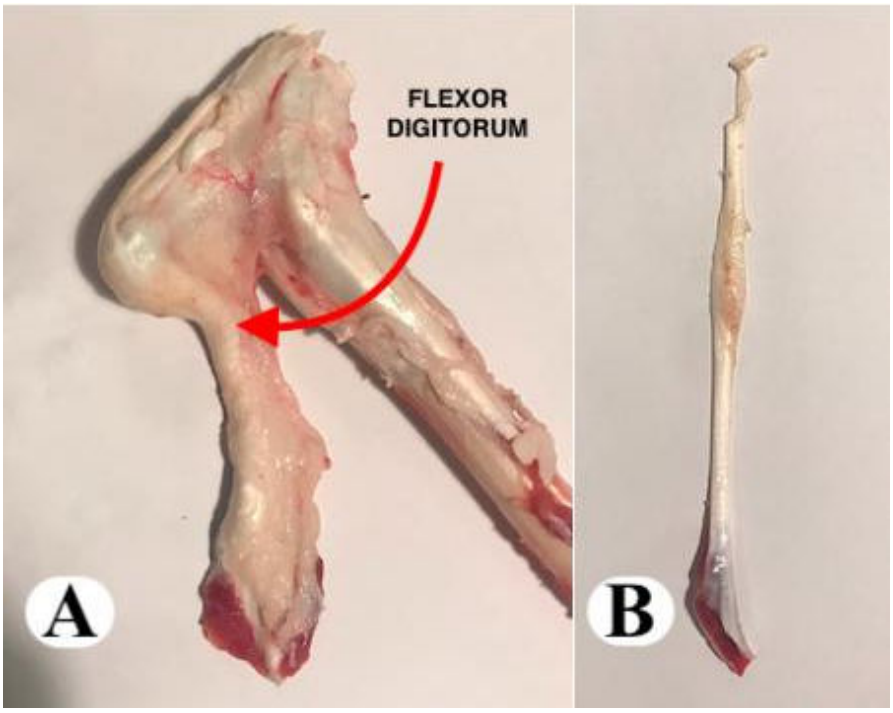

Fig. 3. (A) Hind legs flexor digitorum tendon of a 2-year old sheep, (B) Flexor digitorum tendon, which was used as a graft, after dissection.

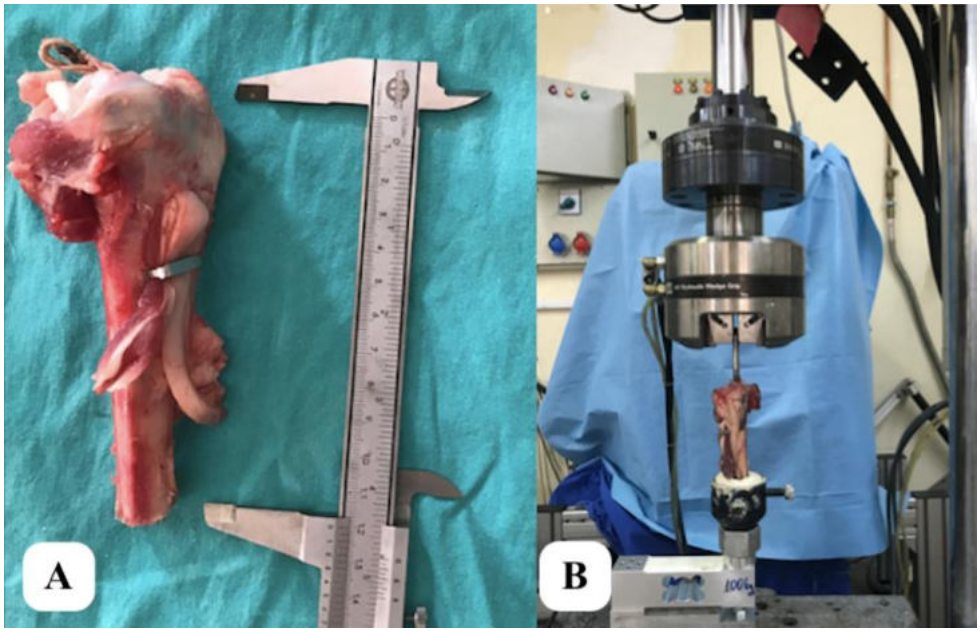

Fig. 4. (A) A 8 mm screw and staple used in the sample after cut which is located 12 cm distal to the knee joint level, (B) The study setup with MTS 858 Mini Bionix 2 tester.

system was discharged up to 10 N. Following this stage, the load-to-failure test was started. After this stage, the load-to-failure test was started by switching the force-controlled protocol to displacement-controlled protocol and traction was applied at a speed of 20 mm/min until the failure occurred. Force displacement values were recorded with MTS Axial Load cell (10000 N / 100Nm). Throughout the test, the values of motion (displacement), strength (stiffness), and ultimate failure load were recorded.

Statistical Analysis

All analyses were performed using SPSS version 22.0 software. Quantitative data were expressed as the mean ± standard deviation (SD). The values of motion (displacement), strength (stiffness), and ultimate failure load displayed nonparametric distribution according to the Kolmogorov-Smirnov test. Mann-Whitney U test was used to assess the between the groups.

RESULTS

In all samples used in the study, it was observed that the grafts were damaged at the screw bone junction on the tibial side, the weakest place in ACL reconstruction surgery [5]. Two samples damaged from the middle of the graft were excluded from the study. Moreover, one sample that ruptured from the apparatus to which it was fixed for the study and one sample broken due to device error during the study were also excluded.

As a result of the statistical analysis, in terms of the necessity of using a staple, there was a significant difference between Group 1 and Group 2 - the former was fixated using a 7 mm screw and the latter with a 7 mm screw and staple – and Group 2 was observed to resist to an average of 398.8N (Table 1). This value was found to be significant compared to the Group 1 samples that could resist to an average of 262.3 N. Group 2 was recorded to be able to resist higher strength than Group 1 ($p = 0.018$). When we compared the values of Group 3 and Group 4, it was seen that additional staple use did not make a statistically significant difference between the two groups (Table 1) ($p = 0.805$).

When the data were analysed in terms of screw diameter, the results of Groups 1 and 3 were compared, in which no additional staple was used. It was statistically determined that the group samples using 8 mm screws had a significantly higher strength with an average of 398 N compared to the average force to which the 7 mm screw could resist (262 N) (Table 2) ($p = 0.011$). Between the Groups 2 and 4 in which an additional staple was used, it was observed that the value of the maximum resistance did not have a statistically

significant difference despite the increased screw diameter (Table 2) (p = 1.00).

DISCUSSION

The main findings of this study are that the fixation strength is higher when fixation was performed with a screw 1 mm larger than the diameter of the tibial tunnel. The fixation strengths are comparable in the samples, which fixation was made with a screw 1 mm wider than the tibial tunnel and the ones fixed with a staple in addition to the screw in the same diameter of the tibial tunnel.

In the study carried out by Micucci *et al*., fixation was respectively provided with 8, 9, 10 and 11 mm screws in the 9 mm tunnel. Maximum strength was found highest in the screw of 11 mm; however the difference was not statistically significant [6]. It has been observed that as the screw width decreases, the maximum strength declines as well. But, when the highest forces, which the grafts were withstanding are considered, all values were found to be higher than the force that the graft would withstand in daily life [6]. The authors also reported that graft motion was less when the screw diameter equal to the tunnel size used [6]. In the study of Suk *et al*. [9], with 8 mm, 9 mm, 10 mm, 11 mm and 12 mm screws, the grafts were fixed to the tibial tunnels drilled with 8 mm drill. As a result of their study, the graft strength is observed to be 20% more in the 11 mm screw compared to the 8 mm screw. It also was reported that there appears no increase in graft fixation strength by increasing interference screw diameter beyond 3 mm of the tunnel diameter [9]. On the other hand, in the study of 37 pig femurs by Morris *et al*. [10], grafts were fixed to 8 mm wide femoral tunnels with 7 mm, 9 mm and 10 mm wide metal interference screws. As a result of thieir study, it was seen that all the tendons fixed with 9 mm wide screws were damaged at the entrance of the femoral tunnel and failed. In fixations with 8 mm screw, it also was determined that there was a risk of 58% graft slippage, 38% graft cut out. According to this study, it was stated that fixation with the same width as the tunnel width or 1 mm smaller screw would be appropriate [10]. Our findings are comparable with Micucci *et al*.'s [6] and Suk *et al*.'s [9] studies. We found that a screw 1 mm wider than the tunnel diameter provides sufficient fixation without the need for staple use.

In the study conducted by Kurosaka *et al*. [14], tibial fixation with only interference screw and that with only a staple were compared and it was concluded that fixation with an only bioabsorbable screw was stronger. Gerich *et al*. [15], who conducted a similar study, found that the maximum strength of the group that was fixated with the interference screw was between 506 N and 758 N and it was only 558 N in the patients who were provided with staple fixation. In the light of these studies, it was concluded that the interference screw alone could withstand the maximum force that the graft will be exposed to in early physical therapy [14, 15]. In the present study, unlike the Gerich *et al*.'s [15] study, the specimens using both bioabsorbable screw and staples in addition to the screw were investigated at the same time. We found that when a screw of the same diameter as the tunnel was used, usage of an additional staple increases the strength of fixation.

In the animal model, Bauer *et al*. [16] did not show significant differences in the samples using interference screw and staple compared with fixation made with an interference screw alone. It might be depending on the samples used, because the swine knee, which used in Bauer *et al*.'s [16], has a stronger cancellous bone compared to the lamb, which is used in the present study. In their study on the knees of 15 pigs, Lee *et al*. [2] examined the difference among using bioabsorbable screws only, bioabsorbable screws with staple use and bioabsorbable screws with Push-lock screws. As a result of the study, they found that additional fixation implants such as staple and screw did not contribute to the structural durability and stiffness of the graft considerably in cyclic loads [2]. However, when they continued the test and the forces that would cause damage to the graft were measured, the samples using additional fixation materials were found to withstand higher forces and as a result of the study. They stated that the use of additional fixation material might be significant in patients with poor bone quality, revision cases, elderly ones and those with metabolic diseases that will affect the bone quality [2]. In the study performed by Hill *et al*. [17], female patients who underwent ACL reconstruction with hamstring graft were observed by dividing them into two groups as metal interference screw and metal in-

terference screw and staple used in tibial fixation. In the study, they found that there was a remarkable difference between the two groups with the Lachman test and KT 2000 arthrometer (MEDmetric, San Diego, CA) in 2 years follow up time [17]. However, when this difference was evaluated with Lysholm score and IKDC (International Knee Documentation Committee assessment), no clinically meaningful difference was found. The authors suggested that staple use is suitable for patients with soft bone in whom the bioabsorbable screw cannot provide sufficient fixation. Also, in this study, they found an increased anterior knee pain due to staple use [17]. In their study on sheep knee, Fabriciani *et al*. [1] compared the metal interference screw used with bone block, absorbable interference screw and staple, metallic screw and washer screw. As a result of the comparison, bioabsorbable screw and staple duo were found as the fixation method that could resist the average highest force. Our findings support these findings [1, 17]. Our results showed that bioabsorbable screw with supplementary fixation with a staple, improve the structural strength for the ultimate tensile load on the tibial side.

In the study performed by Prado *et al*. [18] on the pig knee, fixation with bioabsorbable screw in addition to staple and that with only bioabsorbable screw were compared and no significant difference was found. In the study conducted by Teo *et al*. [19], 31 patients between the ages of 18 and 35 who only used bioabsorbable interference screws and 33 patients who used bioabsorbable interference screws and staple were compared. In the comparison made with the physical examination, arthrometer, IKDC form and Lysholm form 1 year after surgery, no significant difference was found between two groups [20]. The common findings in latter two studies [18, 19], in which they were not able to show any differences in terms of fixation strength on the tibial side, fixation was made with a screw larger than the tibial tunnel. It is well known that wider screw provides better fixation, however, in relation with the insertion angle of the screw used to fix the graft and the patient's bone quality, the graft is quite often damaged at the screw-tendon-bone junction and this is more likely with oversized screw [1, 8]. Therefore, we also tested the specimens fixed with a screw in the same diameter as the tibial tunnel. In our study, we found that when graft fixation was provided with 7mm bioabsorbable screw in the tunnel drilled with 7 mm, staple use increased durability. Therefore, we speculate that the use of a staple in addition to the screw, will increase the reliability of fixation especially in patients with soft bone and fixed with a bioabsorbable screw in the same diameter with the tibial tunnel.

All-inside technique is a safe alternative in which no staple is used and it is gaining popularity in the current practice [20, 21]. We agree with the need for additional fixation particularly in female patients with low bone density [2, 17].

Limitations

One of the limitations of the study is that it was made in the sheep knee and only the tibia was used. Whereas the ideal of the study is to do it in the young cadaver knee. Also, long-term results such as osteolysis could not be evaluated. For this, it is necessary to work with young cadaver and live animals. In addition, the same length bioabsorbable interference screws

(28 mm) were used in this study. Therefore, the effect of the screw length could not be evaluated. However, in this study, we used fresh sheep tendons and bones which was reported that the density of sheep bone is closer to that of young human bone compared to the old cadaver [22]. The animals were slaughtered within 24 hours and were not underwent the freezing process, which makes our study unique. The effect of screw width and staple use in fixation was evaluated with all possible combinations with four different groups.

CONCLUSION

We conclude that, graft fixation with interference screw 1 mm wider than the width of the tibial tunnel provides sufficient stability on the tibial side of specimens with adequate bone quality. As the relevance of clinical practice; by fixation of the graft only with screws, complications caused by staple can be eliminated and the cost of surgery can be reduced. However, in case, we can't afford the risk of injury to the graft with a larger screw, we can use a screw of the same diameter as the tunnel, then a staple should be kept ready during surgery to use if required.

Authors' Contribution

Study Conception: OO; Study Design: OO, DB, EB; Supervision: HB; Funding: OO; Materials: OO; Data Collection and/or Processing: OO, RMO, DB; Statistical Analysis and/or Data Interpretation: OO, EB, SEB; Literature Review: OO; Manuscript Preparation: OO and Critical Review: HB, OO.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Fabbriciani C, Mulas PD, Ziranu F, Deriu L, Zarelli D, Millano G. Mechanical analysis of fixation methods for anterior cruciate ligament reconstruction with a hamstring tendon graft. An experimental study in sheep knees. Knee 2005;12:135-8.
2. Lee JJ, Otarodifard K, Jun BJ, McGarry MH, Hatch GF, Lee TQ. Is supplementary fixation necessary in anterior cruciate ligament reconstructions? Am J Sports Med 2011;39:360-5.
3. Patkowski M, Królikowska A, Reichert P. Comparison of bioabsorbable interference screws composed of poly-l-lactic acid and hydroxyapatite (PLLA-HA) to WasherLoc tibial fixation in patients after anterior cruciate ligament reconstruction of the knee joint. Polim Med 2016;46:53-8.
4. Sun T, Zhang H. Biomechanical comparison of a novel tibial fixation technique versus interference screw fixation for ACL reconstruction using soft tissue grafts. Orthop J Sports Med 2021;9:23259671211028559.
5. Brand J Jr, Weiler A, Caborn DN. Graft Fixation in cruciate ligament reconstruction. Am J Sports Med 2000;28:761-74.
6. Micucci CJ, Frank DA, Kompel J, Muffly M, Demeo PJ, Altman GT. The effect of interference screw diameter on fixation of soft-tissue grafts in anterior cruciate ligament reconstruction. Arthroscopy 2010;26:1105-10.
7. Siebold R, Dejour D, Zaffagnini S. Anterior Cruciate Ligament Reconstruction. A Practical Surgical Guide. Heidelberg: Springer, 2014.
8. Herrera A, Martinez F, Iglesias D, Cegonino J, Ibarz E, Gracia L. Fixation strength of biocomposite wedge interference screw in ACL reconstruction: effect of screw length and tunnel/ screw ratio. A controlled laboratory study. BMC Musculoskelet Disord 2010; 11:139.
9. Suk N, Heywood CS, Bravman JT, Ieyasa K, Kummer FJ, Meislin RJ. The effect of interference screw diameter on soft tissue graft fixation. Bull NYU Hosp Jt Dis 2006;63:153-5.
10. Morris MW, Williams JL, Thake AJ, Lang Y, Brown JN. Optimal screw diameter for interference fixation in a bone tunnel: a porcine model. Knee Surg Sports Traumatol Arthrosc 2004;12:486-9.
11. Simonian PT, Wickiewicz TL, O'Brien SJ, Dines JS, Schatz JA, Warren RF. Pretibial cyst formation after anterior cruciate ligament surgery with soft tissue autografts. Arthroscopy 1998;14:215-20.
12. Hunt P, Scheffler SU, Unterhauser FN, Weiler A. A model of soft-tissue graft anterior cruciate ligament reconstruction in sheep. Arch Orthop Trauma Surg 2005;125:238-48.
13. Steiner ME, Hecker AT, Brown CH Jr, Hayes WC. Anterior cruciate ligament graft fixation: comparison of hamstring and patellar tendon grafts. Am J Sports Med 1994;22:240-6.
14. Kurosaka M, Yoshiya S, Andrish JT. A biomechanical comparison of different surgical techniques of graft fixation in anterior cruciate ligament reconstruction. Am J Sports Med 1987;15:225-9.
15. Gerich TG, Cassim A, Lattermann C, Lobenhoffer HP. Pullout strength of tibial graft fixation in anterior cruciate ligament replacement with a patellar tendon graft: interference screw versus staple fixation in human knees. Knee Surg Sports Traumatol Arthrosc 1997;5:84-8.
16. Bauer LAR, Alberti HAA, Corotti VGP, Franco APGO, Stieven FE, Cunha LAMD. Biomechanical analysis of a double fixation method for tendon graft in porcine tibia using an interference screw plus staple. Rev Bras Ortop 2018;53:564-9.
17. Hill PF, Russell VJ, Salmon LJ, Pinczewski LA. The influence of supplementary tibial fixation on laxity measurements after anterior cruciate ligament reconstruction with hamstring tendons in female patients. Am J Sports Med 2005;33:94-101.
18. Prado M, Martín-Castilla B, Espejo Reina A, Serrano-Fernández JM, Pérez-Blanca A, Ezquerro F. Close-looped graft suturing improves mechanical properties of interference screw fixation in ACL reconstruction. Knee Surg Sports Traumatol Arthrosc 2013; 21:476-84.
19. Teo W, Yeoh C, Wee T. Tibial fixation in anterior cruciate ligament reconstruction. J Orthop Surg (Hong Kong) 2017;25: 2309499017699743.
20. Graf-Alexiou L, Karpyshyn J, Baptiste JJ, Hui C, Sommerfeldt M, Westover L. Biomechanical strength of all-inside ACL reconstruction grafts using side-to-side and backup fixation. Orthop J Sports Med 2021;9:23259671211006521.
21. Bowes J, Mohamed N, Baptiste JJ, Westover L, Hui C, Sommerfeldt M. Biomechanical comparison of graft preparation techniques for all-inside anterior cruciate ligament reconstruction. Orthop J Sports Med 2020;8:2325967120938039.
22. Martini L, Fini M, Giavaresi G, Giardino R. Sheep model in orthopedic research: a literature review. Comp Med 2001;51:292-9.

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The value of FDG PET/CT in the management of malignant melanoma: a retrospective study

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ABSTRACT

Objectives: Positron Emission Tomography with Computed Tomography (PET/CT) is a valuable imaging method for the restaging of malignant diseases as well as the evaluation of treatment outcomes. As melanoma lesions are mostly FDG-avid, whole-body 18F-fluorodeoxyglucose (18F-FDG) PET/CT imaging modality can be used to determine the spread of the tumor. In the present study, we intended to share our clinical experience with FDG PET/CT in malignant melanoma patients under different kinds of treatment.

Methods: In this retrospective study, the data sets of 122 patients who had surgical resection of known primary tumors, all of which were histopathology-proven malignant melanoma, were analyzed. All patients underwent baseline 18F-FDG PET/CT scan no sooner than 45 days and at least once after surgery. Clinical information, radiological imaging, histopathology, and treatment modalities were noted for all patients, and 18F-FDG PET/CT findings were examined.

Results: All patients were histopathology-proven and 47 of them had acral lentiginous, 37 nodular, 23 amelanotic, and 15 atypical malignant melanoma. Local recurrence was detected by 18F-FDG PET/CT in 13 (10.6%; 3 male, 10 female) of the 122 patients, and the mean recurrence time after diagnosis was 3 ± 1.4 years. 10 patients with brain and bone metastases underwent radiotherapy. 19 patients with lung, bone, and hepatic metastases received chemotherapy and 22 patients immunotherapy.

Conclusions: Malignant melanoma is a type of skin cancer that may involve any organ. In localized cases, complete surgical resection may be adequate for a cure. If diagnosed with the latter type, the whole body of the patient must be examined.

Keywords: FDG PET/CT, malignant melanoma, postoperative recurrence, restaging

The worldwide incidence of malignant melanoma has increased over the past decades [1]. Like other malignancies, melanoma is well-known for local recurrence as well as distant metastasis, which occur through lymphatics and the bloodstream. The treatment of melanoma patients has to be based on the metastatic status of the disease.

The incidence of melanoma, the most common type of skin cancer, is increasing faster than any other potentially preventable cancer [1]. Most melanoma cases are diagnosed at an early stage; some patients have metastatic disease at presentation, and others develop metastases after their first definitive treatment. Patients with metastatic melanoma should undergo a detailed evaluation before treatment in order to assess the extent of the disease.

The metastases of malignant melanoma are widespread compared to other tumors, and clinicians

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should be aware of different patterns of metastatic involvement. Skeletal muscle or solitary bone metastases are more frequently observed in patients with advanced melanoma, as evidence of systemic spread.

The combination of metabolic and structural information provided by PET/CT has a significant impact on patient management and survival prediction. The diagnostic value of 18F-FDG PET for the follow-up of patients with malignant melanoma has been reported with a sensitivity and specificity of 96% and 92%, respectively [1, 2]. PET/CT can be used for staging and reassessment of metastatic malignant melanoma.

METHODS

Patient Population

The present study analyzed the data sets of 122 patients who admitted to Gülhane Training and Research Hospital Department of Nuclear Medicine between January 2016 and January 2020. Among those patients, 57.4% (n = 70) were female, 42.6% (n = 52) were male, and the mean age was 46.9 ± 14.2 years. They had surgical resection of known primary tumors, all of which were histopathology-proven malignant melanoma. All patients underwent baseline 18F-FDG PET/CT scan no sooner than 45 days and at least once after surgery. The study was approved by the Ethics Committee of Gülhane Training and Research Hospital, Ankara, Turkey.

Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography Acquisition

Similar to the protocol specified in the study by Malik *et al*. 18F-FDG PET/CT scan was performed on all patients with blood glucose < 200 mg/dl after at least 6 hours of fasting [3]. The scanning was achieved 45–60 min after the IV injection of 370 MBq (~10 mCi) of 18F-FDG using dedicated hybrid scanners (Discovery 710 or Discovery STE-16; GE Healthcare, Milwaukee, Wisconsin, USA). A vertex-to-toe low-dose scout CT (120 kV, 10 mA) was acquired. Contrast enhancement CT followed by 3D-PET scan was performed in caudocranial direction with an acquisition period of 2 min per bed position using the time-of-flight technique. The reconstructed attenuation-corrected PET, CT, and fused images were reviewed.

The scans were retrospectively assessed by two nuclear medicine physicians who were aware of the clinical findings of the patients. Any positive findings in the form of focal tracer uptake on 18F-FDG PET were anatomically localized on contrast-enhanced CT images. The maximum standardized uptake values (SUVmax) for semi-quantitative assessment were obtained by assigning a region of interest over the lesion with the highest tracer uptake.

Statistical Analysis

SPSS version 19.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. The predictive values of 18F-FDG PET/CT in detecting recurrence and metastasis in patients with malignant melanoma were compared at different periods of treatment with regard to localization of the primary tumor, age, and gender.

RESULTS

Patient Characteristics

In the present study, the scans and clinical data of 122 patients (70 female, 52 male, mean age 46.9 ± 14.2 years) were analyzed retrospectively. All patients were histopathology-proven and 47 of them had acral lentiginous, 37 nodular, 23 amelanotic, and 15 atypical malignant melanoma. The primary tumor was localized in 42 patients (34.4%) in the lower extremity, in 17 (13.9%) in the upper extremity, in 20 (16.4%) in the trunk, in 38 (31.1%) in the head or neck, and in 5 (4.1%) in the pelvis. Table 1 and Table 2 represent the lesion types by localization and lesion localization by gender.

Outcome of 18F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography

Clinical information, radiological imaging, histopathology, and treatment modalities were noted for all patients, and 18F-FDG PET/CT findings were examined. The lesions not proven by pathology were excluded. Local recurrence was detected with 18F-FDG PET/CT in 13 (10.6%; 3 male, 10 female) of the 122 patients, and the mean recurrence time after diagnosis was 3 ± 1.4 years. The mean SUVmax of recurrent lesions was 5.08 ± 3.08 g/dl. Local recurrence according to localization is presented in Table 3.

Table 1. Lesion type by lesion localization

	Lesion Localization						
	Lower extremity n (%)	Upper extremity n (%)	Back n (%)	Chest n (%)	Head-neck n (%)	Pelvic n (%)	Total n (%)
Acral lentiginos type	33 (27.00)	11 (9.00)	1 (0.80)	1 (0.80)	1 (0.80)	0 (0.00)	47 (38.50)
Noduler type	7 (5.70)	5 (4.10)	3 (2.50)	5 (4.10)	14 (11.50)	3 (2.50)	37 (30.30)
Amelanocitic type	0 (0.00)	1 (0.80)	3 (2.50)	1 (0.80)	18 (14.80)	0 (0.00)	23 (18.90)
Atypic Melanositic Melanoma	2 (1.60)	0 (0.00)	3 (2.50)	3 (2.50)	5 (4.10)	2 (1.60)	15 (12.30)
Total	42 (34.40)	17 (13.90)	10 (8.20)	10 (8.20)	38 (31.10)	5 (4.10)	122 (100)

The mean follow-up time of patients without recurrence was 4 ±2.4 years. Locoregional lymph nodes were detected in 99 patients, brain metastasis was seen in 10 (8.2%), lung metastasis in 9 (7.4%), bone metastasis in 10 (8.2%), and intramuscular metastatic lesions in 8, all of which were histopathology-proven. 6 patients had distant metastasis to the liver, and 1 to the parotid gland. Multiple peritoneal metastases were detected in 1 patient.

PET/CT imaging identified a) focal, and b) diffuse FDG uptake by the thyroid gland, which were correlated with thyroiditis through ultrasound and thyroid function tests (Fig. 1).

The mean SUVmax of metastatic lung lesions (n = 9) was 5.17 ± 3.89 g/dl, bone lesions (n = 10) 5.9 ± 3.49 g/dl, and muscle lesions (n = 8) 9.18 ± 3.51 g/dl. Ten patients with brain and bone metastases underwent radiotherapy. Nineteen patients who had lung, bone, and hepatic metastases with multiple unresectable lesions given chemotherapy and 22 patients

Table 2. Lesions' localizations by gender

	Lesion Localization						
	Lower Extremity n (%)	Upper Extremity n (%)	Back n (%)	Chest n (%)	Head-neck n (%)	Pelvic n (%)	Total n (%)
Male	21 (17.20)	2 (1.60)	6 (4.90)	3 (2.50)	16 (13.10)	4 (3.30)	52 (42.60)
Female	21 (17.20)	15 (12.30)	4 (3.30)	7 (5.70)	22 (18.00)	1 (0.80)	70 (57.40)
Total	42 (34.40)	17 (13.90)	10 (8.20)	10 (8.20)	38 (31.10)	5 (4.10)	122 (100)

Table 3. Local recurrence according to localization

	Locally recurrence		
Localization	Yes n (%)	No n (%)	Total n (%)
Lower extremity	8 (6.6)	34 (27.9)	42 (34.4)
Upper extremity	0 (0.0)	17 (13.9)	17 (13.9%)
Back	0 (0.0)	10 (8.2)	10 (8.2%)
Chest	2 (1.6)	8 (6.6)	10 (8.2%)
Head-neck	3 (2.5)	35 (28.7)	38 (31.1%)
Pelvic	0 (0. 0)	5 (4.1)	5 (4.1%)
Total	13 (10.7)	109 (89.3)	122 (100%)

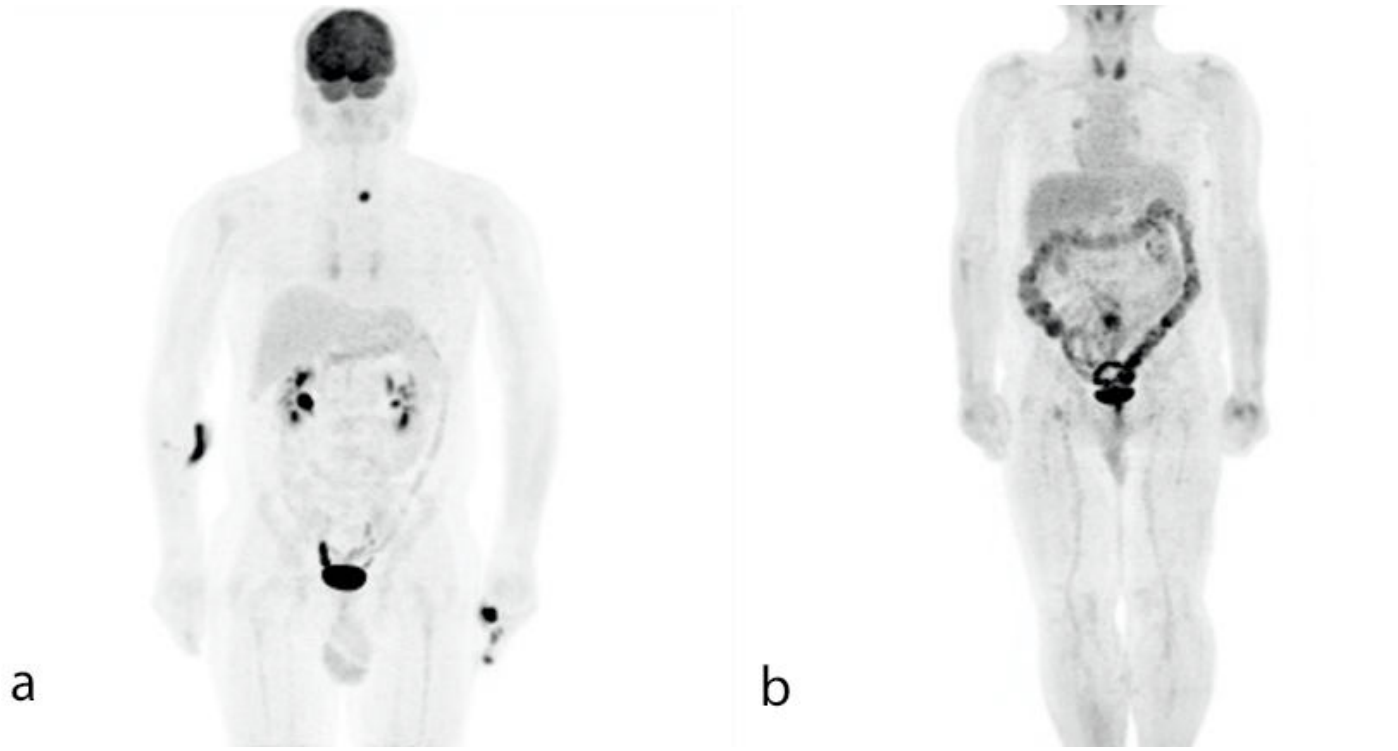

Fig. 1. Malignant melanoma patients who received immunotherapy. PET/CT imaging identified (a) focal, and (b) diffuse FDG uptake by the thyroid gland, which were correlated with thyroiditis through ultrasound and thyroid function tests.

were given immunotherapy. Parotid gland metastases with recurrent tumor foci were surgically resected. After resection, the patients were treated with chemotherapy and immunotherapy protocols.

DISCUSSION

Malignant melanoma patients have follow-up examinations every 3 to 6 months for at least 3 years after surgery. Whole-body 18F-FDG PET/CT is a valuable diagnostic imaging modality for detecting distant metastasis from malignant melanoma [1, 2].

Differentiation between scar tissue and new or recurrent tumor after surgery is challenging. PET/CT provides benefits in estimating tumor extent when considering surgical resection, especially in entities with high glucose consumption, which is often the case in malignant melanoma [4]. FDG is not cancer-specific as it is also trapped in tissues with high glycolytic activity. Due to the high rate of false-positive findings associated with this phenomenon, additional imaging or, if possible, pathological assessment should be considered. In the present investigation, the lesions were histopathology-proven. The SUVmax cutoff value of 2.2 was used to estimate recurrence in patients. All patients underwent surgical excision of the primary melanoma lesions before 18F-FDG PET/CT, and SUVmax was determined for the recurrent lesions. The mean SUVmax of locally recurrent melanoma lesions was 5.08 ± 3.08 g/dl. These results pertained to histopathologically proven lesions, while false-positive FDG PET/CT findings were excluded. Residual metabolic tumor activity in the surgical site could not be ruled out, since the granulation tissue formed in wound healing leads to moderate FDG accumulation but at a lower level than in malignant processes. We recommended reassessment for 17 patients, and 13 turned out true-positive. Correlation with the patient's history and other imaging modalities may be necessary for correct diagnosis.

Cancer patients have an approximately two-fold risk of developing additional primary cancer compared to cancer-free patients of the same sex and age [5]. In our present study, second primary malignancy was present in 5 patients. 4 were free of metastasis from malignant melanoma, such as rectosigmoid, breast, hard palate, and endometrial cancer. Non-small cell lung cancer was detected in 1 patient.

Muscle metastases from malignant tumors are rare

[6]. Solitary skeletal muscle metastases are less common than multiple skeletal muscle metastases on 18F-FDG PET/CT imaging. Herring *et al*. [6]. reported on a series of 15 patients with skeletal muscle metastasis, of which only 2 of the primary tumors were melanoma. Gómez Portilla *et al*. [7] reported a case of isolated rectus abdominis metastasis from melanoma. In the present investigation, 8 patients had intramuscular metastatic lesions; 1 was solitary, and 4 had multiple subcutaneous nodules. The mean SUVmax of muscular metastatic lesions was 9.18 g/dl.

There is limited literature on the relationship between location of lesion and age and gender. Gillgren *et al*. [8] mentioned that melanoma lesions occur more often on the trunks of men and in the lower extremities of women [8]. In the present study, the lower extremity was the most common site, followed by head and neck, and the upper extremity. Gender did not differ significantly in the lower extremity group, but the ratio of female to male was higher for the other regions. In addition, lung (n = 7) and bone (n = 7) metastases were more frequent in women. Our investigation also showed that the majority of the patients had acral lentiginous (38.5%, n = 47) and nodular melanoma (30.3%, n = 37), followed by amelanotic (18.9%, n = 23) and atypical (12.3%, n=15) melanoma.

Bone is a site of metastatic spread from melanoma, but this usually occurs in patients with already-widespread metastasis. Therefore the bone must be considered as a site for late-onset metastasis. Brountzos *et al*. [9] reported on a series of 28 patients with bone metastases from melanoma, and some other clinical studies have shown that bone metastases from malignant melanoma are less frequent than liver or brain metastases. In our investigation, bone metastasis was detected in 9 patients who already had lung metastasis, and 3 of these had liver metastasis as well. All cases of bone metastasis were multifocal, and 1 was solitary spinal metastasis from pelvic malignant melanoma.

Since melanoma pathology features a wide variety of clinical presentations, FDG PET/CT promises improved detection of metastases and better assessment of treatment decisions. Bastiaannet *et al*. [10] reported that FDG PET/CT offered the most accurate combination in diagnosis, restaging a large proportion of patients so as to reduce the number of inappropriate surgical procedures. In the present study, FDG PET/CT findings showed that liver and parotid gland lesions were resected. However, the lung lesions in our case series were multiple and accompanied by mediastinal lymph nodes, compelling the clinicians to non-surgical treatment strategies.

A combination of chemotherapy, radiotherapy, and targeted molecular therapy may result in the most effective treatment modality for metastatic melanomas [11, 12]. Immunotherapy is an efficient therapeutic strategy in melanoma due to the high immunogenicity of that type of tumor. A major feature of immunotherapy is its success in cases with resistance to radiation therapy and cytotoxic chemotherapy [11-13]. Twenty-two of our patient population received immunotherapy with immune checkpoint inhibitors (ICI). All were metastatic melanomas; 8 had lung and bone metastases, 8 had intramuscular and 6 had hepatic metastatic lesions. Immune-related adverse events (such as colitis, thyroiditis, etc.) should be taken into account in the interpretation of FDG PET/CT images, as these are associated with false-positive findings especially in the colon and the thyroid. In the present study, high thyroid metabolic activity was detected in 3 patients among the ones who received immunotherapy. Thyroid ultrasound was performed on these patients, and no thyroid nodules or biochemical thyroid disorders were observed. With regard to colonic activity, it is reported that focal FDG uptake has up to a 70-80% probability of unveiling corresponding abnormal histopathological observations [14]. In our study population, 1 patient had focal uptake of FDG localized to the rectosigmoid junction, for whom colonoscopy was recommended. Rectosigmoid cancer was proven by histopathology in that case.

For patients who are suspected to have metastatic disease the approach to imaging should focus on symptomatic sites. Radiologic evaluation is therefore more likely to be more clinically useful if it is focused on disease site and symptoms. Nodal status and lung metastasis are critical for decision-making regarding adjuvant therapy. Contrast-enhanced CT scanning is a widely used radiographic method for evaluating intrathorasic lesions as well as the metastatic disease of liver. MRI is the preferred imaging modality when brain metastases, soft tissue and bony lesions are suspected. Metastatic lesions involving the gastrointestinal tract are relatively common and abdominopelvic CT scan can evaluate the GI tract but the patients are often asymptomatic.

Our study focused on the implications of metastatic malignant melanoma localization. Anatomic imaging modalities, physical examination and laboratory signs are used in long-term follow-up of these patients. Among those patients, PET/CT imaging is not preferred due to its radiation exposure and high cost until the patients become symptomatic. Anatomical and metabolic status of the whole body lesions can be obtained with PET/CT in one session to define the total body burden of metastases, as well as to provide more specific information regarding specific sites, such as the lung, mediastinum, and abdomen. FDG PET/CT, is a preferred modality for treatment response and in the term of recurrence. Involvement of skin or subcutaneous tissue may be the first sign of metastatic disease; PET/CT can be more accurate for the detection of multifocal abnormalities even in the long-term follow-up these patients.

Limitations

The present study has some limitations: (a) The retrospective design of the study has constrained the patient population. (b) There may have been misjudgments in the evaluation of clinical images by the physicians. (c) The patient cohort was from a single center, which may have influenced the results. (d) The patients' staging FDG PET/CT scans were not included, and this may have affected the recurrence time.

CONCLUSION

Metastatic behavior of malignant melanoma is generally unpredictable because the sites of metastasis are widespread compared to other tumors. The choice of appropriate treatment approach for melanoma requires accurate localization of the site, number, and size of metastases. Our investigation showed that 18F-FDG PET/CT yielded enhanced diagnostic performance in malignant melanoma with suspected recurrence.

Authors' Contribution

Study Conception: AÇ; Study Design: AÇ, Sİ; Supervision: AÇ, Sİ; Funding: AÇ, Sİ; Materials: AÇ, ÜNG; Data Collection and/or Processing: AÇ, Sİ; Statistical Analysis and/or Data Interpretation: AÇ, Sİ; Literature Review: AÇ, ÜNG; Manuscript Preparation: AÇ, Sİ and Critical Review: AÇ, Sİ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Roses DF, Karp NS, Oratz R, Dubin N, Harris MN, Speyer J, et al. Survival with regional and distant metastases from cutaneous malignant melanoma. Surg Gynecol Obstet 1991;172:262-8.
2. Reinhardt MJ, Joe, AY, Jaeger U, Huber A, Matthies A, Bucerius J, et al. Diagnostic performance of whole-body 18FDG PET/CT imaging for N- and M-staging of malignant melanoma: experience with 250 consecutive patients. J Clin Oncol 2006;24:1178-87.
3. Malik D, Sood A, Mittal BR, Basher RK, Bhattacharya A, Singh G. Role of 18F-fluorodeoxyglucose positron emission tomography/computed tomography in restaging and prognosis of recurrent melanoma after curative surgery. World J Nucl Med 2019;18:176.
4. Garcia GA, Fernandez F, Satue E, Buelta L, Val-Bernal J. Metastasis of malignant neoplasms of skeletal muscle. Rev Esp Oncol 1984;31:57-67.
5. Anwar H, Sachpekidis C, Winkler J, Kopp-Schneider A, Haberkorn U, Hassel JC, et al. Absolute number of new lesions on 18F-FDG PET/CT is more predictive of clinical response than SUV changes in metastatic melanoma patients receiving ipilimumab. Eur J Nucl Med Mol Imaging 2018;45:376-83.
6. Herring CL, Harrerlson JM, Scully SP. Metastatic melanoma with an unknown primary. Br J Plast Surg 1990;43:367-8.
7. Portilla G, Cruz A, Juan N, Malo P, Lopez de Heredia E, Larrañaga M, et al. Isolated rectus abdominis metastasis from melanoma, an extremely rare case. Int J Surg Case Rep 2016;26:121-3.
8. Gillgren P, Månsson-Brahme E, Frisell J, Johansson H, Larsson O, Ringborg U. Epidemiological characteristics of cutaneous malignant melanoma of the head and neck--a population-based study. Acta Oncol 1999;38:1069-74.
9. Brountzos E, Panagiotou I, Bafaloukos D, Kelikis D. Bone metastases from malignant melanoma; a retrospective review and analysis of 28 cases. Radiol Oncol 2001;35:209-14.
10. Bastiaannet E, Oyen WJ, Meijer S, Hoekstra OS, Wobbes T, Jager PL, et al. Impact of [18F] fluorodeoxyglucose positron emission tomography on surgical management of melanoma patients. Br J Surg 2006;93:243-9.
11. Schumacher TN, Schreiber RD. Neo antigens in cancer immunotherapy. Science 2015;348:69-74.
12. Sadozai H, Gruber T, Hunger RE, Schenk M. Recent suc-

cesses and future directions in immunotherapy of cutaneous melanoma. Front Immunol 2017;8:1617.
13. Tawbi HAH, Forsyth PA, Algazi AP, Hamid O, Hodi FS, Moschos SJ, et al. Efficacy and safety of nivolumab (NIVO) plus ipilimumab (IPI) in patients with melanoma (MEL) metastatic to the braIn: Results of the phase II study CheckMate 204. J Clin Oncol 2017;35(Suppl):9507.
14. Gutman F, Alberini JL, Wartski, M, Vilain D, Le Stanc E, Sarandi F, et al. Incidental colonic focal lesions detected by FDG PET/CT. AJR Am J Roentgenol 2005;185:495-500.

The European Research Journal 2022;8(3):340-346 Original Article

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Loop ileostomy or loop transverse colostomy for resectable rectal cancers

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ABSTRACT

Objectives: This retrospective study aimed to compare loop ileostomy (LI) with loop transverse colostomy (LTC) as covering stoma regarding the perioperative outcomes in patients who underwent low anterior resection for rectal cancer between 2015 and 2020.

Methods: Data were collected from patient files and the hospital's electronic database. The primary outcome measure was complications related to stoma formation, stoma reversal, and overall complications. Secondary outcome measures were hospital discharge time and readmission rate after discharge.

Results: A total of 90 patients (38 female, 52 male; 56.6 ± 6.8 years) were included in the study. There were two groups considering the technique for covering stoma: Group LI (n = 50) and Group LTC (n = 40). Demographic and perioperative characteristics were similar. Primary outcome measure: Postoperative course was complicated in 49 (54.4%) patients. The complication rate was higher in the group LI than the group LTC (62% vs 45%; $p = 0.03$). Among them, 29 (59.2%) complications were related to the stoma formation related, and 14 (28.6 %) complications were related to the stoma reversal related, and 6 (12.2%) were overall complications. The rate of complications related to the stoma formation was higher in the group LI compared to the group LTC (20 [40%] vs. 9 [22.5%]; $p = 0.01$). The most common complication was periostomal skin irritation (48.3%) followed by dehydration (13.8%), stoma retraction (10.3%) patients, parastomal hernia (10.3%), bleeding (6.9%), anastomotic leak (3.4%), incisional hernia (3.4%), and high-output stoma (3.4%). Complications including incisional hernia, high output stoma, and anastomotic leakage (Grade C; requiring laparotomy) were observed only in the group LI. The morbidity rate in 30 days after the surgery was higher in the group LI compared to the group LTC (16 [32%] vs. 8 [20%]; $p = 0.02$). A total of 14 stoma reversal complications included incisional hernia in 7 (14.3%) patients, wound infection in 5 (10.2%) patients, and rectal bleeding in 2 (4.1%) patients. The rate of complications was not different between groups (16% vs. 15%; $p = 0.41$). Overall complications were similar between study groups (3 complications in each group; $p = 0.73$). Secondary outcome measure: The group LTC patients were discharged earlier compared to the group LI (7.1 ± 2.0 days vs. 9.4 ± 2.5 days; $p = 0.03$). The readmission rate after hospital discharge was higher in the LI group than the group LTC (18% vs. 12.5%; $p = 0.02$).

Conclusions: It was concluded that LTC was superior compared to LI concerning complications after low anterior resection for rectum cancer.

Keywords: It was concluded that LTC was superior compared to LI concerning complications after low anterior resection for rectum cancer.

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Low anterior resection (LAR) is the gold standard for treatment of rectal cancer due to promising survival results. It is also associated with a high incidence of complications, which increase morbidity, hospitalization, and mortality [1]. The anastomotic leak is the most important complication, accounting between 3% and 30% [2]. It is also considered to be a poor prognostic factor irrespective of that the surgery is open or laparoscopic [3]. A covering stoma is widely used to protect the anastomosis by diverting fecal stream and to avoid a contact between the anastomosis and fecal material or a fecal flow through the anastomosis [4]. Two types of re functioning stoma are commonly performed in practice: loop ileostomy (LI) and loop transverse colostomy (LTC). Both are assumed to offer several advantages over LAR without covering stoma, including a lower rate of anastomotic leak, pelvic collection, peritonitis, bowel obstruction or ileus, wound infection, and better resumption of the diet [5]. However, for more than three decades, a continuing debate exists about which technique is more effective in reducing complications, and the superiority has been changed almost every decade, according to the reports in the literature [6, 7]. This present study aimed to compare LI with LTC regarding perioperative outcomes. The primary outcome measures were complications related to the covering stoma techniques classified as stoma formation related complications, stoma reversal related complications, and overall complications. Secondary outcome measures were hospital discharge time and readmission rate after discharge.

METHODS

Study Design and Patient Population

This retrospective and observational study was conducted in a tertiary academic hospital after obtaining the hospital's ethics committee approval (decision number: İ10-626-20). Obtaining informed consent from the patients was waived due to the retrospective nature of the study.

According to the Code of Ethics of the World Medical Association (Declaration of Helsinki), the study was carried out and followed the strengthening of the reporting of observational studies in epidemiology (STROBE) guidelines. Inclusion criteria were patients with American Society of Anesthesiologists (ASA) physical status classification I-III, histopathological confirmed rectal carcinoma, distance from anal verge lower than 4 cm, elective surgery, no metastasis. Exclusion criteria were urgent surgery, metastatic disease, obstructed tumour, permanent colostomy or ileostomy, loss in the follow-up period, and missing data.

Surgical Procedure

Surgical team members have performed surgical procedures under general anesthesia who were experienced in colorectal and tumour surgery. After the tumor was resected and the anastomosis was completed (colorectal or coloanal), it was decided on the covering stoma. LTC was constructed as transverse colostomy, and LI was constructed 20-25 cm proximal to the ileocecal valve. All patients were educated for stoma care during the postoperative period.

Data Collection

The electronic database, patient and anesthesia files were retrospectively evaluated to obtain demographic characteristics, ASA physical status, surgical characteristics, postoperative course, discharge time, overall complications, and specific complications related to stoma formation and stoma closure. Stoma formation related complications were defined as an anastomotic leak, stoma prolapse, retraction, ischemia, bleeding, peristomal skin irritation, incisional hernia, high output stoma, dehydration and parastomal hernia. Stoma reversal complications included anastomotic leak, incisional hernia, wound infection, rectal bleeding, ileus, and fistula. Anastomotic leakage was defined as a defect of the intestinal wall integrity at the anastomotic site, leading to a communication between inta- and extraluminal compartments or a pelvic abscess close to the anastomosis, according to the report by the International Study Group of Rectal Cancer [8]. The severity of the leak was graded as requiring no active therapeutic intervention (Grade A), requiring active intervention, but manageable without re-laparotomy (Grade B), and requiring re-laparotomy (Grade C).

Overall complications were defined as wound infection, bowel obstruction, acute kidney injury, thromboembolism, urinary retention, and other cardiorespiratory problems that occurred in the post-

operative period.

Statistical Analysis

SPSS pocket program (version 21.0; IBM SPSS Inc, Chicago, IL) was used for statistical analysis. Continuous variables were analyzed using descriptive statistics and assessed with the Kolmogorov-Smirnov test when normally distributed. The differences in distributions for categorical variables were analyzed using Pearson's chi-square (χ^2) and Fisher's exact tests between groups. Non-parametric variables were assessed with the Mann-Whitney U test for the distribution in study groups. A p - value < 0.05 was considered statistically significant.

RESULTS

A total of 110 files were evaluated during the study period. Of them, 20 files were excluded from the study due to missing data (n = 12) and lost to follow-up (n = 8). The mean age of the remaining 90 patients were

Table 1. Demographic and preoperative tumor characteristics in study groups

	Group LI (n = 50)	Group LTC (n = 40)	*p* value
Age (years) (mean ± SD)	55.3 ± 5.5	57.5 ± 7.4	0.88
Gender (Female/Male), n (%)	21 (40.2)/29 (59.8)	17 (42.5)/23 (57.5)	
ASA physical status, n (%)			0.72
1	29 (58)	25 (62.5)	
2	21 (52)	15 (37.5)	
3	0 (0)	0 (0)	
Co-morbidity n (%)	6 (12)	5 (12.5)	0.52
Hypertension	3 (6)	3 (7.5)	
Diabetes mellitus	3 (6)	2 (5)	
Coronary artery disease	0 (0)	0 (0)	
Pulmonary disease	0 (0%)	0 (0)	
BMI (kg/m^2) (mean ± SD)	23.3 ± 3.5	22.5 ± 4.2	0.03
Anal verge distance (cm) (mean± SD)	11 ± 3	9 ± 4	0.51
T, n (%)			0.61
0	0 (0)	0 (0)	
1	7 (14)	5 (12.5)	
2	25 (50)	20 (50.0)	
3	12 (24)	10 (25)	
4	6 (12)	5 (12.5)	
N, n (%)			0.42
0	12 (24)	10 (25)	
1	38 (76)	30 (75)	
M, n (%)			0.71
0	44 (88)	36 (90)	
1	6 (12)	4 (10)	
Neoadjuvant chemotherapy (Y/N), n (%)	12 (24)/38 (76)	10 (25)/30 (75)	0.54

LTC = loop transverse colostomy, LI = loop ileostomy, ASA = American Society of Anesthesiologists, BMI = Body Mass Index, T = Tumor, N = Node, M = Metastasis, Y/N = Yes/No, SD = standard deviation, p < 0.05 was considered as statistically significant.

56.6 ± 6.8 years. There were 38 female and 52 male patients assigned to two groups considering the technique for covering stoma. Group LI consisted of 50 patients, and Group LTC consisted of 40 patients. Demographic characteristics were statistically not different between groups ($p > 0.05$) (Table 1). Also, the TNM classification, anal verge distance of the tumour, and the rate of neoadjuvant chemotherapy was similar ($p > 0.05$) (Table 1). Estimated blood loss was lower in the group LT than the group LI (180 ± 65 ml vs 200 ± 70; $p = 0.02$).

Primary Outcome Measure

The postoperative course was complicated in 49 (54.4%) patients. The complication rate was higher in the group LI than the group LTC (62% vs 45%; $p = 0.03$). Among them, 29 (59.2%) complications were related to the stoma formation related complications, 14 (28.6 %) were related to the stoma reversal related complications, and 6 (12.2%) were overall complications.

Complications Related to the Stoma Formation

The complication rate was higher in the group LI compared to the group LTC (20 [40%] vs. 9 [22.5%]; $p = 0.01$), (Table 3). The most common complication was periostomal skin irritation in 14 (48.3%) patients followed by dehydration (13.8%), stoma retraction (10.3%) patients, parastomal hernia (10.3%), bleeding (6.9%), anastomotic leak (3.4%), incisional hernia (3.4%), and high-output stoma (3.4%). All complications were higher in the group LI compared to the group LTC except bleeding. Complications including incisional hernia, high output stoma, and anastomotic leakage (Grade C; requiring laparotomy) were observed only in the group LI. The morbidity rate in 30 days after the surgery was higher in the group LI compared to the group LTC (16 [32%] vs. 8 [20%]; p = 0.02) (Table 3).

Stoma Reversal Complications

A total of 14 complications was related to the stoma reversal. Those included incisional hernia in 7 (14.3%) patients, wound infection in 5 (10.2%) patients, and rectal bleeding in 2 (4.1%) patients. The rate of complications was not different between groups 134 (16% vs 15%; $p = 0.41$).

Overall Complications

A total of 6 complications (3 complications in each group; $p = 0.73$) were recorded. One patient in the group LI suffered from arrhythmia, and one patient from atelectasis. One patient in each group developed deep venous thrombosis. Delirium was diagnosed in two patients (one patient in each group). All those complications were treated successfully in the early postoperative period.

Secondary Outcome Measure

The group LTC patients were discharged earlier

Table 2. Comparing perioperative characteristics between study groups

	Group LI (n = 50)	Group LTC (n = 40)	*p* value
Anastomosis			
Mechanical (stapler), n (%)	45 (90)	35 (87.5)	0.33
Hand-sewn interrupted, n (%)	5 (10)	5 (12.5)	0.92
Operative time (min) (mean ± SD)	150 ± 40	145 ± 45	0.32
Estimated blood loss (ml) (mean ± SD)	200 ± 70	180 ± 65	0.02
Blood transfusion, n (%)	6 (12)	5 (12.5)	0.41
Admission to ICU, n (%)	7 (14)	6 (15)	0.55
Hospital discharge time (day) (mean ± SD)	9.4 ± 2.5	7.1 ± 2.0	0.03
Time to stoma closure (day) (mean ± SD)	78 ± 18	71 ± 17	0.88
Readmission after the discharge, n (%)	9 (18)	5 (12.5)	0.02

LTC = loop transverse colostomy, LI = loop ileostomy, ICU= Intensive care unit, SD = standard deviation, $p < 0.05$ wasconsidered as statistically significant.

Table 3. Comparing complications between study groups

	Group LI (n = 50)	Group LTC (n = 40)	*p* value
Total complications, n (%)	31 (62)	18 (45)	**0.03**
Complications related to the stoma formation, n(%)	20 (40)	9 (22.5)	**0.01**
Periostomal skin irritation	9 (18)	5 (12.5)	**0.01**
Dehydration	3 (6)	1 (2.5)	**0.01**
Stoma retraction	2 (4)	1 (2.5)	**0.02**
Parastomal hernia	2 (4)	1 (2.5)	**0.02**
Bleeding	1 (2)	1 (2.5)	0.31
Incisional hernia	1 (2)	0 (0)	**< 0.01**
High – output stoma	1 (2)	0 (0)	**< 0.01**
Anastomotic leak (Grade C; Requiring laparotomy)	1 (2)	0 (0)	**< 0.01**
30-day morbidity	16 (32)	8 (20)	**0.02**
Complications related to the stoma reversal, n (%)	8 (16)	6 (15)	0.41
Incisional hernia	4 (8)	3 (7.5)	0.53
Wound infection	3 (6)	2 (5)	0.51
Rectal bleeding	1 (2)	1 (2.5)	0.78
Overall complications, n (%)	3 (6)	3 (7.5)	0.73
Cardiorespiratory problems	1 (2)	1 (2.5)	0.31
Deep venous thrombosis	1 (2)	1 (2.5)	0.31
Cognitive dysfunction	1 (2)	1 (2.5)	0.31

LTC = loop transverse colostomy, LI = loop ileostomy. $p < 0.05$ was considered asstatistically significant.

compared to the group LI (7.1 ± 2.0 days vs. 9.4 ± 2.5 days; $p = 0.03$). The readmission rate after hospital discharge was higher in the LI group than the group LTC (18% vs 12.5%; $p = 0.02$).

DISCUSSION

The results of the study showed that LTC was superior to the LI as covering stoma for low anterior resection of rectal cancer for several reasons: a) The total complication rate was reduced, b) The complication rate related to the stoma formation was lower, c) Hospital discharge time was shorter, and d) the readmission rate after the discharge was lower.

These results are compatible with several studies. In a double-blind and prospective study, Abdulmohaymen compared LI with LTC in 70 patients and found that stoma-related complications were significantly higher in the LI group than in the LTC group (75.6% vs. 43.2%), [9]. Peristomal dermatitis was the most frequently recorded complication. Also, the rate of stoma reversal related complications was higher in the LI group (45.4% vs. 13.5%). Diarrhea was the most common complication.

Sun *et al*. also reported that stoma related complications were found higher in the LI group than the LTC group (74.3% vs. 48.7%) [10]. Irritant dermatitis was the most frequent complication. The LI group had a significantly higher stoma reversal complication rate (24.24% vs. 9.01%). Multivariate logistic regression analysis showed that ileostomy was a significant independent risk for stoma-related complications ($p < 0.001$) and stoma reversal perioperative complications ($p < 0.05$). On the other hand, in a meta-analysis study including five randomized and seven non-randomized studies, Chen *et al*. [11] reported that the risks of stoma relapse and LI's wound infection were lower in

the LI patients than LTC. In contrast, no other is statistically significant difference was observed for complications.

Gavriilidis *et al*. [12] reported a meta-analysis with a higher incidence of stoma prolapse, lower incidence of high-output stoma after stoma formation and significantly more complications related to stoma reversal, such as wound infections and incisional hernias in patients receiving LTC than the LI.

Nevertheless, the overall complication rate was similar. Du *et al*. [13] included two randomized controlled trials and six cohort studies with a total of 1451 patients in their recent meta-analysis and systemic review study. Stoma prolapse, stoma retraction, parastomal hernia, surgical site infection, and incisional hernia were higher after LTC, whereas dehydration was lower.

In a review by Geng *et al*. [14], it was found that the prevalence of sepsis, prolapse, parastomal hernia and overall complications were lower after stoma formation in LI patients compared to LTC patients. Also, wound infection and incisional hernia were lower, but overall complications were similar. They concluded that a defunctioning LI might be superior to LC concerning a lower prevalence of surgical complications after low anterior resection of rectum cancer.

In a prospective study including 28 patients, skin excoriation, leaks from the appliance, and parastomal hernia was lower in the LI group, but intestinal obstruction was higher compared to the LTC group [15].

Limitations

Based on the literature, there is still no consensus between LI and LTC about which technique is superior. This study has several limitations. First, the patients were not randomized due to the retrospective nature of the study. This might cause a bias despite the same inclusion and exclusion criteria. Another limitation was the limiting number of patients.

CONCLUSION

Although our results revealed that LTC was superior compared to LI concerning complications after low anterior resection for rectum cancer, it can be stated that both LTC and LI have advantages and disadvantages. The choice of the technique for fecal diversion should be considered for every patient individually.

Authors' Contribution

Study Conception: MAÇ, ŞD; Study Design: MAÇ; Supervision: MAÇ, ŞD; Funding: N/A; Materials: N/A; Data Collection and/or Processing: MAÇ, ŞD; Statistical Analysis and/or Data Interpretation: MAÇ, ŞD; Literature Review: MAÇ, ŞD; Manuscript Preparation: MAÇ, ŞD and Critical Review: SD.

Conflict of interest

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REFERENCES

1. Watanabe T, Miyata H, Konno H, Kawai K, Ishihara S, Sunami E, et al. Prediction model for complications after low anterior resection based on data from 33,411 Japanese patients included in the National Clinical Database. Surgery 2017;161:1597-608.
2. Kim CW, Baek SJ, Hur H, Min BS, Baik SH, Kim NK. Anastomotic leakage after low anterior resection for rectal cancer is different between minimally invasive surgery and open surgery. Ann Surg 2016;263:130-7.
3. Katsuno H, Shiomi A, Ito M, Koide Y, Maeda K, Yatsuoka T, et al. Comparison of symptomatic anastomotic leakage following laparoscopic and open low anterior resection for rectal cancer: a propensity score matching analysis of 1014 consecutive patients. Surg Endosc 2016;30:2848-56.
4. Wu SW, Ma CC, Yang Y. Role of protective stoma in low anterior resection for rectal cancer: a meta-analysis. World J Gastroenterol 2014;20:18031-7.
5. Williams NS, Nasmyth DG, Jones D, Smith AH. Defunctioning stomas: a prospective controlled trial comparing loop ileostomy with loop transverse colostomy. Br J Surg 1986;73:566-70.
6. Gooszen AW, Geelkerken RH, Hermans J, LagaayMB, GooszenHG. Temporary decompression after colorectal surgery: randomized comparison of loop ileostomy and loop colostomy. Br J Surg 1998;85:76-9.
7. Sakai Y, Nelson H, Larson D, Maidl L, Young-Fadok T, Ilstrup D. Temporary transverse colostomy vs loop ileostomy in diver-

sion: a case-matched study. Arch Surg 2001;136:338-42.
8. Rahbari NN, Weitz J, Hohenberger W, Heald RJ, Moran B, Ulrich A, et al. Definition and grading of anastomotic leakage following anterior resection of the rectum: a proposal by the International Study Group of Rectal Cancer. Surgery 2010;147:339-51.
9. Abdulmohaymen AM. Comparative study of loop ileostomy vs loop transverse colostomy as a covering stoma after low anterior resection for rectal cancer. Al-Azhar Assiut Med J 2020;18:136-9.
10. Sun X, Han H, Qiu H, Wu B, Lin G, Niu B, et al. Comparison of safety of loop ileostomy and loop transverse colostomy for low-lying rectal cancer patients undergoing anterior resection: A retrospective, single institute, propensity score-matched study. J BUON 2019;24:123-9.
11. Chen J, Wang DR, Zhang JR, Li P, Niu G, Lu Q. Meta-analysis of temporary ileostomy versus colostomy for colorectal anastomoses. Acta Chir Belg 2013;113:330-9.
12. Gavriilidis P, Azoulay D, Taflampas P. Loop transverse colostomy versus loop ileostomy for defunctioning of colorectal anastomosis: a systematic review, updated conventional meta-analysis, and cumulative meta-analysis. Surg Today 2019;49:108-17.
13. Du R, Zhou J, Tong G, Chang Y, Li D, Wang F, et al. Postoperative morbidity, and mortality after anterior resection with preventive diverting loop ileostomy versus loop colostomy for rectal cancer: A updated systematic review and meta-analysis. Eur J Surg Oncol 2021;47:1514-25.
14. Geng HZ, Nasier D, Liu B, Gao H, Xu YK. Meta-analysis of elective surgical complications related to defunctioning loop ileostomy compared with loop colostomy after low anterior resection for rectal carcinoma. Ann R Coll Surg Engl 2015;97:494-501.
15. Ayman MAA. Loop transverse colostomy versus loop ileostomy after low and ultralow anterior resection. Int Surg J 2018;5:1633-9.

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Prevalence and clinical significance of serum anti-cyclic citrullinated peptide antibodies in primary Sjögren's syndrome

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ABSTRACT

Objectives: Although anti-cyclic citrullinated peptide (anti-CCP) antibody is a specific marker for rheumatoid arthritis (RA), it is also detected positively in some other rheumatological diseases including Sjögren's syndrome (SS) and even in healthy people. Studies have shown that anti-CCP guides the early diagnosis, prognosis and treatment of RA patients. SS and RA overlap syndrome are common. This study was conducted to determine the prevalence and clinical significance of serum anti-CCP in primary SS patients followed in our outpatient clinic.

Methods: Eighty-two primary SS, 100 RA patients and 100 healthy controls applied to rheumatology outpatient clinic were examined. Patients and control groups were compared in terms of demographic characteristics, laboratory results and anti-CCP.

Results: In the present study, anti-CCP was positive in 4 (4.9%) of 82 primary SS patients. In the SS group, among other autoantibodies, ANA was found to be positive at 88.8%, RF 45%, SS-A 64.6%, and SS-B 36.7%. There was no relationship between anti-CCP and joint involvement in patients with SS.

Conclusions: Anti-CCP antibody positivity can be found in SS patients. Acute phase proteins may be higher in primary SS patients with positive anti-CCP antibody. However, studies have found conflicting findings about the prevalence of erosive arthritis and the future development of RA in these patient groups.

Keywords: Primary Sjögren's syndrome, rheumatoid arthritis, anti-cyclic citrullinated peptide

Sjögren's syndrome (SS) is a chronic autoimmune disease characterized by mononuclear cell infiltration in organs, involving all exocrine glands, primarily the salivary and lacrimal glands. The spectrum of the disease ranges from autoimmune exocrinopathy to a systemic process with extraglandular manifestations. Dry mouth and eyes due to glandular involvement are among the most common symptoms of the disease. The disease is generally seen in women between the ages of 40-50. Although the cause of the disease is not known exactly, it can be seen together with other autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis and polymyositis (secondary SS) or it can occur alone (primary SS) [1]. RA is the most common rheumatic disease associated with SS [2].

Anti-CCP antibodies are IgG-type antibodies that develop against citrullinated peptides and are called anti-citrullinated peptide antibodies (ACPA). The most commonly used ACPA tests are anti-cyclic citrulli-

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nated peptide (Anti-CCP) antibodies. Anti-CCP is 97-98% specific and 40-60% sensitive for RA [3]. In addition, they can be detected in a very early period (79%) in RA [4]. It has been reported that patients with positive anti-CCP antibodies develop more joint deformity and radiological damage [5]. Anti-CCP is reported as a much more specific parameter for RA than rheumatoid factor (RF) [6]. Anti-CCP may be also positive in some rheumatologic diseases, including SS, and even in healthy people, except for RA. The most common causes of anti-CCP positivity other than RA, in order of frequency, are psoriatic arthritis (9%), systemic lupus erythematosus (8%), juvenile idiopathic arthritis (8%), scleroderma and CREST syndrome (7%), Sjogren's syndrome (6%), vasculitis (5%); in addition, 3% positive in ankylosing spondylitis, 1% positive in chronic hepatitis C and Wegener granulomatosis [7]. It has been reported that ACPA is detected at a high prevalence in active tuberculosis, except autoimmune diseases [8]. These antibodies are found to be less than 1% positive in the normal population [3].

The aim of this study is to determine the prevalence of anti-CCP in primary SS patients and its relationship with joint and extra-articular involvement associated with the disease.

METHODS

Eighty-two patients with primary SS diagnosis, 100 patients with RA diagnosis and 100 healthy individuals as control group were included. Patients who met the AECG 2002 (American-European Consensus Group) criteria for the diagnosis of primary SS and the ACR/EULAR 2010 (American College of Rheumatology/European League Against Rheumatism) criteria for the diagnosis of RA were included in the study [9, 10].

Patients with secondary SS, patients with autoimmune diseases other than SS, and pregnant women were excluded from the study. Age, gender, symptoms at presentation, joint and extra-articular involvement, onset time of symptoms, time to diagnosis and given treatments were recorded. For each patient diagnosed with primary SS and RA, complete blood count, biochemical parameters, autoimmune markers and serum anti-CCP values available at the time of admission were recorded. Patients and control groups were compared in terms of demographic characteristics, laboratory findings and anti-CCP.

Ethics committee approval was obtained for the study. Anti-CCP serum levels were measured on the Roche cobas-e 601 immunoassay analyzer, a dual modular device, using the Elecsys® Anti-CCP kit from Roche Diagnostics. The measurement was made using the electrochemiluminescence method. The reference range was taken as 0-20 IU/mL.

Statistical Analysis

The compliance of the variables in the study to normal distribution was evaluated using the Shapiro-Wilks test. The median (Interquartile Range-IQR) was used to display descriptive statistics of discrete and non-normally distributed variables. In comparisons of parameters that do not show normal distribution between groups, Kruskall-Vallis H test was used for multiple groups and Mann-Whitney U Test was used for paired groups. Bonferroni-corrected paired comparisons were made for variables with significant differences as a result of the Kruskal-Wallis test. Whether the distribution of categorical variables differed by groups was evaluated using the Chi-square test. In case the number of subjects in the cells is insufficient; differences between groups were analyzed using Fisher's Exact test result. IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) and MS-Excel 2007 programs were used for statistical analysis and ± calculations. Statistical significance level was accepted as $p < 0.05$.

RESULTS

A total of 282 individuals were included in the study. Of these, 82 were primary SS (96.3% female [79/82] and median age 49.0 (IQR = 11.0) years), 100 patients with RA (69% female [69/100] and median age 50.0 (IQR = 21.0) years) and 100 healthy controls (78% female [78/100] and median age 41.5 (IQR = 14.0) years). The mean time elapsed since the onset of symptoms in SS patients was 39.17 ± 28.8 months, and the mean time after diagnosis was 27.04 ± 17.4) months. The average time between the onset of symptoms and diagnosis is 12.12 ± 23.6) months.

Table 1. Clinical features of the SS group

	SS n (%)		SS n (%)
First application complaints		**Joint involvement**	**66 (80.5)**
Dry mouth	24 (29.3)	Large joint (LJ) arthralgia	24
Dry eyes	12 (14.6)	LJ arthritis	4
Dry mouth + eyes	46 (56.1)	Small joint (SJ) arthralgia	5
Minor salivary gland biopsy	**41 (50)**	SJ arthritis	0
none	6	Arthralgia (LJ + SJ)	19
present	35	Arthritis (LJ + SJ)	1
Drugs		Arthritis and arthralgia	13
Methotrexate (Mtx)	9 (11.0)	**Extraarticular involvement**	**26 (31.7)**
Hydroxychloroquine (Hcq)	62 (75.6)	Raynaud syndrome	6
Sulphasalazine (Slz)+Hcq	3 (3.7)	Pulmonary involvement	1
Mtx + Hcq	5 (6.1)	Renal involvement*	14
Mtx + Hcq + Slz	1 (1.2)	Neurological involvement	3
Leflunomide	1 (1.2)	Dermatological + renal involvement	1
Azathioprine	1 (1.2)	Lymphoma	1

SS = Sjögren's syndrome

*According to the 24-hour urine results of SS patients, proteinuria > 150 mg/day was defined as proteinuria, and > 30 mg/day microalbumin detection was defined as microalbuminuria. Renal involvement was accepted in patients with microalbuminuria and proteinuria. 33 patients have 24-hour urine results. Proteinuria was found in 13 patients and microalbuminuria was found in 2 of them.

Extra-articular involvement was present in 26 (31.7%) patients with SS. Among the extra-articular involvement, renal involvement in 14 patients, Raynaud phenomenon in 6, neurological involvement in 3 , lung involvement in 1 and renal and skin involvement were found together in 1 patient. Non-Hodgin Lymphoma (NHL) was observed in only 1 of our patients.

The clinical characteristics of SS patients are shown in Table 1 and the frequency of autoantibodies in Table 2. Anti-CCP value > 20 IU/mL was considered positive. The frequency of anti-CCP positivity shows a significant difference between the SS, RA and control groups ($\chi^2 = 112.176$, $p < 0.001$). In the SS group, 4.9% (n = 4) , in the RA group, 56% (n = 56) were anti-CCP positive. Anti-CCP positivity (n = 0) was not detected in the control group. As a result of binary comparison; Anti-CCP distribution in SS and RA groups ($\chi^2 = 53.283$; $p < 0.001$), SS and control groups ($\chi^2 = 6.488$; $p = 0.011$), RA and control groups ($\chi^2 = 77.778$; $p < 0.001$) was significantly different (Table 2).

Anti-CCP values also differ significantly in the groups ($p < 0.001$). Anti-CCP values are similar in SS and control groups ($p = 0.143$). The median of anti-CCP in the RA group was 68.3 (IQR = 387.4), the median of anti-CCP in the SS and control group was 7.0 (IQR = 0.0), and the median values obtained for RA were significantly different from the other groups ($p < 0.001$) (Tables 3 and 4).

The median value of the erythrocyte sedimentation rate (ESR) variable of the SS group patients is 24.0 (IQR = 26.0), 36.0 (IQR = 36.0) for RA and 14.0 (IQR = 17.0) for the control group. A significant difference was found in terms of ESR variable in SS, RA and control group patients ($p < 0.001$). All pairwise comparison results were significant ($p < 0.05$) (Tables 3 and 4).

C-reactive protein (CRP), rheumatoid factor (RF) variable values differ in the groups. The median of CRP in the RA group was higher than the SS and control group, and the median of CRP in the SS group was higher than the control group ($p < 0.001$). In the RA group, the median of RF was found to be significantly higher than the median obtained for SS and the control

Table 2. The frequency of anti-CCP and other autoantibodies in patients with SS and RA

	SS n (%)	RA n (%)	χ^2	*p* value
Anti-CCP (+)	**4 (4.9)**	**56 (56.0)**	**112.176**	**< 0.001**
ANA (+)	**71 (88.8)**	**30 (33.7)**	**53.080**	**< 0.001**
"anti-dsDNA (+)	**6 (8.1)**	**3 (5.5)**		**0.732***
"Jo1 (+)	**1 (1.4)**	**0 (0.0)**		**1.000***
"Scl70 (+)	**-**	**-**		****
"Sm (+)	**5 (7.1)**	**0 (0.0)**		**1.170***
"SmRnp (+)	**1 (1.4)**	**0 (0.0)**		**1.000***
SS-A (+)	**50 (64.6)**	**2 (5.4)**	**35.531**	**< 0.001**
SS-B (+)	**29 (36.7)**	**1 (2.7)**	**15.198**	**< 0.001**
Anticardiolipin antibody (+)	**1 (3.6)**	**0 (0.0)**		**1.000***
Lupus anticoagulant (+)	**0 (0.0)**	**1 (50.0)**		**1.182***

CCP = cyclic citrullinated peptide, RA = rheumatoid arthritis, SS = Sjögren's syndrome

*Fisher Exact test result. / ** Since the number of subjects is insufficient, the test result cannot be given.

"These values were borderline positive and were not considered significant when evaluated together with the clinic. In the subsequent visits of the patients, especially the anti ds DNA values were found to be negative and no other collagen tissue disease clinic was detected in the patients. The B2 glycoprotein test was not performed at our center at the time of the study.

group ($p < 0.001$ and $p = 0.044$ respectively). RF medians were similar in SS and control groups ($p = 0.142$) (Tables 3 and 4).

No significant relationship between the anti-CCP and the number of joint involvement was detected in patients with SS ($p > 0.05$).

DISCUSSION

Primary SS is associated with many autoantibodies, mainly antinuclear antibody (ANA), anti-SSA, anti-SSB and RF. The frequent association of SS with RA (19.5%) [11] and the high prevalence of RF in SS make it difficult to differentiate RA from SS, especially in patients with arthritis. Anti-CCP is critical in detecting early stage RA patients. Therefore, anti-CCP positive SS patients should be closely monitored for the future development of RA and other overlap syndromes [12, 13]. In our study, the frequency of anti-CCP in primary SS was found to be 4.9%. There are few studies conducted on anti-CCP positivity in primary SS patients.

So far there was only one study in Turkey and it was conducted on a small number of patients [14]. In this study conducted in Istanbul in 2005, the frequency of anti-CCP was examined in 46 RA patients, 32 primary SS and 22 Wegener granulomatosis patient

Table 3. Comparison of ESR, CRP, RF and anti-CCP variables by groups

	Groups				
Variables	**SS Median (IQR)**	**RA Median (IQR)**	**Control Median (IQR)**	χ^2	***p* value**
ESR (mm)	24.0 (26.0)	36.0 (36.0)	14.0 (17.0)	37.475	**< 0.001**
CRP (mg/dL)	0.5 (1.8)	1.6 (3.6)	0.3 (0.5)	67.397	**< 0.001**
RF (IU/mL)	18.1 (25.0)	24.1 (91.8)	20.0 (0.0)	18.559	**< 0.001**
anti-CCP (IU/mL)	7.0 (0.0)	68.3 (387.4)	7.0 (0.0)	113.909	**< 0.001**

CCP = cyclic citrullinated peptide, RA = rheumatoid arthritis, SS = Sjögren's syndrome, ESR = erythrocyte sedimentation rate, CRP = C-reactive protein, RF = rheumatoid factor

Table 4. Pairwise comparison results of ESR, CRP, RF and anti-CCP variables

		RA	Control
		p value	*p* value
ESR	SS	**0.008**	**0.014**
	RA		**< 0.001**
CRP	SS	**< 0.001**	**< 0.001**
	RA		**< 0.001**
RF	SS	**< 0.001**	0.142
	RA		**0.044**
Anti-CCP	SS	**< 0.001**	0.143
	RA		**< 0.001**

CCP = cyclic citrullinated peptide, RA = rheumatoid arthritis, SS = Sjögren's syndrome, ESR = erythrocyte sedimentation rate, CRP = C-reactive protein, RF = rheumatoid factor

groups. Anti-CCP was positive in only 1 patient (3%) of primary SS patients. Our study is the second study conducted in the Turkish population and may reflect the prevalence of anti-CCP in primary SS patients more accurately in terms of the large number of patients. In our study, 4 (4.9%) of 82 primary SS patients were anti-CCP positive and the anti-CCP median was 7.0 (IQR = 0.0).

In the largest of the studies, Ryu *et al*. [15] followed 405 primary SS patients for an average of 60 months. RA developed in 23 (5.6%) patients after follow-up. Similar to our study, the frequency of women was 95.3% and the average age was 52.7 (range: 17-87) years. During the follow-up, 171 (42.2%) patients complained of arthralgia. A total of 73 (18%) patients have arthritis. Anti-CCP was positive in 38 (9.4%) patients. In the follow-up, 42 (10.3%) patients progressed to secondary SS. RA was developed in 23 (5.6%) of secondary SS, SLE in 13 (3.2%), systemic sclerosis in 4 (0.9%) and Behçet's disease in 22 (0.2%). Anti-CCP was positive in all patients who developed RA. Average RA development time was 60 months (7-98 months).

Goeb *et al*. [16] found positive anti-CCP autoantibodies in only 4% of 137 women and 16% of 11 male patients with primary SS. In a study conducted by Gottenberg *et al*. [17] in 134 patients with primary SS, anti-CCP antibodies were found positive with a rate of 7.5% and anti-keratin antibodies with a rate of 5.2%. Other studies have shown that anti-CCP antibodies were detected positively in a minority of primary SS patients, with or without erosive arthritis [14, 18-20].

Thirty-eight percent of primary SS cases have arthralgia and arthritis [21]. Chronic symmetrical arthritis rate in the small joints of the hand and wrist are 16%, and erosion is present on direct radiography in 1.5% of those with chronic arthritis [22]. In clinical practice, in the patient who applied with arthritis; anti-CCP positivity and presence of erosion on plain X-ray are interpreted in favor of RA in the differentiation of primary SS and RA. However, anti CCP can be detected positively in 3-22.1% of primary SS patients. Conflicting results have been found in studies on the clinical importance of anti-CCP positivity in primary SS. While some of them found that the incidence of arthritis increased in those who were anti CCP positive, other studies do not support this [12, 13, 23-25]. In two studies, one with joint ultrasonography in adults and the other in children, a correlation was found between anti-CCP positivity and arthritis frequency in primary SS [23, 24]. A systematic review and meta-analysis showed that anti-CCP positivity is associated with the development of arthritis and further progression to RA [12]. In a prospective study by Haga *et al*. [25], no correlation was found between anti-CCP and arthritis. In this study, only 5 of 62 primary SS cases were found to be anti-CCP positive. In our study, anti-CCP was found only in four of 82 cases, and similarly, no significant relationship was found with anti-CCP in terms of arthritis development.

Anti-CCP positive SS patients should be closely monitored for future risk of development of RA or any other rheumatic disease. In addition, the value of ACPA tests other than anti-CCP (such as anti-citrulline a-enolase antibody and anti-Ro) is gradually increasing [26]. Especially in the treatment of ACPA positive diseases; the blockade of the Peptidyl Arginine Deiminase (PAD) enzyme involved in the citrullination process may affect the course of the disease and prevent joint involvement.

Limitations

Limitation of our study was mean disease duration of sjogren syndrome patients were short (27-28 months). Anti-CCP antibody positivity can be found in SS patients. Acute phase proteins may be higher in

primary SS patients with positive anti-CCP antibody. However, studies have found conflicting findings about the prevalence of erosive arthritis and the future development of RA in these patient groups.

CONCLUSION

Anti-CCP antibody positivity can be found in SS patients. Acute phase proteins may be higher in primary SS patients with positive anti-CCP antibody. However, studies have found conflicting findings about the prevalence of erosive arthritis and the future development of RA in these patient groups.This study is the largest study in determining the prevalence of anti-CCP in primary SS in terms of the number of individuals conducted in the Turkish population. There was no significant relationship between anti-CCP and joint findings. It is necessary to study more patients to determine whether there is a significant relationship between anti-CCP and other clinical findings.

Authors' Contribution

Study Conception: TAK, ŞE; Study Design: İD, OK; Supervision: OK, ŞE; Funding: N/A; Materials: N/A; Data Collection and/or Processing: TAK, İD; Statistical Analysis and/or Data Interpretation: TAK, İD; Literature Review: TAK, İD; Manuscript Preparation: TAK, OK and Critical Review: OK, ŞE.

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REFERENCES

1. Skopouli FN, Dafni U, Ioannidis JP, Moutsopoulos HM. Clinical evolution, and morbidity and mortality of primary Sjögren's syndrome. Semin Arthritis Rheum 2000;29:296-304.
2. Theander E, Jacobsson LT. Relationship of Sjögren's syndrome to other connective tissue and autoimmune disorders. Rheum Dis Clin North Am 2008;34:935-47.
3. Bizzaro N, Mazzanti G, Tonutti E, Villalta D, Tozzoli R. Diagnostic accuracy of the anti-citrulline antibody assay for rheumatoid arthritis. Clin Chem 2001;47:1089-93.
4. Schellekens GA, Visser H, de Jong BAW, van den Hoogen FH, Hazes JM, Breedveld FC, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing anti-cyclic citrulinated peptide. Arthritis Rheum 2000;43:155-63.
5. Visser H, le Cessie S, Vos K, Breedveld FC, Hazes JM. How to diagnose rheumatoid arthritis early? A prediction model for persistent (erosive) arthritis. Arthritis Rheum 2002;46:357-65.
6. Vincent C, Nogueira L, Clavel C, Sebbag M, Serre G. Autoantibodies to citrullinated proteins: ACPA. Autoimmunity 2005;38:17-24.
7. Kwok JS, Hui KH, Lee TL, Wong W, Lau YL, Wong RWS, et al. Anti-cyclic citrullinated peptide: diagnostic and prognostic values in juvenile idiopathic arthritis and rheumatoid arthritis in a Chinese population. Scand J Rheumatol 2005;34:359-66.
8. Kakumanu P, Yamagata H, Sobel ES, Reeves WH, Chan EKL, Satoh M. Patients with pulmonary tuberculosis are frequently positive for anti-cyclic citrullinated peptide antibodies, but their sera also react with unmodified arginine-containing peptide. Arthritis Rheum 2008;58:1576-81.
9. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002;61:554-8.
10. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
11. Alani H, Henty JR, Thompson NL, Jury E, Ciurtin C. Systematic review and meta-analysis of the epidemiology of polyautoimmunity in Sjögren's syndrome (secondary Sjögren's syndrome) focusing on autoimmune rheumatic diseases. Scand J Rheumatol 2018;47:141-54.
12. Molano-González N, Olivares-Martínez E, Anaya JM, Hernández-Molina G. Anti-citrullinated protein antibodies and arthritis in Sjögren's syndrome: a systematic review and meta-analysis. Scand J Rheumatol 2019;48:157-63.
13. Maślińska M, Mańczak M, Wojciechowska B, Kwiatkowska B. The prevalence of ANA antibodies, anticentromere antibodies, and anti-cyclic citrullinated peptide antibodies in patients with primary Sjögren's syndrome compared to patients with dryness symptoms without primary Sjögren's syndrome confirmation. Reumatologia 2017;55:113-9.
14. Kamali S, Polat NG, Kasapoglu E, Gul A, Ocal L, Aral O, et al. Anti-CCP and antikeratin antibodies in rheumatoid arthritis, primary Sjögren's syndrome, and Wegener's granulomatosis. Clin Rheumatol 2005;24:673-6.
15. Ryu YS, Park SH, Lee J, Kwok SK, Ju JH, Kim HY, et al. Follow-up of primary Sjogren's syndrome patients presenting positive anti-cyclic citrullinated peptides antibody. Rheumatol Int 2013;33:1443-6.
16. Goeb V, Salle V, Duhaut P, ouen F, Smail A, Ducroix JP, et al. Clinical significance of autoantibodies recognizing Sjögren's syndrome A (SSA), SSB, calpastatin and alpha-fodrin in primary Sjögren's syndrome. Clin Exp Immunol 2007;148:281-7.
17. Gottenberg JE, Mignot S, Nicaise-Rolland P, Cohen-Solal J,

Aucouturier F, Goetz J, et al. Prevalence of anti-cyclic citrullinated peptide and anti-keratin antibodies in patients with primary Sjögren's syndrome. Ann Rheum Dis 2005;64:114-7.
18. Atzeni F, Sarzi-Puttini P, Lama N, Bonacci E, Bobbio-Pallavicini F, Montecucco C, et al. Anti-cyclic citrullinated peptide antibodies in primary Sjögren syndrome may be associated with non-erosive synovitis. Arthritis Res Ther 2008;10: R51.
19. Iwamoto N, Kawakami A, Tamai M, Fujikawa K, Arima K, Aramaki T, et al. Determination of the subset of Sjogren's syndrome with articular manifestations by anticyclic citrullinated peptide antibodies. J Rheumatol 2009;36:113-5.
20. Mohammed K, Pope J, Le Riche N, Brintnell W, Cairns E, Coles R, et al. Association of severe inflammatory polyarthritis in primary Sjögren's syndrome: clinical, serologic, and HLA analysis. J Rheumatol 2009;36:1937-42.
21. Mariette X, Criswell LA. Primary Sjögren's syndrome. N Engl J Med 2018;378:931-9.
22. Fauchais AL, Ouattara B, Gondran G, Lalloue F, Petit D, Ly K, et al. Articular manifestations in primary Sjögren's syndrome: clinical significance and prognosis of 188 patients. Rheumatology (Oxford) 2010;49:1164-72.
23. Guedes LKN, Leon EP, Bocate TS, Bonfiglioli KR, Lourenço SV, Bonfa E, et al. Characterizing hand and wrist ultrasound pattern in primary Sjögren's syndrome: a case-control study. Clin Rheumatol 2020;39:1907-18.
24. Shabana K, Okamoto N, Sugita Y, Shindo K, Murata T, Tamai H, et al. The findings of musculoskeletal ultrasonography on primary Sjögren's syndrome patients in childhood with articular manifestations and the impact of anti-cyclic citrullinated peptide antibody. Mod Rheumatol 2019;29:821-8.
25. Haga HJ, Andersen DT, Peen E. Prevalence of IgA class antibodies to cyclic citrullinated peptide (anti-CCP) in patients with primary Sjögren's syndrome, and its association to clinical manifestations. Clin Rheumatol 2011;30:369-72.
26. Olivares-Martínez E, Hernández-Ramírez DF, Núñez-Álvarez CA, Liorente L, Hernandez-Molina G. α-enolase is an antigenic target in primary Sjögren's syndrome. Clin Exp Rheumatol 2019;37 Suppl 118:29-35.
27. Tobón GJ, Correa PA, Anaya JM. Anti-cyclic citrullinated peptide antibodies in patients with primary Sjögren's syndrome. Ann Rheum Dis 2005;64:791-2.
28. van Noord C, Hooijkaas H, Dufour-van den Goorbergh BC, van Hagen PM, van Daele PLA, van de Merwe JP. Diagnostic value of anti-cyclic citrullinated peptide antibodies to detect rheumatoid arthritis in patients with Sjögren's syndrome. Ann Rheum Dis 2005;64:160-2.
29. Barcelos F, Abreu I, Patto JV, Trindade H, Teixeira A. Anti-cyclic citrullinated peptide antibodies and rheumatoid factor in Sjögren's syndrome. Acta Reumatol Port 2009;34:608-12.
30. Kim SM, Park E, Lee JH, Lee SH, Kim HR. The clinical significance of anti-cyclic citrullinated peptide antibody in primary Sjögren syndrome. Rheumatol Int 2012;32:3963-7.

The European Research Journal 2022;8(3):354-358 Original Article

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Conjunctival suture internal or external knotting in strabismus surgery: effect on healing and comfort

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ABSTRACT

Objectives: To evaluate the effectiveness of the conjunctival suture tied internally and externally on patient comfort and suture dissolution in the postoperative period in patients who underwent symmetrical strabismus surgery.

Methods: In 50 patients who underwent surgery in the Beyoğlu Eye Training and Research Hospital Strabismus Unit, the conjunctiva was closed by tying the conjunctival suture externally in the right eye and internally tying the left eye. Absorbable 8/0 polyglactin suture was used for conjunctival suturing in all patients. Pain, itching, tearing and, discomfort sensations of the patients were questioned and a slit-lamp examination for conjunctival inflammation and dissolution of the suture were evaluated on the postoperative 1st day, 1st week, 1st month, and 6th weeks.

Results: Twenty-two patients were female and 28 were male. The mean age of the patients was 7.24 ± 4.83 (range: 4-17) years. When the pain, stinging and discomfort of the patients were questioned, 46% of the patients stated that they felt more comfortable in the left eye on the 1st and 10th days, while 48% of the patients stated that they were equal in both eyes. When we evaluated the melting of the suture and conjunctival redness, it was observed that the suture in the left eye was melted prematurely at a rate of 40% and, the conjunctival redness resolved early in the first month. There were equal pain and redness in both eyes at a rate of 54%.

Conclusions: In strabismus surgery, the closure of the conjunctiva by tying the suture internally is thought to increase patient comfort in the postoperative period.

Keywords: Conjunctiva, suture, melting, irritation

The polyglactin 8-0 suture is a frequently used in ocular surgery and is especially preferred for closing the conjunctiva. This suture is preferred for conjunctival closure also in strabismus surgery. It has a average 56-70 days absorption rate. Due to the small diameter of this suture, and its non-antigenic structure, the fact that it causes less tissue reaction compared to other materials, but it can also cause discomforts like itching and foreign body sensation especially in the early postoperative period. These complications include prolonged inflammatory reaction at the suture site, suture granuloma or abscess and, giant papillary conjunctivitis [1, 2]. To avoid these drawbacks, conjunctival suture with internal ligation can be applied.

The aim of this study is to compare the absorbtion rate of the conjunctival suture knotted internally and

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externally and to evaluate its effect on patient comfort.

METHODS

Hundred eyes of 50 patients who underwent bilateral symmetrical surgery were included in the study. Ethics committee approval was obtained from the Okmeydani Training and Research Hospital. This study was conducted in accordance with the principles of the Declaration of Helsinki and written informed consent was obtained from the patients/parents following a detailed explanation of the study objectives and protocol. This study was conducted at our hospital from 2017 to 2019.

The suture material was 8-0 polyglactin 910 (Vicryl®; Johnson & Johnson, Livingston, UK) Externally conjunctival suture tying was used to close conjunctival incisions in the right eye and, internally tying in the left eye of 50 patients during strabismus surgery. At the end of the operation, eyes were closed with antibiotic pomade. Antibiotic and corticosteroid combination eyedrops (tobramycin 0.3%+dexamethasone 0.1% mg) were continued 4 times a day for 3 weeks.

Postoperative follow-ups were done with a slit-lamp examination performed on the 1st day, 1st week, 1st month, and 6th week. The conjunctival redness, inflammation and absorbtion of the suture were determined by slit-lamp examination and recorded. Ocular irritation scores questioning itching, pain and, foreign body reaction were asked to all patients and their parents of incompatible age group patients. The subjective discomfort scale was graded from 0-to-3 according to the answers given by the patient (0, total comfort; 1, minor discomfort; 2, moderate discomfort; 3, severe discomfort) [3-5].

Objective evaluation of conjunctival inflammation was made by the same physician(CG) by examining the operated conjunctival quadrant using a slit-lamp biomicroscope. A modified conjunctival inflammatory index (Table 1) in which hyperemia, chemosis, and discharge were rated on a 0 to 3 scale (0, none; 1, mild; 2, moderate; 3, severe) for a maximum possible inflammation score of 9 used for grading [5-7].

Surgical Technique

Horizontal strabismus surgery under general anesthesia was performed by the same surgical team. After making a fornix-based two radial limbal conjunctival incisions, performed bilateral symmetrical extraocular medial or lateral rectus muscle recession was performed. The conjunctival wound was closed in both eyes with 2-3 stitches of interrupted 8-0 polyglactin suture after completing the muscle surgery. In the right eyes with external knot suture, the conjunctiva was closed with a conventional method. Loose ends of the knot were cut short. In the left eyes with an internal knot, the first pass of the suture started under the conjunctiva, passed through the opposite side conjunctiva from above, and was removed from under the conjunc-

Table 1. Conjunctival inflammation grades

	Scales	Values
Conjunctival hyperemia	0	Absent
	1	Mild hyperemia
	2	Hyperemia around the wounds
	3	Hyperemia around the wounds and entire quadrant
Conjunctival discharge	0	Absent
	1	Small discharge on conjunctiva
	2	Discharge on conjunctiva and cornea
	3	Discharge on conjunctiva, cornea, and eyelid
Conjunctival chemosis	0	Absent
	1	Mild chemosis
	2	Chemosis around the wounds
	3	Chemosis around the wounds and entire quadrant

tiva and tied there. The suture was cut just above the knot without allowing any suture tip to be exposed.

Statistical Analysis

Statistical analysis was performed with SPSS 20 pocket program (SPSS Inc., Chicago, IL, USA). In the evaluation of data, repeated statistical methods as well as paired t-test, variance analysis of multiple groups, Pearson correlation test for the relationship between variables were used. A *p* - value of less than 0.05 was considered statistically significant.

RESULTS

The mean age of the patients was 7.24 ± 4.83 (range: 4-17) years. Twenty-two patients were female and 28 were male. Mean total surgery times were similar in both eyes (17 ± 4 minutes). When the pain, tearing and, discomfort of the patients were questioned, 46% of the patients stated that they felt more comfortable in the left eye on the 1st and 10th days, while 48% of the patients stated that they were equal in both eyes. When we evaluated the dissolution of the suture and conjunctival redness, it was observed that the suture in the left eye was melted prematurely at a rate of 40%, the conjunctival redness resolved early in the first month. There was equal redness in both eyes at a rate of 54%. Table 1 shows the ocular irritation scoring.

Postoperatively at 1st day and 1st week, the pain and tearing scores were lower in the left eyes than in the right eyes, but it was not significant ($p > 0.05$) (Figs. 1 and 2). The mean duration of discomfort was 2.9 ± 0.8 days in the left eye and 3.2 ± 1.2 days in the right eyes ($p > 0.05$). The inflammation was lower in the left eyes for the first month ($p > 0.05$) (Fig. 3).

Conjunctival incisions had successfully closed in both eyes of all patients in the 1st month postoperatively. Tenon's capsule prolapse through the conjunctival incisions, scarring, allergic reaction or infection was not occurred in none of patients.

DISCUSSION

Proper closure of the conjunctiva is essential for successful strabismus surgery. The suture material to be selected is important for this. Polyglactin 8-0 sutures are commonly used. Although this suture is a material that can absorbe, it can cause irritation and discomfort during the early postoperative period. This may delay the patient's return to his/her normal life and cause loss of workforce in adult patients, and difficulty in opening eyes and restlessness in young children [5, 8, 9].

There are a lot of studies about conjunctiva closing with suturation versus fibrin glue [5-14]. These studies had shown although conjunctiva healing pocess takes a similar time course in both procedure; that the conjunctival inflammation and the severity of pain, tearing and discomfort experienced by patients was significantly less for the fibrin glue group especially

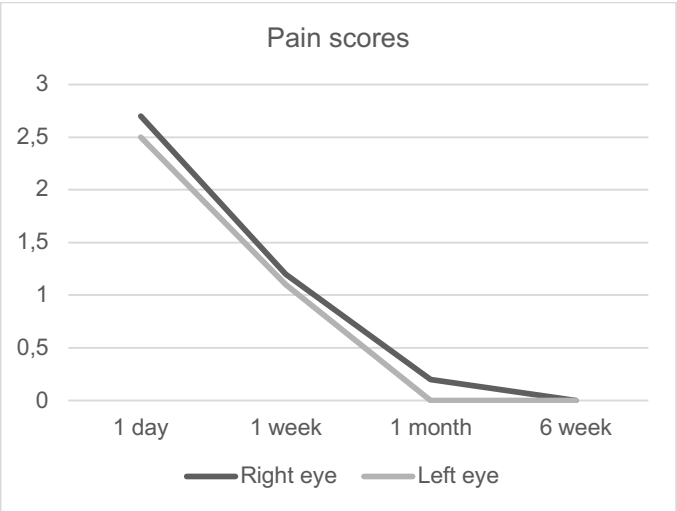

Fig. 1. Mean postoperative pain scores at four follow-up periods. *$p > 0.05$

Fig. 2. Mean postoperative tearing scores at four follow-up periods *$p > 0.05$

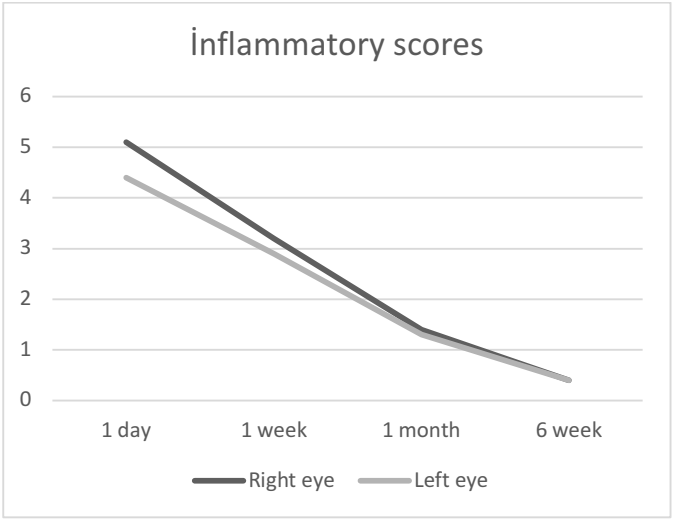

Fig. 3. Mean postoperative inflammatory scores at four follow-up periods. *$p > 0.05$

at the first 24 hours after surgery.

Despite these advantages, fibrin glue could not be used daily in strabismus surgery due to its high cost [12, 15, 16]. And also fibrin glue can be to cause conjunctival retraction [5, 8, 10]. Because of this situation, conjunctival suture with internal knotting can be used for patients' comfort after surgery. This procedure has not statistically significant results, but it can be applied easily and it does not affect the surgery time. Escardo´-Paton and Harrad [17] have shown that the median duration of redness was 9.5 weeks in conjunctiva closed with 6.0 vicryl after adult strabismus surgeries. In other studies have shown that the conjunctival redness persists for an average of 4-5 weeks [3, 5, 11, 12]. The present study found similar results for conjunctival redness in both eyes with 4 weeks. There are no studies in the literature on the internal knotting closure of the conjunctiva. In the present study has been found that the inflammation was lower in the left eyes for the first month.

CONCLUSION

In surgeries that mostly affect the external appearance of patients, such as strabismus surgery, it is important to close the conjunctiva in terms of both providing a visually pleasing image and postoperative patient comfort. Therefore, it is easy to apply and contributes to postoperative patient comfort; we recommend tying the suture internally while closing the conjunctiva in strabismus surgery.

Authors' Contribution

Study Conception: CG, ZH, BG; Study Design: CG, ZH, BG; Supervision: CG, BG; Funding: CG, ZH; Materials: CG, ZH, BG; Data Collection and/or Processing: CG, ZH, BG; Statistical Analysis and/or Data Interpretation: CG, ZH; Literature Review: CG, BG; Manuscript Preparation: CG and Critical Review: CG, ZH, BG.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Coats DK, Olitsky SE. Strabismus surgery and its complications. Berlin: Springer-Verlag; 2007: p.190.
2. Arana LA, Ramos Moreira AT, Grandinetti AA, Moreira H, Moreria Jr CA. Novel vicryl releasable suture technique to close leaking sclerotomies in a transconjunctival vitrectomy. Retina 2019;39 Suppl 1:S108-11.
3. Apt L, Voo I, Isenberg SJ. A randomized clinical trial of the nonsteroidal eyedrop diclofenac after strabismus surgery. Ophthalmology 1998;105:1448-52.
4. Kim TS, Kang NY. Effect of topical nonsteroidal anti-inflammatory drugs versus topical steroids after strabismus surgery. J Korean Ophthalmol Soc 2003;44:115-20.
5. Lee JH, Kang NY. Comparison of fibrin glue and sutures for conjunctival wound closure in strabismus surgery. Korean J Ophthalmol 2011;25:178-84.
6. Alio JL, Gomez J, Mulet E, Bujanda MMM, Martinez JMM, Molina Y. A new acrylic tissue adhesive for conjunctival surgery: experimental study. Ophthalmic Res 2003;35:306-12.
7. Ryu WY, Yoo KW. Comparison of 2-octylcyanoacrylate and suture with 8-0 polyglactin for conjunctival wound closure in rabbits. J Korean Ophthalmol Soc 2005;46:896-903.
8. Erbağcı İ, Bekir N. Sutureless closure of the conjunctiva with a commercial fibrin sealant in extraocular muscle surgery for strabismus. Strabismus 2007;15:89-94.
9. Basmak H, Gursoy H, Cakmak AI, Niyaz L, Yildirim N, Sahin A. Tissue adhesives as an alternative for conjunctival closure in strabismus surgeries. Strabismus 2011;19:59-62.
10. Biender B, Rosenthal G. Conjunctival closure in strabismus surgery: vicryl versus fibrin glue. Ophthalmic Surg Lasers 1996; 27:976.
11. Dadeya S, Kamlesh MS. Strabismus surgery: fibrin glue ver-

sus vicryl for conjunctival closure. Acta Ophthalmol Scand 2001;79:515-7.
12. Yang MB, Melia M, Lambert SR, Chiang MF, Simpson JL, Buffenn AN. Fibrin glue for closure of conjunctival incision in strabismus surgery. A report by the American Academy of Ophthalmology. Ophthalmology 2013;120:1935-41.
13. Graham SL, Murray B, Goldberg I. Closure of fornix based post-trabeculectomy conjunctival wound leaks with autologous fibrin glue. Am J Ophthalmol 1992;114: 221-2.
14. Spierer A, Barequet I, Rosner M, Solomon AS, Matinowitz U. Reattachment of extra-ocular muscles using fibrin glue in a rabbit model. Invest Ophthalmol Vis Sci 1997;38:543-6.
15. Zarbin M. Declining use of sutures for wound closure. Arch Ophthalmol 2012;130:1596.
16. Chaurasia SS, Champakalakshmi R, Angunawela RI, Tan DT, Mehta JS. Optimization of fibrin glue spray systems for ophthalmic surgery. Transl Vis Sci Technol 2012;1:2.
17. Escardo´-Paton JA, Harrad RA. Duration of conjunctival redness following adult strabismus surgery. J AAPOS 2009;13:583-6.

The European Research Journal 2022;8(3):359-367 Original Article

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The comparison of maternal and neonatal outcomes between emergency and planned cesarean deliveries in women with placenta previa

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ABSTRACT

Objectives: To compare maternal and neonatal outcomes concerning emergency or planned cesarean deliveries in pregnancies complicated by placenta previa (PP), and to evaluate factors related to blood transfusion requirement.

Methods: Three hundred sixty-three women with PP with (n = 80) and without (n = 283) placenta accreta spectrum (PAS) who delivered between May 2016 and May 2021 were retrospectively reviewed. The patients were allocated to two main groups as PAS and non-PAS and into two subgroups as emergency cesarean delivery (ECD) and planned cesarean delivery (PCD).

Results: One hundred twenty-eight deliveries were emergency and 155 were planned in non-PAS group. In PAS group 38 patients were delivered urgently and 42 were delivered as planned. General anesthesia was preferred more frequently in emergency cases. Gestational age, birth weight, and the 1st and 5th minute APGAR scores of the infants were significantly lower and neonatal intensive care unit (ICU) admission was significantly higher in the ECD cases ($p < 0.001$) in both PAS and non-PAS groups. The total amount of blood and blood product transfused ($p = 0.005$), length of hospital stay ($p = 0.022$) were higher in the ECD cases and adult ICU admission was significantly higher in the ECD cases in non-PAS group ($p = 0.016$). In multilinear regression analysis, the need for blood transfusion was found to increase with the number of previous cesarean sections, ECD, PP with PAS, general anesthesia, and uterine artery ligation.

Conclusions: In placenta previa, which is an obstetric condition associated with serious maternal and neonatal morbidity and mortality, adverse maternal and neonatal outcomes increase in cases of emergency cesarean delivery.

Keywords: Planned cesarean delivery, emergency cesarean delivery, hysterectomy, placenta previa, placenta accreta spectrum, blood transfusion

Placenta previa (PP), a unique obstetric condition associated with maternal and neonatal morbidity and mortality due to the potential for serious antenatal bleeding, peripartum hemorrhage, and preterm delivery, is defined as the extension and closure of the placental tissue on the internal cervical os [1]. The most accepted explanation in the etiology is the absence of the phenomenon called "trophotropism", atrophy, and

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migration in the natural development of the placenta, due to a defect in the endometrium caused by previous surgery, especially the decreased vascularity in the scar area of a previous cesarean section, which leads the placenta to settle in the lower uterine segment. PP was reported to complicate 0.38% of singleton pregnancies in the 2000s [2], and its prevalence has been reported as 0.56% in recent years [3]. This increasing trend is associated with the increasing rates of cesarean delivery in a dose-response manner [4]. Other risk factors are advanced maternal age, multiparity, smoking, multiple pregnancies, assisted reproductive technology (ART) use, and recurrent pregnancy loss [2, 5]. PP cases are at increased risk of intrapartum blood loss due to the inability of the lower uterine segment, where abnormal placental implantation occurs, to contract as effectively as the normal uterine segment, the possibility of transecting the placenta during uterine incision, and the increased risk of placenta accreta [6].

It should be kept in mind that the possibility of placenta accreta spectrum (PAS) increases if PP is detected in a pregnant woman with a previous cesarean section. PAS (accreta, increta, percreta) is a spectrum disorder ranging from abnormal adhesion to deep invasion, which is clinically important because the placenta does not separate spontaneously at delivery and causes life-threatening bleeding and necessitates blood transfusion, intensive care unit (ICU) admission and even hysterectomy [7]. It has been reported that PP is associated with a three to five-fold increase in the risk of preterm birth, admission to the neonatal intensive care unit (NICU), neonatal and perinatal death [8]. The main causes of neonatal morbidity and mortality are problems related to preterm birth [9].

The aim of our study was to compare the effects of an emergency or planned cesarean delivery on maternal and neonatal outcomes in both PAS and non-PAS PP cases, and secondarily to evaluate factors affecting the need for blood transfusion in these patients.

METHODS

After the approval of Bursa Yüksek İhtisas Training and Research Hospital Clinical Research Ethics Committee (2011-KAEK-25 2021/04-10), 363 women with PP who delivered between May 2016 and May 2021 were retrospectively reviewed. Age, body mass index (BMI), obstetric history (gravida, parity, abortion, previous uterine surgery, number of prior cesarian deliveries), gestational age at delivery, delivery under emergency or elective conditions, type of anesthesia used, surgical maneuvers to control bleeding, intraoperative complications, pathology results in patients who underwent cesarean hysterectomy and lower segment resection, re-laparotomy, amount of blood transfusion, need for follow-up in the adult ICU, hospital length of stay, postoperative complications, infants birth weight, sex, 1st and 5th minute Apgar scores, NICU administration, and neonatal death were noted. Singleton pregnancies complicated by PP after the 24th gestational week were included in the study. Pregnancies with intrauterine fetal death, fetal anomalies, multiple gestations, and insufficient data were excluded.

Patients in whom peripartum ultrasound findings were compatible with an invasion of the placental tissue (loss or irregularity of the retroplacental hypoechoic plane 'clear zone', myometrial thinning < 1 mm, abnormal placental lacunae with turbulent flow, bladder wall interruption, placental bulge) and the invasion was confirmed by the intraoperative difficult manual removal of the placenta or the pathologic examination of the hysterectomy and segmental resection materials were defined as the PAS group. Patients in whom the placenta covered the internal cervical os but without signs of invasion were defined as the non-PAS group. The antenatal diagnosis was obtained from medical records. The definition of emergency and planned cesarean section was made according to the patient's clinic at the time of administration for delivery, regardless of the gestational week (GW), prenatal diagnosis, and the working hours and shift hours of the hospital. The elective surgery of patients who were diagnosed by ultrasound during pregnancy follow-up, were hemodynamically stable, and did not have active vaginal bleeding and uterine contractions was defined as PCD. Emergency surgery was defined as surgery performed for uterine contraction, vaginal bleeding and hemodynamic instability, regardless of prenatal diagnosis.

Gestational age was calculated from the last menstrual period or first-trimester ultrasound. In our clinic, we mostly progress to surgery at 36 0/7-37 6/7 weeks of gestation for women with PP cases without PAS and 34 0/7–35 6/7 weeks of gestation for PP with PAS as

recommended by the American College of Obstetricians and Gyneclogosits (ACOG) [10]. Antenatal corticosteroids were not administered to the majority of the patients because those who were admitted for a planned section were > 34 weeks, and patients who underwent emergency cesarean section did not have time to wait for appropriate efficacy.

Surgical interventions to control bleeding after pharmacologic measures were suture of the placental bed, uterine artery ligation, internal iliac artery ligation (IIAL), balloon tamponade, and B-Lynch suturing according to the depth of invasion. Rapid use of radiologic interventions such as transarterial embolisation was not possible under the conditions of our hospital, thus they were not used in any of the cases. Segmental resection was performed in cases where the depth and surface area of the placenta accreta was limited and the entire placenta implantation area was accessible and visualizable. Hysterectomy was performed in cases where there was deep invasion and surgical maneuvers were insufficient. However, planned or not, patients who completed their fertility, had deep invasion in the preoperative (prior to the delivery) evaluation and were predicted to have massive bleeding underwent with the decision of preoperative cesarean section hysterectomy. The abdomen was entered through a mid-line vertical incision, the baby was delivered by performing a fundal longitudinal hysterotomy, the placenta was left in-situ, the uterine incision was closed, and hysterectomy was performed after bilateral IIAL to minimize blood loss.

Due to the retrospective design of the study and because the majority of patients received intraoperative and postpartum blood and blood product transfusions, the change in hemoglobin levels was not evaluated due to the high probability of bias.

Statistical Analysis

All statistical analyses were performed using the IBM SPSS Statistics version 20 (Chicago, IL). The normality of the distribution of continious variables was assessed using the Kolmogorov-Smirnov test. Categorical variables were assessed using the Chi-square test. Student's t-test was used for normally distributed continuous variables, and the Mann–Whitney U test was used for non-normally distributed variables. Multivariate logistic regression analysis was performed to identify risk factors for blood transfusion requirements. The results are reported as odds ratio (OR) and 95% confidence intervals (95% CI).

RESULTS

In this retrospective study spanning 5 years in our clinic, which is a referral tertiary center where the annual number of deliveries is approximately 12,000, the prevalence of PP was found as approximately 0.6% with 363 PP cases. Further evaluation showed that the rate of PAS cases among patients with PP was 22% (80/363). In this study, which included 128 emergency and 155 planned cesarean sections in non-PAS group and 38 emergency and 42 planned cesarean sections in PAS group, the mean maternal age was 32 years. Although no difference was observed between the groups in terms of demographic and clinical characteristics, general anesthesia was preferred in emergency cases in both groups, whereas spinal anesthesia was used in planned cases (Table 1).

Neonatal outcomes; In both non-PAS and PAS groups, gestational age at delivery, birth weight and 1st and 5th minute APGAR scores were significantly lower and NICU admission was significantly higher in the ECD cases ($p < 0.001$) (Table 2).

Of the 283 non-PAS patients, 131 delivered preterm (< 37th GW) and 152 delivered term (> 37th GW) infants. While 70.2% of preterm deliveries were ECD, 23.7% of term fetuses were delivered urgently ($p < 0.001$). Fourty-nine patients delivered before 37th GW and 31 patients delivered after 37th GW in PAS group. While 61.2% of preterm deliveries were emergency cesarean, 25.8% of term fetuses were delivered urgently ($p = 0.003$). Birth weight, 1st and 5th minute APGAR scores were significantly lower and NICU admission was significantly higher in infants born before 37 weeks of gestation in both PAS and non-PAS groups ($p < 0.001$) (Table 3).

Intraoperative maneuvers performed to control bleeding did not differ in both groups whether the cases were planned or emergency. The need for hysterectomy and re-laparotomy was higher in women with PAS, regardless of whether they were emergencies or planned. Although there was no difference in terms of intraoperative complications between the groups, all of the seven bladder injuries were in PAS group, five in the ECD and two in the PCD cases. The

Table 1. Demographics and baseline characteristics

		PAS negative (n = 283)			PAS positive (n = 80)		
	Total cohort (n = 363)	ECD (n = 128)	PCD (n = 155)	*p* value	ECD (n = 38)	PCD (n = 42)	*p* value
Materal age (year)	32 (16-45)	31.14 ± 5.5	32.19 ± 5.76	0.12	30.39 ± 6.93	32.07 ± 5.37	0.228
BMI (kg/m2)	25 (16.5-42)	24.82 (16.5-42)	25.50 (17.10-41.50)	0.604	25.33 ± 4.21	26.99 ± 5.73	0.148
Gravity	3(1-14)	3(1-8)	3 (1-14)	0.498	3 (1-8)	3 (1-10)	0.167
Parity	2 (0-7)	2 (0-7)	2 (0-7)	0.463	2 (0-5)	2 (0-6)	0.263
No. of curretage	0 (0-6)	0 (0-5)	0 (0-6)	0.909	0 (0-6)	0 (0-3)	0.613
Comorbidity				0.382			0.338
None	344 (94.8)	117 (91.4)	148 (95.5)		38 (100)	41 (97.6)	
GDM	6 (1.7)	3 (2.3)	2 (1.3)			1 (2.4)	
GHT	7 (1.9)	4 (3.1)	3(1.9)				
Hypothyroidy	1 (0.3)	-	1 (0.6)				
Hydronephrosis	1 (0.3)	-	1 (0.6)				
Hearth Disease	1 (0.3)	1 (0.8)	-				
Preeclampcia	2 (0.6)	2 (1.6)	-				
Covid-19	1 (0.3)	1 (0.8)	-				
No. of cesarean	1 (0-5)	0 (0-5)	0 (0-4)	0.469	2 (0-2)	2 (0-4)	0.657
Previous myomectomy				0.527			0.051
Yes	13 (3.6)	5 (3.9)	4 (2.6)		-	4 (9.5)	
No	350 (96.4)	123 (96.1)	151 (97.4)		38 (100)	38 (90.5)	
Anesthesia method				**< 0.001**			**0.026**
Spinal anesthesia	201 (55.4)	65 (50.8)	114 (73.5)		6 (15.8)	16 (38.1)	
General anesthesia	162 (44.6)	63 (49.2)	41 (26.5)		32 (84.2)	26 (61.9)	

Data presented as mean ± SD, n (%) or median (min-max). *P* value < 0.05 was statistically significant. SD = standard deviation, PAS = Placenta accrete spectrum, ECD = emergent cesarean delivery, PCD = planned cesarean delivery, BMI = body mass index, GDM = gestational diabetes mellitus, GHT = gestational hypertension.

Table 2. Neonatal outcomes

		PAS negative (n = 283)			PAS positive (n = 80)		
	Total cohort (n = 363)	ECD (n = 128)	PCD (n = 155)	*p* value	ECD (n = 38)	PCD (n = 42)	*p* value
Gestational age	37 (25-40)	35 (25-39)	37 (34-40)	**< 0.001**	34 (25-40)	37 (25-40)	**< 0.001**
Birth weight (g)	2840 (600-4100)	2557.5 (600-3850)	3000 (1920-4100)	**< 0.001**	2100.13 ± 796.40	2833.21 ± 545.47	**< 0.001**
Fetal sex				0.800			0.535
Female	170 (46.8)	60 (46.9)	75 (48.4)		18 (47.4)	17 (40.5)	
Male	193 (53.2)	68 (53.1)	80 (51.6)		20 (52.6)	25 (59.5)	
1st min Apgar	9 (2-9)	9 (2-9)	9 (7-9)	**< 0.001**	8 (2-9)	9 (4-9)	**< 0.001**
5th min Apgar	10 (5-10)	10 (5-10)	10 (9-10)	**< 0.001**	9 (5-10)	10 (6-10)	**0.01**
Admission to NICU				**< 0.001**			**0.001**
Yes	67 (18.5)	36 (28.1)	7 (4.5)		18 (47.4)	6 (14.3)	
No	296 (81.5)	92 (71.9)	148 (95.5)		20 (52.6)	36 (85.7)	

Data presented as mean ± SD, n (%) or median (min-max). *P* values < 0.05 were statistically significant. SD = standard deviation, PAS = placenta accreta spectrum, ECD = emergency cesarean delivery, PCD = planned cesarean delivery, NICU = neonatal intensive care unit.

total amount of blood and blood product transfused was significantly higher and hospital stay was longer ($p = 0.022$) in patients with ECD with or without PAS ($p = 0.005$) and adult ICU admission was significantly higher in the ECD cases in non-PAS group ($p = 0.016$)(Table 4). There was no maternal death.

Bakri balloon tamponade (BBT) is used effectively and intensively in our clinic. Intraoperative BBT was performed in 55 emergency and 115 planned cesarean deliveries with PP, but it was found to fail in nine patients while the patients were still in the operating room and hysterectomy was performed. There was no need for re-laparotomy in patients who underwent BBT.

Two hundred ten of 363 patients required blood and blood products transfusion, 58.5% of whom were in the ECD patients. In the multilinear regression analysis, the need for blood transfusion was found to increase with the number of previous cesarean sections (OR = 2.21, 95% CI: 0.716-1.396), emergency cesarean section (OR = 9.9, 95% CI: 0.561-1.783), PP with PAS (OR = 6.53, 95% CI: 0.251-3.977), general anesthesia (OR = 2.93, 95% CI: 0.813-1.230), and uterine artery ligation (OR = 2.092, 95% CI: 0.919-1.088) (Table 5).

DISCUSSION

In this retrospective study, the maternal and neonatal outcomes of the 363 cases of PP with PAS and without PAS were compared in terms of whether they were delivered under emergency or planned conditions. Although a high rate of antenatal diagnosis was made (78%), our data demonstrated that 166/363 (45.7%) cases were emergency cesarean sections. To avoid adverse neonatal outcomes due to prematurity, 53 patients who were planned to deliver at late preterm-early term gestational weeks had to deliver urgently before the specified date. General anesthesia was preferred in emergency cases in both PAS and non-PAS patients. In a study conducted by the anesthesia clinic of our hospital, evaluating 4874 patients who underwent emergency cesarean section for 3 years, regional anesthesia rate was 78.5% in all emergency cesarean sections. However it has been reported

Table 3. Neonatal outcomes by gestational week at birth

	PAS negative (n = 283)			**PAS positive (n = 80)**		
	Delivery < 37 weeks (n = 131)	**Delivery > 37 weeks (n = 152)**	***p* value**	**Delivery < 37 weeks (n = 49)**	**Delivery > 37 weeks (n = 31)**	***p* value**
Delivery type			**< 0.001**			**0.003**
ECD	92 (70.2)	36 (23.7)		30 (61.2)	8 (25.8)	
PCD	39 (29.8)	116 (76.3)		19 (38.8)	23 (74.2)	
Birth weight (g)	2470 (600-3800)	3060 (1530-4100)	**< 0.001**	2350 (595-3400)	3100 (2290-3700)	**< 0.001**
Fetal gender			0.722			0.644
Female	64 (48.9)	71 (46.7)		20 (40.8)	15 (48.4)	
Male	67 (51.1)	81 (53.3)		29 (59.2)	16 (51.6)	
1-min Apgar	9 (2-9)	9 (7-9)	**< 0.001**	8(2-9)	9(5-9)	**< 0.001**
5-min Apgar	10 (5-10)	10 (9-10)	**< 0.001**	9(5-10)	10(6-10)	**< 0.001**
Admission to NIC			**< 0.001**			**0.045**
Yes	35 (26.7)	8 (5.3)		19 (38.8)	5 (16.1)	
No	96 (73.3)	144 (94.7)		30 (61.2)	26 (83.9)	

Data presented as mean ± SD, n (%) or median (min-max). *P* values < 0.05 were statistically significant. SD = standard deviation, PAS = placenta accreta spectrum, ECD = emergency cesarean delivery, PCD = planned cesarean delivery, NICU = neonatal intensive care unit.

Table 4. Surgical approach and maternal outcomes

		PAS negative (n = 283)			PAS positive (n = 80)		
	Total cohort (n = 363)	ECD (n = 128)	PCD (n = 155)	*p* value	ECD (n = 38)	PCD (n = 42)	*p* value
Abruptio placenta				-			0.290
Yes	1 (0.3)	-	-		1 (2.6)	-	
No	362 (99.7)	128	155		37 (97.4)	42 (100)	
Uterine rupture				-			0.132
Yes	2 (0.6)	-	-		2 (5.3)	-	
No	361 (99.4)	128	155		36 (94.7)	42 (100)	
B-Lynch suture				0.228			-
Yes	4 (1.1)	3 (2.3)	1 (0.6)		-	-	
No	359 (98.9)	125 (97.7)	154 (99.4)		38 (100)	42 (100)	
Uterine artery ligation				0.847			0.934
Yes	6 (1.7)	2 (1.6)	2 (1.3)		1 (2.6)	1 (2.4)	
No	357 (98.3)	126 (98.4)	153 (98.7)		37 (97.4)	41 (97.6)	
Hypogastric artery ligation				0.677			0.845
Yes	27 (7.4)	1 (0.8)	2 (1.3)		11 (28.9)	13 (31)	
No	336 (92.6)	127 (99.2)	153 (98.7)		27 (71.1)	29 (69)	
Bakri balloon				0.136			0.494
Yes	137 (37.7)	45 (35.2)	68 (43.9)		10 (26.3)	14 (33.3)	
No	226 (62.3)	83 (64.8)	87 (56.1)		28 (73.7)	28 (66.7)	
Uterine lower segment resection				-			0.286
Yes	12 (3.3)	-	-		4 (10.5)	8 (19)	
No	351 (96.7)	128	155		34 (89.5)	34 (81)	
Hysterectomy				0.453			0.165
Yes	58 (16)	2 (1.6)	1 (0.6)		29 (76.3)	26 (61.9)	
No	305 (84)	126 (98.4)	154 (99.4)		9 (23.7)	16 (38.1)	
Relaparotomy				-			0.918
Yes	4 (1.1)	-	-		2 (5.3)	2 (4.8)	
No	359 (98.9)	128	155		36 (94.7)	40 (95.2)	
Blood transfusion	1 (0-12)	1 (0-7)	0 (0-5)	**0.024**	4 (0-12)	2.5 (0-8)	**0.030**
Fresh-frozen Plasma	0 (0-20)	0 (0-7)	0 (0-4)	**0.002**	4 (0-20)	2 (0-8)	**0.01**
Bladder injury				-			0.184
Yes	7 (1.9)	-	-		5 (13.2)	2 (4.8)	
No	356 (98.1)	128	155		33 (86.8)	40 (95.2)	
Bowel injury				0.270			-
Yes	1 (0.3)	1 (0.8)	-		-	-	
No	362 (99.7)	127 (99.2)	155		38	42	
Ureteral injury				0.270			-
Yes	1 (0.3)	1 (0.8)	-		-	-	
No	362 (99.7)	127 (99.2)	155		38	42	
Pulmonary emboli				-			0.290
Yes	1 (0.3)	-	-		1 (2.6)	-	
No	362 (99.7)	128	155		37 (97.4)	42	
Wound infection				-			0.498
Yes	3 (0.8)	-	-		2 (5.3)	1 (2.4)	
No	360 (99.2)	128	155		36 (94.7)	41 (97.6)	
Intensive care unit				**0.016**			0.511
None	210 (57.8)	73 (57)	109(70.3)		11 (28.9)	17 (40.5)	
Intermediate intensive care	109 (30)	45 (35.2)	31(20)		18 (47.4)	15 (35.7)	
Intensive care	44 (12.2)	10 (7.8)	15(9.7)		9 (23.7)	10 (23.8)	
Hospital stay after delivery (day)	3 (1-19)	3 (1-19)	3 (1-19)	**0.039**	7 (2-14)	5 (3-11)	**0.003**

Data presented as mean ± SD, n (%) or median (min-max). *P* values < 0.05 were statistically significant. SD = standard deviation, PAS = placenta accrete spectrum, ECD = emergency cesarean delivery, PCD = planned cesarean delivery.

Table 5. Multiple linear regression analysis for risk factors associated with blood transfusion requirement

	B	S.E.	*p* value	O.R.	95% CI	
					Lower	Upper
Materal age (year)	0.011	0.011	0.351	-0.935	0.955-1.048	
BMI (kg/m^2)	0.015	0.013	0.239	0.239	0.965-1.037	
Nunmber of cesareans	0.146	0.066	**0.028**	2.207	0.716-1.396	
ECD/PCD	1.703	0.172	**< 0.001**	9.928	0.561-1.783	
PAS	2.038	0.312	**< 0.001**	6.529	0.251-3.977	
Anesthesia method	0.418	0.143	**0.004**	2.926	0.813-1.230	
Hysterectomy	0.241	0.341	0.481	0.706	0.262-3.819	
Uterine lower segment resection	0.511	0.429	0.235	-1.191	0.697-1.435	
Hypogastric artery ligation	0.330	0.302	0.275	1.093	0.654-1.529	
Uterine artery ligation	1.090	0.521	**0.038**	2.092	0.919-1.088	
Relaparotomy	1.225	0.623	0.050	1.968	0.855-1.169	

B = standardized regression coefficient, SE = standard error, OR = odds ratio, CI = confidence interval, PAS = placenta accreta spectrum, ECD = emergency cesarean delivery, PCD = planned cesarean delivery. P-values with statistical significance ($p < 0.05$) are shown in bold.

that general anesthesia was applied 51% of class 1 emergency cesarean sections,including placenta previa cases, where there was maternal and fetal life-threatening conditions and high ASA(AmericanSociety of Anesthesiologists) scores [11].

Although ECD was found to be associated with worse neonatal outcomes, it was observed that prematurity (< 37th GW at delivery) was significantly higher in patients who were delivered urgently. In our study ,which conducted in a tertiary referral hospital with an available operating room, blood bank, experienced surgical team and appropriate neonatal care conditions twenty-four-hours-a-day,seven-days-a-week, adverse neonatal outcomes in the emergency cases appeared to be related to prematurity.

Although there was no difference in maternal outcomes in terms of the surgical approach and intraoperative complications, the need for blood transfusion was greater and hospital stay was longer in the ECD cases in both non-PAS and PAS groups. Adult ICU admission was greater in non-PAS group in cases of urgent delivery.

PP complicated approximately 0.6% of deliveries during the study period. Although our rate of PAS cases (22%) was similar to that in the study of Levin *et al*. [12] (PP: 0.6%; PAS: 19.2%), PAS cases were well above the 12,6% rate stated in the literature [13]. We concluded that this might be due to our hospital being an affiliated referral hospital.

Thirty-eight of our patients were nulliparous, one had a history of myomectomy, and three had a history of curettage due to recurrent pregnancy loss, but all were patients with non-PAS. The increase in placenta previa, especially placental invasion anomalies, with increasing cesarean section has been shown in many studies [12, 14]. However, other risk factors also affect the presence of PP. With advanced maternal age, the rate of sclerotic changes on intramyometrial arteries increases, which reduces placental blood flow [15]. In our study, we found that the mean maternal age was 32 years, which is consistent with other studies and very high [16]. Tuzovic *et al*. [17] showed that women aged over 30 years were at 2.5 times higher risk for developing PP. The increase in the rate of patients being multiparous with increasing maternal age may also contribute to the development of PP.

Although studies have been performed to determine the most appropriate week of birth to balance neonatal and maternal risks in PP cases, optimal delivery timing remains controversial [10, 18, 19]. Prolonging pregnancy in women with PP will increase the probability of encountering unscheduled and preterm deliveries. It has been reported that the number of bleeding episodes and the need for blood transfusion

in the antenatal period [20], and the increased number of cesarean sections [21] can predict preterm births in women with PP. Although the appropriate gestational week interval for the delivery of these patients is not specified in the guidelines, it may contribute to individualization in planning the delivery time. The high rates of early gestational age at birth in ECD cases in both non-PAS nad PAS groups in our study was not suprising and suggested that the worse neonatal outcomes in ECD cases was mainly associated with prematurity. In the study of Durukan *et al*. [21], in which similar results were obtained, there was no difference in the NICU need of infants when they evaluated term infants (> 37th GW). Levin *et al*. [12] reported that adverse neonatal outcomes were related to emergency delivery and general anesthesia, and that early gestational age at birth and general anesthesia were independently associated and modifiable factors with neonatal adverse outcomes.

PP is associated with adverse outcomes such as peripartum hysterectomy, blood transfusion, postpartum hemorrhage, and sepsis due to massive antepartum and intrapartum hemorrhage. [22]. Asicioglu *et al*. [13] stated that intraoperative estimated blood loss, vascular and surrounding organ damage and hysterectomy rates were higher in emergency delivery cases with PP, and emergency cases required more blood transfusion.

In the current study, while intraoperative management, complications, hysterectomy, and re-laparotomy rates did not differ between emergency and planned deliveries, adult ICU admission and longer hospital length of stay were more common in the ECD group. The amount of blood and blood products transfused to patients with and without PAS was higher in emergency cases. Durukan *et al*. [21] reported that planned cesarian delivery was associated with higher hemoglobin values, lower rates of blood transfusion, shorter hospital stay, and better maternal outcomes in both PAS and non-PAS cases. In a study limited to patients with non-PAS PP, Erfani *et al*. [19] reported that composite maternal morbidity would not be affected by ECD or PCD in their tertiary center. However, when the results of the aforementioned study were examined in detail, it was seen that there was no difference in terms of intraoperative complications but the lenght of hospital stay was longer in the emergency cesarean section group, similar to our study.

A recent study listed risk factors associated with blood transfusion in PP cases as previous cesarean section, anterior PP, major PP, preoperative bleeding, and emergency cesarean section [23]. In our current study, in which data on antenatal bleeding episodes and estimated blood loss were missing, we found that the need for blood transfusion in PP cases was associated with the increasing number of previous cesarean sections, emergency cesarean section, PAS, uterine artery ligation and general anesthesia. These findings may be useful in the risk assessment of patients and communication with the blood bank during the preoperative preparation process.

Limitations

The limitations of the study were its retrospective design and the fact that antenatal bleeding episodes and estimated blood loss were not evaluated. On the other hand, the large sample size, the evaluation of patients with both PAS and non-PAS, and that all surgeries were performed in the same fashion by an experienced surgical team are the strengths of the study.

CONCLUSION

Emergency cesarean delivery had negative effects on maternal and neonatal outcomes in women with PP. Efforts should be made to prolong pregnancy, as prematurity has a significant effect on adverse neonatal outcomes. It is beneficial to perform planned deliveries with these patients in reference hospitals where experienced surgical teams, consultant subspecialty surgeons, suitable operating room conditions, blood banks, and ICUs are available to care for both mother and infant.

Ethical approval

Ethics approval for this retrospective study was granted by the Clinical Research Ethics Committee of Bursa Yüksek İhtisas Traning and Research Hospital on 28/04/2021 with the Registration Number: 2011-KAEK-25 2021/04-10 The study complied with the principles of the Declaration of Helsinki. All patients signed informed consent forms before undergoing surgery, allowing their medical records to be used for research purposes.

Authors' Contribution

Study Conception: FNT, NNY, NKE, EKİ, FY; Study Design: FNT, NNY, NKE, EKİ, FY; Supervision: FNT, NNY, NKE, EKİ, FY; Funding: FNT, EKİ, FY; Materials: N/A; Data Collection and/or Processing: FNT, EKİ, FY; Statistical Analysis and/or Data Interpretation: FNT, NNY, NKE; Literature Review: FNT, NNY, NKE, EKİ, FY; Manuscript Preparation: FNT, NNY, NKE and Critical Review: FNT, NNY, NKE, EKİ, FY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Reddy UM, Abuhamad AZ, Levine D, Saade GR; Fetal Imaging Workshop Invited Participants. Fetal imaging: Executive summary of a joint Eunice Kennedy Shriver National Institute of Child Health and Human Development, Society for Maternal-Fetal Medicine, American Institute of Ultrasound in Medicine, American College of Obstetricians and Gynecologists, American College of Radiology, Society for Pediatric Radiology, and Society of Radiologists in Ultrasound Fetal Imaging Workshop. J Ultrasound Med 2014;33:745-57.
2. Sheiner E, Shoham-Vardi I, Hallak M, Hershkowitz R, Katz M, Mazor M. Placenta previa: obstetric risk factors and pregnancy outcome. J Matern Fetal Med 2001;10:414-9.
3. Jauniaux E, Grønbeck L, Bunce C, Langhoff-Roos J, Collins SL. Epidemiology of placenta previa accreta: a systematic review and meta-analysis. BMJ Open 2019;9:e031193.
4. Getahun D, Oyelese Y, Salihu HM, Ananth CV. Previous cesarean delivery and risks of placenta previa and placental abruption. Obstet Gynecol 2006;107:771-8.
5. Korosec S, Ban Frangez H, Verdenik I, Kladnik U, Kotar V, Virant-Klun I, et al. Singleton pregnancy outcomes after in vitro fertilization with fresh or frozen-thawed embryo transfer and incidence of placenta praevia. Biomed Res Int 2014;2014:431797
6. Kumru P, Demirci O, Erdogdu E, Arısoy R, Ertekin AA, Tugrul S, et al. The Bakri balloon for the management of postpartum hemorrhage in cases with placenta previa. Eur J Obstet Gynecol Reprod Biol 2013;167:167-70.
7. Tantbirojn P, Crum CP, Parast MM. Pathophysiology of placenta creta: the role of decidua and extravillous trophoblast. Placenta 2008;29:639-45.
8. Vahanian SA, Lavery JA, Ananth CV, Vintzileos A. Placental implantation abnormalities and risk of preterm delivery: a systematic review and metaanalysis. Am J Obstet Gynecol 2015; 213:S78-90.
9. Salihu HM, Li Q, Rouse DJ, Alexander GR. Placenta previa: neonatal death after live births in the United States. Am J Obstet Gynecol 2003; 188:1305-9.
10. ACOG Committee Opinion No. 764: medically indicated late-preterm and early-term deliveries. Obstet Gynecol 2019;133:e151-5.
11. Karaca Ü, Özgünay ÖŞ, Ata F, Kılıçarslan N, Yılmaz C, Karasu D. [Our experiences of anesthesia in emergency cesarean sections]. JARSS 2020;28:275-80. [Article in Turkish]
12. Levin G, Rottenstreich A, Ilan H, Cahan T, Tsur A, Meyer R. Predictors of adverse neonatal outcome in pregnancies complicated by placenta previa. Placenta 2021;104:119-23.
13. Asıcıoglu O, Şahbaz A, Güngördük K, Yildirim G, Asıcıoglu BB, Ülker V. Maternal and perinatal outcomes in women with placenta praevia and accreta in teaching hospitals in Western Turkey. J Obstet Gynaecol 2014;34:462-6.
14. Silver RM, Landon MB, Rouse DJ, Leveno KJ, Spong CY, Thom EA, et al. Maternal morbidity associated with multiple repeat cesarean deliveries, Obstet. Gynecol 2006;107:1226-32.
15. Ananth CV, Wilcox AJ, Savitz DA, Bowes WA Jr, Luther ER. Effect of maternal age and parity on the risk of uteroplacental bleeding disorders in pregnancy. Obstet Gynecol 1996;88:511-6
16. Batmaz G, Molla F, Molla T, Özcan P, Dansuk R, Dane B. [Retrospective analysis of patients with placenta previa]. Med Bull Haseki 2015;53:299-302. [Article in Turkish]
17. Tuzovic L, Djelmis J, Ilijic M. Obstetric risk factors associated with placenta previa development: Case-control study. Croat Med J 2003;44:728-33.
18. Zlatnik MG, Little SE, Kohli P, Kaimal AJ, Stotland NE, Caughey AB. When should women with placenta previa be delivered? A decision analysis. J Reprod Med 2010;55:373-81.
19. Erfani H, Kassir E, Fox KA, Clark SL, Karbasian N, Salmanian B, et al. Placenta previa without morbidly adherent placenta: comparison of characteristics and outcomes between planned and emergent deliveries in a tertiary center. J Matern Fetal Neonatal Med 2019;32:906-9.
20. Ruiter L, Eschbach SJ, Burgers M, Rengerink KO, van Pampus MG, van der Goes BY, et al. Predictors for emergency cesarean delivery in women with placenta previa. Am J Perinatol 2016;33:1407-14.
21. Durukan H, Durukan ÖB, Yazıcı FG. Planned versus urgent deliveries in placenta previa: maternal, surgical and neonatal results. Arch Gynecol Obstet 2019;300:1541-9.
22. Rosenberg T, Pariente G, Sergienko R, Wiznitzer A, Sheiner E. Critical analysis of risk factors and outcome of placenta previa. Arch Gynecol Obstet 2011;284:47-51.
23. Titapant V, Chongsomboonsuk T. Associated factors of blood transfusion for caesarean sections in pure placenta praevia pregnancies. Singapore Med J 2019;60:409-13.

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The problems related to internal medicine consultations in the emergency department

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ABSTRACT

Objectives: Overcrowding in the Emergency Department (ED) is a serious and growing problem during recent years and one of the main causes of it is dysfunctional consultation system. We aimed to determine the problems related to patients who were admitted to the ED and requested consultations from internal medicine (IM) physicians and to contribute to the gap in the literature regarding this isssue.

Methods: In a period of one year, 3601 patients, who were admitted to the ED of a university hospital and were consulted by IM physicians were included in this prospective cross-sectional study. The epidemiological characteristics of patients, length of ED stay and consultation-related problems were investigated.

Results: The most common problems related to consultations were delayed completion of the consultation procedures for 88 (2.4%) patients, and unnecessary consultation requests for 66 (1.8%) patients, and patient referrals with inappropriate indications from IM outpatient clinics to the ED for 53 (1.5%) patients. There were differences of opinion among IM physicians and emergency medicine specialists regarding the need for consultation for 36 (1%) patients. The most important difference was in the various infections seen in patients with histories of hematologic or solid organ malignancies (n = 9).

Conclusions: According to these findings, good collaboration must be established among ED physicians and consultant physicians. Furthermore, physicians must avoid inappropriate referral patients from outpatient clinics to the ED. Additionally, unnecessary consultation requests from the ED must be avoided, and consultation requests must be addressed quickly.

Keywords: Consultation, emergency department, internal medicine

One of the most significant problems of emergency departments (EDs) during recent years is the increasing number of patients. The main causes for this include increased non-emergency admission to the ED, lack of available hospital beds, dysfunctional consultation and referral systems and delay of diagnostic examinations and interventional procedures. Accordingly, patient ED length of stay may be prolonged, treatment of patients with severe disease may be delayed, workload and stress of ED personnel may increase, dissatisfaction of patients and their relatives may also increase and a situation of general chaos and inadequacy may occur in the EDs [1-5].

Patients may be admitted to ED with very different clinical presentations and degrees of urgency. Accurate and rapid management of these very different cases by an ED physician is not possible. Therefore, consultation, is needed in the management of some special patient groups in the ED, for some special diagnostic tests, treatments and interventions, as well as hospi-

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talization or referral of some patients to other hospitals [6-9]. Internal Medicine (IM) is one of the departments that is most frequently requested consultation by ED physicians [10, 11]. The IM department includes the divisions such as Gastroenterology, Oncology, Nephrology, Hematology, Endocrinology and Rheumatology. Accordingly, IM addresses a wide spectrum of diseases. Patients who have various severities of these diseases are frequently admitted to the ED. In the literature, we could not find a detailed research of the problems related to function of the consultation system among ED and IM physicians.

The aim of this study is to analyse in detail the consultation-related problems for patients admitted to the ED of Bursa Uludağ University Hospital in Turkey, who were requested a consultation from IM physicians. It is hoped that this study will address the research gap regarding this issue. Additional aims are to ensure that proactive plans are made to remove the deficiencies in ED consultation procedures in our hospital, and to ensure that a more effective and rapidly working consultation system is established among ED and IM physicians.

METHODS

In this prospective study., the parameters to be evaluated were determined after a one-month pilot study. After approval by the local Medical Research Ethics Committee., the study included 3601 patients who were admitted to the ED and requested consultation from IM physicians within a one-year period between February 2012 and February 2013. Firstly, a senior research assistant of the IM Department first evaluated the patient who was requested the consultation. The procedure was then concluded either by consultation with a specialist from the relevant division(s) of the IM department (Gastroenterology, Oncology, Nephrology, Hematology, Endocrinology and Rheumatology divisions had inpatient clinics during the study period) or by reevaluation of the patient with a specialist. Data were recorded on the pre-prepared form.

Statistical Analysis

All data were analyzed using the SPSS version 13.0 statistical software package. Mann-Whitney U test was used for comparing binary groups when significant differences were present. For descriptive values, mean (+/- standard deviation) or median and range (minimum-maximum) were given for continuous variables according to the distribution structure of data. Number (n) and percent (%) values were given for categorical variables.

RESULTS

In a one-year period, 85,585 patients were admitted to the ED. 3601 (4.2%) patients of them were consulted by IM department physicians. By gender, 2025 patients (56.2%) were male and 1576 (43.8%) were female. Their mean age (± standard deviation) was 57 ± 16.6 years and age range were 18-104.

Among the patients, 1740 (48.3%) were discharged from the ED, 51 (1.4%) died in the ED, 182 (5.1%) were referred to other hospitals because of lack of available beds, and 1628 (45.2%) were transferred to inpatient clinics. Among the last group, 1351 (83%) were hospitalized to different divisions of IM clinics, and 277 (17%) to other medical and surgical clinics

Table 1. Distribution of the patients according to time to completion of IM consultations and ED length of stay

Time	Number of patients	
	Time to completion of IM consultations	ED length of stay
Less than 30 minutes	676 (18.8%)	69 (1.9%)
Less than 3 hours	3048 (84.7%)	1180 (32.8%)
Less than 8 hours	3462 (96.1%)	2652 (73.6%)
Less than 12 hours	3530 (98,0%)	3075 (85.4%)
More than 24 hours	3 (0.08%)	33 (0.9%)

IM = internal medicine, ED = emergency department

Table 2. Distribution of reasons for prolonged ED length of stay

Reasons of prolonged ED length of stay of the patients	Number of patients
Waiting due to lack of available hospital beds	704 (19.6%)
Waiting due to delay in completion of consultation procedure	129 (3.6%)
Due to difficulty in reaching consultant physician or delay in concluding the consultations (n = 88, 2.4%)	
Waiting for completion of consultations in patients with multiple consultation requirements (n = 41, 1.1%)	
Waiting for completion of treatment, replacement therapy and monitoring treatment of patient in ED	68 (1.9%)
Waiting for endoscopy (gastroscopy, colonoscopy)	33 (0.9%)
Waiting for dialysis	15 (0.4%)
Total	949 (26.4%)

ED = emergency department

for their primary diseases. When evaluated according to IM divisions, 1047 (27.4%) patients were consulted by Gastroenterology, 935 (24.5%) by Oncology, 883 (23.2%) by Nephrology, 631 (16.5%) by Hematology, 258 (6.8%) by Endocrinology and 62 (1.6%) by Rheumatology.

In our study, we evaluated the time to completion of IM consultation procedures and observed that consultations were completed in under 3 hours for 84.7% of patients (Table 1). The mean time to completion was 121 min, and the median time was 74 min (range 10 to 2160 minutes). There was no significant difference between the divisions of the IM clinics when the mean time to completion of consultation were compared to patients who were hospitalized in IM division clinics ($p < 0.05$). The mean and median ED length of stay were 403.1 minutes and 284 minutes (range 21 to 2900 minutes), respectively. Although the ED length of stay was not more than 8 hours according to the ED Operating Procedure of our hospital, this target period was exceeded in 949 patients (26.4%). Reasons for these situations are shown in Table 2.

Of the patients who were consulted by IM physicians in the ED, 165 (4.6%) were referred from different outpatient clinics to the ED, and 147 of them were referred from IM outpatient clinics [Oncology (n = 44, 26.6%), Gastroenterology (n = 30, 18%), Nephrology (n = 28, 17%), Endocrinology (n = 19, 11.5%), Hematology (n = 19, 11.5%), Rheumatology (n = 4, 2.4%) and General Internal Medicine (n = 3, 2%)]. ninety-four of these patients were referrred from IM outpatient clinics to the ED for emergent medical reasons such as acute leukosis, gastrointestinal bleeding, urea and creatinine elevation, electrolyte imbalance, impaired general condition, fever and diabetic ketoacidosis. Fifty-three patients were referred to ED for inappropriate reasons (Table 3).

When evaluating inappropriateness related to consultations, we observed that the most common consultation-related problems were delay in completion of consultation procedures by IM physicians (n = 88, 2.4%) and unnecessary consultation requests by ED

Table 3. Distribution of patients according to the appropriateness of referrals from IM outpatient clinics to the ED

Appropriateness of referrals from IM outpatient clinics to the ED	Number of patients
Appropriate referrals - referrals with emergent medical reasons	94 (2.6%)
Inappropriate referrals	53 (1.5%)
For hospitalization purposes to other inpatient clinics through ED (n = 40, 1.1%)	
For some treatments in ED (n = 9, 0.2%)	
For some laboratory and radiologic examinations in ED (n = 4, 0.1%)	
Total	147 (4.1%)

IM = internal medicine, ED = emergency department

physicians (n = 66, 1.8%).

According to IM physicians, there were no indications found for 66 emergent consultations after evaluation of consultation requests. 62 (95%) of these consultation requests were made between 4 PM and midnight, the period when patients were overcrowded in ED. When the files of these 66 patients were subsequently re-examined by an ED specialist, it was found that there was no need to request consultations for 30 of these patients, while it was necessary to ask for consultations for 36 patients (Table 4). Consultation requests that were deemed to be inappropriate both by IM physicians and ED specialist were presence of mild thrombocytopenia with infectious diseases (n = 5), acute calcular cholecystitis (n = 3), postrenal acute renal failure (n = 3), acute abdomen (n = 2), presence of upper respiratory tract infection in patient with solid organ malignity in cure status (n = 2), mild transaminase elevation (n = 2), mild electrolyte disorders (n = 2), presence of mild ketonuria in the patient admitted due to suicide (n = 2), presence of upper respiratory tract infection in patient with compensated chronic renal failure (n = 2), presence of cystitis in the patient with compensated cirrhosis (n = 2), mild creatinine elevation (n = 2), mild hypercalcemia (n = 1), presence of upper respiratory tract infection in the patient with Takayasu arteritis (n = 1), presence of mild thrombocytopenia in the patient with fracture (n = 1).

DISCUSSION

Consultation is frequently used in EDs during patient assessment. According to various studies in different countries, it is notified that the rate of consultation requests ranges between 20% and 56.4% of ED patients [8, 11-13]. In a review of 12 studies, Lee *et al*. [14] reported a consultation rate of between 20% and 40% in the ED. In the studies conducted in different university hospitals of our country, consultation request rates in EDs were reported to be between 19.7% and 39.1% [15-18].

Most patients who are admitted to EDs have primary problems related to internal medicine. It was reported IM consultations (3-12.7%) were the most common of all ED consultations in several studies [10, 11, 15-18]. In another study, Brick *et al*. [11] analyzed the data of 841 patients, who were admitted to ED of a tertiary hospital in Edmonton, Canada in 2010 and reported that the most frequent consultations were requested from the IM and Cardiology departments, respectively, and the most frequently second consultation was requested from IM department. In 2 studies previously performed in the ED of our hospi-

Table 4. Clinical situations in which IM physicians and ED specialist have differing opinions on the appropriateness of consultation requests

Consultation requests	Number of patients
Various infections observed in patients with histories of hematological or solid organ malignancy treatment in the past	9
Mild diabetic ketosis cases	7
Presence of mild cytopenia with different infectious cases	5
Consultation requests which may be requested in clinics, made in ED for patients with internal medicine problems, who were hospitalized other department clinics due to primary disease	3
In order to ensure an early appointment for patient's next clinic control, although his/her internal problem is not urgent	3
Acute noncalculous cholecystitis	2
Mild anemia	2
Presence of ascites in the patient with heart failure	2
Others (corrosive substance admission in small quantities, mild thrombocytopenia in the patient with thalassemia, presence of multinodular goitre in the patient with maculopapular lesions)	3
Total	36 (1%)

IM = internal medicine, ED = emergency department

tal, consultations were requested most frequently from the IM department, with rate of 10.8% and 6.3%, respectively [10, 18].

Emergency service procedures should be fast. In our study, 84.7% and 96.1% of consultations requested from the IM department were completed within 3 hours and within 8 hours, respectively. The completion time for consultations exceeded 24 hours only in the case of 3 patients (0.08%). In a study by Aygencel *et al*. [17] regarding the epidemiology of IM department consultations in the ED of a university hospital in Ankara, Turkey, they reported that 86.6% of IM department consultations were concluded within 48 hours. The main reasons for quicklier completion of IM consultations in our study were as follows: the laboratory tests required for the patient prior to the consultation were substantially performed by ED physicians and IM consultation requests were quickly responded by a senior IM department research assistant whose sole task was to evaluate the consultations.

While the mean time to completion of IM consultations was 121 min, the mean length of stay in ED was 403.1 min and the length of stay was more than 8 hours for 26.4% of patients (n = 949). Although the time to consultation completion was short in our study, the primary cause for prolonged ED stays was the lack of available clinic beds (74.2%). Various studies from the USA, Taiwan and Spain reported that the most common cause of ED patient overcrowding was lack of available clinic beds [19-22].

In our study, 147 of the patients undergoing IM consultations were referred to the ED from IM outpatient clinics. It was determined that 53 of these referrals were inappropriate and most of them (n = 40) were referred to ensure transfer from the ED to other department clinics with available beds when no beds were available in IM clinics. According to a study by Howard *et al*. [23], some of the reasons for ED visits from non-urgent patients include direct referrals by their primary physicians, difficulties obtaining appointments with their physicians and patient belief that they should be examined as soon as possible by emergency services.

Overcrowding of patients in the ED is one of the reasons that ED physicians request many unnecessary consultations. In our study, it was reported that the consultations requested by the ED were unnecessary in 66 cases (1.8%) according to the IM physicians. The most important reason for unnecessary consultations was inadequate assesment of presenting complaints and/or clinic and laboratory examination results by ED physicians during the initial examination of the patient due to the intense ED workload in the evening hours when the number of patients was the highest. In a survey study [24] was conducted of 439 emergency physicians and consultant physicians from 6 hospitals in Turkey to research problems and solutions related to ED consultations. The 3 most significant problems for consultant physicians were "patients who need consultations were not sufficiently examined by an ED physician", "not having enough information in the patients' file" and "invitation of unnecessary consultation". The 3 most significant problems for ED physicians were "trying to complete the diagnostic procedures for patients in the ED", "trying to treat patients who need hospitalization in the ED" and "not finishing the consultation with a definite statement and writing re-consultation forms".

In the case of 36 patients, it was determined that there was difference of opinion between emergency physicians and IM physicians regarding suitability of consultation. The most common reason for these different views included various infections in patients with histories of malignancy, diabetic ketosis cases and cases of mild cytopenia in patients with infectious diseases. Accordingly, consensus meetings between ED and IM physicians should be held regarding these controversial cases so that appropriate decisions can be made.

Limitations

Although we think that it is a suitable example in terms of consultation-related problems in third level EDs in our country, the main limitation of our study was that there was only one center data. There may be differences in health systems according to countries, especially in ED operation procedures and consultation and referral systems. Therefore, it is possible that there may be some differences depending on the countries in consultation-related problems and solution proposals.

CONCLUSION

According to the results of our study, the most impor-

tant consultation-related problems were delay in completion of consultation procedures by IM physicians, unnecessary consultation requests by ED physicians and inappropriate referrals from IM outpatient clinics to ED. Also controversial situations regarding the need for consultation among IM and ED physicians were exemplified. To resolve of these problems is that good collaboration must be established among ED physicians and consultant IM physicians. Additionally, we hope that our study will be a model for similar investigations in other hospitals.

Authors' Contribution

Study Conception: CD; Study Design: CD, VG; Supervision: CD, VG; Funding: CD, VG, ŞAA; Materials: CD, VG, ŞAA; Data Collection and/or Processing: CD, VG, ŞAA; Statistical Analysis and/or Data Interpretation: CD, VG, ŞAA; Literature Review: CD, VG, ŞAA; Manuscript Preparation: CD, VG, ŞAA and Critical Review: CD, VG, ŞAA.

Conflict of interest

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REFERENCES

1. Derlet RW, Richards JR. Overcrowding in the nation's emergency departments: complex causes and disturbing effects. Ann Emerg Med 2000;35:63-8.
2. Trzeciak S, Rivers EP. Emergency department overcrowding in the United States: an emerging threat to patient safety and public health. Emerg Med J 2003;20:402-5.
3. Schiff GD. System dynamics and dysfunctionalities: levers for overcoming emergency department overcrowding. Acad Emerg Med 2011;18:1255-61.
4. Di Somma S, Paladino L, Vaughan L, Lalle I, Magrini L, Magnanti M. Overcrowding in emergency department: an international issue. Intern Emerg Med 2015;10:171-5.
5. Baig MA, Mian A, Najeed F, Shahzad H. Overcrowding in the emergency departments: Challenges and opportunities for improvement. J Pak Med Assoc 2015;65:1344-5.
6. Sears CL, Charlson ME. The effectiveness of a consultation: compliance with initial recommendations. Am J Med 1983;74:870-6.
7. Rosen P. Emergency department disposition and knowledge of other specialties. J Emerg Med 1986;4:325-6.
8. Woods RA, Lee R, Ospina MB, Blitz S, Lari H, Bullard MJ, et al. Consultation outcomes 238 in the emergency department; exploring rates and complexity. CJEM 2008;10:25-31.
9. Kessler CS, Asrow A, Beach C, Cheung D, Fairbanks RJ, Lammers JC, et al. The taxonomy of emergency department consultations-results of an expert consensus panel. Ann Emerg Med 2013;61:161-6.
10. Demircan C, Çekiç C, Akgül N, Odabaşı A, Çalışır N, Kıyıcı S, et al. [Profiles of the patients in the emergency internal medicine unit: one-year experience]. Uludağ Üniv Tıp Fak Derg 2005;31:39-43. [Article in Turkish]
11. Brick C, Lowes J, Lovstrom L, Kokotilo A, Villa-Roel C, Lee P, et al. The impact of consultation on length of stay in tertiary care emergency departments. Emerg Med J 2014;31:134-8.
12. Cortazzo JM, Guertler AT, Rice MM. Consultation and referral patterns from a teaching hospital emergency medicine department. Am J Emerg Med 1993;11:456-9.
13. Geskey JM, Geeting G, West C, Hollenbeak CS. Improved physician consult response times in an academic Emergency Department after implementation of an institutional guideline. J Emerg Med 2013;44:999-1006.
14. Lee RS, Woods R, Bullard M, Holroyd BR, Rowe BH. Consultations in the emergency department: a systematic review of the literature. Emerg Med J 2008;5:4-9.
15. Kılıçaslan İ, Bozan H, Oktay C, Göksu E. [Demographic properties of patients presenting to the emergency department in Turkey]. Turk J Emerg Med 2005;5:5-13. [Article in Turkish]
16. Ay D, Akkas M, Sivri B. Patient population and factors determining length of stay in adult ED of Turkish University Medical Center. Am J Emerg Med 2010;28:325-30.
17. Aygencel G, Nas A, Sarıtaş H, Deryal K, Demircan A. [General characteristics of internal medicine consultations in the emergency service of a university hospital]. Fırat Med J 2012;17:219-22. [Article in Turkish]
18. Aydın T, Aydın ŞA, Köksal O, Özdemir F, Kulaç S, Bulut M. [Evaluation of features of patients attending the emergency department of Uludag university medicine faculty hospital and emergency department practices]. Eurasian J Emerg Med 2010:9:163-8. [Article in Turkish]
19. Graff L. Overcrowding in the ED: an international symptom of health care system failure. Am J Emerg Med 1999;17:208-9.
20. Derlet RW, Richards JR. Emergency department overcrowding in Florida, New York, and Texas. South Med J 2002;95:846-9.
21. Shih FY, Ma MH, Chen SC, Wang HP, Fang CC, Shyu RS, et al. ED overcrowding in Taiwan: facts and strategies. Am J Emerg Med 1999;17:198-202.
22. Sanchez M, Miro O, Coll-Vinent B, Bragulat E, Espinosa G, Gomez-Angelats E, et al. [Emergency department overcrowding: quantification of associated factors]. Med Clin (Barc) 2003;121:161-72. [Article in Spanish]

23. Howard MS, Davis BA, Anderson C, Cherry D, Koller P, Shelton D. Patients' perspective on choosing the emergency department for nonurgent medical care: a qualitative study exploring one reason for overcrowding. J Emerg Nurs 2005;31:429-35.

24. Çıkrıklar Hİ, Yürümez Y, Keleş İ, Özdinç Ş, Selvi F, Engindeniz Z, et al. Emergency room consultations: problems and solutions. Eurasian J Emerg Med 2015;14:167-71.

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The utility of strain elastography in differentiation between healthy thyroid parenchyma and residual/recurrent thyroid parenchyma disorders

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ABSTRACT

Objectives: This study is designed to evaluate the utility of strain elastography in the differentiation between healtly thyroid parenchyma and residual/recurrent thyroid parenchyma after partial thyroidectomy.

Methods: Gray-scale ultrasonography (B-mode US) and strain elastography were performed in patients who had undergone partial thyroidectomy due to benign thyroid diseases and volunteers as a control group without any throid disease. The elasticity scores and strain rate values of thyroid parenchyma were obtained in two groups and compared. The strain elastography of the patient and control groups were analyzed with the Pearson Chi-Square test. Strain rates for both groups were compared with the Manny-Whitney U test. Ethical approval and permissions were obtained from legally authorized representatives and patients.

Results: There were 94 cases in total, 47 cases (4 male, 43 female; mean age: 51 ± 12.9 years, ranging between 25-79) in the patient group and 47 cases (11 male, 36 female; mean age: 36.8 ± 11.5 years, ranging between 19-71) in the control group. The strain rate of the right (2.22 ± 1.9) and left thyroid lobe (1.9 ± 1.9) were significantly higher than control group (1.05 ± 0.6 and 0.8 ± 0.5, respectively; $p = 0.002$ and $p = 0.043$).

Conclusions: The elasticity of thyroid parenchyma after partial thyroidectomy differs from healthy thyroid tissue, which may be helpful in differentiation between residual parenchymal changes in thyroid from malignant lesions with strain elastography.

Keywords: Thyroidectomy, strain elastography, residual, recurrence

Thyroid diseases are fairly common in the population. Diffuse (simple) and nodular goiter may be encountered [1]. The first imaging method of choice in the diagnosis of thyroid diseases is thyroid ultrasonography (US) [2]. The US identifies the location, volume, parenchymal structure, presence, and the number of nodules and may be helpful in differentiation between the cystic-solid nodule and benign-malignant thyroid nodules [3]. Thyroid US is also the mainstay imaging technique in the evaluation of residual or recurrent thyroid disorders.

Despite all the benefits, B-mode US is not specific and sensitive enough to evaluate thyroid disorders. Therefore, an increasing number of studies have been conducted on the use of strain elastography (SE) in evaluating thyroid parenchyma and its pathologies [4]. SE is a method based on measuring the stiffness and elasticity of tissue. In strain elastography, tissue stiff-

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ness is measured by applying external force (compression) [4, 5] while tissue elasticity is measured by qualitative (color coding-elasticity score- ES) and semi-quantitative (strain rate measurement) methods. Diagnostic utility of SE in the assessment of thyroid parenchymal diseases and differentiation between benign and malignant in thyroid nodules was found comparable with fine-needle aspiration biopsy (FNAB) [5].

The recurrence rate of thyroid nodules and the rate of incidental malignancy in thyroid parenchyma after subtotal and total thyroidectomies are not infrequent [6]. The second surgery to be performed is quite risky in terms of the recurrent nerve injury and development of hypoparathyroidism [7]. The B-mode US may be insufficient in the evaluation of residual/recurrent tissue and the differentiation of malignant-benign lesions in the area where thyroidectomy is performed. Thus, it is essential to know if there exists a difference between US-E examination findings in an operated and non-operated thyroid parenchyma. In addition, US-E may be useful in examining the area where post-surgical fibrous tissue development is expected. No studies regarding the utility of US-E in patients who underwent thyroid surgery have been found in the literature. A better understanding of the elasticity changes in the area of thyroidectomy, and revealing masses or visual differences may help to prevent invasive procedures such as FNAB or surgery.

Therefore, this study aims to compare the US-E findings of residual changes and recurrent diseases in postoperative thyroid parenchyma with normal thyroid parenchyma.

METHODS

Study Design and Study Population

Ethical approval of this study was obtained from the Fırat University Medical Faculty "Non-Interventional Clinical Research Ethics Board" of the authorized ethics committee (Number of decision: 2020/06-29). The study was conducted in accordance with the Helsinki declaration and, with informed consent was obtained from the participants. A total of 47 patients aged between 18-70 years who had thyroidectomy (lobectomy, subtotal thyroidectomy, near-total thyroidectomy, total thyroidectomy) due to benign thyroid disease and scheduled for sonographic evaluation of thyroid parenchyma as of January 2020 were included in this study. These were the inclusion criteria; patients with no malignancy as a result of pathology after thyroidectomy, no radioactive iodine treatment and radiation to the neck, no other surgery in the neck, no other malignancy, no collagen tissue disease, no rheumatic disease in the body, with or without thyroid hormone replacement, with thyroid functions of any level (hypothyroid, euthyroid, hyperthyroid). The ones who did not volunteer and were not followed-up were excluded from the study.

The control group of the study consisted of 47 healthy individuals aged between 19-71 years whose thyroid function tests (TSH, free T3 (FT3), free T4 (FT4), thyroid autoantibodies and thyroid the US were normal, who had no chronic and systemic disease (diabetes mellitus, hypertension, coronary heart disease, malignancy, collagen tissue disease, rheumatic disease), and did not use any substance or hormonal drugs.

Whether the control group consisting of individuals who had been examined by the general surgery specialist received any thyroid replacement or suppression treatment was recorded. The patients were directed to the laboratory to evaluate thyroid function tests. The information about the performed operations was obtained and reported from the epicrisis notes and pathology reports available in the hospital records. The results were evaluated by the clinician.

Sonographic Examinations

The procedure was performed using the US equipment (Philips EPIQ 7G) with eL18-4 MHz linear probe while the patient and control groups were in the supine position and the patient head was moderately hyperextended. To prevent interobserver differences, thyroid US and US-E were evaluated by only a single experienced radiologist.

The size (length × width × thickness × 0.52 = volume, mm3, for each lobe), echogenicity (hypoechoic, heterogeneous, isoechoic), vascularity (hypovascular, middle and hypervascular) of residual or recurrent thyroid parenchyma, presence and structure of nodule were interpreted with B-mode and color flow Doppler US.

US-E examination was conducted with freehand compression and decompression technique. The

amount of compression and homogeneity were standardized using a color pressure scale. The assessment was performed after a safe measurement interval of over 50% was achieved on the pressure scale. The tissue elasticity distribution was calculated in real-time, and the results were represented in a color map placed on the B-mode image. The color spectrum ranged from blue (soft-elastic) to red (hard-inelastic) and represented the relative stiffness of the tissue [5]. Elasticity (color) scoring was performed in the residual/recurrent thyroid parenchyma:

Score 1 - Elastic parenchymal structure, almost coded in blue (over 75%).

Score 2 - Predominantly elastic parenchyma (over 50-55%), containing yellow and red coded areas scattered on a blue-green coded ground.

Score 3 - Predominantly inelastic parenchyma (over 50-55%), containing green and yellow coded areas scattered on a red coded ground.

Score 4 - Inelastic parenchyma, almost coded red (over 75%).

In addition, the imaging area was adjusted to include residual/recurrent tissue and surrounding strep muscles in B-mode and elastography examination. After the elastograms were obtained, the residual/recurrent tissue and the strain values of the adjacent strep muscles were measured numerically and proportioned automatically by the device on the static elastogram image with the region of interest (ROI) (mean 4-6 mm^2). The ratio of strain values of strep muscles and residual/recurrent tissues was expressed as strain index (SR, SI). SI was calculated and recorded for residual/recurrent tissue in the left and right lobe separately.

Statistical Analysis

Statistical analysis was performed using the SPSS 22.00 package program. In comparing the measured parameters for the patients in the study, the normality of the data obtained from the patients was evaluated with the Kolmogorov-Smirnov test. Student-T test and Mann-Whitney U-test were used for comparison between groups. chi-square test and Fisher's Exact test were used to evaluate qualitative data. The correlation analysis between variables was performed with Pearson and Spearman methods. P values less than 0.05 were considered statistically significant.

RESULTS

A total of 94 cases were evaluated (47 in the study group and 47 in the control group). The mean age of the patient group was 51 ± 12.9 (range: 25-79) years, and 43 (91.5%) were females. The mean age of the control group was 36.8 ± 11.5 (range: 19-71) years, and 36 (76.6%) were females. There was a statistically significant difference between the two groups in terms of age ($p < 0.001$). In the study group, US-E was performed in recurrent tissue of 5 (11.2%) patients with total thyroidectomy, residual tissue of 38 (81%) patients with subtotal thyroidectomy, thyroid lobe of 3 (5.6%) patients with unilateral lobectomy, and residual tissue of 1 (2.2%) patient with near-total thyroidectomy. The mean time between the time of surgery and participation of the study was 17.6 ± 7.6 years, ranging from 2 to 30 years. Two patients were operated twice; one was 10 and 5 years ago, and the other was 20 and 10 years ago.

The mean volume of the right lobe and left lobe, and isthmus were measured as 8.72 ± 10.4 (range: 0.17-43.6) mm^3 and 7.5 ± 11 (range: 0.32-55) mm^3,

Table 1. The elasticity scores of residual/recurrent thyroid tissue and healthy thyroid parenchyma in the control group

	Right lobe		Left lobe	
Elasticity Scores	**Patient group**	**Control group**	**Patient group**	**Control group**
1	4	3	5	3
2	17	36	17	40
3	11	8	12	4
4	6	0	5	0
***p* value**	**0.003**		**0.001**	

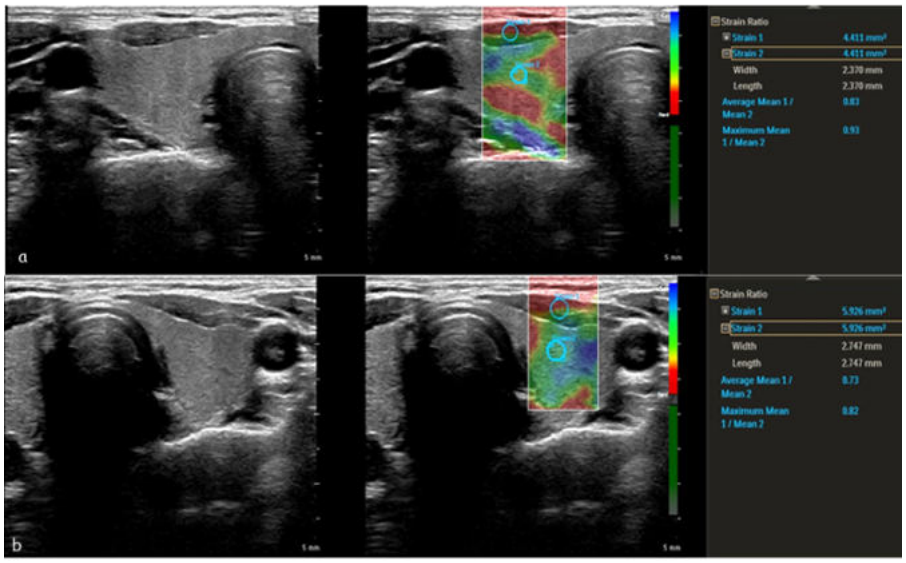

Fig. 1. Elastographic measurement of healthy thyroid parenchyma. (a) Right lobe (ES score 2, mean SR 0.73). (b) Left lobe (ES score 1, mean SR 0.83).
The left side of the windows is a color-coded elastography image; the right side is a gray scale image. The circles show the regions of interest where we measure the SRs. One is in the belt muscle and the other is in the normal thyroid parenchyma. The color scale on the upper left is used to evaluate the ES relatively, and the green color scale on the lower left is used to evaluate the amount of compression and homogeneity. ES = Elasticity Score, SR = Strain Ratio.

and 0.94 ± 0.6 (range: 0.12-2.02) mm³, respectively. Thyroid nodules were detected in 14 (29.8%) of the patients with a mean diameter of 13.8 (range: 2-40) mm.

The echogenicity of the residual/recurrent thyroid tissue was assessed with respect to the echogenicity of the normal thyroid parenchyma. Residual / recurrent thyroid tissue was found hypoechoic in 17 (36.2%) patients, heterogeneous echogenicity in 14 (29.8%), and isoechoic in 16 (34%) patients. Assessment of vascularity in residual/recurrent thyroid tissue yielded hypervascularity in 10 (21.3%) patients, hypovascularity in 24 (51%), and moderate vascularity in 13 (27.7%) patients.

The elasticity scores of residual/recurrent thyroid tissue in patients and healthy thyroid parenchyma in the control group are summarized in Table 1. Elasticity score and Strain ratio measurements in normal thyroid tissue one control subject and one operated patient were showed (Fig. 1 and 2). Most frequently encoun-

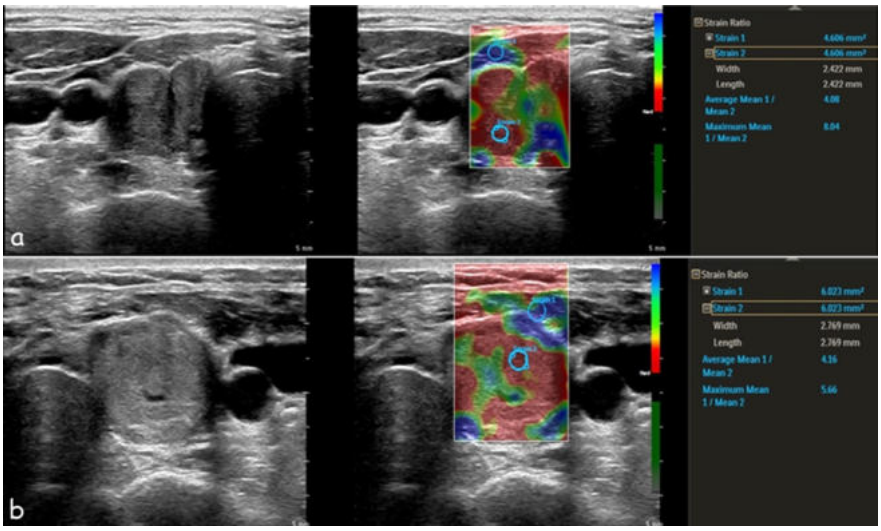

Fig. 2. Images show measurement of ES and SR in residual or recurrent thyroid tissue. (a) Right thyroid lob (ES score 3, mean SR 4.06). (b) Left thyroid lobe (ES score 4, mean SR 4.16). ES = Elasticity Score, SR = Strain Ratio.

Table 2. The mean SR of left and right thyroid lobes in the patient and control groups and comparison the SR between control and patient groups

Strain Ratio (SR)	Patient group	Control group	*p* value
Right lobe	2.22 ± 1.9	1.05 ± 0.6	**0.002**
Left Lobe	1.9 ± 1.9	0.8 ± 0.5	**0.043**

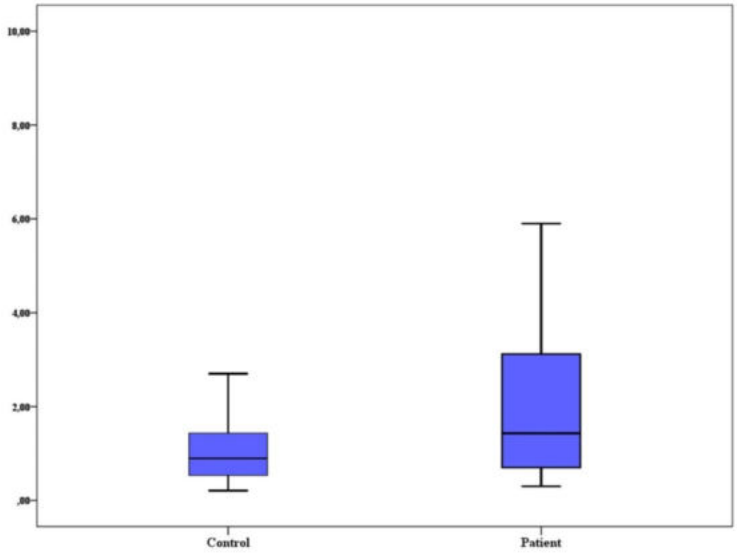

Fig. 3. Histogram graphic shows SR distribution for the right lobe in the patient and control groups. SR = Strain Ratio.

tered ES in the left and right lobe of the patient and control group was score 2.

The elasticity scores were significantly different between the right lobe and left lobe of thyroid parenchyma in patients ($p = 0.003$) and control ($p = 0.001$) groups. In the patient group, both score 3 and score 4 were found higher for the right and left lobes. The mean SR of left and right thyroid lobes in the patient and control groups and comparison the SR between control and patient groups are presented in Table 2. Mean SR of right and left lobes were significantly higher in the patient group than the control group. There was a correlation between the elasticity scores and strain rates (SRs) for both groups ($p < 0.01$). This result was statistically significant and higher in the patient group (Fig. 3 and 4).

Patients who were operated twice were found to have higher SR. (The mean SR was 3.12 and ES was

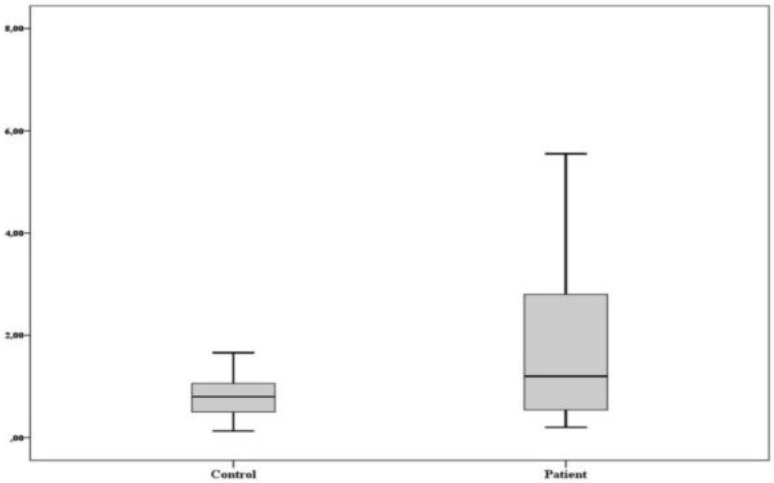

Fig. 4. Histogram graphic shows SR distribution for the left lobe in the patient and control groups. SR = Strain Ratio.

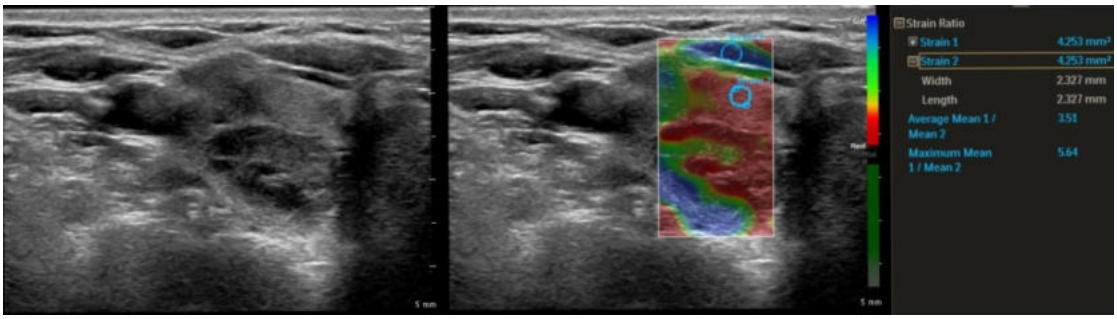

Fig. 5. Images show measurement of ES and SR in residual or recurrent thyroid tissue. (a) Right thyroid lob (ES score 3, mean SR 4.06). (b) Left thyroid lobe (ES score 4, mean SR 4.16). ES = Elasticity Score, SR = Strain Ratio.

4 for right lobe in the first patient, while the mean SR was 2 and ES was 3 for the right lobe, the mean SR was 3.51 and ES was 4 for the left lobe in the second patient) (Fig. 5).

DISCUSSION

The elasticity of recurrent/residual thyroid tissue was found significantly different from healthy thyroid parenchyma. This result implies the importance of elasticity changes in postoperative thyroid tissue. The elasticity of postoperative thyroid tissue differs from healthy thyroid tissue, which should be kept in mind in comparison to elasticity between postoperative thyroid tissue and focal or diffuse thyroid diseases.

Anatomical and morphological changes occur in the neck region after thyroidectomy compared to the preoperative findings. Identifying changes in the thyroidectomy area and distinguishing these changes from malignancy with noninvasive imaging methods is important. Postoperative tissue changes are permanent in some patients. It is more accurate to diagnose if the US technician is aware of the surgical procedure applied to the thyroid in cases where there is residual thyroid tissue after lobectomy or subtotal thyroidectomy. However, in cases with antithyroid drug treatment or thyroiditis or previous radioactive ablation therapy, residual thyroid tissue may not be recognized. The fibrous scar in the surgical area may not be distinguished from a tumor. In such cases, the patient can be evaluated by repeated US follow-ups. The size and shape of the fibrous tissue often remain stable or become smaller [9, 10]. Although these findings have been described in the B-mode US in previous studies, there is no study on US-E evaluating tissue fibrosis and its elasticity in the literature. In this study, residual/recurrent tissue was primarily evaluated with B-mode US and US-E.

In this study, the mean age of the patient group was higher as the incidence of thyroid nodules increases, and the indication for surgery is more common at advanced ages. Therefore, our control group consisted of younger cases. On the other hand, whether there is any elasticity difference in adults that may arise from age difference should be investigated in the future with studies involving patients with similar age groups.

Thyroid diseases are more common in females, which is consistent with our data. In multinodular goiter and benign thyroid diseases, total thyroidectomy has become common in the last decade while subtotal and near-total thyroidectomy were preferred in younger ages [6]. The average time after surgery in the study group was 17.6 years, which explains the high rate of subtotal thyroidectomy. Although the rate of subtotal thyroidectomy was high, the rate of recurrence nodules was 29.8%. As expected from an operated tissue, thyroid tissue was hypovascular in 51% of the patients and moderate vascular in 13% of the patients. When the US-E findings were examined, there was a statistically significant difference between the strain rates of the patient and control groups. The rates of the patient group were higher in both the right and left lobes compared to the control group. It can be understood from this situation that the elasticity of the thyroid tissue decreased in postoperative thyroid parenchyma. In previous US-E studies evaluating the normal thyroid tissue, the thyroid tissue was found to present with high and homogeneous elasticity manifesting as green color code and the elasticity scores of 1 and 2. However, it was also reported that elasticity

might increase towards green/red/yellow direction in cases with parenchymal diseases such as parenchymal hyperplasia. In healthy subjects, the mean SR of thyroid parenchyma was found as 0.76 ± 0.55 [11-13]. Yang *et al*. [14] revealed that the thyroid tissue SR of 43 control group patients without any thyroid disease was found between 0.66-2.70, and the mean was 1.76 ± 0.54. Yurttutan *et al*. [15] found that the mean SR of thyroid tissue was 0.54 ± 0.38 in children without a thyroid disease between the ages of 3-16. However, they did not find any difference between the SR value with age, gender, and body mass index.

In our study, the mean SR values of right and left thyroid lobes in healthy subjects were consistent with the literature. On the other hand, mean SR values of postoperative thyroid parenchyma were found significantly higher than normal parenchyma in both right and left lobes. Elastography scores of the patient and control groups were found statistically significant and the score 3 and score 4 were higher for both lobes in the patient group. While measuring the SR elasticity semi-quantitatively, the elastography score consisting of the color scale is a qualitative measurement method. In our study, as in SR, ES of the patient group was found higher than the control group. These results reveal that the elasticity decreases in tissues that have undergone operation and fibrosis. Although there are no studies in the literature that have performed elastographic measurements in operated thyroid cases, Gode *et al*. [16] measured the elasticity score of preoperative tissue and postoperative fibrous tissue for inferior turbinate process in the lateral nasal cavity, and they found the elasticity score of the postoperative fibrous tissue significantly higher. Korkusuz *et al*. [17] noted decreased tissue elasticity after the procedure in their elastography measurements before and after microwave ablation applied to thyroid nodules. In our study, we found a decrease in postoperative residual/recurrent thyroid tissue elasticity in accordance with the literature.

Studies have shown that the elastographic value of the thyroid parenchyma changes according to the histological state of the tissue. Similarly, it was revealed in our study that the elasticity decreased, and the strain increased in patients who had undergone thyroid operation, changing the histology of the parenchymal structure. The results of the comparison between the elasticity values of diffuse and focal thyroid abnormalities with uninvolved healthy or postoperative thyroid parenchyma are expected to be different due to the results of our study, which reveal the elasticity changes in postoperative thyroid parenchyma.

Limitations

The limitation of this study might be the low number of patients, the age difference between the study and control groups, the difference between the type of surgery and the preoperative diagnosis of the study group.

CONCLUSION

In operated thyroid cases, the US-E values and elasticity of thyroid tissue may be decreased compared to healthy thyroid tissue. Familiarizing with this situation may contribute to minimally invasive intervention as well as being useful in the differential diagnosis of recurrent or residual tissues.

Authors' Contribution

Study Conception: YG, GK; Study Design: YG, ZÖ; Supervision: YG, ZÖ; Funding: GK, ZÖ; Materials: GK, ZÖ; Data Collection and/or Processing: YG, MB; Statistical Analysis and/or Data Interpretation: YG, MB; Literature Review: ZÖ, MB; Manuscript Preparation: YG, ZÖ and Critical Review: YG, GK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Durgun Z, Yazıcı C, Inan AO. [Thyroid hormones and diseases]. Akdeniz Spor Bilimleri Dergisi 2019;2:28-40. [Article in Turkish]
2. Thyroid Study Group, Society of Endocrinology and Metabolism of Turkey. Guideline of Throid Diseases and Treatment. Turkey Clinics Publication, 4. Edition, April 2019. Ankara – Turkey.

3. Cetin M. [Ultrasonographic diagnosis in throid diseases]. SDU Tıp Fakültesi Dergisi. 2009;7:26-9. [Article in Turkish]
4. Dudea SM, Botar-Jid C. Ultrasound elastography in thyroid disease. Med Ultrason 2015;17:74-96.
5. Hefeda MM. Value of the new elastography technique using acoustic radiation force impulse in differentiation between Hashimoto's thyroiditis and Graves' disease. J Clin Imaging Sci 2019;9:17.
6. Kaliszewski K, Strutyńska-Karpińska M, Zubkiewicz-Kucharska A, Wojtczak B, Domosławski P, Balcerzak W, et. al. Should the prevalence of incidental thyroid cancer determine the extent of surgery in multinodular goiter? PloS One 2016;11:e0168654.
7. Cerit MN, Oktar SO. Lenf Bezleri, Tükrük Bezleri ve Boyun Kitlelerinde Elastografi. Türk Radyoloji Seminerleri 2019;7:38-49.
8. Akçakaya A, Koç B, Ferhatoğlu F. [Thyroid anatomy and surgical approach]. Okmeydanı Tıp Dergisi 2012;28(Suppl 1):1-9. [Article in Turkish]
9. Shin JH, Han BK, Ko EY, Kang SS. Sonographic findings in the surgical bed thyroidectomy: comparison of recurrent tumors and nonrecurrent lesions. J Ultrasound Med 2007;26:1359-66.
10. Frates MC. Ultrasound in recurrent thyroid disease. Otolaryngol Clin N Am 2008;41:1107-16.
11. Cantisani V, Lodise P, Grazhdani H, Mancuso E, Maggini E, Di Rocco G, et al. Ultrasound elastography in the evaluation of thyroid pathology. Current status. Eur J Radiol 2014;83:420-8.
12. Menzilcioglu MS, Duymus M, Gungor G, Citil S, Sahin T, Boysan SN, et al. The value of real-time ultrasound elastography in chronic autoimmune thyroiditis. Br J Radiol 2014;87:20140604.
13. Menzilcioglu MS, Duymus M, Avcu S. Sonographic elastography of the thyroid gland. Pol J Radiol 2016;81:152-6.
14. Yang Z, Zhang H, Wang K, Cui G, Fu F. Assessment of diffuse thyroid disease by strain ratio in ultrasound elastography. Ultrasound Med Biol 2015;41:2884-9.
15. Yurttutan N, Gungor G, Bilal N, Kizildag B, Baykara M, Sarica MA. Interpretation of thyroid glands in a group of healthy children: real-time ultrasonography elastography study. J Pediatr Endocrinol Metab 2016;29:933-7.
16. Gode S, Turhal G, Kismali E, Ozturk K, Midilli R. A novel method for comparison of tissue fibrosis after inferior turbinate surgery: ultrasound elastography. Am J Rhinol Allergy 2015;29:e33-6.
17. Korkusuz H, Happel C, Klebe J, Ackermann H, Grünwald F. Diagnostic accuracy of elastography and scintigraphic imaging after thermal microwave ablation of thyroid nodules. Rofo 2015;187:353-9.

The European Research Journal 2022;8(3):383-388 Original Article

DOI: 10.18621/eurj.910755 *General Surgery*

A new, quick, effective and minimally invasive treatment technique applied to ingrown toenails

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ABSTRACT

Objectives: Existing surgical and conservative treatment techniques applied to ingrown toenails remain controversial. In this study, a new minimally invasive technique for the treatment of ingrown toenails is described which was found to provide rapid healing and effective results.

Methods: The presented study includes 39 patients with ingrown toenails treated with the newly developed technique. The technique is a minimally invasive procedure in which a half groove-shaped piece obtained from the intravenous drip set plastic was placed between nail and dermis and fixed on the nail.

Results: The patients treated with this technique were followed up after one week, one month and six months. According to six-month follow-up results of the patients, achievement rate of the presented technique was found to be quite high at 80%.

Conclusions: This new technique has many advantages compared to existing ingrown toenail treatment methods. It was revealed that this method is very effective and successful by not requiring dressing, ensuring low pain, rapid recovery from the first day of the treatment, uninterrupted return to daily life, and low recurrence rate. One of the most important advantages of the technique is that it ensures high patient satisfaction by providing good functional and aesthetic results.

Keywords: Ingrown toenail, toenail, onychocryptosis, minimally invasive technique

Ingrown toenail is a common and often painful nail disease that occurs when the periungual dermis is pierced by the edge of the nail plate [1]. It is also known as onychocryptosis (originating from Greek words *onyx*-nail and *crypto*-secret) or unguis incarnates [2]. It is characterized by foreign body reaction, and in a later stage, inflammation, and the development of granulation tissue. In some cases, the nail plate can penetrate the surrounding soft tissue, or the nail plate can be covered with soft tissue, resulting in infection [3-5]. Although the disease generally occurs in the great toes (hallux nail) (70%), in some unusual cases other fingernails may also be involved after the trauma [6, 7]. Ingrown toenail occurs mostly in the lateral part of the nail, the incidence on the lateral and medial sides of the nail is 2:1 [8]. Ingrown toenail causes walking difficulties, and if not properly treated, it may cause permanent deformities in the nail and toe, or a secondary nail infection of varying severity. There are numerous methods for the treatment of ingrown toenails. The selection of the treatment method depends on the severity and the stage of the disorder. Total nail avulsion, partial nail avulsion or wedge resection, removal or reduction of lateral nail fold, excision of nail bed, surgical and chemical matrixectomies targeting nail matrix have been widely

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used as surgical methods for the treatment. Taping, dental floss, sleeve technique, band-aid method, angle correction, and nail braces, are among the conservative methods [9].

In this study, a new, quick, effective, and minimally invasive technique for treatment of ingrown toenails is presented. It was observed that this technique provides rapid recovery and return to daily life in patients. In the follow-up of patients at the end of first week, first month and sixth month after the treatment, it was observed that a very high recovery rate in the patients was achieved.

METHODS

This study includes the treatment of 39 patients with complaint of ingrown toenails who applied to the General Surgery Department of Sultan Abdulhamid Han Training and Research Hospital located in Istanbul. The period of the study includes the 4-month period between October 2018 and February 2019 when the patients were treated, plus the 6-month follow-up period for each patient.

The new method applied to patients consists of a minimally invasive technique, unlike the commonly applied nail avulsion. In this minimally invasive procedure, firstly, local anesthesia was applied to the toe to be treated. And then the nail edge of ingrown side was slightly lifted until the matrix. A half groove-shaped 3 cm long piece obtained from the tubing part of intravenous drip set plastic was placed between the nail and dermis with the sandwich model. This half groove-shaped piece was fixed to the nail with the help of polypropylene suture, and the process was completed. The fixation method used was suture technique. The piece was not changed or dislodged. The materials used in this new technique are shown in Fig. 1.

The follow-up began immediately after the procedure. The suture was not removed. The suture and groove were cut by the patient along with the nail as the nail grew longer. It was ensured that the nail completely grew on the soft tissue thanks to this barrier in the period of approximately 3-4 months, and the treatment was finalized.

Ethical approval for this study was obtained by Ethics Committee of Health Sciences University, Istanbul, with approval number of 2020/1 and dated 10.02.2020.

Statistical Analysis

Descriptive data were presented as percentages, means and standard deviations.

RESULTS

The number of patients who applied to Sultan Abdulhamid Han Training and Research Hospital with in-

Fig. 1. The materials used in the newly developed technique.

Fig. 2. Before and after treatment images of the patients treated with this new minimally invasive technique. (a) and (b) indicate images of two different patients.

grown toenail complaints was 39, and the mean age was 29.8 years with the range of 18-79. Male to female ratio was 2.9:1 (29 male and 10 female). The number of ingrown toenails treated was 40 because one patient was treated for both right and left toenail ingrown toenails.

The symptoms of the patients treated with this technique were observed as pain, exudate, swelling and erythema. Pain was a single symptom in almost half of the cases (19 patients), while in the other half the pain was accompanied by the other symptoms. These other symptoms accompanying pain were exudate (in 8 patients), erythema (in 4 patients), swelling (in 4 patients), exudate and erythema (in 2 patients), and exudate and swelling (in 2 patients) (Table 1).

The stage of ingrown toenails of the treated patients in this study is stage 2. The presented treatment technique is recommended for the treatment of ingrown toenails with symptoms of similar severity to those described in this study. In other words, with this treatment, it is expected that success will be achieved in the treatment of ingrown toenails with stages 1 and 2.

The patients were treated with the presented minimally invasive treatment technique. The images of two different patients before and after the treatment are shown in Fig. 2 (a and b).

After the treatment of the ingrown toenails, the patients were called for follow-up at the end of the first week, first month, and sixth month.

The results of the follow-up of the patients at the end of the first week, first month and six months demonstrated that the full healing rate was high with 80% at the end of six months (Table 2).

Table 1. The complaints of the patients before the treatment

	Gender		
Complaints	**Man**	**Woman**	**Total**
Pain	15	4	19
Pain, exudate	6	2	8
Pain, erythema	2	2	4
Pain, swelling	4	-	4
Pain, exudate, erythema	-	2	2
Pain, exudate, swelling	2	-	2
Total	**29**	**10**	**39**

DISCUSSION

Ingrown toenail or onychocryptosis is a common health problem that can be observed in any age group in the population, but mostly seen in second or third decades of life, and usually in young adults or teenagers. Ingrown toenails are more common in men than women [10], and most frequently seen in males with an age range of 15 to 50 [11]. The etiology of the disease is multifactorial. In the literature, improper nail cutting and care, narrow shoe selection, abnormal nail structure, hormonal factors, genetic factors, hyperhidrosis or excessive sweating, nail fungal infection, trauma, history of nail surgery, bone abnormalities, and obesity are reported among the risk factors. Among these predisposing factors, improper nail cutting, and wrong shoe selection are more frequent ones [2, 7]. Ingrowing frequently starts at distal end of lateral nail grooves. Narrow shoe wearing compresses the tip of toe, short nail cutting causes the narrowing of the distal nail bed, and consequently it prevents the toenail to find enough space to grow properly. The patient tries to solve this discomfort by

Table 2. Healing rate achieved with the developed minimally invasive technique

	Number of treated ingrown toenails		
Follow-up period	**First week**	**First month**	**Sixth month**
Healing status			
Complaint	6	5	8
No complaint	34	35	32
Total	40	40	40
Healing rate (no complaint)	85%	87.5%	80%

cutting the nail corner more which is difficult due to surrounding soft tissue. As result of this problem, the toenail grows into the nail groove and causes pain. This improper nail cutting and trying to relieve the pain causes a vicious cycle. The fact that wrong nail cutting is one of the most known predisposing factors of ingrown toenails reveals the importance of correct nail cutting. Toenails should be cut straight, not round, also if cut too shortly, it may cause inflammatory reaction in the tissue surrounding the toenail. Diabetes, obesity, having cardiac, renal, and thyroid disorders, and poor foot hygiene are also among the other causes [6]. Hyperhidrosis or excessive sweating increases the flexibility of the nail plate, facilitating skin perforation and thereby contributing to ingrown toenail [11]. Some nail deformities may also cause ingrown toenails such as pincer nail or onychogryphosis. Compared to a flat nail structure, the problem of ingrown toenail is more likely in a more curved nail. The most severe form of this type of nail structure is called 'pincer nail'. Also, an upturned nail structure, or anomalous hallux interphalangeal angles or reduced nail thickness can cause the development of ingrown toenail. Onychogryphosis is one of the toenail deformities with an 'oyster-like' or 'ram's horn nail' appearance, and clinically characterized by a yellow-brown, opaque thickening of the nail plate with increased curvature. It is more common patients with poor personal care and in older adults.

The treatment method for ingrown toenails is selected depending on the clinical stage and severity of the disease. Staging is done according to pain, edema, erythema, infection, and granulation tissue. Most of the patients with ingrown toenails have complaints of pain, erythema and swelling [7]. Initially, pain, erythema, and swelling are seen in patients. In addition to these findings, inflammatory granuloma tissue formation accompanied by seropurulent exudate and infection is observed. Epithelialized granulation tissue and abscess formation are observed in more severe cases. There can be transitions between the stages depending on the patient's self-care [4]. Emmert first described three stages of ingrown toenails [12], later, Heitfiz divided the disease into three phases [13]. In a more recent study, Mozena described four stages. The first stage is called the inflammatory stage and characterized by the presence of slight edema, erythema, and pain in the case of applying pressure to the lateral nail fold. The second stage is the abscess stage which is divided into two substages. Initially, there is an increase in pain, erythema, edema and hyperesthesia, and the nail plate exceeds the nail bed by less than 3 mm. In the second phase of this stage, together with the similar symptoms seen in the previous stage, nail plate exceeds the nail bed by more than 3 mm. The development of granulation tissue and hypertrophy in the nail becomes chronic in the third phase. Untreated ingrown toenail at this stage can progress further, causing significant chronic deformations. In the fourth stage, severe chronic deformities are observed in the toenail, and unlike stage three, distal hypertrophy is observed [14].

Numerous conservative and surgical methods are applied in treatment of ingrown toenails, and there has been a long debate about the best treatment method. In selection of the most appropriate treatment method for ingrown toenails, several factors are evaluated together about the severity and stage of the condition. These factors are pain, and severity of inflammation if any, whether the patient had a history of ingrown toenail or not, and which treatment was applied to ingrown toenail and its success, as well as whether the patient had concomitant disease such as diabetes [9]. Conservative treatment is more reasonable in patients

having ingrown toenails with mild and moderate severity, and reduction in accompanying pain and being a cost-effective approach are considered as the advantages of this method [11]. Main conservative methods for treatment of ingrown toenails are reported as sleeve technique, cotton-wick insertion, band-aid method, dental floss technique, nail wiring, angle correction technique, and other methods such as using nail brace and acrylic artificial nail. Use of antibiotics, foot hygiene measures can also be counted among conservative methods. Surgical treatments include several techniques applied on the nail bed, nail plate and soft tissues surrounding the nail. Common surgical techniques are total nail avulsion, partial nail avulsion or wedge resection, removal or reduction of lateral nail fold, excision of nail bed, surgical segmental matrix excision, segmental matrix horn cauterization. None of the surgical methods applied for the treatment of ingrown toenails can be stated as the best method alone. It was stated by various authors that total nail avulsion has a high general recurrence rate in the range of 64-83% when used as a single technique in the treatment of ingrown toenails [15-17].

Among the various conservative and surgical methods used in the treatment of ingrown toenails, in patients having mild symptoms and those who do not prefer surgery due to the risks of surgical complications, conservative methods are preferred, while in more severe cases and patients who do not respond to conservative treatment, surgical methods can be applied. Elimination of symptoms, minimizing the healing process and patient satisfaction are the results expected from the applied treatment methods. The most appropriate method is the one that provides both functionally and aesthetically high patient satisfaction, has a low recurrence rate, enables the patient to recover rapidly and return to daily activities quickly [9]. The minimally invasive new treatment technique applied to the ingrown toenails presented in this study largely meets the above-mentioned expectations, and it is recommended for the treatment of ingrown toenails of similar severity to the patients included in this study.

CONCLUSION

The new minimally invasive technique presented in this study has many advantages compared to existing ingrown toenail treatment methods. It does not require dressing, ensures low pain, rapid recovery from the first day of the treatment, uninterrupted return to daily life on the day of the procedure, and high healing rate from the first day. The patients treated with this technique were able to wear comfortable shoes and return to their daily activities the day after the treatment. The achievement rate of the technique is also quite high according to the results of the one-week, one-month, and six-month follow-up of the treated patients. The results of this study revealed that the healing rate is high in the six-month follow-up period which demonstrates the effectiveness of this new treatment technique. It is an effective and successful technique due to its advantages such as fast recovery, low recurrence rate, high patient satisfaction by providing good functional and aesthetic results.

Authors' Contribution

Study Conception: ZŞ; Study Design: ZŞ; Supervision: ZŞ; Funding: ZŞ; Materials: ZŞ; Data Collection and/or Processing: ZŞ, TG; Statistical Analysis and/or Data Interpretation: ZŞ; Literature Review: ZŞ; Manuscript Preparation: ZŞ and Critical Review: ZŞ, TG.

Conflict of interest

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REFERENCES

1. DeLauro NM, DeLauro TM. Onychocryptosis. Clin Podiatr Med Surg 2004;21: 617-30.
2. Khunger N, Kandhari R. Ingrown toenails. Indian J Dermatol Venereol Leprol 2012;78:279-89.
3. Blatiere V. [Ingrown nails or onychocryptosis]. Presse Med 2014;43:1230-9. [Article in French]
4. Haneke E. Controversies in the treatment of ingrown nails. Dermatol Res Pract 2012;2012:783924.
5. Cho SY, Kim YC, Choi JW. Epidemiology and bone-related comorbidities of ingrown nail: a nationwide population-based study. J Dermatol 2018;45:1418-24.
6. Park DH, Singh D. The management of ingrowing toenails. Br

Med J 2012;344:e2089.
7. Geizhals, S, Lipner, SR. Review of onychocryptosis: epidemiology, pathogenesis, risk factors, diagnosis and treatment. Dermatol Online J 2019;25:13030/qt9985w2n0.
8. Murtagh, J. Patient education. Ingrowing toenails. Aust Fam Physician 1993;22:206.
9. Martinez-Nova A, Sanchez-Rodriguez R, Alonso-Pena D. A new onychocryptosis classification and treatment plan. J Am PodiatrMed Assoc 2007;97:389-93.
10. Richardson EG, Hendrix CL. Campbell's operative orthopaedics. In: ST C, ed. Disorders of nails and skin. 10th Edition ed. Philadelphia: Mosby; 2003. pp. 4171-87.
11. Heidelbaugh JJ, Lee H. Management of the ingrown toenail. Am Fam Physician 2009;79:303-8.
12. Emmert C. Zur Operation des eingewachsenen Nagels. Archiv fur Klinische Chirurgie 1869;11:266-7.
13. Heifetz CJ. Ingrown toenail. Am J Surg 1937;38:298-315.
14. Mozena JD. The Mozena Classification System and treatment algorithm for ingrown hallux nails. J Am Podiatr Med Assoc 2002;92:131-5.
15. Anderson JH, Greig JD, Ireland AJ, Anderson JR. Randomized, prospective study of nail bed ablation for recurrent ingrowing toenails. J R Coll Surg Edinb 1990;35:240-42.
16. Murray WR. Onychocryptosis: principles of non-operative and operative care. Clin Orthop Relat Res 1979;142:96-102.
17. Bryant A, Knox A. Ingrown toenails: the role of the GP, Am Fam Physician 2015;44:102-5.

The European Research Journal 2022;(3):389-395 Original Article

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Accuracy of Dongjui analyzer for reducing the number of unnecessary urine cultures in an outpatient setting

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ABSTRACT

Objectives: The purpose of this study was to evaluate the diagnostic performance of the Dongjui DJ-8602 urinary analyzer for reducing the number of unnecessary urine cultures in patients with suspected urinary tract infection (UTI).

Methods: This study was designed as a retrospective study performed in patients with suspected UTI from August 1, 2018 to December 1, 2018. Clinical data, C reactive protein, blood hematologic counts were evaluated. Using positive culture results as the gold standard, the cut-off values by the receiver operating characteristic curve technique, sensitivity, and specificity were calculated.

Results: The median values of urine leukocyte levels were 31 cells/high power field (HPF) in the culture-positive group and 5 leukocytes/HPF in the culture-negative group, respectively. The area under the curve for leukocyte and bacteria count were 0.753 (95% Cl, 0.642 to 0.862) and 0.581 (95% Cl, 0.438 to 0.725), respectively. A leukocyte count ≥ 2 cells/HPF, resulting the best sensitivity of 96.3% (95% Cl: 81.03% to 99.48%) and a negative predictive value (NPV) of 96.4% (95% Cl: 79.35% to 99.48%).

Conclusions: The use of the Dongjui DJ-8602 urinary sediment and chemistry analyzer did not accurately predict the outcome of urine cultures with an unsatisfactory sensitivity and NPVs of bacteria counts.

Keywords: Urinalysis, urinary tract infection, urine culture, instrumentation, bacteriuria

Urinary tract infection (UTI) is defined as the presence of microbial pathogens that involve any part of the urinary tract without known functional or anatomical abnormalities of the urinary tract. UTIs are among the most common infections reported in outpatient services [1].

The gold standard for the diagnosis of UTI is midstream urine culture, but it is a laborious and expensive process. Rejection of negative samples with the urinalysis test results has drawn attention to provide reduction in the number of unnecessary cultures [2]. However, there is a variable performance reported in the literature; in some of the studies the absence of cells or bacteria was found to be a useful screen to prevent urine culture, while there are still some concerns in other studies [2-6].

Many manufacturers have developed fully automated, integrated urine analyzers [7]. Automated urinalyzers as a screening system to rule out UTI are evaluated by their diagnostic sensitivity, specificity, accuracy, and area under receiver-operating-characteristic (ROC) curve [8]. Some authors suggest that certain analyzers are controversial to screen urine for UTI [2, 5]. Due to differences in their performance, each

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one needs to be evaluated separately. It is important to know how confident is each prediction outcome, otherwise false positive or false negative results can create diagnostic uncertainty. Manufacturer claims need to be verified independently, with the introduction of a new automated analyzer in a laboratory [2, 9].

Recently, the Dongjui DJ-8602 urinary sediment and chemistry analyzer (Xuzhou Dongjiu Electronic Technology Co., Ltd, China) a fully automatic system that includes one DJ-860 automatic urine sediment analyzer and one DJ-900 automatic urine chemistry analyzer was introduced.

The purpose of this study was to evaluate the diagnostic performance of the Dongjui DJ-8602 urinary analyzer as a means of reducing the number of urine samples requiring culture in patients with suspected UTI.

METHODS

This study was designed as a retrospective study performed in routine care adult patients with suspected UTI admitted to the urology outpatient clinic from August 1, 2018 to December 1, 2018. Data were retrospectively reviewed for the diagnostic performance of Dongui urinalysis system results for excluding culture negative patients. The protocol of the study was conducted in accordance with the Second Declaration of Helsinki.

Only patients with both clinical information and laboratory tests at admission were available were involved. The time for completing the laboratory data in relation to the receipt time was 2 hours after receipt. Exclusion criteria were: catheter urines, urines obtained via invasive procedures, pregnant women, children. Patients were excluded if they had been hospitalized, had been prescribed antimicrobial agents, had been seen by physicians within one month before entering the study. All of these attempts were performed to reduce the number of false-negative culture test results.

Variables recorded to patient files were taken: cloudy urine, foul-smelling urine on examination, any nocturia, dysuria, urgency, urine frequency, flue like symptoms, fever, mid-back pain, lower abdominal cramping, tiredness or fatigue, burning sensation in the bladder, symptom duration/day.

The following markers measured as part of routine care recorded were taken from the laboratory information system (LIS) data: C-reactive protein (CRP) (normal range < 3.0 mg/dl), blood hematologic counts on the Coulter LH 750 cell analyzer (Beckman Coulter, Inc., Miami, USA). The neutrophile-lymphocyte count ratio (NLR) was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. The platelet to lymphocyte count ratio was calculated by dividing the absolute platelet count to lymphocyte count (PLR).

Urine culture results recorded to LIS data from urine samples sent to the laboratory were evaluated. A sample was considered culture-positive if it contained a pure culture of ≥ 105 of colony forming units /mL [10]. Pathogenic microorganisms were identified using the Vitek 2 automated system (BioMerieux, St. Louis, Missouri, USA).

Urinalysis test results by the DJ-8602 urinary sediment and chemistry analyzer (Xuzhou Dongjiu Electronic Technology Co., Ltd, China) recorded to LIS were evaluated. DJ-8602 urinary sediment and chemistry analyzer is a fully automatic system which includes one DJ-860 automatic urine sediment analyzer and one DJ-900 automatic urine chemistry analyzer. The automatic urine sediment analyzer system uses planar flow technology and morphological identification technology applying the support vector machine image processing method.

Microscopic internal quality control results studied with an Urit QC22 Control (level 2; lot 6318600402 and negative control lot, 20170301) during the study period. Urit Strip internal quality control results studied with an IQ-11 (lot 55180007). Our laboratory was participated in the urinalysis external quality control program (KBUDEK, İstanbul, Turkey).

Statistical Analysis

Statistical analyses were performed using SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA), with culture results defined as the 'gold standard.' Samples were divided into two groups: culture negative and culture positive. Parameters with a normal distribution were evaluated using the Kolmogorov-Smirnov test. Comparison of quantitative data and intergroup comparisons of parameters were performed using Student's t-test or the Mann-Whitney U test. The chi-square test was used for the comparison of quali-

Table1. Clinical and laboratory characteristics of patients

	UTI n (%)	No UTI n (%)	*p* value
Age (years) (mean ± SD)	48 ± 15	40 ± 12	0.090
Gender			
Male	21%	9%	
Female	79%	91%	
BMI (kg/m^2) (mean ± SD)	24.8 ± 3.3	25.4 ± 4.8	0.594
Diabetes mellitus	20%	4.5%	**< 0.001**
Clinical predictors of diagnosis			
Foul smelling urine	59.2%	45.7%	0.216
Cloudy urine	44.4%	45.7%	0.905
Dysuria	95.6%	96.8%	0.326
Nocturia	42.9%	44.6%	0.225
Urgency	81%	47%	**0.006**
Urinary frequency	93%	98%	0.101
Flue like symptoms	14.8%	18.0%	0.692
Fever	7.4%	7.4%	0.995
Mid-back pain	40.7%	42.5%	0.867
Burning sensation	77.7%	78.7%	0.916
Lower abdominal cramping	22.2%	35.1%	0.207
Tiredness or fatique	37.0%	29.7%	0.303
Suprapubic warm sensation	59.2%	61.%	0.196
Symptom duration/day (median [IQR])	4 (4)	4(4)	0.778
Laboratory data			
CRP (mg/dl)	4.7 (26)	3.0 (1.6)	**0.030**
Haemogram test			
WBC (×10^9/L)	8.4 (4.1)	7.1 (3.1)	**0.020**
Neutrophil (×10^9/L)	5.4 (3.6)	4.2 (1.7)	0.090
Lymphocyte (×10^9/L)	2.2 (0.7)	2.3 (0.8)	0.228
NLR	2.3 (1.8)	1.7 (0.9)	0.023
Platelet	266 (66)	276 (77)	0.953
PLR	132 (69)	121 (43)	0.144
MPV (fL)	8.1 (1.0)	8.6 (1.4)	0.067
RDW (%)	139 (2.8)	136 (1.7)	0.330
Urine			
Dipstick			
LE ≥ 1+	81.4%	45.7%	**0.004**
NO_2	33.3%	4.2%	**< 0.001**
Microscopy			
Leukocyte (count/HPF)	31 (130)	5 (14)	**< 0.001**
Erythrocyte	8 (36)	4 (12)	**0.019**
Bacteria(count/HPF)	0 (43)	0 (6)	0.198

BMI = body mass index, CRP = C-reactive protein, WBC = white blood cell, NLR = neutrophil to lymphocyte ratio, PLR = platelet to lymphocyte ratio, MPV = mean platelet volume, RDW = red cell distribution width, HPF = high power field, IQR = interquartile range; SD = standard deviation. Chi-square test for Association

tative data. ROC curves were created to identify the cut-off points for variables.

Using positive culture results as the gold standard, sensitivity, specificity, positive predictive value (PPV; the post-test probability of an outcome for positive tests), negative predictive value (NPV; the post-test probability of an outcome for negative tests), accuracy (the proportion of true results in the population), positive likelihood ratio (LR+) (the probability of a person who has the disease testing positive divided by the probability of a person who does not have the disease testing positive), negative likelihood ratio (LR–) (the probability of a person who has the disease testing negative divided by the probability of a person who does not have the disease testing negative), diagnostic odds ratio LR+/LR- and accuracy (true positives + true negatives)/ all cases were calculated.

RESULTS

In total, 121 patients were found to be eligible for study enrollment. Table 1 shows characteristics of patients on admission. The study group ranged in age from 18 to 74 years. Significant bacterial growth was detected in 27 (20.18%) specimens. Escherichia coli grew in 23 (95%) of the positive cultures. The CRP (p = 0.030), WBC (p = 0.020) and NLR (p = 0.023) levels were higher in patients with UTI.

The median values of urine leukocyte levels were 31 cells/HPF and 8 cells/high power field (HPF) for erytrocytes with the DJ-8602 urinary sediment and chemistry analyzer in the culture-positive group and 5 leukocytes/HPF and 4 erytrocytes/HPF in the culture-negative group, respectively (see Table1).

ROC curves for leukocyte and bacterial counts of the urine sediment analyses are presented in Fig. 1. The AUC for leukocyte counts was 0.753 (95% Cl, 0.642 to 0.862), compared with 0.581 (95% Cl, 0.438 to 0.725) for bacteria; showing acceptable discrimination power for UTI only for urine leukocyte count with a Dongjui DJ-8602 urinary sediment and chemistry analyzer (Table 2) [8].

When cut-off points for microscopy results were analysed, a leukocyte count ≥ 2 cells/HPF, resulting in a sensitivity of 96.3% (95% Cl: 81.03 to 99.48) and a NPV of 96.4 (95% Cl: 79.35 to 99.48) (Table 3). An optimum cut-off a leukocyte count ≥ 7 cells/HPF yielded a sensitivity of 70.3% (95% CI: 49.82%-86.25%) and a specificity of 58.51% (95% CI: 47.88%-68.59%). Bacterial counts were not different between UTI and nonUTI groups (p = 0.198) (Fig. 2).

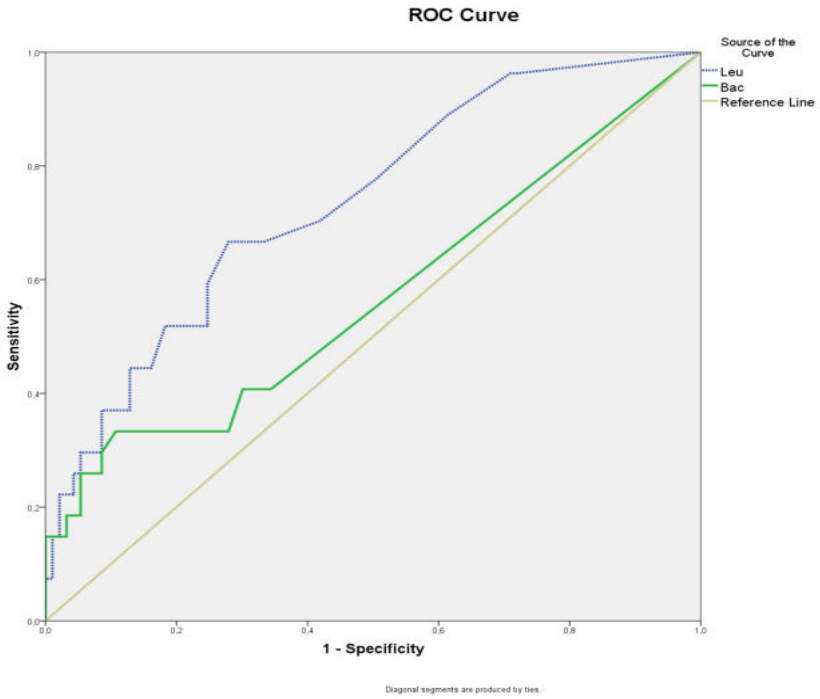

Fig. 1. Receiver operating characteristics (ROC) curve for the whole group to determine the negative culture with urinalysis test results. The optimum cut-off point for WBC was 4 cells/HPF.

Table 2. Diagnostic performances of urinalysis test results for distinguishing culture positive patients from urinalysis

Variables	AUC	SE	Significance	95% CI
Leukocyte	0.753	0.057	**< 0.001***	0.642-0.864
Erythrocyte	0.647	0.059	**0.020**	0531-0.763
Bacteria	0.581	0.073	0.221	0.438-0.725
Cylendir	0.542	0.069	0.532	0.406 -0.677
Crystal	0.518	0. 068	0.789	0.385 -0.650

AUC = area under curve, SE = standard error, CI = confidence interval. *Null hypothesis = true area = 0.5

Table 3. The discriminative and predicting power of the each individual urinalysis variable

Parameter	SE (%) (95% CI)	SP (%) (95% CI)	PPV (%)* (95% CI)	NPV (%)* (95% CI)	LR+ (95% CI)	LR- (95% CI)	Accuracy (%)* (95% CI)
Dipstick							
LE ≥ 1+	81.48 (61.92-93.70)	54.26 (43.66-64.58)	33.85 (27.80-40.47)	91.07 (81.90-95.83)	1.78 (1.34-2.37)	0.34 (0.15-0.77)	60.33 (51.04-69.11)
NO2 ≥ 1+	66.67 (46.04-83.48)	95.74 (89.46-98.83)	81.82 (62.45-92.41)	90.91 (85.41-94.47)	15.67 (5.79-42.39)	0.35 (0.20-0.59)	89.26 (82.33-94.15)
Microscopy							
WBC[1] (≥ 2)	96.3 (81.03-99.91)		27.96 (25.07-31.04)	96.43 (79.35-99.48)	1.35 (1.17-1.57)	0.13 (0.02-0.91)	43.80 (34.80-53.11)
WBC[2] (≥ 7)	70.37 (49.82-86.25)	58.51 (47.88-68.59)	32.76 (25.69-40.70)	87.30 (78.95 to 92.65)	1.70 (1.20-2.39)	0.51 (0.28-0.93)	61.16 (51.87-69.88)
Bacteria[1] (≥ 3)	40.74 (22.39-61.20)	70.21 (59.90-79.21)	28.21 (18.47-40.53)	80.49 (74.61-85.28)	1.37 (0.79-2.37)	0.84 (0.60-1.18)	63.64 (54.40-72.19)
Bakteri[2] (≥ 18)	33.33 (16.52-53.96)	86.17 (77.51-92.43)	40.91 (24.94-59.06)	81.82 (77.30-85.60)	2.41 (1.16-5.02)	0.77 (0.59-1.02)	74.38 (65.65-81.88)

LE = leukocyte esterase, NO_2 = nitrite, SE = sensitivity, SP = specificity, NPV = negative predictive value, PPV = positive predictive value, LR+ = positive likelihood ratio, LR− = negative likelihood ratio, Accuracy = correctly classified %. *These values are dependent on disease prevalence. [1]Highest sensitivity, [2]Cut-off

DISCUSSION

Like others before us, we found that negative urine cultures constitute a large percentage of the clinical laboratory workload in our hospital [2, 3, 5, 11]. Only the ROC analysis of the urinary leukocyte counts of the Dongjui DJ-8602 urine sediment analyzer shows an acceptable AUC value of 0.753. However, this value is not outstanding and when used alone may lead to diagnostic uncertainty [13].

In our study, the sensitivity, specificity, and AUC of leukocyte count were higher than those of bacteria count; similar to Kocer *et al*.'s [12] findings. A screening analyzer to reduce the number of negative urine culture samples is reliable only when a high sensitivity and high negative predictive values are obtained. And ideally, the sensitivity should be > 90-95% to rule out disease [14, 15]. The cut-off point should be low enough to exclude the minimum urine samples.

A detailed analysis showed that the WBC ≥ 2 cells / HPF gave only one false negative result, with a sensitivity of 96.3% and a NPV of 96.4%. This cut-off point will reduce the number of samples sent to the laboratory for culture. However, the harmful effects of false negative findings should be taken into account. On the other hand, predicting negative results may prevent the risk of unnecessary initiation of antibiotic treatment.

Our results were comparable to previous studies with automated microscopy methods such as digital imaging with laminar flow (İQ 200) and verified digital images (sediMAX, FUS-100, Cobas u 700, Atel-

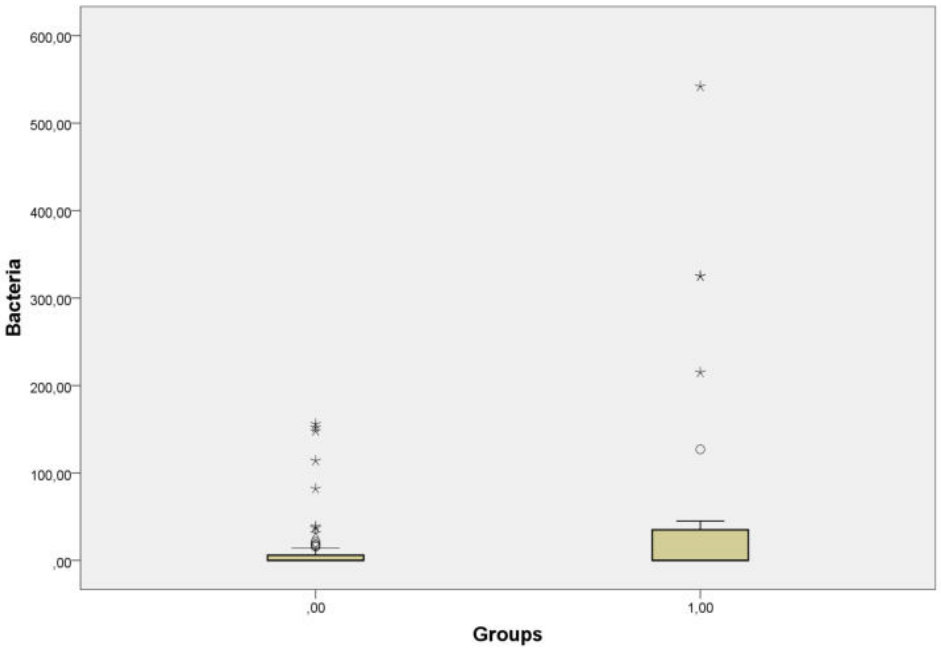

Fig. 2. Comparison of quantitative bacterial counts between groups. Bacteria: count/HPF; Group 0: Patients without UTI, Group 1: Patients with UTI. Box represents the confidence interval (95%).

lica 1500) that leukocyte parameter is better than those of bacteria count [16] . However differences based on the definition of negative urine, variation in cut off levels applied, patient populations and clinical situations makes it difficult to directly compare study results [16].

In our study, we found that the bacteriuria parameter of the Dongjui DJ-8602 urine sediment analyzer, which uses an image analysis method has poor discriminatory power for UTI. Our results are consistent with other studies that the bacteriuria parameter with automated microscopy cannot be used alone to reduce the number of negative urine culture samples [17-20].

The sensitivity of the urine dipstick test for nitrites was low (66.7%) with higher levels of specificity (95.74%) comparable to those of the other studies [2, 21, 22]. The sensitivity of LE was acceptable (81.48%.) [15]. Results produced by dipstick should not be overly relied upon for screening [23, 24].

Limitations

The main limitations of this study include the use of retrospective data, inclusion of data from only one urology department. A single cut off value of 105 colony-forming unit (CFU)/ml was used for the culture. Also, the delay from initiation until publication is a weakness.

CONCLUSION

In conclusion, we found that the use of the Dongjui DJ-8602 urinary sediment and chemistry analyzer did not accurately predict the outcome of urine cultures with low sensitivity and NPVs of bacteria counts.

Authors' Contribution

Study Conception: MÖ, YÜ, KH; Study Design: YÜ, KH; Supervision: MÖ, YÜ, AS, KH; Funding: MÖ, YÜ, AS, KH; Materials: MÖ, YÜ, AS, KH; Data Collection and/or Processing: MÖ, YÜ, AS, KH; Statistical Analysis and/or Data Interpretation: MÖ, YÜ, AS, KH; Literature Review: MÖ, YÜ, AS, KH; Manuscript Preparation: MÖ, YÜ, AS, KH and Critical Review: MÖ, YÜ, AS, KH.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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REFERENCES

1. Grigoryan L, Trautner BW, Gupta K. Diagnosis and management of urinary tract infections in the outpatient setting: a review. JAMA 2014;312:1677-84.
2. Huysal K, Budak YU, Karaca AU, Aydos M, Kahvecioğlu S, Bulut M, et al. Diagnostic accuracy of uriSed automated urine microscopic sediment analyzer and dipstick parameters in predicting urine culture test results. Biochem Med (Zagreb) 2013;23:211-7.
3. Falbo R, Sala MR, Signorelli S, Venturi N, Signorini S, Brambilla P. Bacteriuria screening by automated whole-field-image-based microscopy reduces the number of necessary urine cultures. J Clin Microbiol 2012;50:1427-9.
4. Ma J, Wang C, Yue J, Li M, Zhang H, Ma X, et al. Clinical laboratory urine analysis: comparison of the UriSed automated microscopic analyzer and the manual microscopy. Clin Lab 2013;59:1297-1303.
5. Bignardi GE. Validation and verification of automated urine particle analysers. J Clin Pathol 2017;70:94-101.
6. Shang YJ, Wang QQ, Zhang JR, Xu YL, Zhang WW, Chen Y, et al. Systematic review and meta-analysis of flow cytometry in urinary tract infection screening. Clin Chim Acta 2013;424:90-5.
7. Budak YU, Huysal K. Comparison of three automated systems for urine chemistry and sediment analysis in routine laboratory practice. Clin Lab 2011;57:47-52.
8. Pereiara P. Evaluation of rapid diagnostic test performance. In: Proof and Concepts in Rapid Diagnostic Tests and Technologies. Saxena SK (ed.), 1st ed. Inteck, Croatia; 2016:p.147.
9. Zaman Z, Roggeman S, Verhaegen J. Unsatisfactory performance of flow cytometer F-100 and urine strips in predicting outcome of urine cultures. J Clin Microbiol 2001;39:4169-71.
10. Graham JC, Galloway A. ACP Best Practice No 167: the laboratory diagnosis of urinary tract infection. J Clin Pathol 2001;54:911-9.
11. Brilha S, Proença H, Cristino JM, Hänscheid T. Use of a flow cytometry (Sysmex) UF-100) to screen for positive urine cultures: in search for the ideal cut-off. Clin Chem Lab Med 2010;48:289-92.
12. Kocer D, Sariguzel FM, Ciraci MZ, Karakukcu C, Oz L. Diagnostic accuracy of a new urinalysis system, DongJiu, for diagnosis of urinary tract infection. Ann Clin Lab Sci 2015;45:686-91.
13. McHugh LC, Snyder K, Yager TD. The effect of uncertainty in patient classification on diagnostic performance estimations. PLoS One 2019;14:e0217146.
14. Akobeng AK. Understanding diagnostic tests 1: sensitivity, specificity and predictive values. Acta Paediatr 2007;96:338-41.
15. European Confederation of Laboratory Medicine. European urinalysis guidelines. 2000;231:1-86.
16. Oyaert M, Delanghe J. Progress in automated urinalysis. Ann Lab Med 2019;39:15-22.
17. Stürenburg E, Kramer J, Schön G, Cachovan G, Sobottka I. Detection of significant bacteriuria by use of the iQ200 automated urine microscope. J Clin Microbiol 2014;52:2855-60.
18. Sterry-Blunt RE, S Randall K, J Doughton M, H Aliyu S, A Enoch D. Screening urine samples for the absence of urinary tract infection using the sediMAX automated microscopy analyser. J Med Microbiol 2015;64:605-9.
19. Kocer D, Sarıguzel FM, Karakukcu C. Cutoff values for bacteria and leukocytes for urine sediment analyzer FUS200 in culture-positive urinary-tract infections. Scand J Clin Lab Invest 2014;74:414-7.
20. Zaman Z, Fogazzi GB, Garigali G, Croci MD, Bayer G, Kranicz T. Urine sediment analysis: analytical and diagnostic performance of sediMAX – a new automated microscopy image-based urine sediment analyser. Clin Chim Acta 2010;411:147-54.
21. Ducharme J, Neilson S, Ginn JL. Can urine cultures and reagent test strips be used to diagnose urinary tract infection in elderly emergency department patients without focal urinary symptoms? 2007;9:87-92.
22. Marques AG, Doi AM, Pasternak J, Damascena MDS, França CN, Martino MDV. Performance of the dipstick screening test as a predictor of negative urine culture. Einstein (Sao Paulo) 2017;15:34-9.
23. Dadzie I, Quansah E, Dakorah MP, Abiade V Takyi-Amuah E, Adusei R. The effectiveness of dipstick for the detection of urinary tract infection. Can J Infect Dis Med Microbiol 2019;2019:8642628.
24. Krongvorakul J, Phundhusuwannakul S, Santanirand P, Kunakorn M. A flow cytometric urine analyzer for bacteria and white blood cell counts plus urine dispstick test for rapid screening of bacterial urinary tract infection. Asian Biomed 2012;6:601-8.

The European Research Journal 2022:8(3):396-403 Original Article

DOI: 10.18621/eurj.1099649 *Cardiovascular Surgery*

Protective effect of *Tulbaghia violacea* extract on cardiac damage: deep circulatory arrest rat model

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ABSTRACT

Objectives: Total circulatory arrest (TCA) technique is a method used in cardiac surgery accompanied by cardiopulmonary bypass (CPB). It has been shown that *Tulbaghia violacea* extract increases antioxidant capacity, regulates blood pressure, decreases lipid peroxide, and reduces atherosclerosis. In this experimental study, we aimed to investigate the effect of *T. violacea* extract administration on serum oxidative stress parameters (Total antioxidant status [TAS], total oxidant status [TOS] and oxidative stress index [OSI] and deoxyribonucleic acid [DNA] damage level with 8-Hydroxy-2-Deoxyguanosine [8-OHdG]) and histopathological changes in the heart and other organs in rats with deep circulatory arrest model.

Methods: In this study, 48 Wistar Albino adult rats, 24 female and 24 male, obtained from Harran University Experimental Animals Research Center were used. The average weight of female rats was 250-300 g, and the average weight of male rats was 450-500g. These rats were randomly divided into four groups. 6 male and 6 female rats were used in each group (Group 1 = Sham, Group 2 = Injury, Group 3 = Treatment and Injury, Group 4 = Treatment).

Results: As a result of this experimental study, the changes in the biochemical 8-OHdG, TOS, OSI and TAS levels of the groups were found to be statistically significant ($p < 0.001$). In the subgroup analyzes of the data, 8-OHdG level, which is an oxidative DNA damage marker in Group 2 was higher than the Group 1, Group 3 and Group 4 and there was a statistically significant difference ($p < 0.001$, $p = 0.027$ and $p < 0.001$; respectively). The TOS level of the injury group was higher than Group 1, Group 3 and Group 4 and there was a statistically significant difference ($p < 0.001$, $p = 0.003$ and $p < 0.001$; respectively).

Conclusions: As a result of our study, we revealed that *T. violacea* extract has a protective effect on organ and tissue damage in the TCA model.

Keywords: Circulation, arrest, protective agent, plant derived, *Tulbaghia violacea* extract

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Cardiac diseases have an important place among the causes of morbidity and mortality all over the world [1]. It will continue to be an important health problem in the future [2]. Cardiac surgery has an important role in the treatment of these diseases, and most of cardiac surgeries are performed using cardiopulmonary bypass (CPB). In addition, the total circulatory arrest (TCA) technique is performed in cardiac surgery accompanied by CPB, especially in aortic arch surgery [3]. The deep hypothermic TCA method is the method in which the body temperature is lowered below 20 oC and the blood circulation is temporarily stopped. It is preferred in thoracic aortic lesions associated with aortic arch, complex congenital pathologies, pulmonary thromboembolectomy and sometimes intracerebral aneurysm surgeries other than cardiovascular surgery. After hypothermic circulatory arrest, adverse events related to many organs, especially neurological and cardiac damage, may occur. They are localized ischemic infarcts that develop as a result of global ischemia or embolism as a result of localized stroke, infarction, insufficient flow or protection [4, 5]. In order to prevent these negative situations, cardioplegic arrest for the heart and hypothermia and various medical treatments are used for other organs [6].

Tulbaghia violacea plant is a perennial ornamental plant with green leaves in summer and winter, its leaves and flowers give a garlic odor when rubbed, are edible, have a garlic flavor. This plant contains active compounds such as tannins, terpenoids, flavonoids, saponins, proteins, steroids, cardiac glycosides, phenols and coumarins [7]. It has been shown that *T. violacea* increases antioxidant capacity, regulates blood pressure, decreases lipid peroxide, and reduces atherosclerosis [8].

In this experimental study, we aimed to investigate the effect of *T. violacea* extract administration on serum oxidative stress parameters (Total antioxidant status [TAS], total oxidant status [TOS] and oxidative stress index [OSI] and deoxyribonucleic acid [DNA] damage level with 8-Hydroxy-2-Deoxyguanosine [8-OHdG]) and histopathological changes in the heart and other organs in rats with deep circulatory arrest model.

METHODS

This research was carried out as a doctoral thesis by the Perfusion Technology Program of the Department of Cardiovascular Surgery, Institute of Health Sciences, Harran University. Approval was obtained from Harran University Animal Experiments Local Ethics Committee on 11/12/2020 (Session no: 2020/0006, Decision: 01-17). After the approval of the Ethics Committee, a scientific research project was prepared. The project support of the study was approved by Harran University Scientific Research Projects Coordination Unit on 29/04/2021 (HUBAK Project No: 21130). Experimental study was carried out between 17/06/2021-02/07/2021 in Harran University Experi-

Fig. 1. Controlled ischemia in the heart by applying a cross-clamp to the aorta in a rat who underwent laparotomy and sternotomy.

mental Animals Laboratory. The blood and pathology samples taken as a result of the experimental study were stored under appropriate conditions and the data were obtained by working in the laboratories of Medical Biochemistry and Medical Pathology of Harran University.

Experimental Animals

In this study, 48 Wistar Albino adult rats, 24 female and 24 male, obtained from Harran University Experimental Animals Research Center were used. The average weight of female rats was 250-300 g, and the average weight of male rats was 450-500 g. These rats were randomly divided into four groups. 6 male and 6 female rats were used in each group. Animal experimentation and care of all rats were carried out in accordance with the principles of "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" and "Care Principles of Experimental Animals" shaped by the National Association for Medical Research.

Deep Circulatory Arrest Model

Before the study, rats were fasted for 8 hours. During this time, they were allowed to drink water. All applications were performed under anesthesia. Ketamine (60 mg/kg) (Ketasol® 10%, 10ml, Richter Pharma AG 4600 Wels, Austria) and xylazine (40 mg/kg) intraperitoneally (ip) for anesthesia purposes (Rompun® 2%, 25ml, Bayer Healthcare LLC. Kansas 66201), USA) was applied. If necessary, an additional dose of 3/1 of the application dose was planned. Midline laparotomy and sternotomy were performed on rats whose skin was prepared aseptically (the abdominal skin was cleaned with povidone iodine after shaving), and the heart was reached. Before cardiac arrest, 0.1 ml of heparin (Koparin® 25000 IU/5ml, Koçak Farma İlaç ve Kimya Sanayi A.Ş. Kapaklı, Tekirdağ) was administered to all rats with an insulin injector from the right atrium while the heart was working [9]. Then, controlled ischemia was created in the heart by placing a cross-clamp on the aorta (Fig. 1). Immediately after the application of cross-clamp to the aorta, cardiac arrest was achieved by administering 0.2 ml of potassium chloride (KCL) (Turktipsan Potassium Chlorür® 750 mg/10ml, Turktıpsan Turizm Eğitim ve Tic. AS. Akyurt, Ankara) from the right atrium or aortic root with an insulin injector [10, 11]. In some groups, total circulatory arrest was created for 20 minutes. Cardiac damage was created with this circulatory arrest [12, 13]. Then, blood was drawn from the hearts (right atrium) of all rats and their hearts were surgically removed. Tissues and blood samples were stored at -80 oC for histopathological and biochemical studies until the study.

Working Groups

Group 1 (Sham group, n = 12): They were fed with standard rat chow and tap water for 15 days. On the 16th day, all rats in this group were sacrificed under deep anesthesia (Ketamine (90 mg/kg) and xylazine (10 mg/kg)-i.p) and their blood and all tissues were removed.

Group 2 (Injury group [TCA group], n =12): An experimental cardiac injury model was created with TCA in rats after similar feeding and anesthesia. After TCA, the blood sample and all tissues were removed.

Group 3 (Treatment+Injury group [*T. violacea* extract+TCA group], n = 12): Different from group 2, *T. violacea* extract (500 mg/kg/day) dissolved in 0.9% saline for 15 days was given to this group by oral gavage. administered directly into the stomach.

Group 4 (Treatment group [*T. violacea* extract group], n = 12): In this group, unlike Group 3, blood and tissue samples of the rats were extracted without applying the TCA model.

Histopathological Examination

As a result of the experimental study, the tissues taken from the rats were grouped and numbered, and the heart tissues were fixed separately in 10% buffered neutral formaldehyde solution for histopathological examination. Afterwards, the samples were embedded in paraffin blocks and sections of 5 micron meters were taken. These sections were stained with hematoxylin-eosin dye and examined with Nikon Eclipse® NI DS-FI2, JAPAN microscope. Interstitial edema, inflammatory cell infiltration and presence of necrosis were evaluated in the histopathological examination.

Biochemical Evaluation

As a result of the experimental study, the blood taken from the rats was grouped and numbered and collected in heparinized yellow capped gel-free tubes. Collected blood was centrifuged at 4000 rpm for 10 minutes. The serum portion from the centrifuged

blood was transferred to eppendorf tubes and stored at -80 oC until the study day. Then, changes in oxidative stress parameters (TAS, TOS, OSI) and DNA damage level (8-OHdG) at blood serum levels were examined.

Statistical Analysis

Statistical analyzes were performed using the SPSS® package program (IBM Corp. Released 2012, IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp.). Mean and standard deviations were calculated for continuous and ordinal data. Data were given as mean and standard deviation. Kolmogorov Smirnov test and Shapiro-Wilk test were used to evaluate the normality distribution. One Way ANOVA test was used to compare the four groups. Tukey test was used for subgroup analysis of the data. Statistically $P < 0.05$ values were considered significant.

RESULTS

As a result of this experimental study, the changes in the biochemical 8-OHdG, TOS, OSI and TAS levels of the groups were found to be statistically significant ($p < 0.001$) (Table 1).

In the subgroup analyzes of the data (Table 2), 8-OHdG level, which is an oxidative DNA damage marker in Group 2 was higher than the Group 1, Group 3 and Group 4 and there was a statistically significant difference ($p < 0.001$, $p = 0.027$ and $p < 0.001$; respectively). The TOS level of the injury group was higher than Group 1, Group 3 and Group 4 and there was a statistically significant difference ($p < 0.001$, $p = 0.003$ and $p < 0.001$; respectively).

The OSI level of Group 2 was higher than Group 1, Group 3 and Group 4 and there were statistically significant differences ($p < 0.001$, for all). TAS levels in Group 1, Group 3 and Group 4 were higher than Group 2 and there were statistically significant differences ($p < 0.001$, $p = 0.004$ and $p < 0.001$; respectively).

Cardiac Tissue Histopathological Findings (Fig. 2)

As a result of the experimental study, the regular structure in the heart tissue of the rats was examined in the histopathological examination of the heart tissues taken from the rats. No pathological finding was detected. A regular heart muscle is observed in the sample of Group 1 (Fig. 2A). Histopathological examination of Group 2 reveals localized areas of necrosis and edema in the myocardial tissue in the tissue sections (Figs. 2B and 2C). In the histopathological examination of Group 3 significant improvement was observed in the areas of edema and focal necrosis in the muscle fibers (Fig. 2D). Histopathological examination of Group 4 revealed regular muscle fibers (Fig. 2E).

Histopathological Findings of Liver and Kidney Tissues (Fig. 3)

In Group 1, hepatic (Fig. 3A) and renal parenchyma (Fig. 3B) are observed in a regular histopathological structure. Focal lytic necrosis in the liver tissue of Group 2 is indicated by the green arrow (×400) (Figure 3C). In the histopathological examination of Group 2, hemorrhage in the renal parenchyma, interstitial tissue between the tubules, glomerulosclerosis (Blue arrow), inflammatory infiltration in the stroma (Fig. 3D) (×100), Group 3, significant improvement in periportal fibrosis in liver tissue (Fig. 3E), renal parenchyma (×100) containing regular glomerular structures with minimally thrombosed vascular structures (Fig. 3F).

Table 1. The change of oxidative parameters of the experimental rat groups

	Group 1 (Sham)	Group 2 (Injury)	Group 3 (Treatmet/Injury)	Group 4 (Treatment)	*p* value
8-OHDOG (ng/mL)	1.8 ± 0.59	6.22 ± 1.5	4.58 ± 2.14	1.55 ± 0.47	**< 0.001**
TOS (µmol H2O2 Eqv/1)	10.66 ± 2.15	15.85 ± 1.71	13.13 ± 1.81	10.29 ± 1.32	**< 0.001**
OSİ (aµ)	0.69 ± 0.17	1.4 ± 0.23	0.93 ± 0.18	0.65 ± 0.11	**< 0.001**
TAS (mmol Trolox Eqv/1)	1.57 ± 0.27	1.14 ± 0.12	1.41 ± 0.13	1.6 ± 0.17	**< 0.001**

One Way Anova test was used to compare the four groups. Data were given as mean and standard deviation. 8-OhdG = 8-hydroxy-2'-deoxyguanosine, TOS = Total oxidant status, OSI = Oxidative stress index, TAS = Total antioxidant status.

Table 2. Subgroup analysis of the data of the experimental groups

8-OHDOG (ng/mL)	Group 1 vs Group 2 ***p* < 0.001**	Group 1 vs Group 3 ***p* < 0.001**	Group 1 vs Group 4 *p* = 0.973
	Group 2 vs Group 3 ***p* = 0.027**	Group2 vs Group 4 ***p* < 0.001**	Group 3 vs Group 4 ***p* < 0.001**
TOS (µmol H2O2 Eqv/1)	Group 1 vs Group 2 ***p* < 0.001**	Group 1 vs Group 3 ***p* = 0.007**	Group 1 vs Group 4 *p* = 0.956
	Group 2 vs Group 3 ***p* = 0.003**	Group 2 vs Group 4 ***p* < 0.001**	Group 3 vs Group 4 ***p* = 0.002**
OSI (aµ)	Group 1 vs Group 2 ***p* < 0.001**	Group 1 vs Group 3 ***p* = 0.01**	Group 1 vs Group 4 *p* = 0.949
	Group 2 vs Group 3 ***p* < 0.001**	Group2 vs Group 4 ***p* < 0.001**	Group 3 vs Group 4 ***p* = 0.002**
TAS (mmol Trolox Eqv/1)	Group 1 vs Group 2 ***p* < 0.001**	Group 1 vs Group 3 *p* = 0.175	Group 1 vs Group 4 *p* = 0.988
	Group 2 vs Group 3 ***p* = 0.004**	Group2 vs Group 4 ***p* < 0.001**	Group 3 vs Group 4 *p* = 0.091

Tukey test was used for subgroup analysis of the data. Group 1 = Sham, Group 2 = Injury, Group 3 = Treatment/Injury, Group 4 = Treatment, 8-OhdG = 8-hydroxy-2'-deoxyguanosine, TOS = Total oxidant status, OSI = Oxidative stress index, TAS = Total antioxidant status

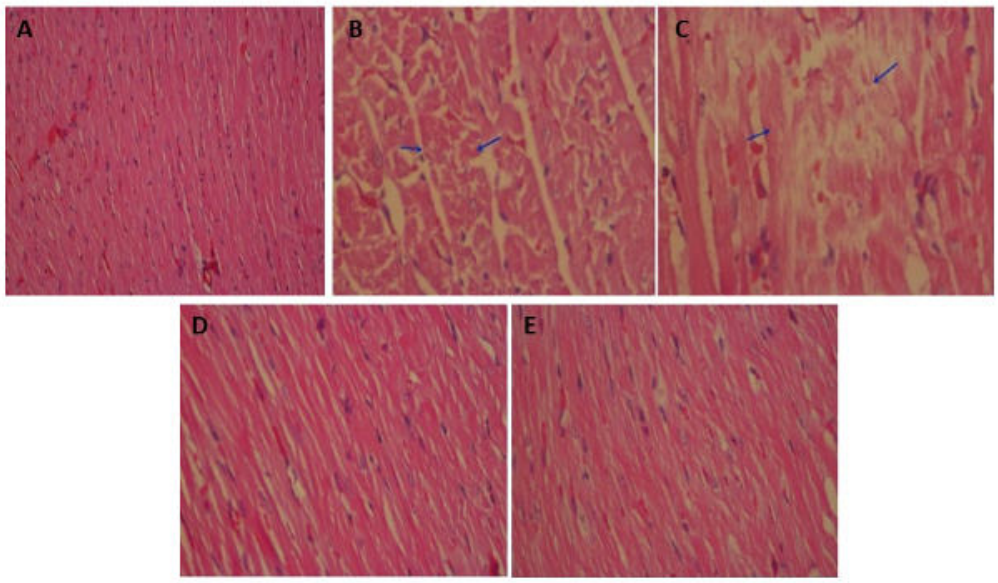

Fig. 2. (A) Regular cardiac muscle is observed in the sample belonging to the Sham group (B, C) Histopathological examination of the injury group (Group 2), necrosis and edema areas in the myocardium in tissue sections in the histopathological examination (D) Histopathological examination of the Treatment+Injury group (Group 3) Significant improvement in edema and focal necrosis in muscle fibers in the examination (E) Regular muscle fibers in the histopathological examination of the treatment group (Group 4).

DISCUSSION

Aortic cross clamp and TCA applications are frequently performed in operations where CPB is used. Oxidative stress occurs as a result of insufficient oxygenated blood to the tissues [14]. CPB is also an im-

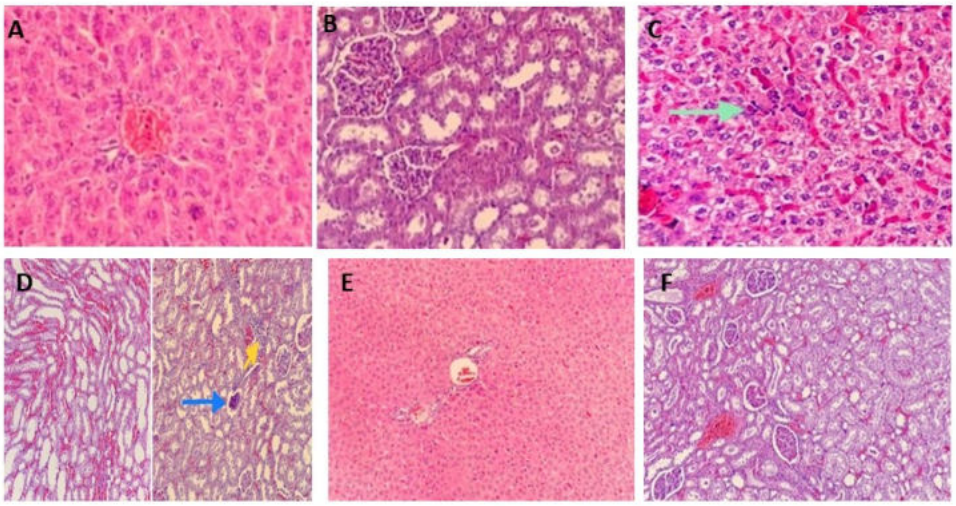

Fig. 3. (A) liver from sham group, (B) kidney from sham group, (C) focal lytic necrosis in liver tissue in injury group, (D) (left side) hemorrhage in intertubular interstitial tissue in renal parenchyma, right side glomerulosclerosis (blue arrow), inflammatory infiltration in the stroma (yellow arrow), (E) reduced periportal fibrosis in the damage and treatment group, (F) renal parenchyma containing regular glomerular structures with minimal thrombosed vascular structures with treatment damage.

portant factor for the formation of oxidative stress. The reason for this is neutrophils, catecholamines, which are activated as a result of contact of blood with nonendothelial circuit elements, activation of the complement system and cytokines released as a result of activation of neutrophils [15]. In these operations, various medical treatments are applied along with hypothermia to ensure the continuation of organ vitality, especially the heart. In this experimental deep circulatory arrest model, we showed that oxidative stress parameters (TAS, TOS, OSI, 8-OHdG), histopathological damage in heart, kidney and liver tissue decreased by administering *T. violacea* extract to rats with organ damage.

It is known that oxidative stress causes damage by causing lesions such as base and sugar modifications, single and double chain breaks, DNA-protein cross-linking on deoxyribo nucleic acid by different mechanisms [16]. In our study, 8-OHdG, a marker of oxidative DNA damage, was found to be higher in the damage group (Group 2) than the other groups. We think that this is due to the insufficiency of existing antioxidant mechanisms as a result of ischemia and TCA. In the groups given *T. violacea* extract, the lower 8-OHdG level can be explained by the external antioxidant effect of T. *violacea* extract [8].

Murugesan *et al*. [17] investigated the protective role of *T. violacea* extract on isoproterenol-induced myocardial necrosis in rats. In their study, they treated rats with *T. violacea* extract (60 mg/kg body weight) daily for 30 days. Myocardial necrosis was induced by subcutaneous injection of isoproterenol (85 mg/kg body weight) on days 29 and 30 in rats. On the 31st day, the rats were sacrificed under anesthesia and their blood and tissues were collected. In the results of myocardial necrosis induced by isoproterenol; stated that cardiac markers, lipid peroxidation products and heart rate levels increased significantly, while plasma enzymatic antioxidants decreased significantly. At the end of the study, they stated that the treatment of myocardial necrosis with *T. violacea* extract showed significant effects on all biochemical and molecular studies. They also found that *T. violacea* extract increased antioxidant production and had a protective effect on isoproterenol-induced myocardial necrosis in rats [17]. In our study, we found positive effects of *T. violacea* extract on cardiac damage (oxidative stress and DNA damage) caused by circulatory arrest.

Modeley *et al*. [18] investigated the cardiovascular effects of TV methanolic extract in Dahl salt sensitive rats. In their study, Dahl salt was administered intraperitoneally in rat groups. After that, distilled water (3 ml/kg) to the control group; captopril (25 mg/kg) to one group; and *T. violacea* methanolic extract (50 mg/kg) to one group were administered for 7 weeks. As a result of their study, they stated that besides many positive effects, *T. violacea* extract, when applied for a long time, showed an antihypertensive effect in rats

sensitive to Dahl salt [18]. Raji *et al*. [19] aimed to investigate the effect of *T. violacea* extract on blood pressure and heart rate in aging normotensive rats and adult spontaneously hypertensive rats. As a result of their study, they stated that *T. violacea* extract significantly and dose-dependently reduced systolic and diastolic blood pressure, and mean arterial pressure in both rat groups [19].

In some studies, it has been shown that *T. violacea* extract has antithrombotic and anticoagulant effects [20, 21]. Davison *et al*. [20] investigated the properties such as platelet aggregation, adhesion and protein secretion in both in vitro and ex vivo rat models to determine the effects of *T. violacea* extract on platelets in their study. In their study, they stated that *T. violacea* extract had a higher inhibition on platelet aggregation and agglutination than aspirin. They stated that in the prothrombin time test, it decreased the coagulation times, but prolonged the coagulation time in the activated partial thromboplastin time (aPTT) experiment in the ex vivo model, which showed its antithrombotic ability. They found that the *T. violacea* extract increased D-dimer and fibrinogen-C concentrations in the in vitro model, but had no effect on D-dimer concentrations. In the ex vivo model, they found that it decreased fibrinogen-C values. As a result, they revealed that *T. violacea* extract has a beneficial effect on the modulation of platelet activation by decreasing fibrinogen levels, increasing the aPTT duration, and through the glycoprotein receptor IIb in platelets [20]. There are also studies showing the protective effect of *T. violacea* extract on other organs (kidney, liver) and tissues (aortic tissue) [22- 24]. In their study, Olorunnisola *et al*. [22] investigated the protective effect of *T. violacea* extract in rats fed on an atherosclerogenic diet (4% cholesterol, 1% cholic acid and 0.5% thiouracil). They also investigated serum lipid profile, tissue antioxidant enzyme depletion, endothelial dysfunction, histopathological changes in the aorta and liver of rats. In the groups treated with *T. violacea* extract compared to the atherogenic control group; There is a significant decrease in the activities of serum markers of liver (Lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin) and kidney (creatinine and bilirubin) damage. They stated that the extract confirmed its protective properties. They also stated that *T. violacea* extract protected against the development of fatty streak plaques (aorta) and fatty changes in hepatocytes in their histopathological evaluation of aortic sections [22]. Similarly, positive effects of *T. violacea* extract were observed in the histopathological evaluation of liver and kidney tissues in our study.

Limitations

The lack of budget-based biochemical parameters in our study is among the limitations of our research. In addition, the mini CPB circuit was not used in our study. Despite this limitation, beneficial effects of *T. violacea* extract have been demonstrated in the arrest model. Our work needs to be supported by new studies using mini CPB circuits.

CONCLUSION

The application of aortic cross-clamp and TCA methods in cases performed with CPB in cardiac surgery causes signs of cardiac damage due to ischemia in the heart tissue. Although cardioplegia solutions form the basis of myocardial protection, it is obvious that a holistic approach is required. For this purpose, all preoperative, intraoperative and postoperative variables should be evaluated. In the light of these results, we think that *T. violacea* extract is protective against cardiac damage and other organ damage and may be a potential therapeutic agent. We also think that these data will form the basis for further experimental and clinical studies.

Authors' Contribution

Study Conception: BA, NK; Study Design: BA, NK; Supervision: BA, NK; Funding: BA, NK; Materials: BA, NK, FG, MSA YÇ, MEG, İK, EZT, ME, ŞY; Data Collection and/or Processing: BA, NK, FG, MSA YÇ, MEG, İK, EZT, ME, ŞY; Statistical Analysis and/or Data Interpretation: BA, NK, FG, MSA YÇ, MEG, İK, EZT, ME, ŞY; Literature Review: BA, NK, FG, MSA YÇ, MEG, İK, EZT, ME, ŞY; Manuscript Preparation: AEY and Critical Review: BA, NK, FG, MSA YÇ, MEG, İK, EZT, ME, ŞY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Ethics Committee Approval

This study was prepared as a doctoral thesis. Our study was approved by Harran University Animal Experiments Local Ethics Committee (Date: 11/12/2020, Session no: 2020/0006, Decision:01-17).

REFERENCES

1. Eriş C, Sanrı US, Engin M, Yavuz Ş. Early postoperative results of on-pump coronary endarterectomy: is it still a controversy? Eur Res J 2021;7:248-55.
2. Ozturk C, Yavuz S. Effect of coronary artery bypass surgery on ventricular functions in patients with poor left ventricular function. Eur Res J 2019;5:502-9.
3. Büket S, Özbaran M, Alayunt A, Hamulu A, Kayaaltı B, Yekginer A, et al. [Deep hypothermic total circulatory arrest application during aortic surgery]. Turk Gogus Kalp Dama. 1994;2:91-4. [Article in Turkish]
4. Ünal EU, Kubat E, Soran Türkcan B, Kiriş E, Demir A, Aytekin B, et al. Visceral oxidative stress during antegrade cerebral perfusion and lower body circulatory arrest. Acta Chir Belg 2019;119:217-23.
5. Ugurlucan M, Yildiz Y, Ulukan MO, Oztas DM, Beyaz MO, Canata E, et al. A surgical technique for ascending aorta, aortic arch and descending aorta replacement without cross-clamp, circulatory arrest or hypothermia. Cardiol Young 2020;30:24-7.
6. Svyatets M, Tolani K, Zhang M, Tulman G, Charchaflieh J. Perioperative management of deep hypothermic circulatory arrest. J Cardiothorac Vasc Anesth 2010;24:644-55.
7. Madike LN, Takaidza S, Pillay M. Preliminary phytochemical screening of crude extracts from the leaves, stems, and roots of Tulbaghia violacea. Int J Pharmacogn Phytochem Res 2017;9:1300-8.
8. Olorunnisola OS, Bradley G, Afolayan AJ. Antioxidant properties and cytotoxicity evaluation of methanolic extract of dried and fresh rhizomes of Tulbaghia violacea. Afr J Pharm Pharmacol 2011;5:2490-7.
9. Lee RH, Couto E Silva A, Possoit HE, Lerner FM, Chen PY, Azizbayeva R, et al. Palmitic acid methyl ester is a novel neuroprotective agent against cardiac arrest. Prostaglandins Leukot Essent Fatty Acids 2019;147:6-14.
10. de Garavilla L, Babbs CF, Tacker WA. An experimental circulatory arrest model in the rat to evaluate calcium antagonists in cerebral resuscitation. Am J Emerg Med 1984;2:321-6.
11. Boening A, Assling-Simon L, Heep M, Boengler K, Niemann B, Grieshaber P. Buckberg's blood cardioplegia for protection of adult and senile myocardium in a rat in vitro model of acute myocardial infarction. Exp Gerontol 2018;104:98-104.
12. Akalın B, Yılmaz Z, Artış T. [The effect of Met-Rantes on liver damage in a controlled experimental non-heart beating donor model]. Erciyes Med J 2012;34:69-72. [Article in Turkish]
13. Vognsen M, Fabian-Jessing BK, Secher N, Løfgren B, Dez fulian C, Andersen LW, et al. Contemporary animal models of cardiac arrest: A systematic review. Resuscitation 2017;113:115-23.
14. Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med 1991;91(3C):14S-22S.
15. Liberman M, Bassi E, Martinatti MK, Lario FC, Wosniak J Jr, Pomerantzeff PM, et al. Oxidant generation predominates around calcifying foci and enhances progression of aortic valve calcification. Arterioscler Thromb Vasc Biol 2008;28:463-70.
16. Atmaca E, Aksoy A. [Oxidative DNA damage and its chromatographic determination]. YYU Vet Fak Derg 2009;20:79-83. [Article in Turkish]
17. Murugesan S, Pandiyan A, Saravanakumar L, Moodley K, Mackraj I. Protective role of wild garlic on isoproterenol-induced myocardial necrosis in wistar rats. J Ethnopharmacol 2019;237:108-15.
18. Moodley K, Mackraj I, Naidoo Y. Cardiovascular effects of Tulbaghia violacea Harv. (Alliaceae) root methanolic extract in Dahl salt-sensitive (DSS) rats. J Ethnopharmacol 2013;146:225-31.
19. Raji I, Obikeze K, Mugabo P. Comparison of the acute effects of Tulbaghia violacea William Henry Harvey (Alliaceae) on blood pressure and heart rate of ageing male normotensive Wistar kyoto rats and adult male spontaneously hypertensive rats. Trop J Pharm Res 2016;15:2429-34.
20. Davison C, Levendal RA, Frost CL. Cardiovascular benefits of an organic extract of Tulbaghia violacea: Its anticoagulant and anti-platelet properties. J Med Plants Res 2012;6:4815-24.
21. Bungu L, van de Venter M, Frost C. Evidence for an in vitro anticoagulant and antithrombotic activity in Tulbaghia violacea. Afr J Biotechnol 2008;7: 681-8.
22. Olorunnisola OS, Bradley G, Afolayan AJ. Protective effect of Tulbaghia violacea Harv. on aortic pathology, tissue antioxidant enzymes and liver damage in diet-induced atherosclerotic rats. Int J Mol Sci 2012;13:12747-60.
23. Moodley K, Joseph K, Naidoo Y, Islam S, Mackraj I. Antioxidant, antidiabetic and hypolipidemic effects of Tulbaghia violacea Harv. (wild garlic) rhizome methanolic extract in a diabetic rat model. BMC Complement Altern Med 2015;15:408.
24. Moodley K, Naidoo Y, Mackraj I. Effects of Tulbaghia violacea Harv. (Alliaceae) rhizome methanolic extract on kidney function and morphology in Dahl salt-sensitive rats. J Ethnopharmacol 2014;155:1194-203.

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Culture and mental health: a Turkish perspective

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ABSTRACT

Culture and mental health are intertwined concepts. Culture should be regarded as the set of distinctive, spiritual, material, intellectual, and emotional properties of a society or a social group, encompassing lifestyles, ways of living together, value systems, traditions, and beliefs, in addition to art and literature (UNESCO, 2002). Culture consists of different components, including ethnicity, race, religion, age, sex, family values, and the location of the country. It should also be noted that culture is not a static phenomenon. Defining a culture specific to a certain ethnicity, country or region is often not a realistic approach. Many factors, such as the location of a certain city of a country or a certain region of the said city, as well as the inhabitants' occupational, economic and family positions, have an impact on lifestyles. Globalization, migration, acculturation, assimilation and transition cultures create significant heterogeneity in the very same society. Mental health can be defined as a state of balance that individuals experience both intra-personally and also with other people within their environment. In modern times, we have come to witness that the world we live in transforms at a speed that is challenging to human nature, rapid changes destroy the usual patterns, social ties evolve into ties established in the virtual world, perception replaces reality, and artificial intelligence-like elements turn into new management tools. The spirit of the new century, while flattering human instincts, compresses authenticity, solidarity and peace into ceremonial sentences.

Keywords: Culture, mental health, Turkish perpective

Culture and mental health are intertwined concepts [1]. Culture should be regarded as the set of distinctive, spiritual, material, intellectual, and emotional properties of a society or a social group, encompassing lifestyles, ways of living together, value systems, traditions, and beliefs, in addition to art and literature (UNESCO, 2002). Culture consists of different components, including ethnicity, race, religion, age, sex, family values, and the location of the country [2]. It should also be noted that culture is not a static phenomenon.

Defining a culture specific to a certain ethnicity, country or region is often not a realistic approach. Many factors, such as the location of a certain city of a country or a certain region of the said city, as well as the inhabitants' occupational, economic and family positions, have an impact on lifestyles. Globalization, migration, acculturation, assimilation and transition cultures create significant heterogeneity in the very same society.

Mental health can be defined as a state of balance that individuals experience both intra-personally and also with other people within their environment [3]. In modern times, we have come to witness that the world we live in transforms at a speed that is challenging to human nature, rapid changes destroy the usual

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patterns, social ties evolve into ties established in the virtual world, perception replaces reality, and artificial intelligence-like elements turn into new management tools. The spirit of the new century, while flattering human instincts, compresses authenticity, solidarity and peace into ceremonial sentences.

Multi-channel, rapid flow of information and changes in family structure and values are dazzling even for people a generation ago. Right at the turn of the new century, we are blown away by the winds of postmodernity, that is, an era "post truth".

Demoralization

It is a subjective feeling of inadequacy and restlessness, a state of boredom, weariness, powerlessness, and discouragement. Current issues, stress, speed, and loneliness are often frustrating. Therefore, not surprisingly, stress is one of the top health risks of the 21st century.

Sometimes, culture can also be a stressor of psychopathology and can activate mental problems in individuals who have a predisposition to a mental pathology [4]. Common causes of demoralization include belonging to disadvantaged classes, poverty, violence, unemployment [5] and authoritarianism [6]. The "tend and befriend" attitude is suggested to have evolved as a typical female response to stress [7].

Chronic Depression

Chronic depression is a long-lasting, fluctuating, low-intensity state of depression that a person experiences as a part of their usual self. Such cases, where the disease/health boundary is not clear, present mental health professionals with a challenge: mental problems intertwined with or originating from personality pathologies and resistant to treatment or even diagnosis [8].

Although its prevalence in the society is limited [9], chronic depression turns into a lifestyle, sometimes a subculture element, and overlaps with human unhappiness. Chronic depression is fairly common in individuals who are exposed to social stress and have a low education level [10].

It can be said that in most cultures, individuals prone to depression at times form a subcultural group or gather in such groups. Since individuals in such situations do not seek help, the gravity of the problem is overlooked. The mental well-being of those who belong to groups of satanism, nihilism, anarchism, political and social violence, as well as followers of arabesque and some metal music, is always controversial. Listening rates of music that imposes violence, aggression, discrimination, nothingness/nihilism can provide clues about the prevalence of psychological problems in the society [11].

Roads to the Blues

First Trauma/First Pain

The arrival in a cold and hostile world from a warm and sheltered mother's womb is defined as a traumatic separation by some psychoanalysts [12]. Mystics talk about the sadness of being cut off from God and thrown to earth. Sufi philosopher Rumi uses the moaning sound of Ney, which was plucked from the reed, as a metaphor for this pain [13].

Family: Great Advantage, Great Risk

While healthy families are a reliable and indispensable line of defense for their members, broken and restless families create significant mental and behavioral problems in children [14].

In our strongly traditional society, families' devotion to their children is widely applauded, however, this exaggerated attention can impair the child's individuation as well as coping abilities. Children's mental health is adversely affected not only by those who are oppressive and disciplined, but also by families who make an effort to ensure that their children do not "stand on their own legs" [15]. This can result in children transform into people who cannot live on their own, who cannot make decisions, who cannot take on responsibilities, who are insecure, weak, and sometimes prone to crime.

The attitude of first glorifying children and later complaining about them is a common Turkish family attitude.

Expectations

Globalization and unlimited communication possibilities raise people's expectations in terms of better living standards. The resulting inconsistency between desires and reality increases the tension. Cultural imperialism, human-made disasters have a potential tsunami effect on our lives. Some real, some distorted consumption displays are reflected to the public, and these can cause new feelings of disappointment, self-

pity or anger in some people every single day. Young people and adolescents are the most at risk in today's era of rapid communication and change, so to speak, they are on a knife-edge in this regard.

Poverty can be a clear cause of depression through triggering social stress [16]. The most suitable segment of society for individuals to feel peaceful, balanced and comfortable is known to be the middle class. On the other hand, most people in vulnerable socio-economic segments are also able to protect their mental health by accepting the conditions in which they were born and raised, by showing solidarity and having realistic and small expectations [8].

Relations, Traditions

Values, sincere human relationships and the effect we have on others always have a significance in terms of mental health. The determinant effect of the rule "it's other people that make people sick" [17] is increased in societies where tolerance and altruism are minimized.

Urbanization negatively affects family structure and support systems. In societies where people's trust in one another is severely damaged, we see that this mental support too, which is of great importance, diminishes. All practices that do not prioritize trust and respect for people are irritating and lead to “diseases/disturbances”.

Cultures and traditions play an important role in identity formation. The middle classes have so far carried the tradition with moderate and humane interpretations. In traditional societies, however, this human intimacy may carry the risk of "lack of ability to keep the appropriate distance", in addition to the psychological benefit it provides.

Solid and irrational cultural-traditional elements can darken the atmosphere in which people live and create an intense spiritual pressure on them. Sexism is practiced with incredible normality and inhumanity in some regions where the means of claiming rights are limited. For women in these places, spiritual growth is out of the question. In particular, the double standard of "honor" code of ethics produces appalling results [18].

Pathological thoughts of guilt and sinfulness may diminish as traditions move to a more rational and gentle plane. In our culture, the depressive personality profile focused on self-sacrifice and the love, wishes and expectations of others overlaps with the definition of being a "good person".

The weakening of moral values was leveled off by the strengthening of law in the West. In underdeveloped countries, however, the law has only been the demand of the weaker groups. Therefore, legal or moral values have remained valid and important only for those with little power.

From a generalist point of view, it can be said that helplessness, dependence, resignation, emotionality and a structure of a “communion” come to the fore in Eastern societies, while self-confidence, ambition for success, independence, objection, rationality and individuality are dominant elements of culture in Western societies.

Religions and Ideologies

Religion can produce many mental health-enhancing cognitions. Moreover, it is often protective against alcohol, substance abuse, and stressful life events that are more commonly seen in the secular world. However, some religious beliefs, such as Catholicism and Judaism, impose guilt in ways that negatively affect mental health. Some religious groups may downplay psychological treatments and interpret these diseases as God's punishment, leading to devastating results for individuals [19].

Some delusional symptoms and compulsive rigidities can be considered as signs of "religiosity" and can have devastating consequences in terms of religion as well. Today, shamanistic practices such as contact with the jinn, casting spells, incense-amulets have turned into a tool for exploiting mental problems. Mystical, mysterious, and sometimes pathological approaches that equate ignorance with belief can confuse people and thus lead them to devalue ways of seeking scientific remedies.

The unhealthy way of interpreting the verses of the Qur'an for one’s own self and the verses of punishment for others is the most harmful epidemic in the Islamic world. Radical/fanatic groups can make religion a tool of separation, polarization and conflict rather than a unifying one. It is possible to call this the "personalized or grouped" form of religion. In every age, many greedy and some mentally ill people who claim to speak for God have used the concept of "sin" as a stick to psychologically beat people, instilling despair, fear and distrust into them.

Letting religion into the Wailing

As in Christianity and Judaism, mourning and grief formed the basis of religious life in some Islamic sects. These groups practice their religion within a sadness that could be found in a funeral. Constantly cited traumas instill a “no laughter” culture in religious people. The saying, which is part of our culture, "to make honey out of pain" is actually a cry from the nooks of traumatic centuries.

The “afflicted” approach not only predisposes people to depression, but also offers depressive individuals an attractive ground where they can perceive their illness as “good deeds”.

Hundreds of obsessional/phobic rituals infiltrating religions, filling and clamping life with superstitions and ghosts, can mask the original religious thought. Hymns, in the form of bad and intensified adaptations of arabesque music, create a deep belief confusion in people, along with arbitrary approaches that distort religious messages with subjective elements of personalities or politics. After all, for a society with a poor education level, it is much more comfortable to "believe what the elders say" than to actually research it. How sad it is that people of the same religion call killing each other jihad (!).

Mysticism: the Comfort of Slowness

Mysticism exists in all religions, but it is dominant in Middle Eastern and Far Eastern beliefs. Emerging as a reaction to an over-attachment to worldly life, it has fostered a culture of renunciation, and of trivializing the world or oneself.

Mysticism sometimes can overlap with laziness, and bless pathological behaviors and folk narratives. Both to consider perceptual changes observed in mystics as psychosis and to consider psychosis as a "saintliness" can lead to dramatic results.

Until the 12th century, the sects have been the centers of solution in the political, economic and social turmoil in the majority of the world. From the eighteenth century onwards, with the acceleration of social turmoil and the creation of an atmosphere of pessimism and despair, the sects regained their functional and reactionary qualities. Although the sects were originally a reaction against the collapse and corruption, they could not stay away from being a part of the corruption themselves [20].

Concepts such as Doomsday, Mahdi, Messiah, fairies, spells, miracles, ghosts, and omens are subjective and vague concepts, to which those who object can be declared as unbelievers. Some people may leave their school, work or home due to scheduled doomsday scenarios.

Religious Communities/Sects

Whether religious or secular, people with similar lifestyles in Turkey are positioned in different “communities” and are alienated from others.

Religious communities are also examples of extraordinary spiritual and social support that emerged with the principle of solidarity in goodness. Naturally, excessive empowerment/growth of these groups can also increase degeneration, thus feeding external or intrapsychic conflict instead of spiritual support.

Some communities have severely criticized approaches to religion that are different from their own. These groups can use cruel expressions such as “infidel, apostate, irreligious, profane, perverted, traitor” against other religious groups, despite not using these against anyone else. It is thought-provoking that in the struggle for power, some religious groups resort to ways that Islam deems as despicable as murder, such as blaming, stigmatizing, envy, backbiting, lying, slandering. What really happens is related to anger and self-interest rather than religion. The justification of violence in the name of religion has thrown the development and chemistry of the mind of the Islamic World into disorder.

Religious discourse with violence, abuse and confusion has decreased in the West due to rationalism and secularism.

Ideologies: Modern Religions

In modern times, we have seen the rise of "false religions" such as militarism, fascism, technology, nationalism, consumerism, fundamentalism, new ageism, asceticism, and psychologism [21].

Although ideologies, i.e. "straitjackets put on our intellect" [22], fascinated mostly anxious and insecure people [23] with their strange prophets and left, several mysterious types of faith still continue to exist with similar followers.

The fact that the world is unfair and will never be fair has turned ideologies into "the religion of the oppressed or the angry". Through their own clergy, they developed a fierce and ruthless super-ego, declared

countless "traitors" and "renegades", instilled in people an excessive sense of guilt, and ruthlessly shed blood. They shook the established orders, but every revolution only spawned new dictators and oppressed every individual whether or not they were different from them.

Turkey: the Battlefield of Prejudies

Centuries of defeats, losses, tensions that are remnants of poverty and ignorance, authoritarian attitudes, thirst for fair share, disappointments, traumas laid the groundwork for the language of anger and hatred, polarization and conflict. This ground seriously damaged both social culture and mental health of individuals. The carriers of the original culture and traditions disappeared. Two different social structures were formed, separated from each other by their value judgments, culture, perception of the world and political preferences [24]. In this vein, a headline in the New York Times was shocking: “In Grief (Great Ankara massacre) and in Triumph, (Nobel Chemistry prize) Turks Remain Divided” [25].

Extremist groups that despised reconciliation within a closed-circuit, bi-communal “community” network disrupted social peace. The broad masses of people who wanted to live together were frightened and in despair. We still live in a shallow intellectuality where we fight over which leader from the past is the most superior.

We have only one condition to love people and to be tolerant (!) towards them: they must be one of us. A language of political confrontation rather than understanding the other, a "victim" psychology full of irrational-emotional reactions... This blind fight darkens our world and our souls.

Turkish society has reached a partial level of democracy awareness with the short periods of freedom it has enjoyed in every ruling period since the foundation of the Republic. However, it has produced a society that operates with new tensions, restrictions of law and human rights and a spiral of "trauma-polarization" due to the administrations that soon became authoritarian. “Not speaking up in order to survive” behavior and submission create a joyless, insecure society. High masculinity shows its depressive effect in Turkey, as in many other countries [26].

Since an effective legal system and sound institutions have not been established, authoritarianism continues to change hands in Asian-Middle Eastern countries. This fuels sociopolitical conflicts and unrest [27].

Modernism: Global Culture/Social Media

Globalization has rapidly blurred the traditional structure of culture. Especially in cultures in transition, rapid acculturation and deculturation caused a return to more defensive and fundamentalist values and increased tensions. Traditional support systems have worn down, and conflict, restlessness, and mental problems have increased between individuals who are acculturated and those who are not, even within the same family [28].

In today's world, people are living in a plastic and narcissistic age that is increasingly associated with destructive competition, greed for consumption, income gap and individualism. A frightening illusion of freedom with no reference... We are no longer exposed to real events and people, but to images in the media.

According to 2021 statistics, 4.2 billion people are social media users, that is, 53% of the world's population [29]. While social media expands individual freedoms, especially the freedom to receive information, it is also an atmosphere that produces fear through false information flows, perception operations directed by different sources, information theft, crime, terrorism, hate speech, myths, distortions, lies and speculation [30].

Turkey ranks high in the world with up to eight hours of internet use per day [31]. It is not very promising that the Turkish society is ranked 104th among 150 countries in the 2021 World Happiness Index [32], and 8th in the Most Angry list [33].

Social media use has been reported to be associated with depression and anxiety in youth and adolescents [34].

Social media affects our sleep, lifestyle and interpersonal relationships, as well as the addiction it creates, the constant distraction with hundreds of messages containing messy information every day, and the fact that it confronts us with problems all over the world. Social media is the world's largest and most complex garbage dump with its information and noise pollution, surprising perspectives, and competition/comparison / show / consumption-oriented structure. At the same time, it is also an arena of "zombification" in which disidentification and plastic phenomena are role models.

In this regard, it is mandatory to take the social media education as a starting point for an evaluation.

Postmodernism: the Comfort of Live Without a Hero

"Anything goes".

A new era began with the realization that heroes are no different from ordinary people. Postmodernism is an objection to Western culture and "grand narratives", "grand projects", "grand principles". It rejects both rationality, positivism/objective reality, value systems and the sanctity of concepts such as universality and freedom. It relativizes not only morality but also science, and is based on self-reference.

Post-modernism emphasizes the disappearance of beliefs and optimism about art, literature and scientific ethics after the Second World War: criticizing everything and everyone, considering almost nothing as correct, being normless and unprincipled.

The characteristics of post-modern consciousness can be defined as emptiness, pessimism, apathy, skepticism (35).

The quest to loosen the restrictive bonds of tradition has put humanity under risk of breaking away from healthy bonds as well.

Unprecedent Speed of Change!

The most important problem of the age is the unprecedented change. The data produced in a single day in recent years is many times more than in the past. Those who dominate the data have also begun to dominate the societies.

We cannot find the time to adapt to this rapid change with our minds, habits and institutions. Many areas, including family, work, school, friendships, customs, trade, have shifted to very different areas. The habits and rules taught as recently as yesterday are falling into disuse in a very short period of time.

Rapid change produces fear and meaninglessness: “We went so fast that our souls are left behind.” I wonder if we completely broke the value system while trying to stretch values? Have we murdered our conscience while softening the super ego?

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REFERENCES

1. Sam DL, Moreira V. The mutual embeddedness of culture and mental illness. Online readings in psychology and culture. Unit 10.2.; 2002. Retrieved from httb://works.bepress.com/david_sam/27.
2. Eshun S, Gurung AR. Introduction to culture and psychopathology. In: Culture and mental health: Socio cultural influences, theory and practice. Eshun S, Gurung AR. Eds. West Sussex: John Wiley & Sons, Ltd., Publication; 2009: pp.3-18.
3. Sartorius N. Foreword. In: Culture and mental health: a comprehensive textbook. Bhui K, Bhugra D, eds.. Edward Arnold Ltd: London; 2007: pp.xv-xvı.
4. Gurung RAR, Andela R-W. Stress and mental health. In:. Culture and Mental Health Sociocultural Influences, Theory, and Practice. Eshun S, Gurung RAR, eds., Blackwell Publishing Ltd: London; 2009: pp.35-54.
5. Kinderman P, Schwannauer M, Pontin E, Tai S. Psychological processes mediate the impact of familial risk, social circumstances and life events on mental health. PloS One 2013;8:e76564.
6. Anuradha. S, Kumar RU. Effects of oppression on mental health. Eur J Mol Clin Med 2020;7:4902-5.
7. Taylor SE, Klein LC, Lewis BP, Gruenewald TL, Gurung RA, Updegraff JA. Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. Psychol Rev 2000;107:411-29.
8. Aşkın R. Kronik Depresyon. Psikiyatri Temel Kitabı. HYB Basım Yayın. Ankara, 2007.
9. Keller MB, Klein DN, Hirschfeld RM, Kocsis JH, McCullough JP, Miller I, et al. Results of the DSM-IV mood disorders field trial. Am J Psychiatry1995;152:843-9.
10. Husain N, Gater R, Tomenson B, Creed F. Social factors associated with chronic depression among a population-based sample of women in rural Pakistan. Soc Psychiatry Psychiatr Epidemiol 2004:39:618-24.
11. Miranda D, Claes M. Musical preferences and depression in adolescence. Int J Adolesc Youth 2007;13:285-309.
12. Otto Rank. Doğum Travması. (Çevirmen Sabir Yücesoy). Metis Yayıncılık, İstanbul, 2017.
13. Mevlana Celaleddini Rumi. Mesnevi. Akçağ Yayınları: İstanbul, 2016.
14. Behere AP, Basnet P, Campbell P. Effects of family structure

on mental health of children: a preliminary study. Indian J Psychol Med 2017;39:457-63.
15. Sümer N, Gündoğdu Aktürk E, Helvacı E. Anne-Baba Tutum ve Davranışlarının Psikolojik Etkileri: Türkiye'de Yapılan Çalışmalara Toplu Bakış. Türk Psikoloji Yazıları 2010;13:42-59.
16. Mossakowski KN. Dissecting the influence of race, ethnicity, and socioeconomic status on mental health in young adulthood. Res Aging 2008;30:649-67.
17. Sullivan HS. The Interpersonel Theory of Psychiatry. WW Norton&Company, New York, 1953.
18. TBMM. Töre ve namus cinayetleri ile kadınlara ve çocuklara yönelik şiddetin sebeplerinin araştırılarak alınması gereken önlemlerin belirlenmesi amacıyla kurulan (10/148, 182, 187, 284, 285) esas numaralı Meclis Araştırma Komisyonu Raporu 2005. https://www.tbmm.gov.tr› sirasayi › donem22 › yil01
19. Goffredo B, Simon D. Religion and mental health . In: Culture and Mental Health. A comprehensive textbook. Bhui K, Bhugra D. eds., Edward Arnold Ltd: London; 2007: pp.47-53.
20. Taş K, Güvendi T. Din ve Dünyevileşme Bağlamında Türk Toplumunda Tarikat ve Cemaatler. Tabula rasa Felsefe & Teoloji dergisi 2020; 34, Online Yayın, 01.03.2021
21. Drazenovich,G, Kourie C. Mysticism and mental health: a critical dialogue. HTS Teologiese Studies / Theological Studies, 2009;66, Art. #845, 8 pages.
22. Meriç C. Bu ülke. İletişim Yayınları: İstanbul, 2004.
23. Stone WF, Schaffner PE. The Psychology of Politics. Springer&Verlag: New York, 1988
24. Çarkoğlu A, Toprak B. Değişen Türkiye'de Din, Toplum ve Siyaset. TESEV Yayınları: İstanbul, 2006.
25. Gazete Vatan. "Ne keder ne de zafer birleştirdi Türkleri''. 13.10.2015.
26. Arrindell, WA, Steptoe A, Wardle J. Higher levels of state depression in masculine than in feminine nations. Behav Res Ther 2003;41:809-17.
27. Prasadarao PSDV. International Perspectives on Culture and Mental Health. In:. SCulture and Mental Health Sociocultural Influences, Theory, and Practice. Eshun S, Gurung RAR, eds., Blackwell Publishing Ltd: London; 2009: pp.149-78.
28. Bhugra. D. Concluding remarks. Globalization, culture and mental health. Int Rev Psychiatry 2014;26:615-6.
29. Bayrak H. Dünya İnternet, Sosyal Medya ve Mobil Kullanım İstatistikleri. Dijilopedi, 3 Şubat 2021.
30. Fuchs C. Sosyal Medya. Eleştirel bir Giriş. Çev. Saraçoğlu D, Kalaycı İ. NotaBene Yayınları: İstanbul, 2016.
31. We are social and Hootside. Digital in 2021 Raport.
32. Kerem Congar. Dünyanın en mutlu ülkeleri listesi açıklandı: Türkiye 11 basamak geriledi. Euronews. 19.03.2021.
33.http://cdn.cnn.com/cnn/2019/images/04/25/globalstateofemotions_wp_report_041719v7_dd.pdf.
34. Keles B, McCrae N, Grealish A. A systematic review: the influence of social media on depression, anxiety and psychological distress in adolescents. Int J Adolesc Youth 2020;25:79-93.
35. Vattimo G, The end of modernity: Nihilism and Hermeneutics in postmodern culture. Johns Hopkins University Press: Baltimore, 1988.

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Pathophysiology of metalloproteinase matrix in relation to morbid obesity and associated pathologies

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ABSTRACT

Objectives: Matrix Metalloproteinases (MMPs), these calcium-dependent zinc-containing endopeptidases play an important role in adipogenesis and angiogenesis by modifying tissues and degrading the extracellular matrix (ECM). Matrix glycoproteins, gelatin, collagens, proteoglycans and elastin are all found in the ECM. Current meta-analysis confirmed the lower levels of IL-6 and CRP was found following bariatric surgery. Several studies have shown correlations between E-selectin levels, BMI, and MMP-9 levels. There was also a strong link between the metalloproteinases MMP-2 and MMP-9. MMP-2 and adiponectin levels are related. MMP-9 levels, on the other hand, were modestly linked with E-selectin and HDL cholesterol levels, as previously stated. Also current observations imply that alterations in the ECM caused by MMP-mediated degradation may be crucial for the differentiation of adipocytes. The most crucial component of this is that MMPs are involved in the remodeling of tissue after gastric bypass surgery, as revealed by these markers (especially MMP-2 and MMP-9). Thus, it is tempting to assume that adipocyte derived MMPs may constitute a novel pharmaceutical target for limiting adipose tissue development through the reduction of adipocyte differentiation and angiogenesis. MMP-2 exhibits far more accurate oscillations than MMP-9 during pre- and post-surgical weight fluctuations, and hence may be used as a predictor for gastric bypass success. The purpose of this paper is to conduct a comprehensive review of the literature with an emphasis on the critical functions that MMPs have in the pathophysiology of obesity and the related diseases.

Keywords: matrix metalloproteinase, morbid obesity, metabolic surgery, extracellular matrix

MMPs are calcium-dependent zinc-containing endopeptidases that play a role in adipogenesis and angiogenesis by modifying tissues and degrading the extracellular matrix (ECM). Matrix glycoproteins, gelatin, collagens, proteoglycans and elastin are all found in the ECM. MMPs are not normally highly produced in normal physiological settings; nonetheless, overexpression of MMPs disrupts the balance of MMP activity and tissue inhibitors of MMPs (TIMPs) resulting in a range of clinical diseases. MMPs are classified into over 25 distinct subtypes [1]. MMPs also break pericellular proteins and surface molecules, depending on the substrate, and are therefore implicated in cell behavior regulation [2].

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Obesity has been linked to an increase in the number of adipocytes and preadipocytes, as well as microvascular endothelial cells [3]. MMP-2 and MMP-9 are two essential enzymes involved in the modulation of the ECM and the production of adipocytes and preadipocytes, respectively. Obese people had higher MMP-2 and MMP-9 levels, which resulted in an altered ECM metabolism [4].

The purpose of this paper is to conduct a comprehensive review of the literature with an emphasis on the critical functions that MMPs have in the pathophysiology of obesity and the related diseases.

METHODS

The article is a narrative review based on a PubMed search of the English-language medical literature for essential elements of the stated goal so that we can create a global picture of the impact that MMPs have on morbid obesity and the conditions that accompany this pathology.

Obesity and Biologic Systemic Status

Systemic inflammation, infiltration of macrophages into adipose tissue, and ECM remodeling mediated by MMPs are all characteristics of obesity. Macrophage infiltration is an indication of adipose tissue inflammation caused by obesity [5]. Macrophage infiltration is also critical for the control of ECM turnover and fibrogenesis, both of which are mediated by various MMPs [6].

The same alterations have been seen in obesity-induced fatty liver macrophages [7]. There is still a need to gather further data to show a link between the expression of MMP in adipose and hepatic tissue. To create regions of fibrosis in an organ, it starts with an area of inflammation and an imbalance between ECM creation (that is, fibrogenesis) [8-10] and ECM breakdown (which is fibrinolysis) [11, 12].

De Meijer *et al*. [13] showed that a 60% fat diet might result in an increased fat index (a measure of obesity) and so promote obesity. Furthermore, it leads to insulin resistance and hepatic steatosis over time, both of which are linked to obesity [13].

Domienik-Karowicz *et al*. [14] demonstrated that the development of diet-induced obesity in an obesity-induced model was linked with an increase in the expression of MMPs in adipose tissue. The relevant MMPs are MMP2, MMP3, MMP8, MMP12, MMP13, ADAM17, TIMP1 and TIMP2 [14]. This result supports the findings of Lijnen *et al*. [15] and Chavey *et al*. [16]. The most consistent finding across most of the research was an elevation in MMP3, MMP8, MMP12, MMP13, and TIMP1 gene expression.

MMPs play an important part in the management of adipogenesis by performing proteolytic activities throughout adipose mass growth [17].

While our meta-analysis confirmed the lower levels of IL-6 and CRP following bariatric surgery, the mean difference for the modification of TNFa did not achieve statistical significance at any of the follow-up intervals. These cytokines are significant because they have a role in the pathophysiology of type 2 diabetes and coronary heart disease [18].

Additionally, MMPs are also involved in glucose homeostasis. Derosa *et al*. [19] discovered elevated MMP-2, MMP-9, TIMP-1, and TIMP-2 plasma levels in diabetic patients, indicating a disruption in ECM metabolism in diabetes. Boden *et al*. [20] observed that hyperinsulinemia enhances membrane MMP-1, MMP-2 and MMP-9 activity in aortic tissues in agreement with this concept.

MMP in Other Related Diseases

It is now well accepted that high adipose tissue formation is a risk factor in and of itself for hypertension, diabetes, atherosclerosis as well as cardiac and vascular dysfunction. Increased calorie intake combined with lower energy expenditure increases adipocyte substrate availability, resulting in hypertrophy [21].

ECM metalloproteinases (MMP-2 and MMP-9), E-selectin, adiponectin, CD40L and PAI-1, all have a role in atherosclerosis. Extracellular MMPs are engaged in ECM remodeling as well as basal membrane remodeling. Increased proteolysis and endothelial injury or matrix constituent buildup are determined by an imbalance between MMPs, their inhibitors, and α-2-macroglobulin. MMPs have been proven to play an important role in the atherosclerotic process, the regulation of adipogenesis, the blood vessels remodeling as well as cardiac muscle walls [6, 7].

Obesity progresses by a process of substantial remodeling of the adipose tissue that includes angiogenesis,

hyperplasia, and hypertrophy.

In contrast to other adipocyte-derived hormones, adiponectin has a negative correlation with visceral fat, BMI, and body weight, according to some authors, however numerous studies contradict this theory. In clinical practice, hypoadiponectinemia is a solid and favorable predictor of type II diabetes, lipid disorders, arterial hypertension, and nonalcoholic fatty liver disease [22].

Several studies have shown correlations between E-selectin levels, BMI, and MMP-9 levels. There was also a strong link between the metalloproteinases MMP-2 and MMP-9. MMP-2 and adiponectin levels are related. MMP-9 levels, on the other hand, were modestly linked with E-selectin and HDL cholesterol levels, as previously stated [4, 5].

The hypertrophy and hyperplasia of adipocytes have been extensively researched. The mechanisms behind these processes are not known at the moment. There is a paucity of evidence on the control of angiogenic processes and also the ECM modification during fat mass accumulation and the resolution of obesity throughout time. Adipocytes perform significant metabolic functions and are capable of producing a variety of substances, including growth factors and cytokines, that are involved in the paracrine control of adipose tissue remodeling. Adipocytes, on the other hand, release proangiogenic substances such as tumor necrosis factor-α, monobutyrin [23], leptin, and vascular endothelial growth factor (VGEF). ECM components are generated and destroyed during adipocyte development, and some investigations have shown that adipocyte differentiation is modulated by the ECM environment [11, 12, 17].

To confirm that the released MMPs originated from mature adipocytes, many tests were done on newly obtained mature adipocytes. RT-PCR study using primers specific for MMP-9 and MMP-2 cDNAs confirmed the existence of both transcripts in human adipocytes, but the expression of MMP-2 was much higher than MMP-9, while MMP-9 also had a significant individual variation. MMP-2 activity was considerably higher in human fat tissue than in MMP-9 activity. Additionally, under the same settings, both mature MMP-2 and pro–MMP-2 were found. MMP-9 increased somewhat throughout the first seven days of differentiation, reaching a peak on day 7, and then dropped [24-26].

As previously stated, MMP-2 and MMP-9 suppression reduce adipocyte development. Preadipocytes may represent the link between MMPs and adipocyte differentiation. To test if the MMP activity, secreted by adipocytes, has a role in differentiation, in the presence of adipogenic media, preadipocytes were treated with batimastat (0.5-10 mmol/l) escalating doses of MMP inhibitors, and captopril (100-1,000 mmol/l).

Bouloumie *et al*. [27] established for the first time that the adipose tissue of humans produces and secretes MMP-2 and MMP-9. Further investigation of MMPs secreted by adipocytes on the mouse preadipocyte indicates that MMP-9 and MMP-2 production and the release are higher throughout adipocyte development. These findings demonstrate unequivocally that MMPs are required for adipocyte development [27].

Despite what was previously stated, adipocytes demonstrated substantial interindividual variability, indicating that adipocyte-derived MMP production, secretion, and activity may be regulated by as-yet-unidentified modifying variables [28].

While the extracellular network of fibronectin and the network structures of laminin, as well as type IV collagen are damaged throughout the differentiation process of adipocyte, the network of type I collagen, which is the last to mature, remained intact. To understand why skin elasticity is affected both by obesity and weight reduction, it is important to understand the structural degradations in elastin, fibronectin and subcutaneous collagen [29, 30].

Taken together, the current observations imply that alterations in the ECM caused by MMP-mediated degradation may be crucial for the differentiation of adipocytes. Furthermore, given that TIMP inhibited angiogenesis induced by adipocyte conditioned medium, it is tempting to infer that MMPs may represent novel, intriguing therapeutic targets for monitoring adipose tissue growth by decreasing adipocyte differentiation and angiogenic processes [31].

There is evidence to support the production and release of MMP-9 and MMP-2 by human adipose tissue. Indeed, whereas MMP-2 activity was much greater than MMP-9 activity, both were detectable [32, 33].

The levels of MMP-9 were shown to be considerably greater in individuals with metabolic syndrome and obesity (BMI values) as compared to those with

no metabolic disorders as well as normal body weight. Derosa *et al*. [19], and other studies established a link between MMP-9 and body weight parameters in individuals with severe obesity before to and after bariatric surgery [34-36].

Not only are the advantages of bariatric surgery biological in nature, but they also seek to enhance one's quality of life. Several studies (Mirica *et al*. [12, 17]) reveal that the majority of patients who have bariatric surgery, increases their athletic performance and frequency, as well as their sexual status ($p < 0.05$). Additionally, 44% of patients improved their libido and intercourse quality. Bariatric surgery's less invasive method enables a rapid reintegration of the patient into regular activities [37, 38]. Bariatric surgery should be seen in terms of the benefits it provides to daily life [39-43].

MMPs in Diabetes

Along with weight loss, bariatric surgery may significantly improve glycemic control in obese with type II diabetes patients. As a result, it is also known as metabolic surgery. Additionally, the impact on type II diabetes and body weight varies according to the kind of bariatric surgery performed, although the precise processes behind these effects are unclear [44].

Numerous biological variables are considered to have a significant role in diabetes pathogenesis. Fetuin-A, for example, is a secretory protein made by the liver that binds to the insulin receptor blocking the insulin signaling and ultimately inducing in vitro insulin resistance. In humans, it is also connected with diabetes and insulin resistance. MMP-7 is another indicator of diabetes since it is capable of digesting ECM structural proteins. Additionally, it is required for the development of cancer, innate immunity, and inflammatory illnesses such as scleroderma. MMP-7 levels were also shown to be elevated in individuals with type II diabetes, diabetic diastolic dysfunction, diabetic renal illness. However, the precise process remains a mystery [45].

As shown in multiple investigations, the preoperative blood MMP-7 level correlates with preoperative age, obesity-related metabolic diseases and indicators of central obesity. Serum MMP-7 levels are increased in patients with liver steatosis, type II diabetes, cardiovascular disease, and abdominal obesity [46].

Diabetes affects the blood levels of fetuin-A and MMP-7 in obese persons. At one year following surgery, Roux-en-Y gastric bypass (RYGB), mini gastric bypass MGB, and sleeve gastrectomy (SG) all lowered circulating fetuin-A levels while simultaneously lowering glycemic levels and antidiabetic therapy dosages. On the contrary, as Yang *et al*. [22] demonstrated, after RYGB, MGB, or SG the circulating MMP-7 levels were constant.

MMPs in Vascular Remodeling

The production of new blood arteries (angiogenesis) is essential for tissue development, tissue repair, and, unfortunately, solid tumor growth. MMPs may play a role in angiogenesis in three ways: they can facilitate the migration of endothelial cells into adjacent tissues by removing the barrier of ECM, they can encourage it by freeing stored angiogenic substances, and they can deny it by creating-angiogenic breakdown products. Furthermore, during the development of newly created blood vessels, the equilibrium between MMPs and their inhibitors must be reestablished to promote basement membrane formation, endothelial cell differentiation, as well as quiescence [47-50].

MMPs may potentially suppress angiogenesis by producing anti-angiogenic peptides. Angiostatin is a plasminogen breakdown product produced by MMP-2, MMP-3, MMP-7, MMP-9, and MMP-12 [51-55]. However, the role of MMPs in inhibiting angiogenesis is unknown, even though they are unquestionably significant positive regulators of angiogenesis.

MMPs in Cancer

MMPs are abundant and are triggered more frequently in malignancies than in benign, normal, or even premalignant tissues, with the greatest levels of expression occurring near the tumor-stroma interface during an active invasion. MMP expression has been shown to correlate significantly positively with a variety of indications of poor prognosis in practically all kinds of cancer. Increased MMP levels may be regarded as an independent predictor of decreased disease-free survival as well as an overall survival factor. It has been proven that when MMP activity is elevated or TIMP activity is decreased, benign cells develop malignant features. Malignant cells may become less aggressive by lowering their MMP levels or limiting their activity.MMP-1, MMP-2, MMP-3, MMP-9, and

MMP-14 have all been associated as agonists of angiogenesis, tumor invasion, and metastasis, as was reviewed by Sternlicht *et al*. [56].

Because endothelial cells cannot penetrate the ECM if it is not degraded, malignant cells cannot spread above it if the ECM is not inhibited or degraded. Recent data shows that MMPs have a greater influence on cancer than simply removing physical barriers; several MMPs have been demonstrated to promote early cancer formation, with MMP-3 promoting late epithelial-mesenchymal phenotypic changes associated with more severe malignant behavior [57]. These findings support the hypothesis that MMPs may play a role in practically every stage of cancer development, both early and late. Certain MMPs may inhibit cancer development, whereas others may not be involved in cancer but clearly perform basic physiologic functions. These alternatives must be explored, and the processes behind MMPs' impact on cancer must be fully understood, in order to maximize the efficacy while limiting toxicity of treatment medicines.

DISCUSSION

MMPs play an important part in adipogenesis through their proteolytic activity during adipose tissue remodeling. Hanusch-Enserer [7] established in 2009 that MMPs and ECM play a direct role in adipogenesis. In 2015, Domienik-Karowicz [14] demonstrated that the development of diet-induced obesity is associated with an increase of MMP expression in adipose tissue [14]. Regarding the diet used to induce obesity in rats, de Meijer *et al*. [13, 31, 32] demonstrated that a high fat diet, 60%, can not only increase the fat index and cause obesity, but if used for a lengthy period of time, it can also increase insulin resistance and hepatic steatosis, both of which are directly associated to obesity.

The diet administered in the research from Mirica *et al*. [12] was a combination of a hypercaloric-hyperlipidemic diet as described by other authors, a diet currently consumed by most of the people in medium as well as high-developed countries, and a diet based on food that is hypercaloric, sweetened beverages and sweetened carbonated beverages. To achieve obesity, the rats were given 82 percent butter and glucose-enriched chocolate. This was supplemented with rat meal at the discretion of the researcher and ad libitum water enhanced with 35 g of sugar/100 g of water. Fast food and sugary carbonated beverages consumption are rapidly increasing in nowadays society, so the same circumstances were reproduced. The statistically significant difference in food intake between the research groups and the control group on a regular diet saw an increase with 162 grams, more than 50% the body weight of the control rats.

The literature is replete with data on numerous diets that produce comparable effects, the critical factor being the end weight at which records are kept. It is well established that if a patient is obese for an extended length of time, he will get diabetes as a direct result of the food-induced obesity. Mirica *et al*. [17] revealed statistically significant variations in preoperative blood glucose levels and weight between the control and study groups. The research demonstrates that cormparable discrepancies exist even between triglycerides (TGL) and cholesterol (CHO) levels. Postoperative weight and blood glucose measurements, as well as CHO and TGL values, all demonstrate a significant decrease in values compared with the preoperative ones. It is worth mentioning that, in addition to the surgical operation that accounts for the majority of these variations, all rats in the research groups were restored to their usual diets. The BS group weight loss was significantly different; weight loss was also significantly different in group B but did not approach the levels of the BS group. As a result, it was theorized that the difference in postoperative weight reduction between the B and BS groups was due to sulodexide administration. However, more investigations would be required to evaluate this hypothesis.

Sulodexide is not known to have a significant function in weight loss, and it was hypothesized that its effect on tissue remodeling via altering the state of MMPs is what accounts for the advantage group BS had in weight loss. Mannello *et al*. [58] further illustrate the role of sulodexide in the metabolism of MMP. All groups consuming a hyperglycemic diet had preoperative glycemic levels of more than 190 mg/dl, except the C group, which had a value of 121 mg/dl. Blood glucose levels in group B decreased statistically significantly from 207 ± 23 mg/dl preoperatively to 123 ± 10 mg/dl postoperatively measured at 28 days, demonstrating once again the tight relationship between glycemic status and obesity and the ability of

weight reduction to normalize the levels of blood glucose.

Cases of complete diabetic remission have also been described in the literature barely one month following surgery. A special remark should be made of group S, where the reduction in glycemia 28 days postoperatively was not statistically significant (reduction was from 198.4 ± 13.19 mg/dl to only 191.7 ± 8.71 mg/dl). This is because this group has not undergone weight-loss surgery and the sulodexide administered did not initially reduce blood glucose levels, albeit it did return to normal diet. It seems as if the standard diet is insufficient to alter the glycemic status or weight curve. The control group saw a little rise in glycemic mean, which is regarded to be typical for this measure. While group S had the highest pre and postoperative TGL levels, any suggestion that this was related to sulodexide administration is presently without scientific basis, since there is no clear relationship between TGL status and sulodexide. Following the procedure, TGL decreased in groups S, B and BS, with the largest and statistically significant declines occurring in groups B and BS.

In a 2014 research on Wistar rats, Lilis et. al. [59] obtained a maximum reduction of 70 mg/dl. Because CHO demands the same level of care and relevance as TGL, preoperative values were recorded across batches, but without statistical significance, the highest levels being reported in the S group. Following surgery, the B and BS levels reduced significantly, but only in the BS group it was a statistical significance difference. Postoperative CHO levels correlate favorably with postoperative TGL, glycemia and the postoperative weight in BS group, respectively. Kawano *et al*. [60] and Kalaivani et. al. [61] both showed substantial reductions in TGL and CHO after bariatric surgery, which were all connected with postoperative estimated body weight loss (% EBWL).

A massive meta-analysis consisting of 6131 patients revealed that bariatric/metabolic surgery was 9.8 to 15.8 times more effective than conventional treatment in terms of weight loss, CHO, TGL, fasting blood glucose reduction and glycosylated hemoglobin (HbA1C). These findings indirectly support the conclusion that surgical treatment of obese patients is successful for weight reduction and also for type II diabetes mellitus remission [62].

The research on MMPs roles in obesity is highly varied in terms of the MMPs implicated and their mechanics. The majority of research concur that MMP-1, MMP-2, MMP-8 and MMP-9 are the primary MMPs involved with obesity. If preoperative MMP-2 values in groups S, B and BS were larger than those in the C group, postoperative MMP-2 values in both B and BS groups declined dramatically, even reaching levels below those in the control group, C group. While both MMPs exhibited measurable levels, MMP-9's dynamics were very different from those of MMP-2, in that it progressed from a greater preoperative value to a decreased postoperative value for B and BS groups. The values in group S are comparable to those in group C.

Some studies in the literature have shown that corroborated this dynamic after bariatric surgery, regardless of the participants' gender or their association with hyperlipidemia, insulin resistance or hypertension. MMP-2 and MMP-9 both have a favorable correlation with weight reduction in the B and BS groups, but there is not any difference between them. Sulodexide has no statistically significant effect on MMP-2, but has a substantial but not statistically significant effect on MMP-9, with values in BS group being higher than group B.

Additionally, MMP-2 had a statistically significant positive correlation with the value of TGL both pre- and post-operatively, which did not apply for MMP-9, a biomarker that is less affected by CHO, TGL as well as glycemic alterations. However, a greater effect had the administration of sulodexide. Tchernof *et al*. [63] showed that in obese individuals, there are correlations between MMP status and the lipid state.

Although not totally free of risks, gastric bypass has a high weight loss efficiency of about 82% and a small complication rate of roughly 1%.

CONCLUSION

Metabolic surgery has been to be the most successful mean of weight reduction and improvement, if not complete remission, of obesity-related diseases. The most crucial component of this is that MMPs are involved in the remodeling of tissue after gastric bypass surgery, as revealed by these markers (especially MMP-2 and MMP-9). Thus, it is tempting to assume that adipocyte derived MMPs may constitute a novel

pharmaceutical target for limiting adipose tissue development through the reduction of adipocyte differentiation and angiogenesis. MMP-2 exhibits far more accurate oscillations than MMP-9 during pre- and post-surgical weight fluctuations, and hence may be used as a predictor for gastric bypass success. Improving the quality of life is strongly connected to weight loss, % EBWL, and hence postoperative BMI. Additionally, there is a link between an enhanced quality of life and an improved sexual life or an increased frequency of physical activity. Metabolic surgery's less invasive method enables a rapid reintegration of the patient into regular activities. Metabolic surgery should be seen in terms of the beneficial improvements it brings to daily life.

Authors' Contribution

Study Conception: RMM; Study Design: RMM, AM; Supervision: LZ, NI; Funding: ABV, DCC; Materials: ABV, DCC; Data Collection and/or Processing: RI, AR; Statistical Analysis and/or Data Interpretation: AR, RMM; Literature Review: OG, RMM; Manuscript Preparation: ABV, DCC, RMM and Critical Review: LZ, NI.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Lee YJ, Heo YS, Park HS, Lee SH, Lee SK, Jang YJ. Serum SPARC and matrix metalloproteinase-2 and metalloproteinase-9 concentrations after bariatric surgery in obese adults. Obes Surg 2014;24:604-10.
2. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 2001;17:463-516.
3. Ress C, Tschoner A, Ciardi C, Laimer MW, Engl JW, Sturm W, et al. Influence of significant weight loss on serum matrix metalloproteinase (MMP)-7 levels. Eur Cytokine Netw 2010;21:65-70.
4. Deng ZB, Liu Y, Liu C, Xiang X, Wang J, Cheng Z, et al. Immature myeloid cells induced by a high-fat diet contribute to liver inflammation. Hepatology 2009;50:1412-20.
5. Popov Y, Schuppan D. Targeting liver fibrosis: strategies for development and validation of antifibrotic therapies. Hepatology 2009;50:1294-306.
6. Guldiken S, Demir M, Arikan E, Turgut B, Azcan S, Gerenli M, et al. The levels of circulating markers of atherosclerosis and inflammation in subjects with different degrees of body mass index: soluble CD40 ligand and high-sensitivity C-reactive protein. Thromb Res 2007;119:79-84.
7. Hanusch-Enserer U, Zorn G, Wojta J, Kopp CW, Prager R, Koenig W, et al. Non-conventional markers of atherosclerosis before and after gastric banding surgery. Eur Heart J 2009;30:1516-24.
8. Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008;134:1655-69.
9. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005;115:209-18.
10. Schuppan D, Afdhal NH. Liver cirrhosis. Lancet 2008;371:838-51.
11. Mirica RM, Ionescu M, Mirica A, Ginghina O, Iosifescu R, Rosca A, et al. Quality of life assessment after bariatric surgery-- a single-center experience. Indian J Surg 2018;80:435-41.
12. Mirica RM, Ionescu M, Mirica A, Ginghina O, Iosifescu R, Rosca A, et al. The important roles of matrix metalloproteinases in the pathophysiology of obesity. Rev Chim 2017;68:1481-4.
13. de Meijer VE, Sverdlov DY, Le HD, Popov Y, Puder M. Tissue-specific differences in inflammatory infiltrate and matrix metalloproteinase expression in adipose tissue and liver of mice with diet-induced obesity. Hepatol Res 2012;42:601-10.
14. Domienik-Karłowicz J, Rymarczyk Z, Dzikowska-Diduch O, Lisik W, Chmura A, Demkow U, et al. Emerging markers of atherosclerosis before and after bariatric surgery. Obes Surg 2015;25:486-93.
15. Lijnen HR, Demeulemeester D, Van Hoef B, Collen D, Maquoi E. Deficiency of tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) impairs nutritionally induced obesity in mice. Thromb Haemost 2003;89:249-55.
16. Claffey KP, Wilkison WO, Spiegelman BM. Vascular endothelial growth factor. Regulation by cell differentiation and activated second messenger pathways. J Biol Chem 1992;267:16317-22.
17. Mirica RM, Ionescu M, Mirica A, Ginghina O, Iosifescu R, Rosca A, et al. Matrix metalloproteinases in obesity after gastric bypass surgery -- an experimental study . Indian J Surg 2020. DOI: 10.1007/s12262-020-02462-x
18. Rao SR. Inflammatory markers and bariatric surgery: a meta-analysis. Inflamm Res 2012;61:789-807.
19. Derosa G, D'Angelo A, Scalise F, Avanzini MA, Tinelli C, Peros E, et al. Comparison between metalloproteinases-2 and -9 in healthy subjects, diabetics, and subjects with acute coronary syndrome. Heart Vessels 2007;22:361-70.
20. Boden G, Song W, Pashko L, Kresge K. In vivo effects of insulin and free fatty acids on matrix metalloproteinases in rat

aorta. Diabetes 2008;57:476-83.
21. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res 2002;90:251-62.
22. Yang PJ, Ser KH, Lin MT, Nien HC, Chen CN, Yan WS, et al. Diabetes associated markers after bariatric surgery: fetuin-A, but not matrix metalloproteinase-7, is reduced. Obes Surg 2015;25:2328-34.
23. Wilkison WO, Choy L, Spiegelman BM. Biosynthetic regulation of monobutyrin, an adipocyte-secreted lipid with angiogenic activity. J Biol Chem 1991;266:16886-91.
24. Kubo Y, Kaidzu S, Nakajima I, Takenouchi K, Nakamura F. Organization of extracellular matrix components during differentiation of adipocytes in long-term culture. In Vitro Cell Dev Biol Anim 2000;36:38-44.
25. Nakajima I, Yamaguchi T, Ozutsumi K, Aso H. Adipose tissue extracellular matrix: newly organized by adipocytes during differentiation. Differentiation 1998;63:193-200.
26. Kuri-Harcuch W, Argüello C, Marsch-Moreno M. Extracellular matrix production by mouse 3T3-F442A cells during adipose differentiation in culture. Differentiation 1984;28:173-8.
27. Bouloumié A, Sengenès C, Portolan G, Galitzky J, Lafontan M. Adipocyte produces matrix metalloproteinases 2 and 9: involvement in adipose differentiation. Diabetes 2001;50:2080-6.
28. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994;94:2493-503.
29. Newby AC. Matrix metalloproteinases regulate migration, proliferation, and death of vascular smooth muscle cells by degrading matrix and non-matrix substrates. Cardiovasc Res 2006;69:614-24.
30. Newby AC. Do metalloproteinases destabilize vulnerable atherosclerotic plaques? Curr Opin Lipidol 2006;17:556-61.
31. de Meijer VE, Sverdlov DY, Popov Y, Le HD, Meisel JA, Nose V, et al. Broad-spectrum matrix metalloproteinase inhibition curbs inflammation and liver injury but aggravates experimental liver fibrosis in mice. PLoS One 2010;5:e11256.
32. de Meijer VE, Le HD, Meisel JA, Akhavan Sharif MR, Pan A, Nosé V, et al. Dietary fat intake promotes the development of hepatic steatosis independently from excess caloric consumption in a murine model. Metabolism 2010;59:1092-105.
33. Ailhaud G, Grimaldi P, Négrel R. Cellular and molecular aspects of adipose tissue development. Annu Rev Nutr 1992;12:207-33.
34. Zangani D, Darcy KM, Masso-Welch PA, Bellamy ES, Desole MS, Ip MM. Multiple differentiation pathways of rat mammary stromal cells in vitro: acquisition of a fibroblast, adipocyte or endothelial phenotype is dependent on hormonal and extracellular matrix stimulation. Differentiation 1999;64:91-101.
35. Szczęsny W, Kuligowska-Prusińska M, Dąbrowiecki S, Szmytkowski J, Reśliński A, Słupski M. Activity of metalloproteinases and adiponectin in obese patients-a possible factor of incisional hernias after bariatric procedures. J Zhejiang Univ Sci B 2018;19:65-70.
36. Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S. Adipokines: molecular links between obesity and atheroslcerosis. Am J Physiol Heart Circ Physiol 2005;288:H2031-41.
37. Krebs M, Geiger M, Polak K, Vales A, Schmetterer L, Wagner OF, et al. Increased plasma levels of plasminogen activator inhibitor-1 and soluble vascular cell adhesion molecule after triacylglycerol infusion in man. Thromb Haemost 2003;90:422-8.
38. Iribarren C. Lipoprotein-associated phospholipase A2 and cardiovascular risk: state of the evidence and future directions. Arterioscler Thromb Vasc Biol 2006;26:5-6.
39. Freedman JE. CD40 ligand--assessing risk instead of damage? N Engl J Med 2003;348:1163-5.
40. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87-91.
41. Grzechocińska B, Dąbrowski FA, Sierdzinski J, Cyganek A, Wielgoś M. The association between serum metalloproteinase concentration, obesity, and hormone levels in reproductive-aged women. Endokrynol Pol 2019;70:49-56.
42. Blann AD, Lip GY. Hypothesis: is soluble P-selectin a new marker of platelet activation? Atherosclerosis 1997;128:135-8.
43. Marx N, Imhof A, Froehlich J, Siam L, Ittner J, Wierse G, et al. Effect of rosiglitazone treatment on soluble CD40L in patients with type 2 diabetes and coronary artery disease. Circulation 2003;107:1954-7.
44. Varo N, Vicent D, Libby P, Nuzzo R, Calle-Pascual AL, Bernal MR, et al. Elevated plasma levels of the atherogenic mediator soluble CD40 ligand in diabetic patients: a novel target of thiazolidinediones. Circulation 2003;107:2664-9.
45. Bermudez EA, Rifai N, Buring J, Manson JE, Ridker PM. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. Arterioscler Thromb Vasc Biol 2002;22:1668-73.
46. Henn V, Slupsky JR, Gräfe M, Anagnostopoulos I, Förster R, Müller-Berghaus G, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature 1998;391:591-4.
47. Déchanet J, Grosset C, Taupin JL, Merville P, Banchereau J, Ripoche J, et al. CD40 ligand stimulates proinflammatory cytokine production by human endothelial cells. J Immunol 1997;159:5640-7.
48. Slupsky JR, Kalbas M, Willuweit A, Henn V, Kroczek RA, Müller-Berghaus G. Activated platelets induce tissue factor expression on human umbilical vein endothelial cells by ligation of CD40. Thromb Haemost 1998;80:1008-14.
49. Zhou L, Stordeur P, de Lavareille A, Thielemans K, Capel P, Goldman M, et al. CD40 engagement on endothelial cells promotes tissue factor-dependent procoagulant activity. Thromb Haemost 1998;79:1025-8.
50. Silva SA, Gobbo MG, Pinto-Fochi ME, Rafacho A, Taboga SR, Almeida EA, et al. Prostate hyperplasia caused by long-term obesity is characterized by high deposition of extracellular matrix and increased content of MMP-9 and VEGF. Int J Exp Pathol 2015;96:21-30.
51. André P, Prasad KS, Denis CV, He M, Papalia JM, Hynes RO, et al. CD40L stabilizes arterial thrombi by a beta3 integrin--dependent mechanism. Nat Med 2002;8:247-52.
52. Henn V, Steinbach S, Büchner K, Presek P, Kroczek RA. The inflammatory action of CD40 ligand (CD154) expressed on ac-

tivated human platelets is temporally limited by coexpressed CD40. Blood 2001;98:1047-54.
53. Kräling BM, Wiederschain DG, Boehm T, Rehn M, Mulliken JB, Moses MA. The role of matrix metalloproteinase activity in the maturation of human capillary endothelial cells in vitro. J Cell Sci 1999;112 (Pt 10):1599-609.
54. Owolabi US, Amraotkar AR, Coulter AR, Singam NSV, Aladili BN, Singh A, et al. Change in matrix metalloproteinase 2, 3, and 9 levels at the time of and after acute atherothrombotic myocardial infarction. J Thromb Thrombolysis 2020;49:235-44.
55. Tuomainen AM, Nyyssönen K, Laukkanen JA, Tervahartiala T, Tuomainen TP, Salonen JT, et al. Serum matrix metalloproteinase-8 concentrations are associated with cardiovascular outcome in men. Arterioscler Thromb Vasc Biol 2007;27:2722-8.
56. Sternlicht MD, Bergers G. Matrix metalloproteinases as emerging targets in anticancer therapy: status and prospects. Emerging Ther Targets 2000;4:609-33.
57. Lochter A, Galosy S, Muschler J, Freedman N, Werb Z, Bissell MJ. Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. J Cell Biol 1997;139:1861-72.
58. Mannello F, Medda V, Ligi D, Raffetto JD. Glycosaminoglycan sulodexide inhibition of MMP-9 gelatinase secretion and activity: possible pharmacological role against collagen degradation in vascular chronic diseases. Curr Vasc Pharmacol 2013;11:354-65.
59. Lillis J, Kendra KE. Acceptance and Commitment Therapy for weight control: Model, evidence, and future directions. J Contextual Behav Sci 2014;3:1-7.
60. Kawano Y, Ohta M, Hirashita T, Masuda T, Inomata M, Kitano S. Effects of sleeve gastrectomy on lipid metabolism in an obese diabetic rat model. Obes Surg 2013;23:1947-56.
61. Kalaivani A, Uddandrao VVS, Parim B, Saravanan G, Nivedha PR, Sushma CK, et al. Reversal of high fat diet-induced obesity through modulating lipid metabolic enzymes and inflammatory markers expressions in rats. Arch Physiol Biochem 2019;125:228-34.
62. Esquivel AL, Pérez-Ramos J, Cisneros J, Herrera I, Rivera-Rosales R, Montaño M, et al. The effect of obesity and tobacco smoke exposure on inflammatory mediators and matrix metalloproteinases in rat model. Toxicol Mech Methods 2014;24:633-43.
63. Tchernof A, Després JP. Pathophysiology of human visceral obesity: an update. Physiol Rev 2013;93:359-404.

The European Research Journal 2022;8(3):420-427 Case Report

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Two male patients from an extended seven generation Turkish family diagnosed with Renpenning syndrome: identifying the causative mutation and review of the literature

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ABSTRACT

Intellectual disability (ID) is a lifelong condition that begins during the developmental period, and characterized by significant limitations in intellectual functioning and adaptive behavior including social, conceptual and practical skills. In these case series, we aimed to identify the genetic etiopathogenesis of two male patients with ID from a seven-generation large-Turkish family. Two affected boys with syndromic ID were evaluated. Genome-wide auto zygosity mapping was performed on affected individuals and other available healthy family members for identifying shared chromosomal segments between affected individuals. Critical region co-segregating with the disease was confirmed and narrowed down by short tandem repeat polymorphism markers. Whole exome sequencing was performed to identify the responsible genes and mutations. Sanger sequencing was performed for segregation analysis. We performed a comprehensive genetic analysis to reveal the underlying genetic aetiology of the patients and identified a mutation on *PQBP1* gene (NM_005710.2:c.459-462delAGAG) that is associated with Renpenning syndrome. Considering the clinical findings and genetic data of the affected probands, the patients were diagnosed with Renpenning syndrome and this is the first report for Renpenning syndrome with attention deficit and hyperactivity disorder comorbidity.

Keywords: Renpenning syndrome, PQBP1, intellectual disability, microcephaly

Intellectual disability (ID) is a lifelong condition beginning during the developmental period, and characterized by significant limitations in intellectual functioning and adaptive behavior (DSM-V). There are tremendous genetic heterogeneity for ID caused by single-gene mutations [1]. It is estimated that approximately more than 2 000 genes of the genome can be associated with ID [2]. IDs are more frequent in males than females and that X-linked gene defects have major roles in the aetiology of X-linked IDs (XLID) [3].

Renpenning syndrome (OMIM #309500) is an XLID syndrome characterized by ID ranging from mild to severe. These patients have a variety of phenotypic abnormalities including microcephaly, short stature, small testes and facial dysmorphisms includ-

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ing tall narrow facies, nasal anomalies, short philtrum and cupped ears [4-6]. Almost all of the patients are male but recently a female proband has been reported by Raymond *et al*. [7] due to complete skewing of the X chromosome inactivation.

In these case series, we aimed to identify the genetic etiopathogenesis of two male patients that belong to a seven-generation extended Turkish family. The probands had syndromic ID (S-ID) with mild dysmorphic findings. Since S-ID is a very heterogeneous syndrome in terms of both phenotype and genotype we aimed to perform genome-wide autozygosity mapping to map and identify all shared chromosomal segments. Then, in order to identify the causative mutant gene that shared between affected probands we performed whole exome sequencing (WES). We herein review and discuss the findings in view of the literature.

CASES PRESENTATION

Patients

Two affected individuals of a large Turkish family with S-ID were evaluated (Fig. 1). The study protocol was approved by the Ethics Committee of Karadeniz Technical University, Faculty of Medicine (approval

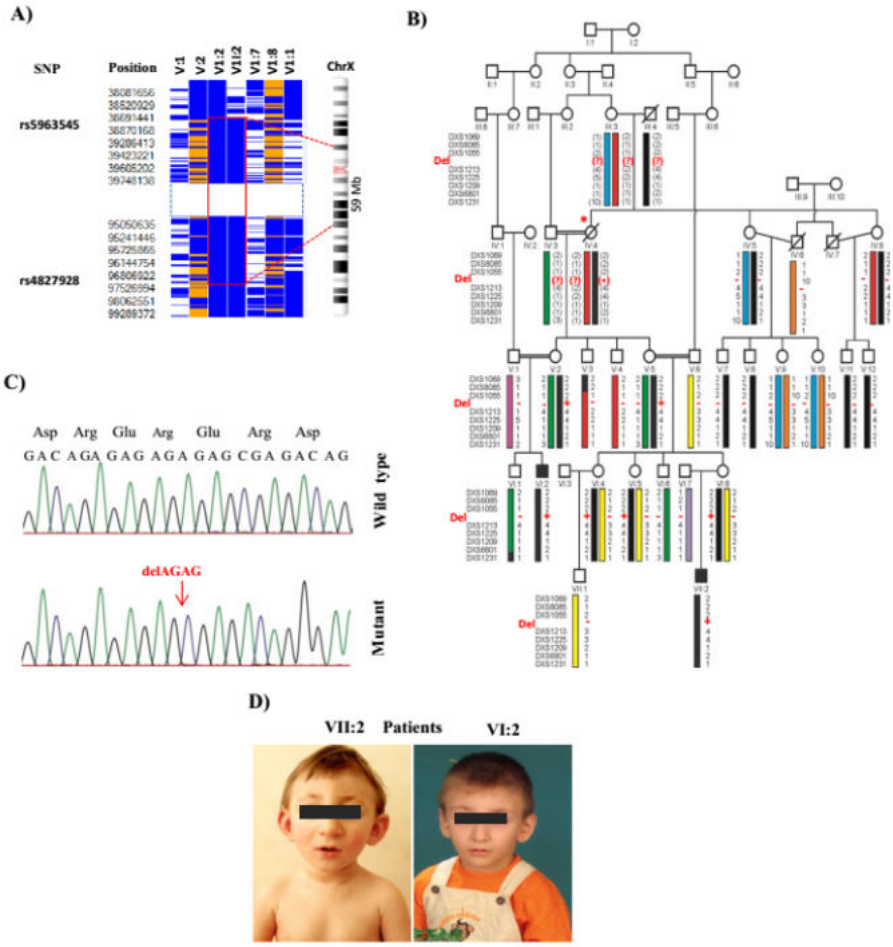

Fig. 1. Genome-wide autozygosity mapping and haplotype analysis. (A) Schematic representation of the genome-wide homozygosity mapping data. Homozygous genotypes identical to the genotype data obtained from the affected individuals VI:2 and VII:2 are shown in blue, non-homozygous genotypes are shown in white and heterozygous genotypes appear in orange. A single homozygote segment, which is marked as a vertical rectangle, is approximately 55 Mb in size. SNPs correspond to borders of a hemizygous segment, are marked as horizontal rectangle and shown on the left side of the image. The location of the homozygote segment on the X chromosome ideogram is shown on the right side of the image. (B) Pedigree and haplotype analysis of critical chromosomal region with selected STR markers on X chromosome shown at the left side of the pedigree. The mutation segregation is shown in red. The mutant delAGAG allele was originated from the IV:4 female subject as de novo and inherited to her descents (red star). (C) Wild type and mutant allele Sanger sequencing chromatogram. The mutation region is shown in red. (D) Two patients' photographs.

number: 2013/73). An informed consent was obtained from the guardians of the patients for publication.

DNA Isolation, PCR Reactions and Sanger Sequencing

Genomic DNA isolation was performed from peripheral blood of patients and controls by using standard protocol (salting-out). All PCR primers were designated by primer3 program [8]. Sanger sequencing was performed on ABI 3130 platform by using

Table 1. Clinical findings of both patients in the family

General	Features	Patient VII:2	Patient VI:2
Age	Year of birth	2010	2003
	Age at diagnosis	1	8
Physical	Height	73 cm (10p)	120 cm (10p)
	Head circumference	41.5 cm (<3p) -3.81SD	47.4 cm (<3p) -4SD
Dysmorphic findings	Long face	+	-
	Triangular face	+	+
	Micrognathia / Mandibular prognathia	≠	+ / -
	Large cupped ears	+	+
	Prominent nasal bridge	-	-
	Wide nasal bridge	+	+
	Cleft Palate / Lip	- / -	- / -
	High Palate	+	+
	Thin upper lip	-	+
	Thin hair	*	+
	Laterally sparse eyebrows	+	+
Genital anomalies	Small testis	-	Unspecified
Skeletal system findings	Clinodactyly of the 5th fingers	-	-
	Camptodactyly	-	-
	Scoliosis	-	-
	Talipes equinovarus (TEV)	-	+ (Orthosis treatment)
Neurological findings	Global developmental delay	+	+
	Intellectual disability / IQ	? / Unspecified	+ / Mild (IQ=50)
	Hyperreflexia	-	Unspecified
	Spasticity	-	Unspecified
	MRI	-	The third, fourth and lateral ventricular enlargement
	EEG	-	+ (normal)
	Seizures	-	-
Psychiatric findings	Autistic features	?	-
	Attention deficit hyperactivity disorder (ADHD)	-	+
	Emotional lability	-	+

≠Lower jaw growth continues until the age of 4.

*Permanent terminal hair occurs after 2 years of age.

?These diagnoses cannot be done at this age.

BigDye™ Terminator v3.1 Cycle Sequencing Kit according to manufacturer's protocol.

Genome-Wide Autozygosity Mapping and Whole Exome Sequencing

Genome-wide autozygosity mapping was carried out by using Illumina HumanCytoSNP-12 v2.1® platform. To identify the disease segregating genomic segments between affected families, raw data of the Illumina Iscan® platform was transferred to the VIGENOS (Visual Genome Studio, Hemosoft, Ankara) program which facilitates visualization of the large quantity of genomic data in comprehensible visual screens. WES performed by the Illumina HiSeq® platform. Reads were aligned to human genome reference consortium build 37 (GRCh37/hg19) and subsequently, the WES analysis pipeline was performed according to Genome Analysis Toolkit (GATK) best practices [9]. Sanger sequencing was used to confirm the presence of the identified mutation in affected individuals and to perform segregation analysis.

Clinical Findings

We studied a seven-generation Turkish family in which two affected males (VI:2 and VII:2) were born with S-ID from consanguineous parents (Figs. 1A and 1B). Common shared clinical findings in both patients include microcephaly, triangular face, highly arched palate, beaked nose, cupped ears, laterally sparse eyebrows and developmental delay (Table 1).

Case 1 (VII)

He was first evaluated by child neurology due to developmental delay and dysmorphic findings. Because the age of the proband is under of two psychiatric evaluations was not conducted.

Case 2 (VI)

He was first evaluated by child psychiatry and child neurology with speech delay at the age of 3 and referred to special education due to developmental delay. During the follow-up, the patient had complaints of restlessness, not paying attention to the course and irritability and diagnosed with attention deficit and hyperactivity disorder (ADHD), comorbid oppositional and defiant disorder based on DSM-IV-based disruptive behavioral disorders screening and evaluation scale [10]. The form was completed by the patient's caregivers and teachers. The clinical interview was completed by a child-adolescent psychiatrist.

Genome-Wide Autozygosity Mapping

Critical region co-segregating with the disease was confirmed and narrowed down by short tandem repeat polymorphism markers genotyping on the X chromosome of two affected individuals and their family members because the pedigree of the family was consistent with the X-chromosomal inheritance pattern of the disease (Figs. 1A and 1B), and narrowed the disease co-segregating region to a 55 Mb region. But, unexpectedly we saw that the critical region on X-chromosome was shared with unaffected male family members (Fig. 1B). Thus, we decided to perform WES as trio (VI:7, VI:8, and VII:2).

Whole Exome Sequencing

We found a four base-pair deletion mutation on polyglutamine-binding protein 1 (PQBP1) (NM_005710.2:c.459-462delAGAG;p.(Arg153SerfsX41)) gene (generally known as delAGAG). This gene and mentioned mutation were associated with Renpenning syndrome, before [11].

Confirming Mutation and Performing Mutant Allele Segregation Analysis

This finding was verified by Sanger sequencing and it was shown that this particular mutation was present exclusively in affected individuals and in their obligate carrier mothers (V:2, VI:8) and sisters (V1:4, V1:5, and V:5). We also performed mutant PQBP1 allele segregation analysis on all available family members by Sanger sequencing (Figs. 1B and 1C). When we analyzed the pedigree, shared haplotypes and mutation segregation we saw that the mutant delAGAG allele was originated from the IV:4 female subject as de novo and inherited to her descents. The sisters of the female subject of the IV:4 (those are IV:5 and IV:8) and their progeny did not have the mutant delAGAG allele (Fig. 1B).

DISCUSSION

Renpenning syndrome is an ultra-rare (estimated incidence < 1:1 000 000) [12] subgroup of XLID and is characterized by a variety of phenotypic findings in-

Table 2. Comparison of clinical findings in reported families with Renpenning syndrome

Findings	Renpenning *et al.* [11]	Golabi *et al.* [24]	Sutherland *et al.* [25]	Porteous *et al.* [4]	Hamel *et al.* [26]	Lenski *et al.* [27]	MRX55 [4]	Family N9 [4]	Family N45 [4]	Stevenson et al.[4]	Present study	Total
Ancestry	Dutch	?	English	Scottish	Dutch?	?	Moroccan	Dutch	Dutch	?	Turkish	
Affected males	20	3	8	6	4	2	3	3	3	4	2	58
Short stature < 3rd centile	6/10	3/3	5/8	1/2	1/1	1/1	1/1	2/3	1/3	0/4	0/2	21/38 (55%)
Head circumference < 3rd centile	13/14	3/3	7/8	0/2	4/4	2/2	0/1	3/3	3/3	3/4	2/2	40/46 **(86%)**
Long/Triangular face	0/6	3/3	2/8	2/2	2/2	1/1	1/1	2/2	3/3	3/4	2/2	21/34 (61%)
Mandibular prognathia	2/4	-	2/8	1/2	1/2	0/2	-	1/3	2/3	0/4	0/2	9/30 (30%)
Large ears	1/10	-	1/8	1/2	-	-	-	0/3	0/3	1/4	2/2	6/30 (20%)
Cupped ears	0/5	3/3	-	1/2	3/3	-	-	1/3	1/3	4/4	2/2	15/25 (60%)
Dystopia canthorum / Wide nasal bridge	0/5	2/3	-	0/2	-	-	-	-	-	0/4	2/2	4/16 (25%)
Cleft Palate	0/20	0/3	0/8	0/2	2/4	0/2	-	2/3	0/3	1/4	0/2	5/51 (9 %)
High Palate	0/5	-	0/8	0/2	1/4	-	-	3/3	3/3	0/4	2/2	9/31 (29%)
Thin upper lip/Short philtrum	5/5	-	-	1/2	2/2	1/1	1/1	2/2	2/3	0/4	1/2	15/22 **(68%)**
Thin/ brittle hair	0/5	2/3	-	0/2	0/2	-	-	0/3	0/3	0/4	1/2	3/24 (12%)
Small testis	4/9	0/2	4/5	0/2	0/1	-	-	0/3	0/3	2/3	-	10/28 (35%)
Clinodactyly of the 5th fingers	-	-	0/8	0/2	2/2	-	-	0/3	0/3	0/4	0/2	2/24 / (8%)
Global developmental delay	20/20	3/3	5/8	6/6	3/3	2/2	3/3	3/3	3/3	4/4	2/2	54/57 **(94%)**
Intellectual disability / Mild (IQ 50-70)	1/14	0/1	1/8	6/6	0/3	0/2	-	0/2	0/3	0/2	1/2	9/43 (20%)
Spasticity	0/5	3/3	6/8	0/6	3/3	1/1	-	0/3	0/3	0/4	-	13/33 (36%)
Seizures	3/20	1/2	0/8	0/2	-	1/2	-	0/3	0/3	0/4	0/2	5/46 (10%)
Attention deficit hyperactivity disorder (ADHD)	-	-	-	-	-	-	-	-	-	-	1/2	-

cluding microcephaly, short stature, dysmorphic facies, lean body, small testes, and a varying degree of ID [4, 11-14]. It is stated that in this syndrome, as being for most genetic syndromes, phenotypic variability is the rule rather than the exception [4]. Thus, in accordance with this already reported phenotypic variability, our patients with Renpenning syndrome shared some common findings as microcephaly, triangular face, large cupped ears and IDs while some of their clinical findings were discordant (Tables 1 and 2). Our literature reviews about clinical findings of reported patients with Renpenning syndrome are summarized in Table 2. Accordingly the most common clinical findings are global developmental delay (94%), microcephaly (86%) and thin upper lip/short philtrum (68%), respectively.

The *PQBP1* gene contains nine transcripts and two conserved coding sequences (CCDS) (Fig. 2A). The delAGAG mutation leads to a premature stop codon, resulting in a truncated version of the protein that partially lacks proline rich domain (PRD) and completely lacks nuclear localization sequence (NLS) and carboxy terminal domain (CTD) domains of the *PQBP1* protein (Figs. 2B and 2C).

Musante *et al*. [15] showed that in their patient-derived cells, the mRNAs carrying indel mutations

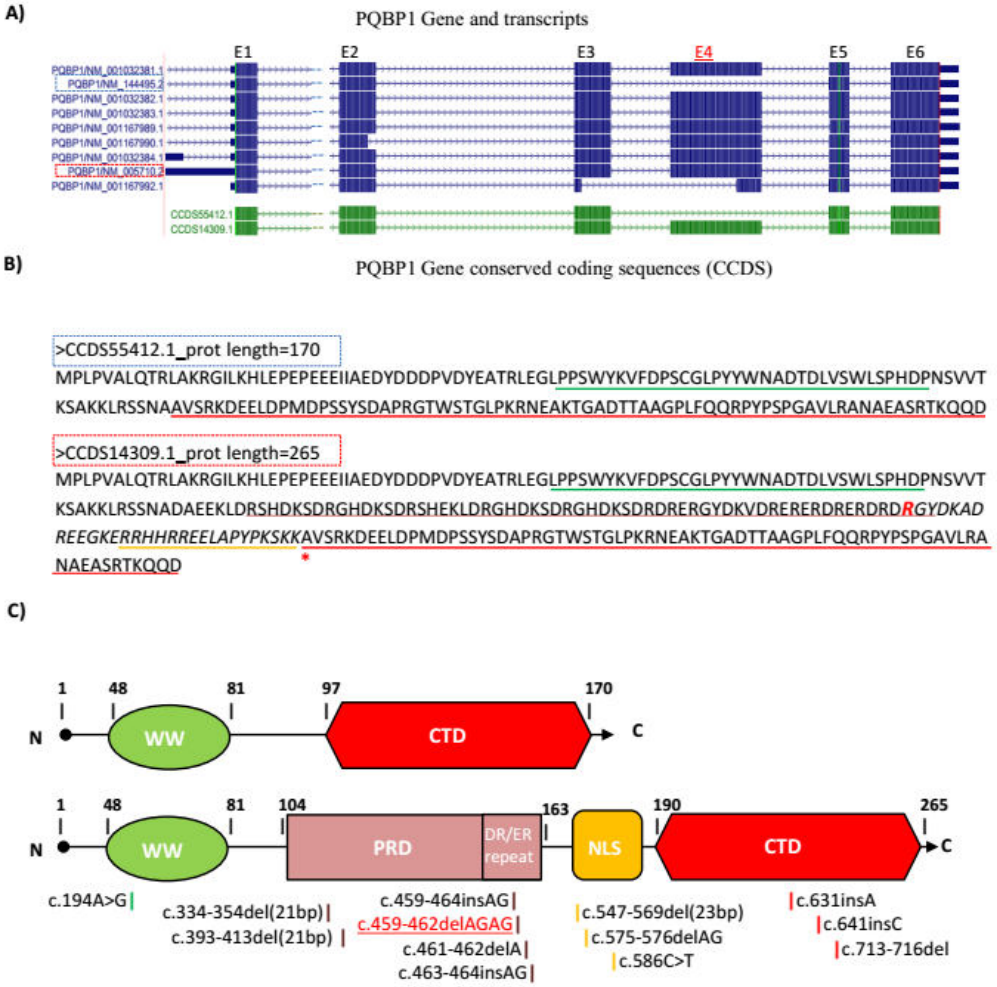

Fig. 2. The gene model, transcripts, reported mutations, and protein products of the PQBP1 gene. (A) A UCSC genome browser screenshot of the PQBP1gene and transcripts. Horizontal blue rectangles and the E symbol upside of the rectangles show exons and exon numbers, respectively. Horizontal green rectangles show conserved coding sequences. The delAGAG mutation localized exon (E4) is depicted as red. The blue and red dotted rectangles show the 19 kDa and 37 kDa protein-coding transcripts of the PQBP1gene, respectively. (B) Protein amino acid sequences of the two conserved coding sequences. Corresponding amino acid sequences to PQBP1 protein domains are underlined with colors as depicted in C, and the effect of delAGAG mutation on amino acid sequences depicted as italic. The star symbol at the bottom of Alanine (A) depicts the stop codon position. (C) Schematic drawing of domains of the PQBP1 protein. All available reported mutations causing Renpenning syndrome is shown at the bottom of the corresponding domains. Our mutation was shown as red. PRD = Proline rich domain, NLS = Nuclear localization sequence, CTD = Carboxy terminal domain, WW: WW domain.

that cause premature stop codon on the PRD domain including delAGAG, incur nonsense mediated decay (NMD) and result in decreased expressions of the mutant mRNAs and associated protein truncated variants. Further, they showed the existence of the 19 kDa protein isoform (Figs. 2B and 2C), which is an unaffected product from delAGAG and other indel mutations located on PRD/NLS domains, in control and in patient-derived cells. Many functional studies were done in relation to microcephaly and ID causes of these mutations on *PQBP1* to date. One of these showed that indel mutant forms of the protein (delAG and delAGAG) binds to Fragile X-mental retardation protein (FMRP) by a gain-of-function pathogenic mechanism and promotes its degradation [16]. Because FMRP is an important regulator of homeostatic synaptic scaling, dysregulated FMRP degradation might cause disruption of neuronal development that leads to intellectual disability. In addition, *PQBP1* regulates nuclear events such as splicing and gene transcription [17, 18].

To our knowledge present study is the second report for Renpenning syndrome from Turkey. Kurt Colak *et al*. [19] reported recently a patient they suspected of having Renpenning syndrome, but in addition, they also suspected from the *PACS1* related Schuurs-Hoeijmakers syndrome for their reported patient. Their reported second de novo mutation on the PACS1 gene (c.640C>T; p.(Arg214Trp)) is a well-known and published disease-causing mutation in medical literature for Schuurs-Hoeijmakers syndrome [20-23]. De novo *PACS1* pathogenic variant and phenotypic findings were thought to be compatible with Schuurs-Hoeijmakers. There was no phenotype in favor of blended phenotype and/or dual diagnosis, and can be regarded genotypically as pathogenic variant *PACS1*.

CONCLUSION

In this study, we have performed a comprehensive genetic analysis to reveal the underlying genetic etiology of two male patients from a large extended Turkish family and identified that the responsible mutation is a known delAGAG on the *PQBP1* gene and patients are affected from Renpenning syndrome. It is clear from the functional studies mentioned above that the delAGAG mutation results in truncated protein and causes Renpenning syndrome. In addition, it is worth analyzing further the cellular function of the 19 kDa protein isoform of the *PQBP1* gene and its effect on heterogeneity and clinical severity of the Renpenning syndrome.

Authors' Contribution

Study Conception: BT; Study Design: HK, EK; Supervision: HK, EK; Funding: EK; Materials: SÇB, HK, EK; Data Collection and/or Processing: BT, HK, EK; Statistical Analysis and/or Data Interpretation: BT, TD, GYB; Literature Review: BT; Manuscript Preparation: BT and Critical Review: TD, HK, EK.

Informed Consent

Written informed consent was obtained from the families of the patients for publication of these cases and any accompanying images or data.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Stevenon RE, Procopio-Allen AM, Schroer RJ, Collins JS. Genetic syndromes among individuals with mental retardation. Am J Med Genet A 2003;123A:29-32.
2. Chiurazzi P, Pirozzi F. Advances in understanding – genetic

basis of intellectual disability. F1000Research 2016;5:599.
3. Ropers H-H. X-linked mental retardation: many genes for a complex disorder. Curr Opin Genet Dev 2006;16:260-9.
4. Stevenson RE, Bennett CW, Abidi F, Kleefstra T, Porteous M, Simensen RJ, et al. Renpenning syndrome comes into focus. Am J Med Genet A 2005;134:415-21.
5. Germanaud D, Rossi M, Bussy G, et al. The Renpenning syndrome spectrum: new clinical insights supported by 13 new PQBP1-mutated males. Clin Genet 2011;79:225–235.
6. Mameesh MM, Al-Kindy A, Al-Yahyai M, Ganesh A. Microphthalmos-anophthalmos-coloboma (MAC) spectrum in two brothers with Renpenning syndrome due to a truncating mutation in the polyglutamine tract binding protein 1 (PQBP1) gene. Ophthalmic Genet 2019;40:534-40.
7. Cho RY, Peñaherrera MS, Du Souich C, Huang L, Mwenifumbo J, Nelson TN, et al. Renpenning syndrome in a female. Am J Med Genet Part A 2020;182:498-503.
8. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, et al. Primer3-new capabilities and interfaces. Nucleic Acids Res 2012;40:e115.
9. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010;20:1297-303.
10. Silva RR, Alpert M, Pouget E, Silva V, Trosper S, Reyes K, et al. A rating scale for disruptive behavior disorders, Based on the DSM-IV Item Pool. Psychiatr Q 2005;76:327-39.
11. Renpenning H, Gerrard JW, Zaleski WA, Tabata T. Familial sex-linked mental retardation. Can Med Assoc J 1962;87:954-6.
12. Rappaport N, Nativ N, Stelzer G, et al. MalaCards: an integrated compendium for diseases and their annotation. Database 2013;2013:bat018.
13. Ropers H-H, Hamel BCJ. X-linked mental retardation. Nat Rev Genet 2005;6:46-57.
14. des Portes V. X-linked mental deficiency. In: Handbook of clinical neurology. 2013:pp. 297-306.
15. Musante L, Kunde S-A, Sulistio TO, Fischer U, Grimme A, Frints SGM, et al. Common pathological mutations in PQBP1 induce nonsense-mediated mRNA decay and enhance exclusion of the mutant exon. Hum Mutat 2010;31:90-8.
16. Zhang X-Y, Qi J, Shen Y-Q, Liu X, Liu A, Zhou Z, et al. Mutations of PQBP1 in Renpenning syndrome promote ubiquitin-mediated degradation of FMRP and cause synaptic dysfunction. Hum Mol Genet 2017;26:955-68.
17. Waragai M, Lammers CH, Takeuchi S, Imafuku I, Udagawa Y, Kanazawa I, et al. PQBP-1, a novel polyglutamine tract-binding protein, inhibits transcription activation by Brn-2 and affects cell survival. Hum Mol Genet 1999;8:977-87.
18. Wang Q, Moore MJ, Adelmant G, Marto JA, Silver PA. PQBP1, a factor linked to intellectual disability, affects alternative splicing associated with neurite outgrowth. Genes Dev 2013;27:615-26.
19. Kurt Colak F, Eyerci N, Aytekin C, Eksioğlu AS. Renpenning syndrome in a Turkish patient: de novo variant c.607C>T in PACS1 and hypogammaglobulinemia phenotype. Mol Syndromol 2020;11:157-61.
20. Schuurs-Hoeijmakers JHM, Landsverk ML, Foulds N, Kukolich MK, Gavrilova RH, Greville-Heygate S, et al. Clinical delineation of the PACS1-related syndrome--Report on 19 patients. Am J Med Genet A 2016;170:670-5.
21. Stern D, Cho MT, Chikarmane R, Willaert RW, Retterer K, Kendal F, et al. Association of the missense variant p.Arg203Trp in PACS1 as a cause of intellectual disability and seizures. Clin Genet 2017;92:221-3.
22. Hoshino Y, Enokizono T, Imagawa K, Tanaka R, Suzuki H, Fukushima H, et al. Schuurs-Hoeijmakers syndrome in two patients from Japan. Am J Med Genet A 2019;179:341-3.
23. Schuurs-Hoeijmakers JHM, Oh EC, Vissers LELM, Swinkwls MEM, Gilissen C, Willemsen MA, et al. Recurrent de novo mutations in PACS1 cause defective cranial-neural-crest migration and define a recognizable intellectual-disability syndrome. Am J Hum Genet 2012;91:1122-7.
24. M Golabi, M Ito BH. A new X-linked multiple congenital anomalies/ mental retardation syndrome. Am J Med Genet 1984;17:367-74.
25. Sutherland GR, Gedeon AK, Haan EA, Woodroffe P, Mulley JC, et al. Linkage studies with the gene for an X-linked syndrome of mental retardation, microcephaly and spastic diplegia (MRX2). Am J Med Genet 1988;30:493-508.
26. Hamel BCJ, Mariman ECM, van Beersum SEC, Schoonbrood-Lenssen AM, Ropers HH. Mental retardation, congenital heart defect, cleft palate, short stature, and facial anomalies: a new X-linked multiple congenital anomalies/mental retardation syndrome: clinical description and molecular studies. Am J Med Genet 1994;51:591-7.
27. Lenski C, Abidi F, Meindl A, Gibson A, Platzer M, Kooy RF, et al. Novel truncating mutations in the polyglutamine tract binding protein 1 gene (PQBP1) cause Renpenning syndrome and X-linked mental retardation in another family with microcephaly. Am J Hum Genet 2004;74:777-80.